# HORIZON 2030: INNOVATIVE APPLICATIONS OF HEART RATE VARIABILITY

EDITED BY: Sylvain Laborde, Julian F. Thayer, Emma Mosley and Clint Bellenger

PUBLISHED IN: Frontiers in Neuroscience, Frontiers in Physiology, Frontiers in Neurology and Frontiers in Sports and Active Living

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-978-2
DOI 10.3389/978-2-88974-978-2

## About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

# HORIZON 2030: INNOVATIVE APPLICATIONS OF HEART RATE VARIABILITY

Topic Editors:
**Sylvain Laborde,** German Sport University Cologne, Germany
**Julian F. Thayer,** The Ohio State University, United States
**Emma Mosley,** Southampton Solent University, United Kingdom
**Clint Bellenger,** University of South Australia, Australia

**Citation:** Laborde, S., Thayer, J. F., Mosley, E., Bellenger, C., eds. (2022). Horizon 2030: Innovative Applications of Heart Rate Variability.
Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-978-2

# Table of Contents

frontiers | Frontiers in Neuroscience

EDITORIAL
published: 03 June 2022
doi: 10.3389/fnins.2022.937086

# Editorial: Horizon 2030: Innovative Applications of Heart Rate Variability

*Sylvain Laborde*[1,2]*, *Emma Mosley*[3], *Clint Bellenger*[4,5] and *Julian Thayer*[6]

[1] Department of Performance Psychology, Institute of Psychology, German Sport University Cologne, Cologne, Germany, [2] Normandie Université Caen, Unité de Formation et de Recherche des Sciences et Techniques des Activités Physiques et Sportives, Caen, France, [3] Solent University, Southampton, United Kingdom, [4] Allied Health and Human Performance Unit, Alliance for Research in Exercise, Nutrition and Activity (ARENA), University of South Australia, Adelaide, SA, Australia, [5] South Australian Sports Institute, Adelaide, SA, Australia, [6] Department of Psychological Science, University of California, Irvine, Irvine, CA, United States

**Keywords: heart rate variability (HRV), parasympathetic nervous system (PNS), vagus nerve, vagus nerve (VN) stimulation, wearable**

**Editorial on the Research Topic**

**Horizon 2030: Innovative Applications of Heart Rate Variability**

## INTRODUCTION

The Guest Editors are delighted to showcase 39 papers for this Frontiers Research Topic (RT) "Horizon 2030: Innovative Applications of Heart Rate Variability". We are thankful to all 139 contributors, representing institutions from 16 countries (See **Table 1**). There is a growing genuine interest in this topic as there have been over 175,000 views as of April 2022. This is also a reflection of the growing use of heart rate variability (HRV) in scientific publications. The number of studies mentioning HRV since the 1970s has grown significantly and is now reaching almost 2000 papers per year (see **Figure 1**). This rapid growth makes it challenging to see and understand the broader interpretation of research findings. This Editorial provides a contextual overview to our RT, building upon previous influential Frontiers RT on HRV (e.g., Billman et al., 2015, 2019; Drury et al., 2019), with a view to highlight conceptual considerations for HRV in the future.

Prior to discussing the contributions, the Guest Editors will put this RT into a broader context, anchoring it within classical work, and showcasing how it can advance current HRV knowledge. HRV, the variation in the time intervals between adjacent heartbeats (Malik, 1996; Berntson et al., 1997; Laborde et al., 2017), has become one of the most popular psychophysiological measures in recent times. Its use by researchers and practitioners spans across many different fields, encompassing medicine, the health sciences, psychology, exercise and sport sciences, and other disciplines. The growing popularity of HRV is perhaps due to its desirable characteristics: non-invasive, low cost, and its recording feasibility across a large range of settings. Above all, HRV is capable of indexing the activity of the vagus nerve - the main nerve of the parasympathetic nervous system - regulating cardiac functioning, through cardiac vagal activity (also referred to as cardiac vagal tone) (Malik, 1996; Berntson et al., 1997; Laborde et al., 2017). Cardiac vagal activity is associated with many functions in self-regulation, adaptation, and health (Thayer and Lane, 2009; Thayer et al., 2009; Shaffer et al., 2014; Laborde et al., 2017, 2018b; Smith et al., 2017), thus making it a key marker of interest to researchers.

OPEN ACCESS

***Edited and reviewed by:***
*Joel C. Bornstein,*
*The University of Melbourne, Australia*

***Correspondence:**
*Sylvain Laborde*
*s.laborde@dshs-koeln.de*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *05 May 2022*
***Accepted:*** *13 May 2022*
***Published:*** *03 June 2022*

***Citation:***
*Laborde S, Mosley E, Bellenger C and Thayer J (2022) Editorial: Horizon 2030: Innovative Applications of Heart Rate Variability. Front. Neurosci. 16:937086. doi: 10.3389/fnins.2022.937086*

**TABLE 1 |** List of countries represented in the Research Topic, based on first author's institution.

| 1st author institution country | Number |
|---|---|
| United States | 11 |
| Italy | 9 |
| Germany | 5 |
| Netherlands | 2 |
| United Kingdom | 2 |
| Australia | 1 |
| Austria | 1 |
| Canada | 1 |
| China | 1 |
| France | 1 |
| Israel | 1 |
| Japan | 1 |
| New Zealand | 1 |
| South Korea | 1 |
| Spain | 1 |

*A 16th country was also represented within the affiliation of co-authors, Switzerland.*

In order to guide readers through the broad diversity of the papers constituting this RT, we organize and integrate the contributions in relation to theoretical, methodological, and applied considerations related to HRV.

## HRV THEORY: THE NEED TO BASE ONE'S RESEARCH ON SOLID THEORETICAL FOUNDATIONS

One of the primary goals of this RT was to address an aspect often neglected in HRV research, namely the theoretical foundations supporting it. Given the accessibility of HRV measurement, it is tempting to use it for any application researchers may deem interesting. However, given the dozens of HRV parameters that one can calculate based on RR interval data (for an overview of HRV parameters and their physiological underpinning, see Laborde et al., 2017, **Table 1**), this therefore increases the likelihood of significant statistical relationships when all variables are explored. Consequently, to interpret collected data, and to understand phenomena associated with HRV, we need theories. Researchers often highlight the importance for more theoretically driven work and developing overarching theoretical frameworks in order to develop research hypotheses and draw comprehensive conclusions (Muthukrishna and Henrich, 2019). As a metaphor, instead of randomly accumulating bricks on a building site, theories allow us to build the foundations and structure of a house.

The main influential theories in HRV research have been reviewed in previous summary works (Shaffer et al., 2014; Laborde et al., 2017), and the most comprehensive theoretical perspective to date has been the Neurovisceral Integration Model (NIM; Thayer et al., 2009; Smith et al., 2017). In the present RT, 60% of papers use the NIM framework. Importantly, all theories related to HRV have a core component: the ability of HRV to index cardiac vagal activity, which enables the understanding of the role of HRV in phenomena related to self-regulation (Holzman and Bridgett, 2017).

A recent additional consideration to the NIM was the Vagal Tank Theory (Laborde et al., 2018b), which stresses the need to go beyond only considering resting HRV levels, to also take into account the reactivity to the task/event, and the recovery from the task/event, to achieve a comprehensive understanding of cardiac vagal activity as indexed by HRV. In the present RT, HRV reactivity was investigated by Condy et al., who found different associations for HRV at rest and HRV reactivity with the outcomes investigated; and by Borges et al., who controlled for resting HRV to interpret the relationship between reactivity HRV, transcutaneous vagus nerve stimulation (tVNS), and cognitive functioning.

After considering which theoretical approach is adequate to provide a solid understanding background for their research questions, researchers need to pay close attention to HRV methodological guidelines, given the influence methodological choices may have on HRV data interpretation.

## METHODS: THE IMPORTANCE OF FOLLOWING HRV METHODOLOGICAL GUIDELINES

Methodological aspects have to be considered when conducting HRV research, in order to ensure correct interpretation of the data (Laborde et al., 2017). A range of papers in this RT contribute to methodological development in HRV research. As we mentioned above, HRV theories focus on the ability of HRV to index cardiac vagal activity, and we will now refer to these parameters as vagally-mediated HRV (vmHRV). Examples of parameters reflecting vmHRV are the root mean square of successive differences (RMSSD), or high-frequency HRV when the respiratory frequency is comprised between 9 and 24 cycles per minute (Malik, 1996; Berntson et al., 1997; Laborde et al., 2017).

A common query surrounds the duration of HRV measurement. Seminal work of the HRV Task Force (Malik, 1996) pointed toward the standardized use of both short-term (5 min) and long-term (24 h) measurements in order to allow comparison across research labs. However, some phenomena may not necessarily fit those durations and researchers have started to examine shorter measurements opportunities. Within this RT, Shaffer et al. performed a critical review of ultra-short-term (UST) HRV measurements ($\leq$5 min). The overarching message from this paper was that the validity of UST measurements remains questionable at this point in time. Specifically, the research reviewed lacked rigorous investigation of criterion validity (which indicates that a novel measurement procedure produces comparable results to a currently validated measurement tool). Another recurring issue is the correction of artifacts, given a single false heartbeat can dramatically alter HRV metrics (Berntson and Stowell, 1998). Those challenges

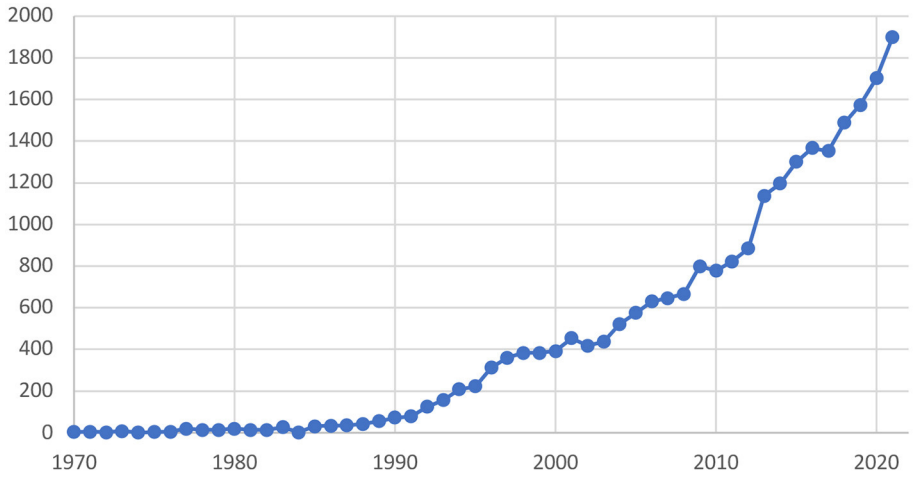



**FIGURE 1 |** Yearly frequency of publications mentioning "heart rate variability" in PubMed.

remain to be addressed before a widespread use of UST HRV measurements can be recommended.

Kim et al. further investigated the validity of UST HRV in non-static conditions, which would provide greater application in activities of daily life. However, they found that longer measurements are needed in dynamic conditions in comparison to static conditions to obtain reliable results. It is important to note that dynamic conditions also give rise to interpretational challenges, since metabolic influences on HRV have to be taken into account in this context.

This question was investigated by Brown et al., while assessing six methods to optimally detect episodes of non-metabolic vmHRV reduction as an indicator of psychological stress in everyday life. They found that a 24 h measurement detected the largest percentage of episodes of reduced additional vmHRV that matched self-reported stress levels. While this method was the most promising, using the first 10 min from three consecutive hours was also a good indicator. Another illustration of the use of 24 h measurement is found in Jarczok et al., where the authors describe how 24 h HRV measurements can be used as a communication tool for a personalized psychosomatic consultation in occupational health.

The devices used to measure HRV can also influence the interpretation of HRV data. While electrocardiogram (ECG) measurement remains the gold standard (Laborde et al., 2017), chest belts (e.g., Bellenger et al.) and photoplethysmography measurements (Mejia-Mejia et al.) enable HRV measurements in a broader range of contexts. Mejia-Mejia et al. investigated pulse-rate variability, which refers to HRV information obtained from pulse wave signals such as photoplethysmography measurements. They found that pulse-rate variability may quantify different, more localized information about the autonomic nervous system in the periphery of the body, which was thought to be due to the location of the pulse-rate variability monitor when compared to more central HRV measurements.

In the consideration of HRV parameters, we recommend researchers select them in line with theorical underpinning of the research. Researchers should also be mindful of the purported physiological mechanisms they are suggested to reflect. While editing the papers submitted to this RT, we noticed many studies still rely on the low-frequency/high-frequency (LF/HF) ratio, and the so-called autonomic or sympathovagal balance. This interpretation was originally based on the premise that LF provided an index of sympathetic nervous activity, a claim that has now long been disproved in the literature, in particular using blockade studies (for a recent overview, see Ackermann et al., 2021).

While HRV alone cannot provide a full overview of the autonomic nervous system, its pairing with impedance cardiography for this purpose seems promising. Indeed, impedance cardiography enables assessment of the preejection period (PEP, the time elapsed between the electrical depolarization of the left ventricle and the beginning of ventricular ejection), which has been recognized as the gold standard index of sympathetic nervous activity (Sherwood et al., 1990). In the present RT, Wiley et al. provided evidence for the use of the left-ventricular ejection time (LVET) instead of PEP for the same purpose. Like HRV, LVET is indeed a measure of chronotropic influence (reflecting the control of the heart via the sinoatrial node), while PEP is a measure of inotropic influence (reflecting myocardial contractility).

One way to further develop HRV theory is to investigate HRV together with other psychophysiological measurement techniques, such as with functional near-infrared spectroscopy (fNRIS), as performed by Condy et al.. This combined methodological approach based on neuroimaging allows for direct testing of the NIM. More specifically, this enables the investigation of pre-frontal activation via oxygenated hemoglobin to provide a critical test of the assumptions of the NIM, with vmHRV, which helps illustrate how task demands

and environmental contexts have to be taken into account for accurate interpretation of HRV.

Finally, in addition to *in vivo* measurements, *in silico* investigation (i.e., based on computer modeling) also prove to be beneficial in answering specific research questions. For example, *in silico* investigation was used to clarify the different impact of HRV in the deep cerebral and central hemodynamics at rest (Scarsoglio et al.). One of the interesting findings was that an increase in HRV per se does not seem to be sufficient to trigger a better cerebral hemodynamic response.

After showcasing the diversity of methodological advances addressed within our RT, we now move toward the domains of applications that emerged from the studies of this RT.

## HRV HORIZON 2030: DIVING INTO TOMORROW'S HRV

The Guest Editors certainly share a juvenile enthusiasm for the fantastic opportunities and insights offered by HRV research, however, they are also among the first ones to recommend extreme care regarding the claims that are made about HRV. This position is brilliantly illustrated by Karemaker in his opinion piece. Karemaker, an experienced HRV researcher who published his first HRV paper shortly after the first Guest Editor of this Research Topic was born (DeBoer et al., 1984), provides us with a stimulating and witty reflection about exaggerated claims made with HRV: "*The heart may be a mirror of the soul, but the human mind is more than its heart rate variability*". Karemaker uses a fictional case study taking place in 2030, illustrating the issues that may stem from a society geared toward preventive medicine. In this scenario, implanted biochips tracking HRV and other biomarkers watch over the health of the global population, with artificial intelligence analyzing the massive data flow to support the diagnostic process. Considering this insight into the future and warnings regarding over interpretation of HRV data, we now look to this peer reviewed RT to discover the exciting applications of HRV.

### HRV Across the Lifespan

HRV measurement has relevance at any age. At the beginning of the lifespan, Chiera et al. reviewed the literature to support the use of HRV monitoring in neonatal intensive care units. Specifically, they elaborate on a routine measurement integrating infants' HRV metrics, vital signs, and past history, in order to develop models capable of efficiently monitoring and predicting the infant's clinical conditions, enabling healthcare to improve in every stage of the perinatal period (from conception to the first years of life).

At the other end of the lifespan continuum, Hernandez-Vicente et al. focused on extreme longevity. They showed a reduction of vmHRV (and other HRV parameters) with age, which could be representative of a natural exhaustion of allostatic systems related to age. HRV may thus be considered an indicator of healthy aging.

### Psychology

HRV proves to be useful in the investigation of many psychophysiological phenomena, specifically when considering vmHRV. vmHRV was found to help further understand psychological concepts, for example compassion (Di Bello et al.). In their study, Di Bello et al. found vmHRV to be associated distinctively to compassion components, lower vmHRV for the empathic component (i.e., having the pain resonate in oneself), and higher vmHRV for the action component (i.e., engaging in actions aimed to alleviated self or others suffering), which helped to shed light on the different nature of compassion components.

Zammuto et al. investigated HRV together with the theory of mind (ToM), which is the human ability to infer the mental states of others to understand their behaviors and plan their own actions. The results preliminarily suggest that resting vmHRV might be used as an indicator of the ability to understand the content of mind of others. Furthermore, vmHRV may also be connected to morality. Lischke et al. found a positive association between individuals' vmHRV and moral rule adherence, implying that individuals with efficient integration abilities were more inclined to follow moral rules than individuals with inefficient integration abilities.

Regarding interoceptive accuracy, Lischke et al. found a positive association with vmHRV. Given the role played by our interoceptive ability to perceive and interpret changes in our autonomic nervous system, which then influence our emotional experiences, this finding is of high importance to psychological wellbeing. Furthermore, vmHRV can also be used to index the cardiorespiratory response to environmental challenges associated with specific personality characteristics. For example, Martino et al. investigated the cardiorespiratory response to moderate hypercapnia in female college students expressing behaviorally inhibited temperament. Despite baseline differences in behaviorally inhibited individuals (lower LF-HRV), no differences were found with the non-behaviorally inhibited individuals in response to hypercapnia, both groups showing an increase in vmHRV, reflecting an adaptation mechanism.

Investigating vmHRV as a potential biomarker for coping with constant discrimination, Rosati et al. investigated the cardiovascular conundrum in sexual minorities. The cardiovascular conundrum is a paradoxical profile of greater elevated sympathetic vasoconstriction (increased total peripheral resistance) and increased vmHRV, which has been reported in African Americans both at rest and in response to orthostasis. The authors found a similar pattern of response in sexual minorities, another group frequently exposed to constant discrimination.

When considering the relationship between vmHRV, depression, and positive affect, Spangler et al. found that they were influenced by gender. This study also provides guidance for researchers to look beyond linear relationships between HRV and specific outcomes, to consider also the possibility for quadratic relationships. Investigating worries and general anxiety disorder symptoms, Fishback et al. found that worriers who have higher levels of top-down control capacity (as indexed by vmHRV) may initiate and persist in worry, at least initially, because they value it.

In organizational psychology, vmHRV was suggested to be an important marker of work exhaustion, in combination with other psychological variables, such as self-esteem (De Longis et al.). Finally, Lohani et al. used HR and vmHRV to investigate arousal

and cognitive demands in manual and partially automated driving, and no differences were found in drivers new to partial automation.

To summarize, the research presented in this RT showcases the diverse implementation of HRV, specifically vmHRV, in psychological research.

## Medicine

Contributions to this RT illustrate that HRV can be very useful in the medical field, as a prevention and diagnostic tool.

HRV assessment was proposed to be valuable in the assessment of autonomic dysfunction linked to the motor activity of the human colon (Ali et al.). More specifically, during propulsive motor patterns, an overall shift in autonomic activity with an increase in parasympathetic control was found.

In cardiology, HRV was found to have prognostic value after acute myocardial infarction (Hayano et al.). The authors found that mortality risk in post-acute patients with low left ventricular ejection fraction is predicted by indices reflecting decreased HRV or HR responsiveness and cardiac parasympathetic dysfunction, whereas in patients without low left ventricular ejection fraction, the risk is predicted by a combination of indices that reflect decreased HRV or HR responsiveness and an indicator that reflects large abrupt HR changes, suggesting sympathetic involvement.

Regarding temporal lobe epilepsy (TLE), resting HRV measurement was used to identify the influence of lateralization on cardiac dysfunctions (Dono et al.), showing that left TLE is associated with higher vmHRV than right TLE. Left TLE patients may consequently have a lower risk of developing cardiac dysfunctions, and hence be less susceptible to develop Sudden Death for Epilepsy.

Finally, HRV may prove useful in cancer monitoring. Wu et al. found an association between HRV and breast tumor stage, and HRV parameters may help construct an effective early diagnostic and clinical prognostic model.

To summarize, routine assessment of HRV appears beneficial to improve the prevention and diagnostic of a large range of medical conditions.

## Large-Scale Health Assessment

One of the attractive characteristics of HRV is the possibility to measure it remotely with a diversity of devices, that become more accessible to a larger audience each day, enabling integration of HRV measurements to large-scale health monitoring strategies.

The role of HRV in the future of remote digital biomarkers is considered by Owens. Specifically, remote HRV assessment has potential as an adjunct digital biomarker in neurovisceral digital phenotyping that can add continuously updated, objective and relevant data to existing clinical methodologies, aiding the evolution of current "diagnose and treat" care models to a more proactive and holistic approach that pairs established markers with advances in remote digital technology. Remote HRV assessment also enables 24 h measurements, which can then be used as a communication tool for a personalized psychosomatic consultation in occupational health (Jarczok et al.).

Greater accessibility makes HRV very important during the COVID-19 pandemic, given vmHRV can provide a sensitive measure of inflammatory processes and immunomodulation. Drury et al. provides a nice illustration of this concept with the use of the Oura ring, a ring able to monitor vmHRV continuously via PPG.

We understand that the interested reader may desire field-based HRV measurements, and we therefore provide some suggestions for further reading around additional devices based on PPG beyond the Oura ring that would also enable large scale vmHRV assessment, in particular via smartphone apps using chest belts (e.g. Polar, Garmin, Suunto), smartwatches (e.g., Apple Watch, Fitbit), or smartphone apps like Elite HRV, Kenkou, and HRV4Training (Plews et al., 2017; Altini and Plews, 2021). An overview of the accuracy of popular commercial technologies that measure resting HRV can be found in Stone et al. (2021).

In summary, we are intrigued by the possibilities offered by HRV measurements applied to a large scale, facilitated by the use of remote measurement technologies.

## Sport and Exercise

Sport and exercise science is a growing area within HRV research, given athletes and coaches have discovered the benefits of monitoring their HRV to adjust training load and optimize recovery to achieve best performance (Stanley et al., 2013; Buchheit, 2014; Bellenger et al., 2016).

HRV may help to reduce the cost of testing and training (Rogers et al.). Rogers et al. presented a new detection method based on HRV defining the aerobic threshold for endurance exercise and training prescription. This detection method may substitute existing procedures (i.e. formal gas exchange testing or invasive blood lactate sampling) which have the characteristics of being costly, requiring special test equipment, trained operators, as well as ongoing calibration and verification.

Considering the monitoring of training-induced adaptations, Bellenger et al. investigated the impact of functional overreaching on post-exercise parasympathetic reactivation in runners. Importantly, they showed increased post-exercise vmHRV following heavy training in functionally overreached athletes, which may seem paradoxical, given increases in post-exercise vmHRV are also observed in response to improvements in performance. Consequently, this study very nicely illustrates the need to consider additional measures to provide more context to vmHRV measurements, such as subjective training tolerance in the case of athletes.

Regarding the monitoring of stress-recovery status, Barrero et al. used HRV during a Cycling Grand Tour (female version of the 2017 Tour de France), with a specific test, the so-called orthostatic test (transitioning from lying down to standing up). Specifically, a low HR and vmHRV index change between supine and standing positions was found to reflect a maladaptive training stress-recovery status, with HR change having a higher predictive value than vmHRV change.

Finally, in terms of training planning, we know that individualization is crucial. Hottenrott et al. used HRV for coaching an elite endurance athlete during and after viral

infection. Specifically, they showed the extent to which vmHRV and HR can be used to individualize training recommendations instead of following general rules. In a similar vein to Barrero et al., they used the orthostatic test, together with HR and vmHRV indicators, and found that this procedure was useful for detecting viral diseases early when implemented in daily routines.

In summary, HRV, and specifically vmHRV appears a promising way for athletes and coaches to monitor and help improve performance and wellbeing.

## VMHRV ENHANCEMENT

Given vmHRV is associated with a large range of positive outcomes, understanding how to enhance it is of great interest for researchers and practitioners (Fatisson et al., 2016; Laborde et al., 2018a,c).

One of the most effective methods to increase vmHRV is the voluntary slowing and pacing of one's breath, slow-paced breathing, to a frequency around 6 cycles per minute (Laborde et al., 2021, 2022; Sevoz-Couche and Laborde, 2022), a technique that has also been referred to as HRV biofeedback (Lehrer et al., 2020).

Three papers focus on slow-paced breathing in this RT which showcase noteworthy advances of the traditional paradigm. Shaffer and Meehan provide a practical guide to resonance frequency assessment for HRV biofeedback. If a respiratory frequency of 6 cycles per minute was found to be beneficial across individuals (Lehrer et al., 2020), performing slow-paced breathing at the individual resonance frequency may provide even higher benefits (see also the corrigendum to this article). Further, Patron et al. showed that the benefits of slow-paced breathing with biofeedback could be enhanced introducing competitive settings, in a sample comprising highly competitive individuals (manager). Finally, Tatschl et al. examined how implementing slow-paced breathing with biofeedback as an adjunctive therapy during inpatient psychiatric rehabilitation. They found that slow-paced breathing facilitates recovery of depressive symptoms and enhances autonomic functioning short-term, with a 1-year pre-post intervention follow-up study. This unique longitudinal study nicely illustrates how slow-paced breathing with biofeedback can be used as an adjunct, safe and non-invasive complementary treatment to help individuals with depression.

Regarding other techniques aimed at stimulating the vagus nerve, we find ostheopatic treatment, where HRV analysis is proposed to evaluate the effectiveness of osteopathic manipulative treatment as a preventive or complementary strategy in clinical and non-clinical conditions characterized by autonomic dysfunction (Carnevali et al.). Furthermore, non-invasive brain stimulation also offers options to stimulate the vagus nerve, specifically with tVNS, showing that it can increase cognitive flexibility in a set-shifting paradigm (Borges et al.). However, the mechanism in which tVNS influences vmHRV is less conclusive.

In summary, techniques aimed at increasing vmHRV are promising, given the positive outcomes linked to increased vmHRV.

## ANIMAL RESEARCH

As a last domain of application, beyond human research, it appears meaningful to investigate HRV in animals, to develop animal models which may help to better understand HRV in humans. Piantoni et al. investigated HRV together with selective pharmacological autonomic blockades, to document an age-related impairment in cardiac vagal modulation in mice, which is consistent with the human condition. Given their short life span, mice could be further utilized as an aged model for studying the trajectory of vagal decline with advancing age using HRV measures.

Finally, Shemla et al. investigated the beating rate variability of isolated mammal (rabbit and mouse) sinoatrial node tissue, providing insight into its contribution to HRV. Different trends were found between beating rate and beat rate variability or HRV in isolated sinoatrial node tissue vs. recordings collected under *in vivo* conditions, respectively, implying a complex interaction between the sinoatrial node and the autonomic nervous system in determining HRV *in vivo*.

In summary, animal HRV models, especially developed in mammals, may provide very interesting insights that would help us to better understand HRV in humans.

## CONCLUSION

This RT showcased the current richness and diversity of HRV, as well as novel approaches in emerging research. Many challenges remain open at the Horizon 2030, and we are glad to have contributed to the evolution of the field with this RT, respectfully standing on the shoulders of the giants from the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Malik, 1996). We recognize the influence of our colleagues who started this revolution in HRV research more than three decades ago, as well as of those who relentlessly pursued these endeavors since the end of the 20$^{th}$ century. Exciting HRV challenges are ahead of us, as illustrated by the contributions to this RT summarized in this Editorial.

Keeping in mind the warning of Karemaker expressed above, the Guest Editors would like to encourage researchers and practitioners to bravely start or continue their journey to integrate HRV to our lives. Finally, given individuals cannot solely be surmised by their HRV, we trust that HRV can provide a nice starting point toward helping making the world in which we live more ***para****sympathetic*.

## AUTHOR CONTRIBUTIONS

SL wrote the first draft of this manuscript. EM, CB, and JT provided very useful critical feedback. All authors contributed to the article and approved the submitted version.

## ACKNOWLEDGMENTS

We gratefully acknowledge the contribution and effort of the 139 authors, and look enthusiastically forward to further new HRV discoveries.

## REFERENCES


Ackermann, S., Laborde, S., Borges, U., and Mosley, E. (2021). Commentary: Photoplethysmography for quantitative assessment of sympathetic nerve activity (SNA) during cold stress. *Frontiers in Physiology* 12, 602745. doi: 10.3389/fphys.2021.602745

Altini, M., and Plews, D. (2021). What is behind changes in resting heart rate and heart rate variability? A large-scale analysis of longitudinal measurements acquired in free-living. *Sensors (Basel)* 21, 7932. doi: 10.3390/s21237932

Bellenger, C. R., Karavirta, L., Thomson, R. L., Robertson, E. Y., Davison, K., and Buckley, J. D. (2016). Contextualizing parasympathetic hyperactivity in functionally overreached athletes with perceptions of training tolerance. *Int. J. Sports Physiol Perfor.* 11, 685–692. doi: 10.1123/ijspp.2015-0495

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., and Stowell, J. R. (1998). ECG artifacts and heart period variability: dont miss a beat! *Psychophysiology* 35, 127–132. doi: 10.1111/1469-8986.3510127

Billman, G. E., Huikuri, H. V., Sacha, J., and Trimmel, K. (2015). An introduction to heart rate variability: methodological considerations and clinical applications. *Front. Physiol.* 6, 55. doi: 10.3389/fphys.2015.00055

Billman, G. E., Sacha, J., Werner, B., Jelen, P. J., and Gasior, J. S. (2019). Editorial: heart rate variability and other autonomic markers in children and adolescents. *Front. Physiol.* 10, 1265. doi: 10.3389/fphys.2019.01265

Buchheit, M. (2014). Monitoring training status with HR measures: do all roads lead to Rome? *Front. Physiol.* 5, 73. doi: 10.3389/fphys.2014.00073

DeBoer, R. W., Karemaker, J. M., and Strackee, J. (1984). Comparing spectra of a series of point events particularly for heart rate variability data. *IEEE Trans. Biomed. Eng.* 31, 384–387. doi: 10.1109/TBME.1984.325351

Drury, R. L., Porges, S., Thayer, J., and Ginsberg, J. P. (2019). Editorial: heart rate variability, health and well-being: a systems perspective. *Front. Public Health.* 7, 323. doi: 10.3389/fpubh.2019.00323

Fatisson, J., Oswald, V., and Lalonde, F. (2016). Influence diagram of physiological and environmental factors affecting heart rate variability: an extended literature overview. *Heart Int.* 11, e32–e40. doi: 10.5301/heartint.5000232

Holzman, J. B., and Bridgett, D. J. (2017). Heart rate variability indices as bio-markers of top-down self-regulatory mechanisms: a meta-analytic review. *Neurosci. Biobehav. Rev.* 74, 233–255. doi: 10.1016/j.neubiorev.2016.12.032

Laborde, S., Allen, M. S., Borges, U., Dosseville, F., Hosang, T. J., Iskra, M., et al. (2022). Effects of voluntary slow breathing on heart rate and heart rate variability: A systematic review and a meta-analysis. *Neurosci. Biobehav. Rev.* doi: 10.1016/j.neubiorev.2022.104711. [Epub ahead of print].

Laborde, S., Allen, M. S., Borges, U., Iskra, M., Zammit, N., You, M., et al. (2021). Psychophysiological effects of slow-paced breathing at six cycles per minute with or without heart rate variability biofeedback. *Psychophysiology.* 59, *e*13952. doi: 10.1111/psyp.13952

Laborde, S., Mosley, E., and Mertgen, A. (2018a). A unifying conceptual framework of factors associated to cardiac vagal control. *Heliyon* 4, e01002. doi: 10.1016/j.heliyon.2018.e01002

Laborde, S., Mosley, E., and Mertgen, A. (2018b). Vagal tank theory: the three rs of cardiac vagal control functioning – resting, reactivity, and recovery. *Front. Neurosci.* 12, 458. doi: 10.3389/fnins.2018.00458

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Physiol.* 8, 213. doi: 10.3389/fpsyg.2017.00213

Laborde, S., Mosley, E., and Ueberholz, L. (2018c). Enhancing cardiac vagal activity: factors of interest for sport psychology. *Progress in Brain Research* 240, 71–92. doi: 10.1016/bs.pbr.2018.09.002

Lehrer, P. M., Kaur, K., Sharma, A., Shah, K., Huseby, R., Bhavsar, J., et al. (2020). Heart rate variability biofeedback improves emotional and physical health and performance: a systematic review and meta analysis. *Appl. Psychophysiol. Biofeedb.* 45, 109–29. doi: 10.1007/s10484-020-09466-z

Malik, M. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task force of the european society of cardiology and the north american society of pacing and electrophysiology. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Muthukrishna, M., and Henrich, J. (2019). A problem in theory. *Nat. Hum. Behav.* 3, 221–229. doi: 10.1038/s41562-018-0522-1

Plews, D. J., Scott, B., Altini, M., Wood, M., Kilding, A. E., and Laursen, P. B. (2017). Comparison of heart-rate-variability recording with smartphone photoplethysmography, polar H7 chest strap, and electrocardiography. *Int. J. Sports Physiol. Perfor.* 12, 1324–1328. doi: 10.1123/ijspp.2016-0668

Sevoz-Couche, C., and Laborde, S. (2022). Heart rate variability and slow-paced breathing:when coherence meets resonance. *Neurosci Biobehav Rev* 135, 104576. doi: 10.1016/j.neubiorev.2022.104576

Shaffer, F., McCraty, R., and Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front. Psychol* 5, 1040. doi: 10.3389/fpsyg.2014.01040

Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Lovallo, W. R., and van Doornen, L. J. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology* 27, 1–23. doi: 10.1111/j.1469-8986.1990.tb02171.x

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Stanley, J., Peake, J. M., and Buchheit, M. (2013). Cardiac parasympathetic reactivation following exercise: implications for training prescription. *Sports Med.* 43, 1259–1277. doi: 10.1007/s40279-013-0083-4

Stone, J. D., Ulman, H. K., Tran, K., Thompson, A. G., Halter, M. D., Ramadan, J. H., et al. (2021). Assessing the accuracy of popular commercial technologies that measure resting heart rate and heart rate variability. *Front. Sports Active Living.* 3, 37. doi: 10.3389/fspor.2021.585870

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004


**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 25 May 2020
doi: 10.3389/fnins.2020.00523

# Transcutaneous Vagus Nerve Stimulation May Enhance Only Specific Aspects of the Core Executive Functions. A Randomized Crossover Trial

*Uirassu Borges[1]*, Laura Knops[2], Sylvain Laborde[1,3], Stefanie Klatt[4,5] and Markus Raab[1,6]*

*[1] Institute of Psychology, German Sport University, Cologne, Germany, [2] Institute of Clinical Neuroscience and Medical Psychology, Heinrich Heine University, Duesseldorf, Germany, [3] UFR STAPS, Université de Caen Normandie, Caen, France, [4] Institute of Exercise Training and Sport Informatics, German Sport University, Cologne, Germany, [5] Institute of Sports Science, University of Rostock, Rostock, Germany, [6] School of Applied Sciences, London South Bank University, London, United Kingdom*

OPEN ACCESS

***Edited by:***
*Vitor Engracia Valenti, São Paulo State University, Brazil*

***Reviewed by:***
*Lorenza S. Colzato, Ruhr University Bochum, Germany*
*Jiliang Fang, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, China*

****Correspondence:***
*Uirassu Borges*
*u.borges@dshs-koeln.de*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *03 January 2020*
***Accepted:*** *27 April 2020*
***Published:*** *25 May 2020*

***Citation:***
*Borges U, Knops L, Laborde S, Klatt S and Raab M (2020) Transcutaneous Vagus Nerve Stimulation May Enhance Only Specific Aspects of the Core Executive Functions. A Randomized Crossover Trial. Front. Neurosci. 14:523. doi: 10.3389/fnins.2020.00523*

**Background:** Individuals are able to perform goal-directed behaviors thanks to executive functions. According to the neurovisceral integration model, executive functions are upregulated by brain areas such as the prefrontal and cingulate cortices, which are also crucially involved in controlling cardiac vagal activity. An array of neuroimaging studies already showed that these same brain areas are activated by transcutaneous vagus nerve stimulation (tVNS). Despite evidence toward effects of tVNS on specific executive functions such as inhibitory control, there have been no studies investigating what type of inhibition is improved by tVNS by systematically addressing them within the same experiment. Furthermore, the effect of tVNS on another core executive function, cognitive flexibility, has not yet been investigated.

**Objective:** We investigated the effects of tVNS on core executive functions such as inhibitory control and cognitive flexibility.

**Methods:** Thirty-two participants (nine women, $M_{age}$ = 23.17) took part in this study. Vagally mediated heart rate variability parameters (root mean square of successive differences, RMSSD, and high frequency, HF) were measured while participants performed four different cognitive tasks that mainly rely on different aspects of both the aforementioned executive functions.

**Results:** Despite clear conflict effects in the four tasks, only performance on the task used to measure set-shifting paradigm was improved by tVNS, with switch costs being lower during tVNS than during sham stimulation. Furthermore, HF increased during each of the cognitive flexibility tasks, although HF during tVNS did not differ from HF during sham stimulation.

**Conclusion:** The results indicate for the first time (a) that tVNS can increase cognitive flexibility in a set-shifting paradigm, and (b) that tVNS may exert a stronger effect on

cognitive flexibility than inhibition. The present study provides only partial evidence for the neurovisceral integration model. Future studies should address further paradigms that demand cognitive flexibility, thus investigating this new hypothesis on the specificity of the tVNS effects on cognitive flexibility.

**Keywords: tVNS, vagus nerve stimulation, HRV, heart rate variability, cardiac vagal activity, executive functions, neurovisceral integration model**

## INTRODUCTION

Transcutaneous vagus nerve stimulation (tVNS) is a technology used to electrically and non-invasively modulate vagal activity through the auricular branch of the vagus nerve. There has been an increasing amount of studies using tVNS to enhance cognitive processes that rely on prefrontal activity. An array of these studies addressed specific aspects of inhibitory control separately (e.g., Keute et al., 2018a; Ventura-Bort et al., 2018), whereas others investigated more complex cognitive functioning such as creativity (Colzato et al., 2018b) and implicit spiritual self-representation (Finisguerra et al., 2019). Attempts motivated by theory-driven hypotheses to systematically investigate the effects of tVNS on different aspects of basic cognitive functions are still scarce. Based on the predictions outlined in the neurovisceral integration model (Thayer et al., 2009), the current study aimed at investigating the effects of tVNS on the core executive functions inhibitory control and cognitive flexibility (Diamond, 2013). Furthermore, and also in line with the neurovisceral integration model, we measured cardiac vagal activity during tVNS and cognitive performance, a parameter suggested to reflect the effectiveness of executive functioning.

Executive functions refer to top–down mental processes that serve goal-directed behavior (Diamond, 2013). Inhibitory control and cognitive flexibility are considered core executive functions, meaning that they are necessary components for building higher-order executive functions (Miyake and Friedman, 2012; Diamond, 2013). Inhibitory control involves the ability to override dominant or prepotent responses by controlling one's attention and behavior, and can be distinguished between selective attention and response inhibition (Diamond, 2013). Selective attention is expressed by the inhibitory cognitive control of attention, which occurs by suppressing prepotent mental representations on the level of perception. Response inhibition is a behavioral inhibition that keeps a person from acting impulsively. Cognitive flexibility consists in quickly and flexibly switching between tasks or mental sets (Diamond, 2013). It can be broken down into task switching and set shifting. Task switching differs from set shifting in the type of conflict: task switching is related to switching between tasks with different instructions involving different stimuli. Set shifting, in turn, consists of shifting attention between different features of the same stimuli to follow a given instruction (Dajani and Uddin, 2015).

Executive functioning is linked to prefrontal activity (Arnsten and Li, 2004). According to the neurovisceral integration model (Thayer et al., 2009; Smith et al., 2017), cardiac vagal activity–the activity of the vagus nerve regulating cardiac functioning–reflects the output of the central autonomic network, which links the prefrontal cortex to the heart (Thayer et al., 2009). The optimal activation of the neural pathways within this network is crucial for performing a given task that requires cognitive effort and for showing flexible responses to a changing environment (Thayer et al., 2009). Because cardiac vagal activity and executive functioning share common underlying neurovisceral self-regulation mechanisms, higher cardiac vagal activity is associated with improved executive functioning. Cardiac vagal activity can be indexed via heart rate variability (HRV), the difference in the time interval between adjacent heartbeats (Malik et al., 1996), and specifically by the root mean square of the successive differences (RMSSD) and by high frequency (HF).

There is a large body of empirical evidence linking higher levels of cardiac vagal activity to higher executive performance (Inhibitory control: Alderman and Olson, 2014; cognitive flexibility: Johnsen et al., 2003; Colzato et al., 2018a). Based on the evidence of the relationship between executive functioning and cardiac vagal activity as indexed by HRV (RMSSD and HF), in the present study we will consider the executive functions described here to investigate if tVNS can improve different types of inhibitory control and cognitive flexibility as well as cardiac vagal activity.

The expected link between tVNS and executive functions can be understood by considering the neuroanatomical pathways of the vagus nerve. The electrical signal, starting in the auricular branch of the vagus nerve (ABVN), reaches the nucleus tractus solitarius, which is a crucial structure that projects to a variety of brain areas, including cortical regions such as the anterior cingulate cortex and the prefrontal cortex (Aihara et al., 2007). As shown by several functional magnetic resonance imaging (fMRI) studies (Kraus et al., 2013; Frangos et al., 2015; Yakunina and Kim, 2017; Badran et al., 2018), tVNS evoked, in contrast to sham stimulation, higher activity in the nucleus tractus solitarius (Frangos et al., 2015; Yakunina and Kim, 2017), in the left prefrontal cortex and in cingulate areas (Badran et al., 2018). Importantly, these brain areas affected by tVNS correspond to the areas described by the neurovisceral integration model as regulating both executive and cardiac regulation, such as the prefrontal cortex and cingulate areas (Thayer et al., 2009, 2012).

So far, there are studies showing that tVNS affects the types of inhibitory control (**Table 1**). These studies used varying cognitive paradigms, which comprise different dependent variables, and addressed the inhibitory control types only separately and in different study designs (see **Table 1** for an overview of design-related characteristics of studies investigating inhibitory control using tVNS). Thus, an integrating, evidence-based discussion on the interplay between tVNS and these types of inhibitory control has not been possible.

**TABLE 1 |** Summary of the studies with tVNS addressing different types of inhibitory control.

| Study | Dependent variable | Cognitive paradigm | Study design | Sample size | Results |
|---|---|---|---|---|---|
| Beste et al., 2016 | Response inhibition and working memory | Backward inhibition and mental workload inhibition paradigm | Between-subjects | 51 | Higher response inhibition processes only when working memory processes are needed |
| Fischer et al., 2018 | Selective attention, N2 and P3 amplitudes | Simon | Within-subject | 21 | Adaptation to conflict was enhanced, N2 amplitude higher |
| Keute et al., 2019a | Automatic motor response inhibition, readiness potentials | Subliminal motor priming | Within-subject | 16 | Increased NCE; effects on readiness potentials only in compatible trials |
| Steenbergen et al., 2015 | Response selection as a consequence of response inhibition | Stop-change | Between-subjects | 30 | Faster responses when two actions were executed in succession |
| Ventura-Bort et al., 2018 | Selective attention, sAA, P3a and P3b amplitudes | Oddball | Within-subject | 20 | Increased sAA after tVNS; easy trials produced larger P3b amplitudes |

*NCE, negativity comparability effect; sAA, salivary alpha-amylase; tVNS, transcutaneous vagus nerve stimulation.*

As stated above, executive functions and cardiac vagal activity share overlapping neurological structures, with both being upregulated by cortical areas, including the prefrontal cortex (Thayer et al., 2009). Given that the tVNS signal is sent afferently to the prefrontal cortex via ABVN, cardiac vagal activity has also been thought to be affected by tVNS (Murray et al., 2016). Using RMSSD to measure the effect of tVNS on cardiac vagal activity, different studies did not find any differences between active and sham stimulation (Burger et al., 2016, 2019; De Couck et al., 2017). One study showed in three experiments that tVNS consistently increased RMSSD; however, this increase was similarly observed during both active and sham stimulation, with this possibly indicating that tVNS sends non-specific signals at the brainstem level that similarly influence cardiac vagal activity in both active and sham stimulation (Borges et al., 2019). Nonetheless, this study did not take any cognitive paradigm into account, which might have contributed to understanding if this possible signal non-specificity-identified as an increase in cardiac vagal activity during both active and sham stimulation-can also be observed in cognitive functions. This possibility would challenge the use of earlobe sham stimulation, which has widely been used in current research with tVNS. Therefore, further studies on the effect of active as well as sham tVNS on cardiac vagal activity are still needed.

To summarize, there is evidence toward the modulation of inhibitory control by tVNS; however, these findings refer to different cognitive phenomena that have been found in different samples and in the context of different study designs. So far, there is no study that has systematically investigated the effects of tVNS on different aspects of core executive functions, and importantly, there is a lack of studies whose hypotheses were explicitly motivated by a theory. To address different aspects of executive functioning in an integrative way, it is crucial to use the same study design and setup. This way it is possible to control for possible experimental variations such as length of resting and of stimulation periods, daytime, and other factors that might influence measurement of cardiac vagal activity. Confounders related to study design, e.g., instructions, laboratory setup, and differences in sample size, can also be considered. Thus, going beyond existing literature, the present study aims at investigating the effects of tVNS on inhibitory control, cognitive flexibility, and cardiac vagal activity. To achieve this, it uses an integrative theoretical background, namely the neurovisceral integration model (Thayer et al., 2009), and applies the same study design across these target executive functions. Based on the evidence on neurophysiological pathways related to tVNS, addressing cognitive processes that mainly rely on different executive functions might help to further understand how tVNS affects basic cognitive processes involved in goal-directed behavior.

Against this background, it was hypothesized that the performance on the four cognitive tasks is higher during active tVNS, compared to sham stimulation (H1a for selective attention, H1b for response inhibition, H1c for task switching, and H1d for set shifting; this assignment of the subtypes of executive functions to the letters is also valid for the next hypotheses). Furthermore, we expected that cardiac vagal activity increases relatively to the resting phase only during active stimulation and not during sham stimulation, with cardiac vagal activity during the tasks being higher in the active tVNS condition (H2a–d). Moreover, we hypothesized that cardiac vagal activity during tVNS and before each cognitive task is positively associated with task performance only in the active tVNS condition (H3a–d). Finally, we expected cardiac vagal activity during the tasks to have a more strongly positive relationship to task performance in the active condition than in the sham condition (H4a–d).

# MATERIALS AND METHODS

## Participants

As it is not possible to run power analyses for multi-factorial repeated-measures designs with G*Power 3.1 (Faul et al., 2007), we followed the same procedure found in previous studies with similar study design (e.g., Liepelt et al., 2019). Accordingly, we matched the average number of participants in the studies that investigated executive functions with tVNS

using a within-subject design (summarized in **Table 1**). Since we also measured cardiac vagal activity, we additionally considered the average sample size in Borges et al. (2019), because this study systematically investigated the effect of tVNS on cardiac vagal activity in different experiments. Twenty-nine participants were calculated to be necessary to find an effect. Anticipating possible exclusions due to drop-outs and after data cleaning, we recruited 35 participants. Thirty-two participants (nine female) were included in the analysis due to technical problems with the electrocardiogram (ECG) signal of three participants. Mean age was 23.17 years old ($SD$ = 4.08), whereby female participants had $M_{age}$ = 21.11, $SD$ = 1.27, and male participants had $M_{age}$ = 24.87, $SD$ = 5.87. Consort flowchart (Dwan et al., 2019) is presented in **Figure 1**.

The sample consisted of healthy students at the local university. Participants were eligible if they were not pregnant at the time of the experiment and free of cardiovascular or neurological diseases, or major mental disorders, for example severe depression or anxiety disorder. They were asked not to smoke, exercise, or consume food, alcohol, or caffeine for at least 2 h before participation. These potentially confounding variables as well as tVNS safety-related questions were assessed by means of an adapted version of the demographics questionnaire for experiments using HRV developed by Laborde et al. (2017). All participants gave written informed consent prior to the experiment, which was approved by the local ethical committee (ethics approval number 120/2018).

## Transcutaneous Vagus Nerve Stimulation

We employed the NEMOS tVNS device developed by Cerbomed (Erlangen, Germany). Two titan electrodes found in a structure similar to an earphone are placed in the cymba conchae of the left ear, an area thought to be exclusively innervated by the ABVN (Peuker and Filler, 2002), in order to electrically stimulate these vagal fibers (Ellrich, 2011). In the sham stimulation, the electrodes are placed on the left earlobe, which is thought to be free of vagal innervation (Peuker and Filler, 2002) and has abundantly been used as a sham stimulation in research with tVNS (van Leusden et al., 2015). The tVNS device delivers a stimulation with a pulse width of 200–300 μs at 25 Hz and an on–off cycle of 30 s. Regarding the adjustment of the stimulation intensity, cardiac vagal activity may be similarly influenced by electrical afferent stimuli that are triggered by different methods to stipulate stimulation intensity (Borges et al., 2019). Therefore, we followed procedures found in previous research with tVNS that allow participants to choose their individual intensity (Fischer et al., 2018; Ventura-Bort et al., 2018). Accordingly, in each session participants received increasing and decreasing series of 10-s stimulation trials, and rated the subjective sensation of the stimulation on a 10-point scale, ranging from nothing (0), light tingling (3), strong tingling (6), to painful (10). The increasing series of trials started from an intensity of 0.01 mA and increased by 0.01 mA on a trial-by-trial basis until participants reported a tingling sensation of 9. Before starting the decreasing series, the same intensity was repeated and then reduced trial by trial in 0.01 mA until a subjective sensation of 6 or below was experienced. This procedure was repeated a second time. The final stimulation intensity used for the experimental procedure was calculated based on the average of the four intensities rated as 8 (two from the increasing and two from the decreasing series). The average chosen stimulation intensity in the active condition was $M$ = 2.19 mA ($SD$ = 0.93) and $M$ = 2.20 mA ($SD$ = 1.06) in the sham condition. These stimulation intensities did not differ significantly from each other, $t(31) = 0.063$, $p = 0.950$.

## Cardiac Vagal Activity

To assess cardiac vagal activity, we used the Faros 180° device from Mega Electronics (Kuopio, Finland) with a set sampling rate of 500 Hz. This device enables users to measure the ECG signal as recommended by current guidelines on HRV measurement for psychophysiological experiments (Laborde et al., 2017). We placed two disposable ECG pre-gelled electrodes (Ambu L-00-S/25, Ambu GmbH, Bad Nauheim, Germany) on the chest, the positive electrode on the right infraclavicular fossa and the negative one on the left anterior axillary line below the 12th rib.

RMSSD, as well as HF (0.15–0.40 Hz band) transformed with autoregressive modeling, were chosen as indicators of cardiac vagal activity in the main analyses (Malik et al., 1996). From ECG recordings, we extracted HRV with Kubios software (University of Eastern Finland, Kuopio, Finland), visually inspected the full ECG recording, and manually corrected artifacts (Laborde et al., 2017). Since HF is only influenced by breathing when breathing cycles are between nine cycles per minute (0.15 Hz) and up to 24 cycles per minute (0.40 Hz) (Malik et al., 1996), participants with a respiration rate of less than nine cycles per minute and more than 24 cycles per minute were excluded from analyses with HF. The respiration rates (the number of respiratory cycles per minute) was obtained multiplying the ECG-derived respiration value obtained via the Kubios algorithm by 60 (Tarvainen et al., 2013) and was also separately analyzed. We considered for analysis measurements in blocks of 4 min, which is in accordance with the range suggested by recommendations for experiment planning in psychophysiological research (Laborde et al., 2017). Given that the cognitive tasks differed greatly from one another regarding time length, with the tasks lasting between 5 and 13 min, for the analysis within task blocks we chose a time window of the last 4 minutes respectively for each cognitive task.

## Cognitive Tasks

In order to standardize the tasks and therefore avoid response mistakes, all tasks used the keys "S" and "K" as responses for left and right, respectively. The participants were instructed to press the buttons with their index fingers, and the stimuli were presented in white against a gray background (except for the set-shifting task). We used a 24-in. flat-screen monitor (1,920 × 1,080 pixels at 60 Hz) at a viewing distance of 60 cm to present the tasks and ran all of them with PsychoPy3 Version 3.0.0 (Peirce et al., 2019). The participants performed four tasks which are thought to mainly rely on inhibitory control (selective attention and response inhibition), and cognitive flexibility (task switching and set shifting). These tasks were chosen according to two criteria: First, we followed recommendations from influential reviews on executive functions (Miyake and Friedman, 2012; Diamond, 2013). For the choice of the cognitive task, we

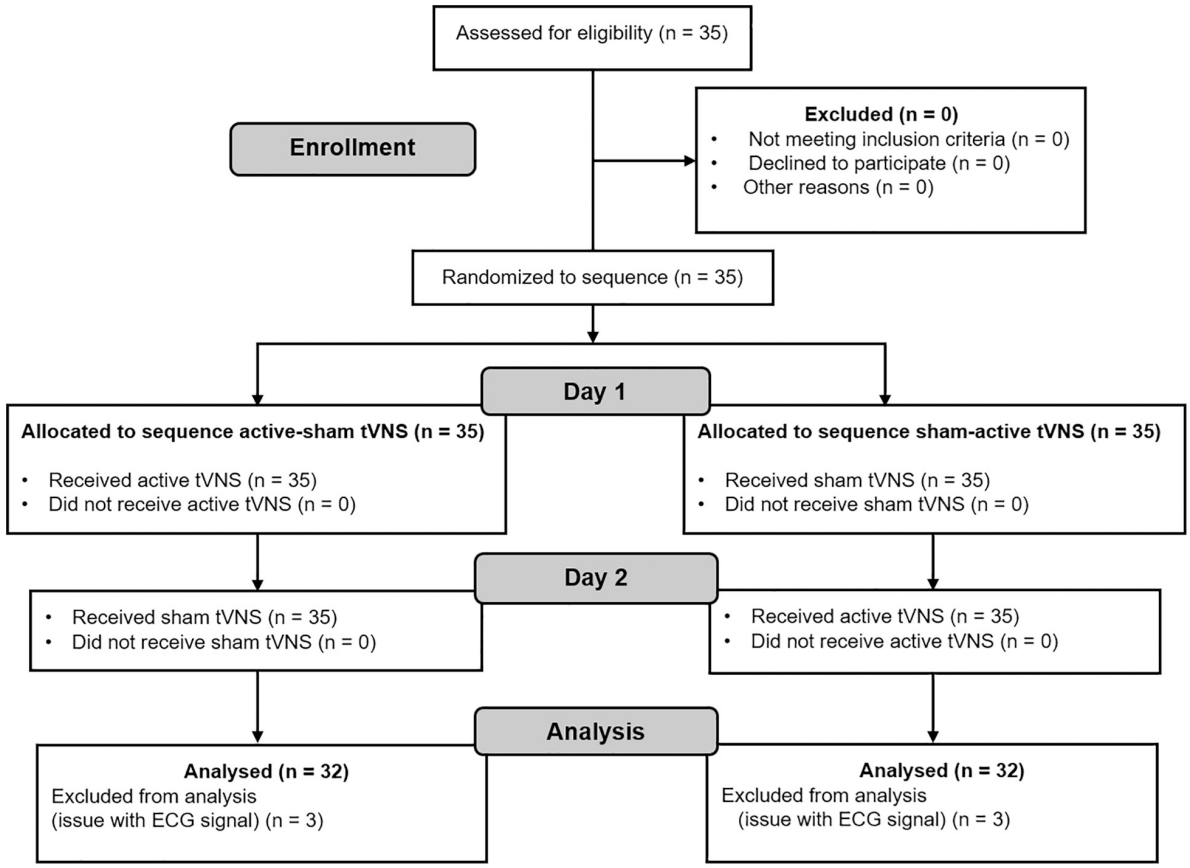



**FIGURE 1 |** Consort (2019) diagram.

considered the task impurity problem: according to Miyake and Friedman (2012), because executive functions necessarily manifest themselves by operating on other cognitive processes, any executive task strongly implicates other cognitive processes that are not directly relevant to the target executive function. Consequently, we chose the tasks that are thought to minimize demands of other executive functions (Diamond, 2013). Second, we performed a literature search to find studies that used the tasks recommended by the aforementioned reviews and also provided evidence on the relationship with (a) tVNS, (b) cardiac vagal activity, and (c) prefrontal activity (imaging studies). The tasks chosen are the following:

## Flanker Task

Following recommendations from Diamond (2013), to measure selective attention we used a modified version of the Flanker task (Eriksen and Eriksen, 1974). We used the Flanker task as reported by Alderman and Olson (2014). With this version, it could be shown that individuals with higher fitness levels expressed higher HF values during the task, and that these individuals had lower RT than the less fit group. A trial consists of five arrows in which the third one is the target arrow. Participants were asked to press the left key on the computer keyboard when the target arrow pointed to the left and the right key when the target arrow pointed to the right. Participants were instructed to respond as quickly and accurately as possible for each trial. After a practice block of 30 trials, two experimental blocks of 120 trials each were presented, each separated by 30 s. Each block consisted of congruent and incongruent stimuli presented in random order. The congruent trials consisted of the target arrow being flanked by arrows facing the same direction, while incongruent trials involved the target arrow being flanked by arrows facing the opposite direction. Each stimulus was presented for 100 ms (to increase task difficulty) with a response window of 1,500 ms. A random inter-stimulus time interval of 1,100, 1,300, or 1,500 ms was also used between each 50 ms visual fixation (+) and the stimulus in order to increase task difficulty (**Figure 2A**).

## Spatial Stroop Task

The task for measuring response inhibition was the Spatial Stroop task, as this task is thought to minimize memory demands compared to other classical tasks such as the Simon task (Diamond, 2013). This response inhibition task was designed according to Marotta et al. (2018), from which we only took the arrow part of the task, and consisted of a practice and two experimental blocks. During the practice block, 15 trials were presented, and feedback was provided. The practice block was followed by two experimental blocks of 64 experimental

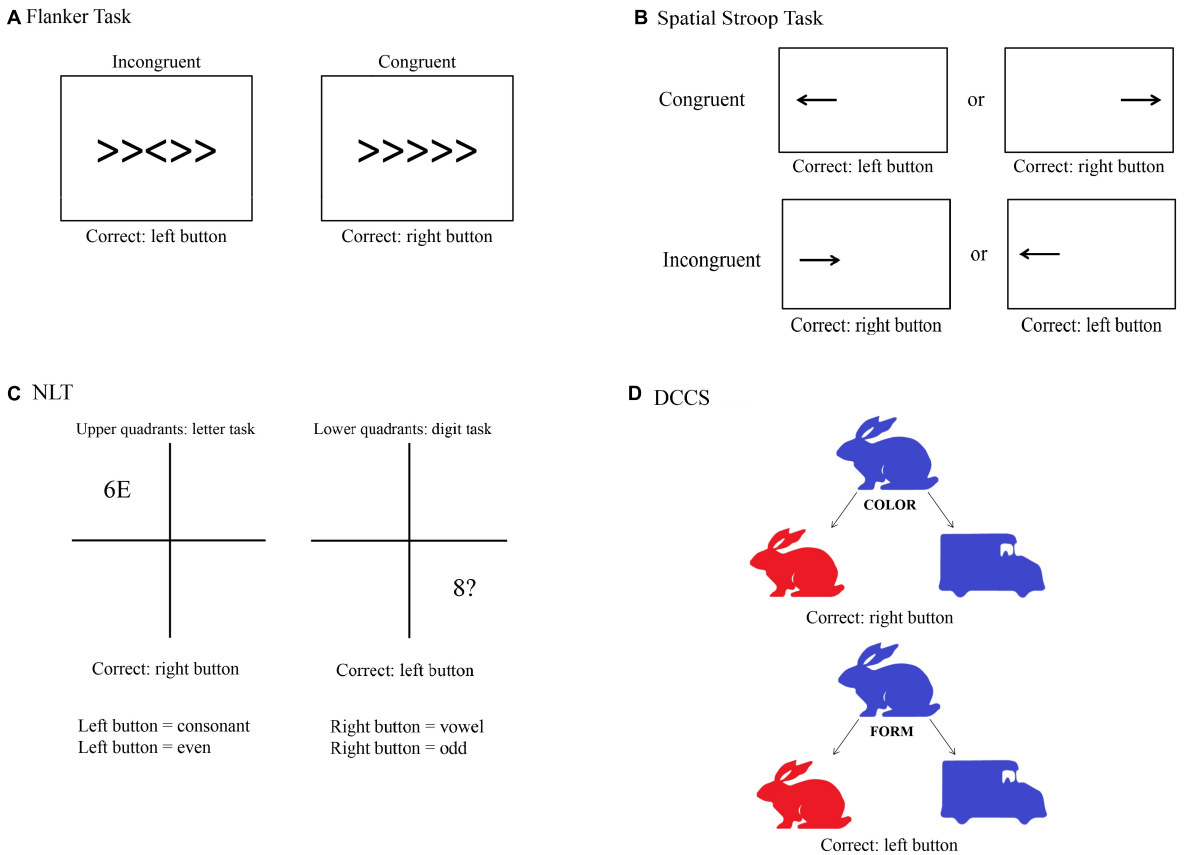



**FIGURE 2 |** Visual depiction of the four cognitive tasks used in the study. **(A)** Flanker task; **(B)** Spatial Stroop task; **(C)** Number–Letter task (NLT); **(D)** Dimensional Change Card Sorting task (DCCS).

trials each. Participants were instructed to fixate a fixation cross presented in the center of the screen. A directional arrow appears randomly on the left or on the right side of the fixation point, and this arrow points randomly to the right or the left side. Participants are required to indicate the direction of the arrow by pressing the left key if the arrow points to the left and the right key if the arrow points to the right, while ignoring its location. They were instructed to respond as quickly and accurately as possible for each trial. The arrow was presented either left or right of the fixation cross for 2,000 ms. Feedback for incorrect key presses was provided to participants in the form of a 220-Hz tone presented for 1,500 ms. This design produced trials that were congruent (e.g., a right-indicating target presented on the right) or incongruent (e.g., a left-indicating target presented on the right, see **Figure 2B**).

## Number–Letter Task

We used the Number–Letter task (NLT) as described in Colzato et al. (2018a), which found that participants with higher resting-state cardiac vagal activity showed greater flexibility than individuals with lower resting-state cardiac vagal activity. Throughout the task, a 10-cm square divided into four quadrants was displayed on the computer screen. During each trial, a character pair consisting of letters, numbers or symbols was presented in the center of one quadrant. Participants had to either perform a letter task in which they classified the letter in the stimulus pair as a consonant or a vowel, or they had to perform a number task in which they classified the number in the pair as odd or even. They were instructed to respond as quickly and accurately as possible for each trial. After their response or after 2,000 ms had passed, a new stimulus pair was displayed in the next quadrant following a clockwise pattern. The upper quadrants were assigned to the letter task and the lower quadrants to the digit task, so that the display location served as a task cue and the task changed predictably. Depending on the task, the relevant character in the stimulus pair was either a letter or a digit, whereas the second and irrelevant character was either a member of the other category, so that the response afforded by this character could be congruent or incongruent with the task-relevant response, or was drawn from a set of neutral characters. This design produced switch trials in Quadrants 1 and 3, and non-switch trials when the stimuli appeared in Quadrants 2 and 4. Consonants were sampled randomly from the set < G, K, M, R >, vowels from the set < A, E, I, U >, even numbers from the set < 2, 4, 6, 8 >, odd numbers from the set < 3, 5, 7, 9 >, and neutral characters from the set < #, ?, *, % >, with the restriction that a stimulus could not be repeated on successive trials. The position of the task-relevant character within a pair (left or

right) was randomly determined on each trial. The participants pressed the left key to indicate "even" or "consonant" and the right key to indicate "odd" or "vowel." Participants completed a practice set of 9 blocks, each with 16 trials, before entering the experimental phase. This consisted of a set of 15 blocks, with each block again consisting of 16 trials. A short response stimulus interval (RSI) of 150 ms was chosen which remained constant within a given set. A short RSI, the so-called preparation component, has been shown to provoke more pronounced switch costs than long RSI, also known as residual component. This is because shorter intervals usually hamper the reconfiguration process before the stimulus is presented (Colzato et al., 2018a). Stimuli were response-terminated or presented for a maximum duration of 2,000 ms (**Figure 2C**).

### Dimensional Change Card Sorting Task

The Dimensional Change Card Sorting task (DCCS) based on Zelazo et al. (2014) was used in the present study to measure set shifting, as recommended by Diamond (2013). This version is part of the NIH Toolbox Cognition Battery and was validated with 268 adults (Zelazo et al., 2014). DCCS makes use of two different styles of bivalent cards, displaying a red rabbit on the left and a blue truck on the right side at the bottom of the screen throughout the task. The participants are then asked to respond to a centrally presented bivalent stimulus (blue/red rabbit/truck) regarding either its shape or color. Pressing the left key sorts the stimulus to the location of the left target (i.e., the red rabbit); pressing the right key sorts the stimulus to the location of the right target (i.e., the blue truck). The DCCS task consists of four blocks (practice, pre-switch, post-switch, and mixed). During the practice block with 24 trials (12 for each dimension), participants receive a feedback whether the response was correct or false. At the beginning of each trial, a fixation cross was shown for 1,000 ms, being followed by the cue (the word "color" or "shape") they had to respond to. This cue was presented for 1,000 ms. The stimulus was then presented and disappeared only after a response was recorded. Test trials started with a pre-switch block consisting of 15 trials that had the same sorting dimension (color or shape) that was used in the preceding practice block. After that, participants were cued to the other dimension, and a post-switch block with 15 trials took place. When those two blocks are finished, the mixed block begins. Participants are then instructed to sort the stimuli to the dimensions and they are presented with 50 mixed trials that are presented in a pseudorandomized order. This mixed block includes 40 "dominant" and 10 "non-dominant" trials. The dominant dimension, which could be shape or color, was always the sorting dimension that participants were presented to in the post-switch block. The arrangement for all three test blocks is the same as for practice trials, but no feedback is provided. The order of the pre- and post-switch blocks as well as the task version with one of the dominant dimensions was counterbalanced across participants (**Figure 2D**).

### Procedure

The experiment had a sham-controlled, single-blinded, randomized crossover within-subject design. For each stimulation condition (active or sham stimulation), the participants underwent all tasks within one session. The order of the tasks was randomized for each participant beforehand. After determining the individual stimulation intensity (familiarization phase), a total of four task blocks were presented, one per task. Each block consisted of one cognitive task and a total of three measurements: The first one was done to take only resting cardiac vagal activity into account (resting period, 4-min measuring interval), the second to measure cardiac vagal activity during the stimulation (tVNS period, 4-min period), and the third to measure cardiac vagal activity during the stimulation simultaneously with the cognitive tasks (task period, 4 min). The tVNS period was included because there is a lack of evidence on the temporal latency of the effects of tVNS (Borges et al., 2019). Thus, a build-up period of four minutes of the effects of tVNS and sham stimulation was used, as done in previous studies (e.g., Burger et al., 2019). Between each test block, the participants could take a 30-s break and were then asked to continue with the next task (**Figure 3**).

The data collection took place on two different dates with approximately 1 week between the two sessions. During the sessions, either active or sham stimulation was administered to each participant. According to the crossover design, all participants underwent both stimulation conditions. The order of stimulation condition (active-sham; sham-active) was counterbalanced across participants. After taking a seat, signing the informed consent, and answering questions from a body check which included questions related to the exclusion criteria, the ECG and the tVNS electrodes were positioned. The participants then performed the four cognitive tasks across the four blocks. The HRV resting measure was taken in a sitting position with the eyes looking at a gray screen, knees at 90°, and hands on the thighs. The same body position was kept for all measurement periods, and the participants were asked to move as little as possible during the experiment. The order of the tasks was counterbalanced, however, the course of events in both conditions was identical. At the end of the second testing session, the participants were debriefed and thanked.

## Data Analysis

Outliers in the HRV data (less than 1% of the data) were winsorized, meaning that values higher/lower than two standard deviations from the mean were transformed into a value of two standard deviations from the mean. Since the HRV data as well as the tasks data were afterward still positively skewed, they were log-transformed to obtain a normal distribution. We ran the analyses with the log-transformed values; however, we indicate the raw data as descriptive values, given that they can be more easily interpreted. We excluded incorrect and missed responses for all RT analyses, and for all error percentage analyses, incorrect and missed responses were included. We defined the same cut-off values to exclude outliers in the four cognitive tasks, namely responses faster than 200 ms and greater than 2,000 ms.

To test H1a–d, we ran 2 × 2 repeated-measures analyses of variance (rmANOVAs) with stimulation condition (active vs. sham stimulation) and congruency (congruent vs. incongruent trial) for inhibitory control tasks, and stimulation condition (active vs. sham stimulation) and trial type (switch vs. non-switch

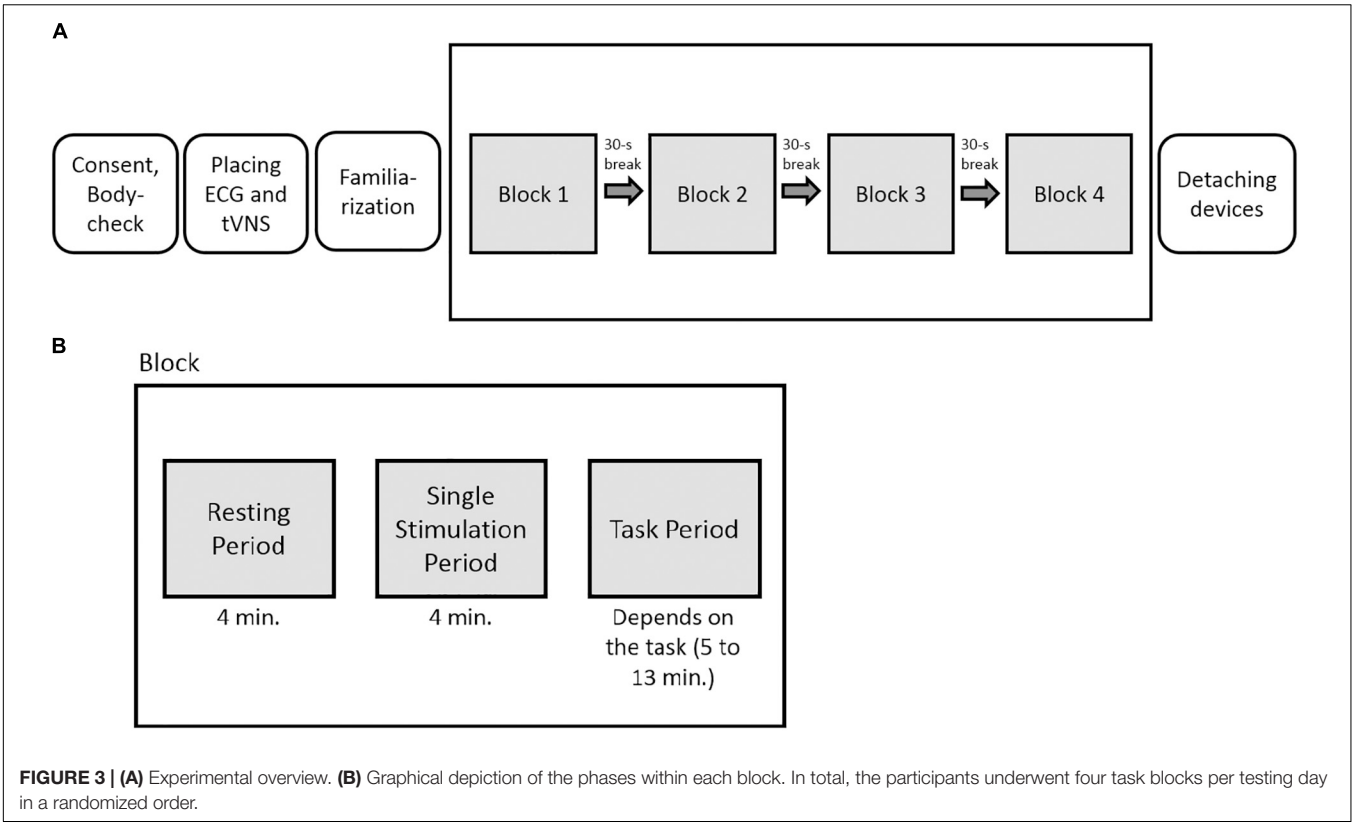



**FIGURE 3 | (A)** Experimental overview. **(B)** Graphical depiction of the phases within each block. In total, the participants underwent four task blocks per testing day in a randomized order.

trial) for cognitive flexibility tasks as within-subject factors. The relevant task parameters are RT and percentage error for all four tasks, and additionally switch costs for the cognitive flexibility tasks. Only for the effect of tVNS on switch costs (RT on switch trials minus RT on repeated trials), paired samples *t*-tests were run. To investigate H2a–d, we ran a 2 (active and sham stimulation) × 3 (resting, single tVNS, and task period) rmANOVA for each task block. Relevant dependent variables were RMSSD, HF, and respiratory frequency. To address H3a–d, we ran separated Pearson product-moment correlation matrices, one for active and one for sham stimulation, for all tasks. We investigated the correlation between RMSSD and HF during the single tVNS period and RT and percentage error, while controlling HF for respiration. In the analysis of the cognitive flexibility tasks, we additionally included switch costs. Finally, to test H4a–d, we did the same analysis as for H3a–d, but considering RMSSD and HF during the tasks instead of during the single tVNS period. To control for false discovery rate (FDR) due to multiple correlation testing, for all correlation matrices we applied the Benjamini–Hochberg procedure which adjust the *p*-value (Benjamini and Hochberg, 1995). For all rmANOVAs, Greenhouse–Geisser correction was used when sphericity was violated. In the case of a significant main or interaction effect, *post hoc* paired sample t-tests with aggregated means were conducted using Bonferroni correction. To quantify evidence for the hypotheses found, we ran Bayesian statistics using Bayesian information criteria (Wagenmakers, 2007) for all analyses. Terms used to discuss the reported Bayes factors are based on Wetzels et al. (2011) recommendations. Accordingly, values higher than 1 provide evidence for alternative hypotheses, whereas values lower than 1 provide evidence for null hypotheses. The Bayes factor can have the following meanings: anecdotal or worth no more than a bare mention ($0.333 < B_{10} < 3$), substantial ($0.100 < B_{10} \leq 0.333$ or $3 \leq B_{10} < 10$), strong ($0.033 < B_{10} \leq 0.100$ or $10 < B_{10} < 30$), very strong ($0.010 < B_{10} \leq 0.033$ or $30 \leq B_{10} < 100$), and decisive ($B_{10} \leq 0.010$ or $B_{10} \geq 100$) evidence. To control for carry-over effects on RMSSD and HF, which potentially arose in the current block due to the previous block, we tested the effect of position (i.e., first, second, third and fourth resting periods arranged chronologically) on each testing day. We also took the testing days (Day 1 and Day 2) into account in the same analysis and checked if there was a difference in RMSSD and HF from the first to the second day. We ran two separated 2 (Day 1 and Day 2) × 4 (Resting period 1, Resting period 2, Resting period 3, and Resting period 4) rmANOVAs, one for each vmHRV parameter. Furthermore, we checked whether there was a learning effect in the cognitive tasks from one testing day to the other by running 2 (Day 1 and Day 2) × 2 (congruent and incongruent or non-switch and switch trials, depending on the task) rmANOVAs, one for each behavioral measurement. Finally, to check whether tVNS affects task performance more strongly when its trials are novel, we split the trials of the tasks into first and second half, whereby first half would correspond to novel trials, and collapsed the congruent/non-switch with the incongruent/switch trials. We then ran 2 × 2 rmANOVAs with stimulation (active and sham stimulation) and novelty (first and second half of the task) as

factors, and RT and percentage error of all tasks as dependent variables. The results of these additional analyses can be found as a **Supplementary Table 1**. To report the results of the present study, we followed the CONSORT statement, which stands for Consolidated Standards of Reporting Trials (Dwan et al., 2019). We used IBM SPSS Statistics 26 to prepare the data and JASP 0.11.1 to analyze it. Significance level was $\alpha = 0.05$.

# RESULTS

## Effects of tVNS on Executive Functions

Descriptive statistics are presented in **Table 2**, and complete results of the hypothesis testing can be found in **Table 3** (inhibitory control tasks) and **Table 4** (cognitive flexibility tasks), here we will mainly focus on significant results as well as on results of Bayesian estimations for effects of stimulation. The rmANOVAs revealed that, regarding RTs in the Flanker task, there was an effect of congruency, $F(1,31) = 95.788$, $p < 0.001$, $\eta_p^2 = 0.755$, with RTs in the congruent trials ($M = 475.93$ ms, $SD = 52.14$) being significantly shorter than in the incongruent trials ($M = 555.38$ ms, $SD = 72.28$), $t(31) = 9.100$, $p < 0.001$, $d = 1.609$. No effect of active stimulation compared to sham stimulation could be found ($p = 0.283$). Regarding percentage error in the Flanker task, there was an effect of congruency, $F(1,31) = 8.202$, $p = 0.007$, $\eta_p^2 = 0.209$, with congruent trials ($M = 4.40\%$, $SD = 4.40$) presenting less errors than incongruent trials ($M = 6.80\%$, $SD = 7.12$), $t(31) = 3.157$, $p = 0.004$, $d = 0.558$. No effect of active stimulation compared to sham stimulation could be found ($p = 0.760$). According to the estimated Bayes factors (alternative/null), data provided substantial evidence for null effects of stimulation condition on RT ($B_{10} = 0.311$) and substantial evidence of null effects in percentage error ($B_{10} = 0.196$).

For RT in the Spatial Stroop task, there was an effect of congruency, $F(1,31) = 39.001$, $p < 0.001$, $\eta_p^2 = 0.557$, with RTs in the congruent trials ($M = 504.08$ ms, $SD = 51.73$) being significantly shorter than in the incongruent trials ($M = 531.64$ ms, $SD = 56.21$), $t(31) = 6.245$, $p < 0.001$, $d = 1.104$. No effect of active stimulation compared to sham stimulation could be found ($p = 0.361$). Regarding percentage error, there was an effect of congruency, $F(1,31) = 37.673$, $p < 0.001$, $\eta_p^2 = 0.549$, with congruent trials ($M = 1.47\%$, $SD = 1.48$) presenting less errors than incongruent trials ($M = 4.39\%$, $SD = 3.63$), $t(31) = 6.138$, $p < 0.001$, $d = 1.085$. No effect of active stimulation compared to sham stimulation could be found ($p = 0.756$). According to the estimated Bayes factors, data provided anecdotal evidence against the alternative hypothesis for stimulation condition regarding RT ($B_{10} = 0.344$) and substantial evidence against evidence for effects of stimulation on percentage error ($B_{10} = 0.201$). Furthermore, Bayesian estimation indicated substantial evidence for an interaction effect ($B_{10} = 3.047$).

For NLT, an effect of trial type (switch trial vs. non-switch trial) could be found on RT, $F(1,31) = 225.365$, $p < 0.001$, $\eta_p^2 = 0.879$, with non-switch trials ($M = 969.73$ ms, $SD = 130.41$) having shorter RT than switch trials ($M = 1{,}209.02$ ms, $SD = 127.84$), $t(31) = 15.012$, $p < 0.001$, $d = 2.654$. No effect of active stimulation compared to sham stimulation could be found regarding RT ($p = 0.505$). Switch costs during active stimulation ($M = 225.23$ ms, $SD = 107.14$) and during sham stimulation ($M = 251.08$ ms, $SD = 97.47$) did not differ from each other, $p = 0.140$. Regarding percentage error, there was an effect of trial type, $F(1,31) = 59.615$, $p < 0.001$, $\eta_p^2 = 0.658$, with non-switch trials ($M = 22.68\%$, $SD = 2.91$) presenting more errors than switch trials ($M = 20.39\%$, $SD = 3.22$), $t(31) = 7.721$, $p < 0.001$, $d = 1.365$. There was no main effect of stimulation ($p = 0.168$). Bayes factor indicates substantial evidence against the alternative hypothesis for stimulation condition regarding RT ($B_{10} = 0.210$), anecdotal evidence supporting the effect of stimulation on percentage error ($B_{10} = 1.097$), and anecdotal evidence against the effect of tVNS on switch costs ($B_{10} = 0.529$).

For DCCS, an effect of trial type on RT could be found, $F(1,31) = 14.720$, $p = 0.001$, $\eta_p^2 = 0.322$, with non-switch trials ($M = 969.73$ ms, $SD = 130.41$) having shorter RT than switch trials ($M = 1{,}209.02$ ms, $SD = 127.84$), $t(31) = 15.012$, $p < 0.001$, $d = 2.654$. There was no effect of stimulation on RT ($p = 0.904$), but there was an interaction effect between trial type and stimulation conditions, $F(1,31) = 11.106$, $p = 0.002$, $\eta_p^2 = 0.264$. *Post hoc* analyses (Bonferroni-corrected $p = 0.0125$) revealed that RT in non-switch trials during the sham stimulation condition ($M = 557.51$ ms, $SD = 113.56$) was significantly lower than RT in switch trials during the sham condition ($M = 614.01$ ms, $SD = 138.65$), $t(31) = 4.767$, $p < 0.001$, $d = 0.843$. Regarding percentage error, there was an effect of trial type, $F(1,31) = 15.343$, $p < 0.001$, $\eta_p^2 = 0.331$, with non-switch trials having a lower percentage error ($M = 17.49\%$, $SD = 11.39$) than switch trials ($M = 28.00\%$, $SD = 17.30$), $t(31) = 3.917$, $p < 0.001$, $d = 0.692$. There was no effect of stimulation on RT ($p = 0.677$). Active and sham stimulation differed significantly regarding switch costs, with switch costs during active stimulation ($M = 4.77$ ms, $SD = 39.75$) being lower than during sham condition ($M = 37.54$ ms, $SD = 45.39$), $t(31) = 2.797$, $p = 0.009$, $d = 0.494$. Bayes factor indicates substantial evidence against any effects of stimulation condition on RT ($B_{10} = 0.192$), against the alternative hypothesis for percentage error ($B_{10} = 0.233$), and substantial evidence for the differences in switch costs ($B_{10} = 4.916$). Furthermore, Bayesian estimation indicated substantial evidence for an interaction effect ($B_{10} = 3.047$).

## Effects of tVNS on Cardiac Vagal Activity

Descriptive statistics are presented in **Table 5**, and complete results of the hypothesis testing can be found in **Table 3** (inhibitory control tasks) and **Table 4** (cognitive flexibility tasks), here we will mainly focus on significant results as well as on results of Bayesian estimations for effects of stimulation. Regarding changes of cardiac vagal activity within the test blocks (i.e., between resting, single tVNS, and tVNS with task periods, as well as between active and sham stimulation), for Flanker task there was neither a main effect of stimulation condition ($p = 0.621$), nor of time on RMSSD ($p = 0.065$). The same applies to the main effects on HF (stimulation

**TABLE 2 |** Mean scores and standard deviations for the performance-relevant parameters of the four cognitive tasks used in the study.

| | | RT (ms) | | Percentage error (%) | | Switch costs (ms) | |
|---|---|---|---|---|---|---|---|
| | | **Active Stimulation** | **Sham Stimulation** | **Active Stimulation** | **Sham Stimulation** | **Active Stimulation** | **Sham Stimulation** |
| Flanker Task | Congruent trials | 482.29 (68.19) | 469.57 (48.91) | 4.50 (4.68) | 4.64 (4.45) | | |
| | Incongruent trials | 562.54 (88.48) | 548.21 (73.63) | 7.65 (10.59) | 5.95 (5.32) | | |
| Spatial | Congruent trials | 501.55 (52.93) | 506.60 (60.88) | 1.13 (1.40) | 1.80 (2.26) | | |
| Stroop Task | Incongruent trials | 526.08 (60.86) | 537.20 (64.90) | 4.45 (3.98) | 4.32 (4.24) | | |
| NLT | Non-switch trials | 984.11 (164.33) | 955.35 (126.00) | 21.96 (4.25) | 23.41 (2.44) | | |
| | Switch trials | 1, 212.09 (148.21) | 1, 205.95 (141.31) | 20.12 (4.20) | 20.65 (3.85) | | |
| | | | | | | 225.23 (107.14) | 251.08 (97.47) |
| DCCS | Non-switch Trials | 600.16 (138.69) | 577.51 (113.56) | 18.31 (16.01) | 16.68 (15.48) | | |
| | Switch Trials | 603.90 (137.04) | 614.01 (138.65) | 28.24 (23.92) | 27.76 (24.32) | | |
| | | | | | | 4.77 (39.75) | 37.54 (45.39) |

*RT, reaction times; NLT, Number Letter task; DCCS, Dimensional Card Sorting task.*

**TABLE 3 |** Inhibitory control tasks: results of repeated measures analyses of variance for the performance-related as well as heart rate variability parameters, with Bayesian analyses ($B_{10}$).

| | Flanker task | | | | Spatial Stroop task | | | |
|---|---|---|---|---|---|---|---|---|
| | ***F*-value** | ***p*-value** | $\eta_p^2$ | $B_{10}$ | ***F*-value** | ***p*-value** | $\eta_p^2$ | $B_{10}$ |
| **RT** | | | | | | | | |
| Congruency | 95.788 | <0.001 | 0.755 | 2.018E+13 | 39.001 | <0.001 | 0.557 | 2,732.297 |
| Stimulation condition | 1.192 | 0.283 | | 0.311 | 0.860 | 0.361 | | 0.344 |
| Stimulation × congruency | 0.001 | 0.992 | | 0.280 | 0.754 | 0.392 | | 3.047 |
| **Percentage error** | | | | | | | | |
| Congruency | 8.202 | 0.007 | 0.209 | 3.796 | 37.673 | <0.001 | 0.549 | 4.204E+7 |
| Stimulation condition | 0.095 | 0.760 | | 0.196 | 0.098 | 0.756 | | 0.201 |
| Stimulation × congruency | 0.511 | 0.480 | | 0.278 | 2.626 | 0.115 | | 0.596 |
| **RMSSD** | | | | | | | | |
| Stimulation condition | 0.250 | 0.621 | | 0.215 | 0.009 | 0.926 | | 0.189 |
| Time measurements | 2,862 | 0.065 | | 0.220 | 2.576 | 0.084 | | 0.154 |
| Time × condition | 0.351 | 0.645 | | 0.048 | 3.845 | 0.027 | 0.110 | 0.372 |
| **HF** | | | | | | | | |
| Stimulation condition | 1.669 | 0.211 | | 0.664 | 0.012 | 0.915 | | 0.196 |
| Time measurements | 2.291 | 0.135 | | 0.632 | 2.146 | 0.132 | | 0.726 |
| Time × condition | 3.038 | 0.059 | | 0.158 | 0.681 | 0.512 | | 0.203 |
| **Respiratory frequency** | | | | | | | | |
| Stimulation condition | 0.714 | 0.405 | | 0.617 | 0.213 | 0.648 | | 0.227 |
| Time measurements | 3.518 | 0.047 | 0.102 | 0.102 | 2.917 | 0.062 | | 0.099 |
| Time × condition | 0.855 | 0.430 | | 0.010 | 0.109 | 0.897 | | 0.087 |

*RT, reaction times; RMSSD, root mean square of the successive differences; HF, high frequency.*

condition: $p = 0.135$; time: $p = 0.221$). There was no effect of stimulation on respiratory frequency ($p = 0.405$), but an effect of time, $F(1.587,49.206) = 3.518$, $p = 0.047$, $\eta_p^2 = 0.102$. However, *post hoc* analyses (Bonferroni-corrected $p = 0.017$) revealed no significant mean differences. According to the estimated Bayes factors, data provided substantial evidence against the alternative hypothesis for stimulation condition regarding RMSSD ($B_{10} = 0.215$), and anecdotal evidence regarding HF ($B_{10} = 0.664$).

For the Spatial Stroop task, neither a main effect of stimulation on RMSSD ($p = 0.926$), nor of time ($p = 0.084$), was found. There was an interaction effect between the stimulation condition and RMSSD, $F(2,62) = 3.845$, $p = 0.027$, $\eta_p^2 = 0.110$, however, *post hoc* analyses revealed no effects after Bonferroni correction ($p = 0.006$). There was no effect of stimulation ($p = 0.915$), and time ($p = 0.132$) on HF and no effects on respiratory frequency (stimulation: $p = 0.648$, time: $p = 0.062$). Bayes factor indicates substantial evidence against the alternative hypothesis for stimulation condition regarding RMSSD ($B_{10} = 0.189$), HF ($B_{10} = 0.196$), and respiratory frequency ($B_{10} = 0.227$).

For the NLT, there was neither an effect of stimulation on RMSSD ($p = 0.991$), nor on time ($p = 0.599$). Regarding HF, no

**TABLE 4 |** Cognitive flexibility tasks: results of repeated measures analyses of variance for the performance-related as well as heart rate variability parameters, with Bayesian analyses ($B_{10}$).

| | NLT | | | | DCCS | | | |
|---|---|---|---|---|---|---|---|---|
| | ***F*-value** | ***p*-value** | $\eta_p^2$ | $B_{10}$ | ***F*-value** | ***p*-value** | $\eta_p^2$ | $B_{10}$ |
| **RT** | | | | | | | | |
| Trial type | 225.365 | < 0.001 | 0.879 | 1.446E+22 | 14.720 | 0.001 | 0.322 | 0.314 |
| Stimulation condition | 0.454 | 0.505 | | 0.210 | 0.015 | 0.904 | | 0.192 |
| Stimulation x congruency | 1.670 | 0.206 | | 0.411 | 11.106 | 0.002 | 0.264 | 0.339 |
| **Percentage error** | | | | | | | | |
| Trial type | 59.615 | < 0.001 | 0.658 | 602.764 | 15.343 | < 0.001 | 0.331 | 0.491 |
| Stimulation condition | 1.996 | 0.168 | | 1.097 | 0.177 | 0.677 | | 0.233 |
| Stimulation × congruency | 3.214 | 0.083 | | 0.382 | 0.552 | 0.463 | | 0.250 |
| **Switch costs**[1] | 1.513 | 0.140 | | 0.529 | 2.797 | 0.009 | 0.494 | 4.916 |
| **RMSSD** | | | | | | | | |
| Stimulation condition | < 0.001 | 0.991 | | 0.152 | 0.024 | 0.877 | | 0.160 |
| Time measurements | 0.517 | 0.599 | | 0.073 | 1.590 | 0.212 | | 0.133 |
| Time × condition | 0.810 | 0.449 | | 0.011 | 1.269 | 0.288 | | 0.150 |
| **HF** | | | | | | | | |
| Stimulation condition | 0.324 | 0.575 | | 0.216 | 0.217 | 0.646 | | 0.186 |
| Time measurements | 4.689 | 0.014 | 0.039 | 12.853 | 6.821 | 0.002 | 0.078 | 260.327 |
| Time × condition | 1.061 | 0.355 | | 0.163 | 0.391 | 0.679 | | 0.130 |
| **Respiratory frequency** | | | | | | | | |
| Stimulation condition | 0.021 | 0.885 | | 0.159 | 0.010 | 0.920 | | 0.168 |
| Time measurements | 0.657 | 0.522 | | 0.078 | 1.516 | 0.228 | | 0.078 |
| Time x condition | 0.508 | 0.604 | | 0.100 | 0.545 | 0.582 | | 0.083 |

[1] *The depicted results are from t-tests. Consequently, for switch costs, instead of F and $\eta_p^2$, the results are for t-values and Cohen's d, respectively. NLT, Number Letter task; DCCS, Dimensional Card Sorting test; RT, reaction times; RMSSD, root mean square of the successive differences; HF, high frequency.*

**TABLE 5 |** Mean scores and standard deviations for the heart rate variability parameters over time in the four cognitive task blocks.

| | | RMSSD (ms) | | HF (ms$^2$) | | Respiratory frequency (cycles per minute) | |
|---|---|---|---|---|---|---|---|
| | | **Active Stimulation** | **Sham Stimulation** | **Active Stimulation** | **Sham Stimulation** | **Active Stimulation** | **Sham Stimulation** |
| Flanker Task | Resting | 48.43 (22.38) | 52.34 (26.56) | 13.81 (8.78) | 13.71 (12.45) | 12.36 (2.06) | 12.19 (2.68) |
| | tVNS | 52.56 (28.53) | 54.66 (25.02) | 15.27 (11.26) | 19.59 (13.94) | 12.51 (2.40) | 12.10 (2.91) |
| | Task | 55.44 (29.81) | 55.26 (24.66) | 14.44 (9.60) | 16.16 (11.56) | 12.23 (2.33) | 11.66(3.03) |
| Spatial | Resting | 52.38 (27.64) | 53.48 (21.52) | 12.97 (10.05) | 14.12 (10.80) | 14.70 (9.61) | 15.85 (10.79) |
| Stroop Task | tVNS | 54.47(25.99) | 58.85 (26.31) | 18.74(13.19) | 17.31(13.60) | 19.60(11.82) | 19.16(15.58) |
| | Task | 55.93(26.89) | 50.70(19.28) | 15.65 (8.45) | 17.45 (13.91) | 16.25 (9.12) | 20.32 (16.48) |
| NLT | Resting | 51.82(24.75) | 50.07 (22.2) | 18.06(12.22) | 13.83(10.98) | 12.20 (2.03) | 12.02(2.33) |
| | tVNS | 49.91(21.12) | 51.82(20.44) | 18.51(12.56) | 18.85 (15.07) | 12.27 (2.05) | 12.38(2.64) |
| | Task | 50.28(25.77) | 48.78(18.45) | 17.78(12.13) | 17.547(9.40) | 12.06 (1.88) | 12.17(2.48) |
| DCCS | Resting | 54.26(24.46) | 51.82(22.46) | 14.93(10.11) | 16.24(14.98) | 13.52 (8.82) | 15.66(13.96) |
| | tVNS | 54.90(25.86) | 57.4 (24.75) | 17.56(12.57) | 19.59(13.11) | 17.95(12.18) | 19.23(12.80) |
| | Task | 56.36(24.52) | 55.41(23.24) | 19.83(13.16) | 17.55(11.14) | 20.76(11.75) | 19.90(10.07) |

*RMSSD, root mean square of the successive differences; HF, high frequency; tVNS, transcutaneous vagus nerve stimulation (single stimulation phase); NLT, Number Letter task; DCCS, Dimensional Change Card Sorting task.*

effect of stimulation ($p$ = 0.575), but a main effect of time was found, $F(2,46) = 4.689$, $p = 0.014$, $\eta_p^2 = 0.039$. *Post hoc* analyses (Bonferroni-corrected $p$ = 0.017) revealed that HF during the resting period ($M$ = 12.92, $SD$ = 8.25) was significantly lower than during the task period ($M$ = 18.31, $SD$ = 9.39), $t(31) = 4.108$, $p < 0.001$, $d = 0.726$. According to the estimated Bayes factors, there is substantial evidence against the alternative hypothesis for stimulation condition regarding RMSSD ($B_{10} = 0.152$), regarding HF ($B_{10} = 0.216$), and respiratory frequency ($B_{10} = 0.159$).

For the DCCS, there was neither a main effect of stimulation condition on RMSSD ($p$ = 0.877), nor of time ($p$ = 0.212). Regarding HF, there was no effect of stimulation, ($p$ = 0.646),

but a main effect of time, $F(1.613,38.708) = 6.821$, $p = 0.002$ $\eta_p^2 = 0.078$. *Post hoc* analyses (Bonferroni-corrected $p = 0.017$) revealed that HF increased from resting ($M = 13.36$, $SD = 9.42$) to single stimulation phase ($M = 16.71$, $SD = 11.20$), $t(31) = 3.205$, $p = 0.003$, $d = 0.566$, and from resting to task phase ($M = 19.71$, $SD = 8.96$), $t(31) = 4.708$, $p < 0.001$, $d = 0.832$. According to the estimated Bayes factors, data provided substantial evidence against the alternative hypothesis for RMSSD regarding stimulation condition ($B_{10} = 0.160$), regarding HF ($B_{10} = 0.186$), and regarding respiratory frequency ($B_{10} = 0.168$).

## Correlations Between Cardiac Vagal Activity and Cognitive Performance

We ran Pearson product-moment correlations to investigate if vmHRV parameters that were measured during the single stimulation phase and the task phase predicted performance on the cognitive tasks. Complete correlation matrices can be found in **Table 6** (for inhibitory control tasks) and **Table 7** (for cognitive flexibility tasks), here we will only present significant results. None of the vmHRV parameters measured during the Flanker task correlated with the cognitive parameters. Regarding the Spatial Stroop task, there was only significant correlations between the parameters measured in the sham condition: RT in both congruent ($r = -0.42$, $p = 0.018$) and incongruent trials ($r = -0.39$, $p = 0.027$) correlated negatively with RMSSD during the single stimulation phase. HF correlated negatively with RT in the congruent trials during the single stimulation phase ($r = -0.43$, $p = 0.038$), and positively with percentage error of the incongruent trials during the single stimulation phase ($r = 0.43$, $p = 0.032$). In the NLT, RMSSD correlated positively with percentage error of non-switch trials during the active condition ($r = 0.40$, $p = 0.025$). In the active condition, HF during the single stimulation phase correlated negatively with RT of both non-switch ($r = -0.44$, $p = 0.015$) and switch trials ($r = -0.50$, $p = 0.005$), and HF during the task phase correlated negatively with switch costs ($r = -0.42$, $p = 0.019$). In the sham condition, HF correlated positively with percentage error during the task phase ($r = 0.48$, $p = 0.015$). In the DCCS, switch costs in the active condition correlated positively with RMSSD during the single stimulation phase ($r = 0.40$, $p = 0.024$), with RMSSD during the task phase ($r = 0.37$, $p = 0.035$), and negatively with HF during the task phase ($r = -0.42$, $p = 0.019$). HF during the task phase correlated positively with RT of both non-switch ($r = -0.40$, $p = 0.026$) and switch trials ($r = -0.42$, $p = 0.018$). Importantly, after adjusting the *p*-values using the FDR correction, none of these correlations remained significant.

# DISCUSSION

The aim of this study was to investigate the effect of tVNS on performance in tasks commonly used to measure inhibitory control and cognitive flexibility, core executive functions on which higher-order executive functions rely. Based on the neurovisceral integration model (Thayer et al., 2009), we hypothesized that executive performance would be better during the active stimulation condition compared to the sham stimulation condition (H1a–d). Conflict effects were found in all four tasks used. However, among the four tasks, only in the DCCS a better performance could be directly linked to tVNS, with switch costs being lower in the active condition than in the sham condition. For this reason, among the H1 hypotheses, only H1c was supported. On the physiological level, we expected vmHRV to be higher in the active condition during both the single stimulation period and the task period (H2a–d). During both cognitive flexibility tasks, HF increased from resting phase to task phase, but no difference between active and sham stimulation could be detected. Therefore, H2a–d were not supported. Moreover, it was hypothesized that higher cardiac vagal activity in the single stimulation phase (H3a–d) and in the task phase (H4a–d) would be associated with better task performance only in the active condition. Because none of the correlations remained significant after adjusting the *p*-values, none of these hypotheses could be confirmed.

In the present study, we could provide a conceptual replication (Walker et al., 2017) of the conflict effects previously observed in tasks that are thought to mainly demand selective attention like the Flanker task (Alderman and Olson, 2014) and response inhibition with the Spatial Stroop task (Marotta et al., 2018). In the same sense, findings toward dual-task interference evoked by a task used to measure task switching with NLT (Colzato et al., 2018a), as well as by a task thought to measure set shifting with DCCS (Zelazo et al., 2014) could be replicated with large effect sizes. However, an effect of tVNS could be found only on set shifting with DCCS. First, smaller switch costs during tVNS were observed compared to the sham condition. Second, RT in non-switch trials did not differ from RT in switch trials during active stimulation, but in the sham stimulation RT in switch trials were higher than in non-switch trials. Possibly tVNS diminished the dual-task interference, whereas sham stimulation did not, and this would explain this difference in switch costs between tVNS and sham stimulation. Importantly, some results referring to a lack of difference between active and sham stimulation were not substantially supported by Bayesian estimations, namely for RT in the Spatial Stroop task, HF and respiratory frequency in the Flanker task, and percentage error and switch costs in the NLT. Consequently, these findings should be interpreted carefully.

The mixed nature of the results and the lack of correlation between cognitive performance and cardiac vagal activity provide evidence against a generability of the neurovisceral integration model (Thayer et al., 2009). These findings can be interpreted in various manners. First, the present study indicates that tVNS may exert a circumscribed influence on core executive functions. This suggests that the neurovisceral integration model may be less generally applicable than previously outlined (Thayer et al., 2009; Smith et al., 2017). This specificity is in line with previous findings involving executive functions and cardiac vagal activity (Jennings et al., 2015). Jennings et al. (2015) found that cardiac vagal activity was not directly related to resting state activity of intrinsic brain networks but rather to more localized connectivity. This implies

**TABLE 6 |** Pearson product-moment correlations between cognitive performance-relevant parameters and vagally-mediated heart rate variable parameters during the single stimulation phase (tVNS) and the task phase (task) for active and sham conditions.

| | | | Active Stimulation | | | | Sham Stimulation | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | RT | | Percentage error | | RT | | Percentage error | |
| | | | Congruent trials | Incongruent trials | Congruent trials | Incongruent trials | Congruent trials | Incongruent trials | Congruent trials | Incongruent trials |
| **Flanker task** | | | | | | | | | | |
| RMSSD | tVNS | Pearson's *r* | 0.02 | −0.05 | −0.21 | −0.22 | −0.29 | −0.23 | 0.26 | 0.197 |
| | | *p*-value | 0.935 | 0.768 | 0.243 | 0.233 | 0.114 | 0.207 | 0.144 | 0.280 |
| | Task | Pearson's *r* | −0.06 | −0.08 | −0.25 | −0.28 | −0.24 | −0.24 | 0.16 | 0.27 |
| | | *p*-value | 0.760 | 0.666 | 0.171 | 0.115 | 0.193 | 0.189 | 0.369 | 0.140 |
| HF | tVNS | Pearson's *r* | −0.20 | −0.19 | 0.16 | 0.23 | −0.24 | 0.01 | 0.06 | 0.03 |
| | | *p*-value | 0.288 | 0.334 | 0.395 | 0.237 | 0.262 | 0.991 | 0.785 | 0.906 |
| | Task | Pearson's *r* | −0.25 | −0.30 | −0.09 | −0.17 | −0.34 | −0.18 | −0.01 | 0.07 |
| | | *p*-value | 0.189 | 0.119 | 0.647 | 0.378 | 0.109 | 0.394 | 0.987 | 0.760 |
| **Spatial Stroop task** | | | | | | | | | | |
| RMSSD | tVNS | Pearson's *r* | 0.06 | −0.04 | −0.22 | −0.15 | −0.42* | −0.39* | −0.12 | −0.01 |
| | | *p*-value | 0.755 | 0.845 | 0.227 | 0.403 | 0.018 | 0.027 | 0.532 | 0.987 |
| | Task | Pearson's *r* | −0.02 | −0.13 | −0.18 | −0.34 | −0.34 | −0.31 | 0.19 | 0.12 |
| | | *p*-value | 0.907 | 0.485 | 0.318 | 0.054 | 0.059 | 0.088 | 0.311 | 0.514 |
| HF | tVNS | Pearson's *r* | −0.25 | −0.27 | −0.11 | 0.28 | −0.43* | −0.32 | 0.17 | 0.45* |
| | | *p*-value | 0.175 | 0.151 | 0.579 | 0.131 | 0.038 | 0.142 | 0.437 | 0.032 |
| | Task | Pearson's *r* | −0.20 | −0.07 | −0.17 | 0.12 | −0.27 | −0.24 | 0.11 | 0.10 |
| | | *p*-value | 0.302 | 0.715 | 0.376 | 0.539 | 0.219 | 0.264 | 0.623 | 0.642 |

*Coefficients for the inhibitory control tasks. *$p < 0.05$. Non-adjusted p-values. RT, reaction times; RMSSD, root mean square of successive differences; HF, high frequency; tVNS, transcutaneous vagus nerve stimulation (single stimulation phase).*

that the integration between autonomic and cognitive control is more limited than the general integration originally suggested. Consequently, the neurovisceral integration model (Thayer et al., 2009) might not apply to the full range of executive functions, but rather to specific cognitive functions (Jennings et al., 2015).

It is not clear, however, whether the specificity of the integration between autonomic and cognitive regulation shown in the present study is valid for executive functions in general–i.e., independently of the method used to manipulate them–or whether tVNS affects only specific cognitive regulation processes. One of the reasons for this possible specificity related to tVNS might lie in the level of neurotransmission: tVNS sends a signal to the locus coeruleus (Kraus et al., 2007; Dietrich et al., 2008), the primary source of norepinephrine in the brain (Foote et al., 1983). Norepinephrine has been thought to be engaged by tVNS (Steenbergen et al., 2015; van Leusden et al., 2015; Beste et al., 2016). Locus coeruleus plays an important role in reorienting attention and cognitive flexibility, and those neurons have been shown to have a task-related activation (Sara, 2015). Noradrenergic α-1 and α-2 receptors act in distinct cognitive processes: whereas α-2 receptors engage at moderate rates of norepinephrine release, thus promoting working memory, α-1 receptors are activated at higher rates, promoting both focused and flexible attention (Berridge and Spencer, 2016). It is not clear whether DCCS demands more flexible attention than NLT, and whether the difference between the two could only be observed because tVNS evokes a stronger release of norepinephrine, engaging α-1 receptors that were necessary for the DCCS but less so for the NLT. Hence, it is recommended for future studies to address the possible specific efficacy of tVNS by considering an on-line measurement of norepinephrine such as pupillary responses (Warren et al., 2018; Keute et al., 2019b; Burger et al., 2020). This approach might complement and further specify the hypotheses based on the neurovisceral integration model (Thayer et al., 2009).

Second, despite all efforts in taking well-acknowledged recommendations into account, task impurity (Miyake et al., 2000) may not have been ruled out. Consequently, the question remains whether other cognitive processes underlying the specific task used to measure set shifting, and not set shifting *per se*, are influenced by tVNS. For instance, inhibitory processes have been thought to take place in cognitive flexibility. Accordingly, for the efficient activation of another set in the context of set shifting, the inhibition of the previous, no longer relevant task, is required. Therefore, backward inhibition is a process highly involved in cognitive flexibility (Mayr and Keele, 2000). It remains unclear if a comparable amount of backward inhibition is required for both tasks used to measure cognitive flexibility. Similarly, rather than Spatial Stroop task being considered a good index of response inhibition, possibly interference control, i.e., control at the level of perception, is measured by means of this task (Tafuro et al., 2019). To overcome these concerns, it is necessary to develop cognitive

**TABLE 7 |** Pearson product-moment correlations between cognitive performance-relevant parameters and vagally mediated heart rate variable parameters during the single stimulation phase (tVNS) and the task phase (task) for active and sham conditions. Coefficients for the cognitive flexibility tasks.

| | | | Active Stimulation | | | | | Sham Stimulation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RT | | Percentage error | | | RT | | Percentage error | | |
| | | | Non-switch trials | Switch trials | Non-switch trials | Switch trials | Switch costs | Non-switch trials | Switch trials | Non-switch trials | Switch trials | Switch costs |
| **NLT** | | | | | | | | | | | | |
| RMSSD | tVNS | Pearson's *r* | −0.28 | −0.24 | 0.40* | 0.14 | 0.13 | 0.12 | 0.15 | 0.19 | −0.06 | −0.02 |
| | | *p*-value | 0.132 | 0.179 | 0.025 | 0.434 | 0.434 | 0.513 | 0.430 | 0.308 | 0.727 | 0.934 |
| | Task | Pearson's *r* | −0.06 | −0.03 | 0.33 | 0.31 | 0.13 | 0.10 | 0.29 | 0.22 | −0.02 | 0.28 |
| | | *p*-value | 0.732 | 0.860 | 0.070 | 0.081 | 0.475 | 0.595 | 0.113 | 0.238 | 0.909 | 0.115 |
| HF | tVNS | Pearson's *r* | −0.44* | −0.50** | 0.37* | 0.31 | 0.14 | 0.09 | −0.10 | 0.11 | −0.23 | −0.28 |
| | | *p*-value | 0.015 | 0.005 | 0.046 | 0.099 | 0.463 | 0.677 | 0.626 | 0.599 | 0.279 | 0.170 |
| | Task | Pearson's *r* | −0.39* | −0.24 | 0.28 | 0.22 | 0.42* | −0.10 | −0.02 | 0.48* | 0.07 | 0.15 |
| | | *p*-value | 0.034 | 0.204 | 0.129 | 0.242 | 0.020 | 0.621 | 0.914 | 0.015 | 0.748 | 0.482 |
| **DCCS** | | | | | | | | | | | | |
| RMSSD | tVNS | Pearson's *r* | −0.27 | −0.17 | 0.24 | 0.29 | 0.40* | 0.09 | 0.04 | −0.03 | −0.01 | −0.10 |
| | | *p*-value | 0.134 | 0.351 | 0.180 | 0.103 | 0.024 | 0.623 | 0.837 | 0.869 | 0.973 | 0.603 |
| | Task | Pearson's *r* | −0.25 | −0.14 | 0.16 | 0.23 | 0.37* | 0.06 | 0.01 | 0.03 | 0.02 | −0.08 |
| | | *p*-value | 0.177 | 0.440 | 0.385 | 0.212 | 0.035 | 0.741 | 0.953 | 0.858 | 0.920 | 0.660 |
| HF | tVNS | Pearson's *r* | −0.40* | −0.42* | 0.29 | 0.31 | −0.08 | 0.05 | 0.04 | −0.19 | −0.14 | 0.03 |
| | | *p*-value | 0.026 | 0.018 | 0.110 | 0.089 | 0.684 | 0.796 | 0.835 | 0.356 | 0.483 | 0.900 |
| | Task | Pearson's *r* | 0.07 | −0.01 | −0.27 | −0.19 | −0.42* | −0.05 | −0.03 | 0.02 | −0.06 | 0.10 |
| | | *p*-value | 0.715 | 0.970 | 0.150 | 0.314 | 0.019 | 0.819 | 0.905 | 0.931 | 0.761 | 0.619 |

*$p < 0.05$, **$p < 0.01$. Non-adjusted p values. RT = reaction times; RMSSD = root mean square of successive differences; HF = high frequency; NLT = Number Letter task; DCCS = Dimensional Change Card Sorting task; tVNS = transcutaneous vagus nerve stimulation (single stimulation phase).*

tasks that minimally vary from one another in the sense that the additional cognitive processes necessary for performing a cognitive task can be minimized or at least kept constant. This would enable a more accurate integrative assessment of the core executive functions in future research with tVNS investigating executive performance.

Third, the lack of a difference between tVNS and sham stimulation regarding cardiac vagal activity, which is in line with previous findings (Burger et al., 2016, 2019; De Couck et al., 2017; Borges et al., 2019), could have contributed to the heterogeneity of the findings. Despite ample evidence on the effects of tVNS on cognition (e.g., Steenbergen et al., 2015; Sellaro et al., 2017), the evidence provided by the present study on cardiac vagal activity substantiates the arguments against the suitability of the earlobe as a sham stimulation, as discussed lately (Keute et al., 2018b; Rangon, 2018; Borges et al., 2019). At present, there is only one detailed description of the nerve distribution of the human auricle and it shows that the earlobe is free from vagal innervation (Peuker and Filler, 2002). However, it lacks substantial evidence that electrical stimulation on the earlobe cannot stimulate brain center nuclei that trigger an increase in cardiac vagal outflow (Rangon, 2018). This is especially relevant because the boundaries between particular dermatomes often overlap (Butt et al., 2019), so that a clear understanding of the nerve distribution of the human auricle is needed. Regardless of the suitability of the earlobe, it has also been discussed whether vmHRV parameters are sensitive to afferent vagal changes triggered by tVNS; it is not yet clear whether the electrical signal produced by tVNS is strong enough to overcome body-related barriers such as skin and blood vessels, and therefore to trigger vagal afferent firing in a way that would robustly increase prefrontal activity, thus indirectly affecting cardiac vagal activity (Borges et al., 2019).

In the present study, the cognitive tasks themselves did not seem to have an impact on the vmHRV parameters, since neither RMSSD nor HF decreased during the tasks when compared to before the tasks. It is not clear whether this lack of a decrease–which would be expected based on the neurovisceral integration model (Thayer et al., 2009; Smith et al., 2017), given the conflict effects elicited by the tasks–was due to tVNS or not. Possibly, the tasks were not cognitively demanding enough to evoke a decrease in cardiac vagal activity. The lack of cognitive demand could also explain why we found no effect of tVNS on inhibitory control, whereas an array of previous studies provided evidence in this direction (see **Table 1**). Importantly, none of these previous studies used the same paradigms that were used in the present study. It is possible that the paradigms for measuring inhibitory control used here, at least concerning the amount of trials and instructions used in the present study, are not sensitive to effects that might otherwise be elicited by tVNS. Moreover, none of the previous studies investigating the effects of tVNS on inhibitory control found overall enhanced performance, measured by means of RT and percentage error (see **Table 1**). Instead, they addressed inhibitory control in specific contexts, such as backward inhibition when working memory is more strongly demanded (Beste et al., 2016), or response selection during action cascading (Steenbergen et al., 2015). Regarding cognitive demand, future studies should incorporate measures of the cognitive demand of the tasks, for instance by means of subjective questionnaires or imaging techniques such as functional near-infrared spectroscopy (fNIRS) and fMRI to measure prefrontal activity during task performance.

As the only vmHRV parameter to show changes in the present study, HF increased during the NLT and DCCS when compared to the resting phase. Since both tasks are cognitively demanding due to the dual-task interference, based on the neurovisceral integration model (Thayer et al., 2009) HF should decrease compared to both resting and single stimulation phases. At the same time, this increase of HF was not associated with a better performance in the DCCS, as it would be predicted by the neurovisceral integration model. Although there was no difference between tVNS and sham stimulation regarding HF in the present study, the increase in HF during the DCCS might be linked to the positive effect of tVNS found on switch costs. So far, there has been no other study investigating the effect of tVNS on respiration, and whether respiration, when affected by tVNS, moderates executive performance. Future studies should address this question in order to further investigate the mechanisms of action behind tVNS.

## Limitations

There are limitations to our study that should be mentioned. First, RMSSD increased within the experimental sessions (see **Supplementary Material**). It is not clear, however, whether this carry-over effect emerged from the stimulation itself, or simply from the fact that the participants were sitting during the experiment. Thus, this increase during the experimental sessions may represent a relevant confounder that renders it difficult to interpret cardiac vagal activity measurements. Second, despite considering inhibitory control and cognitive flexibility differentially by taking different aspects into account, the present study did not consider other types of cognitive flexibility. Creatively thinking outside the box, seeing something from different perspectives (Diamond, 2013), or stochastic reversal learning (Colzato et al., 2018a) could be aspects of cognitive flexibility prone to be influenced by tVNS. Third, respiratory frequency was obtained via a dedicated algorithm from Kubios (Tarvainen et al., 2013). However, a more precise assessment of respiratory frequency such as a respiration belt or a pneumotachograph is recommendable (Quintana et al., 2016). Fourth, the sample has a misbalance regarding gender, with male participants being vast majority. Given that sex differences can influence cardiac vagal activity (Koenig and Thayer, 2016), this misbalance may have been an issue for the analysis. Finally, as stated above, the tasks are not comparable to each other. For example, the Flanker task used here has, when compared to the Spatial Stroop task, a shorter stimulus presentation time and random intertrial interval. This may provoke different cognitive processes that deviate from the ones we aimed at measuring. A further difference is the length of the tasks, ranging from five (DCCS) to 13 (Flanker task) min. The amount of trials also greatly varies between the tasks. Due to a lack of measurement of task difficulty, it was not possible to investigate whether

the difficulty level differed strongly between the tasks, as stated above. Furthermore, the DCCS uses colorful pictures, whereas all other tasks are bicolored and involve time pressure. The impact of these differences on the cognitive tasks should be considered when using them in future studies with tVNS.

## CONCLUSION

The present study is the first to investigate different core executive functions with their different subtypes in an integrative manner. Additionally, this is the first study to investigate the effect of tVNS on cognitive flexibility. On the one hand, it was shown that tVNS can lead to less switch costs in set shifting, possibly explained by diminished dual-task interference due to tVNS. On the other hand, the present study provided evidence that tVNS may have only very specific effects on cognitive processes. By addressing the different aspects of core cognitive functions in one standardized study design, the present study contributes to a better understanding of the effects of tVNS by further delineating what kind of cognitive and physiological mechanisms might be influenced by this neuroenhancement tool. Future studies investigating the effect of tVNS on executive functions should further investigate cognitive flexibility and consider task characteristics as well as address different types of executive functions.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

## ETHICS STATEMENT

This study was reviewed and approved by the Ethics Committee of the German Sport University Cologne (120/2018). The participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

UB and SL contributed to conceiving the design of the study. LK led the data collection with the help of UB. UB realized the statistical analysis with the help of SL. UB wrote the first draft of the manuscript. SL, MR, and SK provided critical comments to improve it. SL and MR suggested the final adjustments on the manuscript. All authors agreed on the final version.

## ACKNOWLEDGMENTS

We are thankful to the colleagues of the Performance Psychology Group for their helpful comments, Fabian Vermum for his help in collecting the data, and Esther van Schwartzenberg, Patricia Faust, and Niklas Piontek for their help in preparing the cognitive tasks and the data.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2020.00523/full#supplementary-material

## REFERENCES

Aihara, M., Ida, I., Yuuki, N., Oshima, A., Kumano, H., Takahashi, K., et al. (2007). HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. *Psychiatry Res. Neuroimaging* 155, 245–256. doi: 10.1016/j.pscychresns.2006.11.002

Alderman, B. L., and Olson, R. L. (2014). The relation of aerobic fitness to cognitive control and heart rate variability: a neurovisceral integration study. *Biol. Psychol.* 99, 26–33. doi: 10.1016/j.biopsycho.2014.02.007

Arnsten, A. F. T., and Li, B. (2004). Neurobiology of executive functions?: catecholamine influences on prefrontal cortical functions. *Biol. Psychiatry* 57, 1377–1384. doi: 10.1016/j.bps.2004.08.019

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57, 289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Berridge, C. W., and Spencer, R. C. (2016). Differential cognitive actions of norepinephrine a2 and a1 receptor signaling in the prefrontal cortex. *Brain Res.* 1641, 189–196. doi: 10.1016/j.brainres.2015.11.024

Beste, C., Steenbergen, L., Sellaro, R., Grigoriadou, S., Zhang, R., Chmielewski, W., et al. (2016). Effects of concomitant stimulation of the gabaergic and norepinephrine system on inhibitory control – a study using transcutaneous vagus nerve stimulation. *Brain Stimul.* 9, 811–818. doi: 10.1016/j.brs.2016.07.004

Borges, U., Laborde, S., and Raab, M. (2019). Influence of transcutaneous vagus nerve stimulation on cardiac vagal activity: not different from sham stimulation and no effect of stimulation intensity. *PLoS One* 14:e0223848. doi: 10.1371/journal.pone.0223848

Burger, A. M., van der Does, W., Brosschot, J. F., and Verkuil, B. (2020). From ear to eye? No effect of transcutaneous vagus nerve stimulation on human pupil dilation: a report of three studies. *Biol. Psychol.* 152:107863. doi: 10.1016/j.biopsycho.2020.107863

Burger, A. M., Van der Does, W., Thayer, J. F., Brosschot, J. F., and Verkuil, B. (2019). Transcutaneous vagus nerve stimulation reduces spontaneous but not induced negative thought intrusions in high worriers. *Biol. Psychol.* 142, 80–89. doi: 10.1016/j.biopsycho.2019.01.014

Burger, A. M., Verkuil, B., Van Diest, I., Van der Does, W., Thayer, J. F., and Brosschot, J. F. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. *Neurobiol. Learn. Mem.* 132, 49–56. doi: 10.1016/j.nlm.2016.05.007

Butt, M. F., Albusoda, A., Farmer, A. D., and Aziz, Q. (2019). The anatomical basis for transcutaneous auricular vagus nerve stimulation. *J. Anat.* 236, 588–611. doi: 10.1111/joa.13122

Colzato, L. S., Jongkees, B. J., de Wit, M., van der Molen, M. J. W., and Steenbergen, L. (2018a). Variable heart rate and a flexible mind: higher resting-state heart rate variability predicts better task-switching. *Cogn. Affect. Behav. Neurosci.* 18, 730–738. doi: 10.3758/s13415-018-0600-x

Colzato, L. S., Ritter, S. M., and Steenbergen, L. (2018b). Transcutaneous vagus nerve stimulation (tVNS) enhances divergent thinking. *Neuropsychologia* 111, 72–76. doi: 10.1016/j.neuropsychologia.2018.01.003

Dajani, D. R., and Uddin, L. Q. (2015). Demystifying cognitive flexibility: implications for clinical and developmental neuroscience. *Trends Neurosci.* 38, 571–578. doi: 10.1016/j.tins.2015.07.003

De Couck, M., Cserjesi, R., Caers, R., Zijlstra, W. P., Widjaja, D., Wolf, N., et al. (2017). Effects of short and prolonged transcutaneous vagus nerve stimulation on heart rate variability in healthy subjects. *Auton. Neurosci. Basic Clin.* 203, 88–96. doi: 10.1016/j.autneu.2016.11.003

Diamond, A. (2013). Executive functions. *Annu. Rev. Psychol.* 64, 135–168. doi: 10.1146/annurev-psych-113011-143750.Executive

Dietrich, S., Smith, J., Scherzinger, C., Hofmann-Preiß, K., Freitag, T., Eisenkolb, A., et al. (2008). A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI. *Biomed. Tech.(Berl.)* 53, 104–111. doi: 10.1515/BMT.2008.022

Dwan, K., Li, T., Altman, D. G., and Elbourne, D. (2019). CONSORT 2010 statement: extension to randomised crossover trials. *BMJ* 366:l4378. doi: 10.1136/bmj.l4378

Ellrich, J. (2011). Transcutaneous vagus nerve stimulation. *Eur. Neurol. Rev.* 6:254. doi: 10.17925/ENR.2011.06.04.254

Eriksen, B. A., and Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Percept. Psychophys.* 16, 143–149. doi: 10.3758/BF03203267

Faul, F., Erdfelder, E., Lang, A.-G., and Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. doi: 10.3758/BF03193146

Finisguerra, A., Crescentini, C., and Urgesi, C. (2019). Transcutaneous vagus nerve stimulation affects implicit spiritual self-representations. *Neuroscience* 412, 144–159. doi: 10.1016/j.neuroscience.2019.05.059

Fischer, R., Ventura-Bort, C., Hamm, A., and Weymar, M. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances conflict-triggered adjustment of cognitive control. *Cogn. Affect. Behav. Neurosci.* 18, 680–693. doi: 10.3758/s13415-018-0596-2

Foote, S. L., Bloom, F. E., and Aston-Jones, G. (1983). Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol. Rev.* 63, 844–914. doi: 10.1152/physrev.1983.63.3.844

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: FMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Jennings, J. R., Allen, B., Gianaros, P. J., Thayer, J. F., and Stephen, B. (2015). Focusing neurovisceral integration: cognition, heart rate variability, and cerebral blood flow. *Psychophysiology* 52, 214–224. doi: 10.1111/psyp.12319. Focusing

Johnsen, B. H., Thayer, J. F., Laberg, J. C., Wormnes, B., Raadal, M., Skaret, E., et al. (2003). Attentional and physiological characteristics of patients with dental anxiety. *J. Anxiety Disord.* 17, 75–87.

Keute, M., Boehrer, L., Ruhnau, P., Heinze, H.-J., and Zaehle, T. (2019a). Transcutaneous vagus nerve stimulation (tVNS) and the dynamics of visual bistable perception. *Front. Neurosci.* 13:227. doi: 10.3389/fnins.2019.00227

Keute, M., Demirezen, M., Graf, A., Mueller, N. G., and Zaehle, T. (2019b). No modulation of pupil size and event-related pupil response by transcutaneous auricular vagus nerve stimulation (taVNS). *Sci. Rep.* 9:11452. doi: 10.1038/s41598-019-47961-4

Keute, M., Ruhnau, P., Heinze, H., and Zaehle, T. (2018a). Behavioral and electrophysiological evidence for GABAergic modulation through transcutaneous vagus nerve stimulation. *Clin. Neurophysiol.* 129, 1789–1795. doi: 10.1016/j.clinph.2018.05.026

Keute, M., Ruhnau, P., and Zaehle, T. (2018b). Reply to "Reconsidering sham in transcutaneous vagus nerve stimulation studies.". *Clin. Neurophysiol.* 129, 2503–2504. doi: 10.1016/j.clinph.2018.09.001

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability?: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Kraus, T., Hösl, K., Kiess, O., Schanze, A., Kornhuber, J., and Forster, C. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *J. Neural Trans.* 114, 1485–1493. doi: 10.1007/s00702-007-0755-z

Kraus, T., Kiess, O., Hösl, K., Terekhin, P., Kornhuber, J., and Forster, C. (2013). CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal – a pilot study. *Brain Stimul.* 6, 798–804. doi: 10.1016/j.brs.2013.01.011

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 08:213. doi: 10.3389/fpsyg.2017.00213

Liepelt, R., Porcu, E., Stenzel, A., and Lappe, M. (2019). Saccadic eye movements do not trigger a joint Simon effect. *Psychon. Bull. Rev.* 26, 1896–1904. doi: 10.3758/s13423-019-01639-0

Malik, M., Camm, A. J., Bigger, J. T., Breithart, G., Cerutti, S., and Cohen, R. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the european society of cardiology and the North American society of pacing and electrophysiology. *Circulation* 93, 1043–1065.

Marotta, A., Román-Caballero, R., and Lupiáñez, J. (2018). Arrows don't look at you?: qualitatively different attentional mechanisms triggered by gaze and arrows. *Psychon. Bull. Rev.* 25, 2254–2259. doi: 10.3758/s13423-018-1457-2

Mayr, U., and Keele, S. W. (2000). Changing internal constraints on action: the role of backward inhibition. *J. Exp. Psychol. Gener.* 129, 4–26. doi: 10.1037//0096-3445.129.1.4

Miyake, A., and Friedman, N. P. (2012). The nature and organization of individual differences in executive functions: four general conclusions. *Curr. Dir. Psychol. Sci.* 21, 8–14. doi: 10.1177/0963721411429458

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., and Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "' Frontal Lobe "' tasks?: a latent variable analysis. *Cogn. Psychol.* 41, 49–100. doi: 10.1006/cogp.1999.0734

Murray, A. R., Atkinson, L., Mahadi, M. K., Deuchars, S. A., and Deuchars, J. (2016). The strange case of the ear and the heart: the auricular vagus nerve and its influence on cardiac control. *Auton. Neurosci. Basic Clin.* 199, 48–53. doi: 10.1016/j.autneu.2016.06.004

Peirce, J., Gray, J. R., Simpson, S., MacAskill, M., Höchenberger, R., Sogo, H., et al. (2019). PsychoPy2: experiments in behavior made easy. *Behav. Res. Methods* 51, 195–203. doi: 10.3758/s13428-018-01193-y

Peuker, E. T., and Filler, T. J. (2002). The nerve supply of the human auricle. *Clin. Anat.* 15, 35–37. doi: 10.1002/ca.1089

Quintana, D. S., Alvares, G. A., and Heathers, J. A. J. (2016). Guidelines for reporting articles on psychiatry and heart rate variability (GRAPH): recommendations to advance research communication. *Transl. Psychiatry* 6, e803–e810. doi: 10.1038/tp.2016.73

Rangon, C.-M. (2018). Reconsidering sham in transcutaneous vagus nerve stimulation studies. *Clin. Neurophysiol.* 129, 2501–2502. doi: 10.1016/j.clinph.2018.08.027

Sara, S. J. (2015). Locus coeruleus in time with the making of memories. *Curr. Opin. Neurobiol.* 35, 87–94. doi: 10.1016/j.conb.2015.07.004

Sellaro, R., Gelder, B. De, Finisguerra, A., and Colzato, L. S. (2017). Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex* 99, 213–223. doi: 10.1016/j.cortex.2017.11.007

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Steenbergen, L., Sellaro, R., Stock, A.-K., Verkuil, B., Beste, C., and Colzato, L. S. (2015). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during action cascading processes. *Eur. Neuropsychopharmacol.* 25, 773–778. doi: 10.1016/j.euroneuro.2015.03.015

Tafuro, A., Ambrosini, E., Puccioni, O., and Vallesi, A. (2019). Brain oscillations in cognitive control: a cross-sectional study with a spatial stroop task. *Neuropsychologia* 133:107190. doi: 10.1016/j.neuropsychologia.2019.107190

Tarvainen, M. P., Niskanen, J., Lipponen, J. A., Ranta-aho, P. O., and Karjalainen, P. A. (2013). Kubios HRV – heart rate variability. *Comput. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

van Leusden, J. W. R., Sellaro, R., and Colzato, L. S. (2015). Transcutaneous vagal nerve stimulation (tVNS): a new neuromodulation tool in healthy humans? *Front. Psychol.* 6:102. doi: 10.3389/fpsyg.2015.00102

Ventura-Bort, C., Wirkner, J., Genheimer, H., Wendt, J., Hamm, A. O., and Weymar, M. (2018). Effects of transcutaneous vagus nerve stimulation (tVNS) on the P300 and alpha-amylase level: a pilot study. *Front. Hum. Neurosci.* 12:202. doi: 10.3389/fnhum.2018.00202

Wagenmakers, E.-J. (2007). A practical solution to the pervasive problems of p values. *Psychon. Bull. Rev.* 14, 779–804. doi: 10.3758/BF03194105

Walker, R. M., James, O., and Brewer, G. A. (2017). Replication, experiments and knowledge in public management research. *Public Manag. Rev.* 19, 1221–1234. doi: 10.1080/14719037.2017.1282003

Warren, C. M., Tona, K. D., Ouwerkerk, L., van Paridon, J., Poletiek, F., Bosch, J. A., et al. (2018). The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3 event-related potential. *Brain Stimul.* 12, 635–642. doi: 10.1016/j.brs.2018.12.224

Wetzels, R., Matzke, D., Lee, M. D., Rouder, J. N., Iverson, G. J., and Wagenmakers, E. (2011). Statistical evidence in experimental psychology: an empirical comparison using 855 t Tests. *Perspect. Psychol. Sci. ?* 6, 291–298. doi: 10.1177/1745691611406923

Yakunina, N., and Kim, S. S. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Zelazo, P. D., Anderson, J. E., Richler, J., Wallner-Allen, K., Beaumont, J. L., Conway, K. P., et al. (2014). NIH toolbox cognition battery (CB): validation of executive function measures in adults. *J. Int. Neuropsychol. Soc.* 20, 620–629. doi: 10.1017/S1355617714000472

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 21 July 2020
doi: 10.3389/fnins.2020.00738

# Implementing Mobile HRV Biofeedback as Adjunctive Therapy During Inpatient Psychiatric Rehabilitation Facilitates Recovery of Depressive Symptoms and Enhances Autonomic Functioning Short-Term: A 1-Year Pre–Post-intervention Follow-Up Pilot Study

*Josef M. Tatschl[1]*, Sigurd M. Hochfellner[2] and Andreas R. Schwerdtfeger[1,3]*

*[1] Health Psychology Unit, Institute of Psychology, University of Graz, Graz, Austria, [2] Privatklinik St. Radegund Betriebs GmbH, St. Radegund, Austria, [3] BioTechMed-Graz, Graz, Austria*

OPEN ACCESS

**Edited by:**
*Sylvain Laborde, German Sport University Cologne, Germany*

**Reviewed by:**
*Cristina Ottaviani, Sapienza University of Rome, Italy*
*Martin Gerbert Frasch, University of Washington, United States*

***Correspondence:**
*Josef M. Tatschl*
*josef.tatschl@uni-graz.at*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

**Received:** *31 March 2020*
**Accepted:** *22 June 2020*
**Published:** *21 July 2020*

**Citation:**
*Tatschl JM, Hochfellner SM and Schwerdtfeger AR (2020) Implementing Mobile HRV Biofeedback as Adjunctive Therapy During Inpatient Psychiatric Rehabilitation Facilitates Recovery of Depressive Symptoms and Enhances Autonomic Functioning Short-Term: A 1-Year Pre–Post-intervention Follow-Up Pilot Study. Front. Neurosci. 14:738. doi: 10.3389/fnins.2020.00738*

**Objective:** New treatment options for depression are warranted, due to high recurrence rates. Recent research indicates benefits of heart rate variability biofeedback (HRVBF) on symptom recovery and autonomic functioning in depressed individuals. Slow-paced breathing-induced amplification of vagus nerve activity is the main element of HRVBF. Thus, the latter represents a safe and non-invasive complementary depression treatment. However, its efficacy in patients undergoing inpatient psychiatric rehabilitation receiving highly comprehensive treatments has not been evaluated.

**Methods:** Ninety-two inpatients were randomly assigned to an intervention group (IG) or control group (CG). While the latter received the standard treatment only, adjunctive HRVBF was provided to the IG over 5 weeks. Depression severity and heart rate variability (HRV) were assessed before (pre) and after 5 weeks (post). Moreover, 1-year follow-up depression scores were available for 30 participants.

**Results:** Although depression improved in both groups, the IG exhibited significantly larger improvements at post-assessment ($\eta_p^2 = 0.065$) and significant increases in resting LF-HRV ($d = 0.45$) and cardiorespiratory coherence ($d = 0.61$). No significant effects for RMSSD, SDNN, HF-HRV, or HR were found ($ps > 0.05$). Additionally, the IG showed a medium- to large-sized reduction in resting respiratory rate from 13.2 to 9.8 breaths per minute ($p < 0.001$, $d = 0.86$), with the CG exhibiting only a small decrease from 13.5 to 12.4 ($p = 0.49$; $d = 0.35$). While the IG exhibited significantly lower depression scores at post-assessment ($p = 0.042$, $d = 0.79$), this effect decreased during follow-up ($p = 0.195$, $d = 0.48$).

**Conclusion:** HRVBF as adjuvant therapy during inpatient psychiatric rehabilitation facilitated depression recovery. Additionally, amplified LF-HRV as well as

cardiorespiratory coherence at rest and a decrease in resting breathing frequency was observed in the HRVBF group. These findings emphasize HRVBF's value as complementary therapy regardless of concurrent treatments. Moreover, these incremental benefits could serve as resource even after the actual training period. However, the additional antidepressant gains vanish during the long-term follow-up, indicating the need for more intense training or regular practice afterward, respectively. Thus, future studies are warranted to examine how the initial benefits of HRVBF during inpatient psychiatric rehabilitation can be preserved post discharge.

**Keywords: biofeedback, slow-paced breathing, depression, heart rate variability, psychiatric rehabilitation, resonance frequency, vagus nerve stimulation**

## INTRODUCTION

Depression has been identified as the leading cause of disability worldwide, affecting approximately 300 million people globally (World Health Organization, 2017; James et al., 2018). While antidepressants are still the standard treatment for depression, a debate regarding their efficacy has been emerging in recent years (Davidson, 2010; Ormel et al., 2020). A recent meta-analysis suggests only minor benefits compared to placebo treatments (Cipriani et al., 2018). Importantly, taking antidepressants seems to increase suicidality and all-cause mortality (Baldessarini et al., 2017; Maslej et al., 2017). Due to these obvious limitations of pharmacotherapy, alternative and safer treatment options are considered worthwhile. Importantly, the high recurrence rates among those affected by this debilitating disease indicate the need to complement conventional therapeutic approaches to improve depression prognosis (Burcusa and Iacono, 2007).

Of note, autonomic functioning is shifted toward increased sympathetic activity in depression (Koschke et al., 2009; Schumann et al., 2017). Importantly, autonomic activity can be reliably and non-invasively assessed through heart rate variability (HRV), which refers to the fluctuation of subsequent beat-to-beat intervals of the heart rate, with mathematical analysis of HRV permitting inferences onto the underlying vagal modulations (Berntson et al., 1997). HRV can be assessed in time-domain and frequency-domain measures (Shaffer and Ginsberg, 2017). A sensitive indicator of vagally mediated HRV is the respiratory sinus arrhythmia (RSA), which reflects the concomitant increase in heart rate with inspiration and decrease with expiration, with the exact phase relationship between respiration and heart rate depending on the breathing frequency (Berntson et al., 1997; Vaschillo et al., 2002). Additionally, the root mean square of successive differences between normal heartbeats (RMSSD) is an established marker of vagally mediated HRV (vmHRV; Schwerdtfeger et al., 2019). Noteworthily, a recent meta-analysis shows attenuated vagal functioning in depressed individuals, manifesting in decreased heart rate variability, including vmHRV (Koch et al., 2019).

The neurovisceral integration model (NIM), first postulated by Thayer and Lane (2000), provides a framework for a possible explanation regarding the link between depression and HRV. The NIM proposes that the regulation of affect, attention, and autonomic activity shares neural circuits, and therefore, vmHRV could index the efficacy of central–peripheral neural feedback loops (Thayer and Lane, 2009). Importantly, the prefrontal cortex, central to executive functions, is considered as a major effector regarding autonomic functioning, exhibiting top-down inhibition on sympathetic activity (Thayer and Lane, 2009). Thus, dysfunctional cognitions and emotions, respectively, could trigger the release of the prefrontal vagal brake, manifesting in decreased vmHRV (Thayer and Lane, 2009; Smith et al., 2017). Accordingly, perseverative cognition like rumination is associated with attenuated vmHRV (Gerteis and Schwerdtfeger, 2016; Ottaviani, 2018).

Importantly, enhancing HRV is hypothesized to increase cerebral oscillations, supposedly strengthening functional connectivity in brain areas relevant to emotion regulation, including prefrontal areas, which in return should improve mental well-being (Mather and Thayer, 2018). Hence, increasing HRV via heart rate variability biofeedback (HRVBF) could constitute an alternative treatment for alleviating depressive symptoms. HRVBF is based on the phenomenon of maximum RSA amplification occurring at a specific respiratory frequency, which on average is approximately 5.5 (0.09 Hz) breaths per minute (Vaschillo et al., 2002; Lehrer et al., 2003). Due to the cardiovascular resonance in response to this specific respiratory pattern, it has also been labeled resonant breathing (Vaschillo et al., 2006). HRVBF supposedly amplifies autonomic reflexes, like the baroreflex, ultimately enhancing autonomic functioning, which eventually increases HRV (Vaschillo et al., 2002; Lehrer et al., 2003; Lehrer and Gevirtz, 2014). Noteworthily, breathing at such a slow rate (i.e., 0.09 Hz) shifts the RSA from the high-frequency (HF; 0.15–0.4 Hz) to the low-frequency (LF; 0.04–0.15 Hz) domain of HRV, which seems primarily vagally mediated (Lehrer et al., 2003; Kromenacker et al., 2018).

Importantly, several studies have shown benefits of HRVBF on depression recovery and HRV in clinical depression (Karavidas et al., 2007; Siepmann et al., 2008; Hartogs et al., 2017; Caldwell and Steffen, 2018; Lin et al., 2019). Although compelling, small sample sizes and lack of control groups in previous research limit interpretation and long-term outcomes of HRVBF have not been evaluated yet. Thus, the present work aims to expand prior research by evaluating for the first time the short- and long-term efficacy of HRVBF in individuals undergoing inpatient psychiatric rehabilitation. Importantly, the main intent of this study was to assess the general feasibility of HRVBF to improve

depressive symptoms in patients already receiving a highly comprehensive treatment program. Since aiming at elucidating HRVBF's antidepressant efficacy on a more global level and in stationary psychiatric rehabilitation *per se*, patients with diagnoses other than depression were included.

Since inpatients are exposed to the same environmental factors during the 6-week in-clinic rehabilitation period, this provides an excellent context to investigate HRVBF's efficacy, especially as HRV seems sensitive to external influences like diet, exercise, and even air quality (Levy et al., 1998; Haberfellner et al., 2008; Pieters et al., 2012; Kingsley and Figueroa, 2016; Young and Benton, 2018). Therefore, any HRV or depression differences occurring between the intervention group (IG) and the control group (CG) are likely due to HRVBF.

Based on the proposed benefits of HRVBF on depression recovery and autonomic functioning, we hypothesized that inpatient psychiatric rehabilitation supplemented by HRVBF will yield greater improvements in depressive symptoms and HRV than the standard treatment alone. Specifically, we expected that practicing HRVBF enhances vagal and baroreflex functioning, which should result in increased RMSSD, HF-HRV, and LF-HRV, respectively. Finally, the cumulative effect of increased vagal activity as well as improved baroreflex should manifest in improved overall variability and therefore increased SDNN. On an exploratory basis, we also evaluated whether HRVBF during rehabilitation affects 12-month recovery from depressive symptoms.

# MATERIALS AND METHODS

## Ethics Statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013. This study was approved by the institutional ethics committee (GZ. 39/43/63 ex 2017/18). From all participants, oral and written informed consent was obtained.

## Participants and Design

Participants were recruited from a local inpatient psychiatric rehabilitation clinic, where they stay on average for 42 days. Patients taking antidepressants, anxiolytics, and other medication like anti-hypertensives or supplements, respectively, were included only if intake had been started at least 3 months prior study admission. The sampling protocol is shown in **Figure 1**. Patients diagnosed with a substance-use disorder were excluded. Initially, 48 participants were randomly assigned to the intervention group (IG) and 44 participants to the control group (CG). Final sample size was reduced due to dropouts (IG = 8; CG = 5), diagnosed substance-use disorder (IG = 3; CG = 2), acute illness at post-assessment (IG = 1), severe side effects due to new medication (IG = 1), missing items in the BDI-II (IG = 1; CG = 3), artifacts in the electrocardiogram (ECG; IG = 1), and missing ECG assessments (CG = 2). Thus, the depression pre–post analyses included 68 participants (IG = 34; CG = 34) aged 26–66 ($M$ = 48.7; $SD$ = 9.4; **Table 1**). Pre–post data for HRV were available from 69 participants (see **Table 1**). The 12-month follow-up questionnaires were returned by 30 participants (IG = 14; CG = 16).

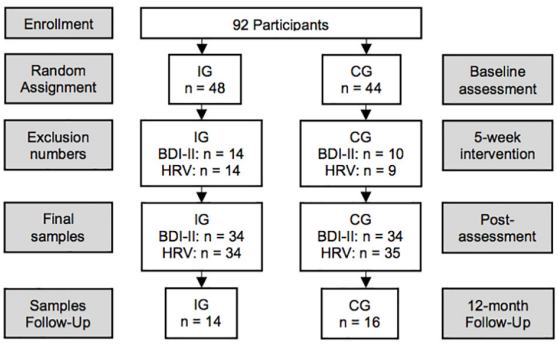



**FIGURE 1 |** Study design. $N$ = 92 in-patients enrolled in the study and were randomly assigned to the intervention (IG) or control group (CG). Due to dropouts, missing items in the BDI-II, side effects from medication, illness, artifacts in the ECG, and missing assessments, the final sample size was reduced to 68 for the depression pre–post and to 69 for the HRV pre–post analyses, respectively. Overall, 30 participants (IG = 14; CG = 16) returned the follow-up questionnaires.

A 2 × 2 pre–post design was applied with group (IG vs. CG) as between-subject factor, time (pre–post; post-follow-up) as within-subject factor, and depression as well as various HRV measures as dependent variables. The IG practiced HRVBF in addition to the standard treatment. The CG received standard treatment only, provided with the opportunity to receive a brief HRVBF training after the study.

## Procedure

On admission day, inpatients received an overview of the study and were assured about the confidentiality, anonymity, and possibility to withdraw from the study without negative consequences. They completed psychometric testing and two separate short-term HRV recordings, prior and after the 5-week intervention phase. After completing the baseline assessments, participants were randomly assigned to the IG or the CG, respectively (**Figure 1**). After post-assessment, a 12-month follow-up regarding depressive symptoms was conducted in written form. During the follow-up period, no further support was provided.

## Measures

### Demographics and Confounders

Participants filled out questionnaires regarding demographic/control variables at study entry. At admission, diagnoses and medication including supplement intake were obtained from the patient documentations. Medications and supplements were also assessed from the demographic/control questionnaires to record potential unregistered intake. Supplements were assessed, since various substances like vitamin $D^3$ or probiotics seem to have mood-altering effects (Shaffer et al., 2014; Mocking et al., 2016; Schefft et al., 2017;

**TABLE 1 |** Demographic information for the BDI-II and HRV pre–post analyses, between groups.

| | BDI-II | | | HRV | | |
|---|---|---|---|---|---|---|
| | IG *n* = 34 | CG *n* = 34 | *p* | IG *n* = 34 | CG *n* = 35 | *p* |
| Age in years (*M*, *SD*) | 48.5 (8.2) | 48.9 (10.6) | 0.858 | 49.03 (7.7) | 49.43 (10.4) | 0.857 |
| Female sex (%) | 67.6 | 61.8 | 0.612 | 64.7 | 60.0 | 0.687 |
| Body mass index (*M*, *SD*) | 25.6 (4.9) | 27.9 (6.6) | 0.105 | 25.7 (4.9) | 27.7 (6.6) | 0.165 |
| **Primary psychiatric diagnosis ICD-10 (%)** | | | | | | |
| Schizophrenia and schizotypal and delusional disorders F20–29 (%) | 0.0 | 5.9 | 0.151 | 0.0 | 8.6 | 0.081 |
| Affective disorders F30–39 (%) | 76.5 | 73.5 | 0.779 | 76.5 | 71.4 | 0.633 |
| Neurotic, stress-related, and somatoform disorders F40–48 (%) | 23.5 | 20.6 | 0.770 | 23.5 | 20.0 | 0.722 |
| Psychiatric comorbidity (%) | 35.3 | 32.4 | 0.798 | 32.4 | 37.1 | 0.676 |
| Burnout symptoms Z73.0 (%) | 20.6 | 14.7 | 0.525 | 17.6 | 14.3 | 0.703 |
| Psychotropics (%) | 85.3 | 91.2 | 0.452 | 85.3 | 88.6 | 0.686 |
| Psychotropics *N* (*M*, *SD*) | 2.9 (1.9) | 2.8 (1.5) | 0.945 | 3.0 (2.0) | 2.7 (1.5) | 0.507 |
| Antidepressants (%) | 85.3 | 82.4 | 0.742 | 85.3 | 80.0 | 0.562 |
| Antidepressants *N* (*M*, *SD*) | 1.8 (1.1) | 1.8 (1.1) | 0.914 | 1.9 (1.2) | 1.7 (1.2) | 0.563 |
| SSRI (%) | 64.7 | 79.4 | 0.177 | 64.7 | 71.4 | 0.549 |
| SNRI (%) | 38.2 | 17.6 | 0.059 | 41.2 | 17.1 | 0.028 |
| NaSSA (%) | 11.8 | 17.6 | 0.493 | 14.7 | 11.4 | 0.686 |
| Tricyclic (%) | 5.9 | 0.0 | 0.151 | 5.9 | 0.0 | 0.145 |
| Neuroleptics (%) | 20.6 | 23.5 | 0.770 | 23.5 | 25.7 | 0.833 |
| Atypical neuroleptics (%) | 26.5 | 5.9 | 0.021 | 23.5 | 8.6 | 0.090 |
| Antiepileptics (%) | 5.9 | 26.5 | 0.021 | 5.9 | 28.6 | 0.013 |
| Anxiolytics (%) | 23.5 | 29.4 | 0.582 | 26.5 | 28.6 | 0.845 |
| Anxiolytics *N* (*M*, *SD*) | 0.3 (0.5) | 0.3 (0.5) | 0.804 | 0.3 (0.5) | 0.3 (0.5) | 0.944 |
| Somatic comorbidity (%) | 55.9 | 55.9 | 1.00 | 55.9 | 60.0 | 0.729 |
| CVD (%) | 17.6 | 17.6 | 1.00 | 17.6 | 20.0 | 0.803 |
| Chronic pain (%) | 11.8 | 17.6 | 0.493 | 11.8 | 17.1 | 0.526 |
| Respiratory disease (%) | 8.8 | 5.9 | 0.642 | 8.8 | 5.7 | 0.618 |
| Anti-hypertensives (%) | 26.5 | 23.5 | 0.779 | 29.4 | 25.7 | 0.731 |
| Supplements (%) | 29.4 | 26.5 | 0.787 | 29.4 | 28.6 | 0.939 |
| Vitamin $D^3$ (%) | 11.8 | 20.6 | 0.349 | 11.8 | 20.0 | 0.375 |
| Probiotics (%) | 0.0 | 2.9 | 0.314 | 0.0 | 2.9 | 0.321 |
| Other (%) | 2.9 | 2.9 | 1.00 | 2.9 | 2.9 | 0.983 |
| **Behavioral data** | | | | | | |
| Nicotine (%) | 32.4 | 48.5 | 0.178 | 33.3 | 47.1 | 0.252 |
| Alcohol (%) | 41.2 | 51.5 | 0.396 | 44.1 | 47.1 | 0.808 |
| Aerobic training (%) | 84.8 | 75.0 | 0.321 | 84.4 | 78.8 | 0.562 |
| Weekly aerobic training (*M*, *SD*) | 230 (192) | 160 (152) | 0.114 | 230 (195) | 171 (150) | 0.182 |
| Strength (%) | 6.1 | 6.5 | 0.949 | 6.3 | 12.5 | 0.391 |
| Weekly strength (*M*, *SD*) | 0.2 (0.7) | 0.1 (0.6) | 0.618 | 0.2 (0.7) | 0.2 (0.8) | 0.840 |
| In-clinic breathing training (%) | 0.0 | 20.6 | 0.005 | 0.0 | 20.0 | 0.006 |

*BDI-II, demographics between groups for depression pre–post comparisons; HRV, demographics between groups for HRV pre–post comparisons; variables tested by chi-square tests and by t-tests. CVD, cardiovascular disease. Aerobic training, total number and percentage of participants practicing aerobic exercise regularly. Weekly aerobic training, mean weekly volume of aerobic exercise in minutes. Strength, total number and percentage of participants practicing strength training regularly. Weekly strength, mean frequency of weekly strength sessions. N, total number. %, percentage. M, mean. SD, standard deviation.*

Nikolova et al., 2019). Additionally, the care report of every participant was reviewed to control for changes in medication, occurring illness and extraordinary incidents.

## Primary Outcomes

### Depressive Symptoms

Depression severity was assessed with the Beck–Depression Inventory II (BDI-II) (Beck et al., 1996), which seems particularly sensitive to detect changes among psychiatric patients (Wang and Gorenstein, 2013). The BDI-II consists of 21 items and is a self-report measure, assessing cognitive, affective, and neurovegetative symptoms of depression (Beck et al., 1996; Steer and Clark, 1997). In addition to an overall depression score, BDI-II distinguishes between a cognitive and somatic–affective subscale (Huang and Chen, 2015). Cronbach's alphas for the BDI-II overall score and the cognitive and somatic–affective subscales

were 0.95, 0.91, and 0.92 at baseline; 0.95, 0.90, and 0.93 at post-assessment; and 0.95, 0.91, and 0.92 at follow-up, respectively, indicating high internal consistency (Peterson, 1994).

## Heart Rate Variability

### HRV Data Analysis

Heart rate variability was acquired by means of the "HRV Scanner," a one-channel ECG with a sampling rate of 500 Hz (BioSign GmbH, D-85570, Ottenhofen, Germany). The signal was obtained from two limb clamps, placed at participants' wrists. Data analysis was performed with the HRV-Scanner Software (BioSign GmbH, D-85570, Ottenhofen, Germany). Participants completed a 3-min short-term electrocardiogram (ECG), from which time-domain and frequency-domain measures were assessed. The ECG signal was automatically controlled for artifacts by the HRV-Scanner software, and only data containing less than five percent of artifacts were included for further analyses. Additionally, the ECG was visually controlled by two experienced examiners. One participant from the IG was excluded due to excessive artifacts (baseline: 5.04%; post-assessment: 8.65%). Of note, both groups showed similar mean artifact ratios at baseline (IG: $M = 0.16$, $SD = 0.55$; CG: $M = 0.09$, $SD = 0.28$) and post-assessment (IG: $M = 0.15$, $SD = 0.77$; CG: $M = 0.04$, $SD = 0.25$).

### HRV Measures

Heart rate variability parameters in the time domain encompass heart rate (HR), root mean square of successive differences (RMSSD), and standard deviation of RR intervals (SDNN). RMSSD is considered as a cardinal marker of parasympathetic activity and SDNN a global measure of all autonomic influences on HRV (Umetani et al., 1998; Shaffer F. et al., 2014). Frequency-domain analysis classifies HRV into low (LF; 0.04–0.15 Hz) and high frequencies (HF; 0.15–0.4 Hz), expressed as $ms^2$. HF power primarily reflects parasympathetic (i.e., vagal) activity (Berntson et al., 1997). Although regarded as a marker of cardiac sympathetic control (Berntson et al., 1997), a major vagal influence on LF power is proposed (Billman, 2013; Reyes del Paso et al., 2013). Of note, during resting conditions, LF-HRV seems predominantly influenced by baroreflex and vagal activity, with only minor sympathetic contributions, compared to ambulatory settings, where sympathetic efference could be more dominant (Shaffer et al., 2014). Accordingly, Kromenacker et al. (2018) showed that increases in LF power due to slow breathing were predominantly vagally mediated.

We analyzed HR, SDNN, RMSSD, HF, and LF from the 3-min ECG recordings. Additionally, as an indicator of RSA the grade of rhythmization (GR) was calculated, which aims to quantify HRVBF success. This index integrates fluctuations of LF-HRV and HF-HRV. Specifically, changes in LF and HF are weighted against each other, with HF assigned a higher weight, thus quantifying the ratio of peak amplitude power compared to the remaining signals in the spectral analysis. This is due to the well-known phenomenon, that during states of enhanced cardiorespiratory coherence, an elevated peak and a narrower distribution of power can be observed in the spectrogram, shifting from HF to LF frequency. Therefore, GR increments correspond to an increase in the peak amplitude power, including a higher signal density centered around the peak and less power within the remaining frequencies, indicating a high RSA state. On the contrary a distribution of the power across a wider frequency range and a lower power peak, respectively, should indicate a lower GR and therefore a low RSA state. Hence, the GR aims at describing the quantity (i.e., height of amplitude) and quality of the RSA (i.e., presence of non-respiratory influences on the RSA), indicating the degree of cardiorespiratory coherence (e.g., Druschky and Druschky, 2015). It should be noted, though, that the GR is of explorative nature, since published validation studies are lacking. Frequency-domain HRV parameters were analyzed applying fast-Fourier transformation.

At the day of testing, participants were instructed to abstain from alcohol, nicotine, and exercise until HRV measurements were completed. Individuals were also instructed to fast at least 2 h prior to their appointments, as food intake potentially influences HRV (Hayano et al., 1990; Lu et al., 1999; Cornelissen et al., 2010; Romanowicz et al., 2011; Kingsley and Figueroa, 2016). Due to circadian HRV fluctuations, participants' pre–post measurements were taken within the same 3 h of the day (Bonnemeier et al., 2003). The ECG was taken in a supine position after participants had rested for 10 min. Pre–post ECG measurements were conducted in the same climatized room.

### Breathing Frequency

Resting breathing frequency was analyzed pre- and post-intervention from the ECG. The HRV Scanner Software analyzes respiratory rate from the ECG signal, which is highly correlated with the actual breathing rate (Schrumpf et al., 2016). Thus, ECG-derived breathing frequency has been suggested as an accurate measure of respiration (Tong et al., 2014).

## Secondary Outcomes

### HRVBF Training Compliance

We assessed three compliance measures. First, we documented participants' number of attended group trainings, and second, self-practice frequency was analyzed from the portable HRVBF devices. Third, an overall compliance score was calculated adding up group and self-practice frequencies.

### HRVBF Training Performance

To measure participants' HRVBF training performance, we assessed the relative grade of rhythmization (relGR). The relGR describes the mean achieved percentage of the set target GR (i.e., RSA amplitude required to receive a perfect feedback) during HRVBF sessions. Thus, the relGR objectifies the difficulty of the HRVBF while simultaneously measuring training success. For example, a relGR of 76 corresponds to producing on average 76% of the set target GR, while a relGR of 108 equals a mean GR, 108% of the target GR. Hence, the relGR can exceed 100% if the achieved values are higher than the set target GR, thus indicating superior performance. Additionally, respiratory rates were estimated from the biofeedback data, calculating the power peak in the frequency domains (Karlen et al., 2011).

### Technical Details HRVBF

Heart rate variability biofeedback was delivered through a portable device named Qiu (BioSign GmbH, D-85570, Ottenhofen, Germany), which allowed participants to practice HRVBF at any time. The sphere-shaped device is battery-powered, has the size of a tennis ball, and measures heart rate by an optical sensor (i.e., photoplethysmography) at the palm, second digit, or thumb. Alternatively, an ear clip can be used to sense pulse rate. The Qiu provides the option to guide the practitioner's BF by moving blue LED lights, which can be set individually. The Qiu records date, time, and the RR intervals of every session. Once heart rate is detected, the luminescent upper half of the Qiu visualizes the current relGR through a continuous visual feedback, which ranges from dark red (i.e., low relGR) to bright green (i.e., high relGR). Accordingly, the optical feedback displays the degree to which practitioners achieve their target GR, which can be set individually, based on the participants' individual values. Importantly, the Qiu applies an algorithm controlling for error variance in the GR during the biofeedback, which ensures accuracy of the short feedback latency, necessary for the HRVBF.

In general, the HRVBF protocol used in this study differs from the original procedure (i.e., Lehrer et al., 2000, 2013). The original protocol assesses the precise resonance frequency with a rather time intensive procedure as a basis for the actual HRVBF (Lehrer et al., 2000, 2013). On the contrary, a 60-s deep breathing HRV test (DBT) is used to estimate the target HRV amplitude for the Qiu-HRVBF. In the DBT, participants breathe at 6 breaths per minute, which corresponds to the approximate resonance frequency, with inspiration and expiration lasting 5 s each, guided by a visual signal (Ewing and Clarke, 1982; Lehrer et al., 2003; Shields, 2009). Hence, instead of assessing the individual resonance frequency, the approximate maximum HRV amplitude is assessed from the DBT.

Precisely, the HRV Scanner software calculates the GR from the 60-s DBT, which is used by the Qiu as reference for the HRVBF. Importantly, Qiu's target GR is set higher than the actual maximum GR amplitude achieved in the DBT. Therefore, enough margin is provided to enable practitioners to achieve their actual peak HRV during the HRVBF practice. Thus, participants have to adapt their breathing pattern in response to the visual feedback to achieve their maximum HRV (i.e., GR) amplitude. Accordingly, practitioners determine their precise resonance frequency during every training session in order to achieve a positive feedback.

## Treatments

### Standard Treatment (ST)

The ST consisted of 240 min of daily multifaceted therapies during the week and 80 min of therapy on Saturdays. These treatments included psychotherapy, psychoeducation, music therapy, physical and exercise therapy, and relaxation methods, including progressive muscle relaxation. Importantly, inpatients have to adhere to a strict treatment curriculum, which is equal for all inpatients, with non-adherence leading to early discharge. Hence, the IG and CG were comparable regarding treatment regiments independent of the HRVBF intervention. Of note, the clinic also provided breathing training by physical therapists, as additional individual therapy. Only the CG could participate in the in-clinic breathing training to avoid any confluent effects with the HRVBF on the study outcome.

### Details HRVBF Training Procedure

The IG received a 2-h introduction to the HRVBF, consisting of hierarchical steps: First, participants were taught nasal abdominal and pursed-lip breathing according to Lehrer et al. (2000). We emphasized nasal inspiration, as recent literature indicates improved entrainment of cerebral activity as compared to oral inspiration (Zelano et al., 2016; Herrero et al., 2018; Piarulli et al., 2018). In addition, switching from thoracic to abdominal breathing could improve vagal activation via slowly adapting stretch receptors during deep breathing (Noble and Hochman, 2019). Pursed-lip breathing is supposed to improve breathing economy through decreasing air turbulences during exhalation and mechanically dilating the airways (e.g., Lehrer et al., 2000). Also, participants were instructed to focus the mind on the Dan Tian, a supposed "energy center" in the mind–body technique of Qi Gong, allegedly located three centimeters below the navel inside the belly (Chan et al., 2008). We integrated this idea as focusing on the Dan Tian while breathing seems to facilitate slow, deep breathing, which eventually is a prerequisite to successfully modulate HRV (Lehrer et al., 2003; Chan et al., 2008). Importantly, we disentangled this concept from its dogmatic valence and instructed participants to focus on the center of their abdomen to facilitate deep breathing. Second, participants were familiarized with the Qiu. Third, they were trained to use the taught techniques to modify their breathing and to adopt the latter according to the Qiu's visual feedback to optimize their HRV. Thus, the goal was to maximize the GR, rather than rigidly execute a specific technique. Fourth, participants received written instructions of the breathing techniques and details regarding Qiu usage, including self-practice.

The self-practice consisted of a 10-min HRVBF twice a day. Since participants had to attend the various standard treatments during the day, we recommended to do the first session in the morning and the second in the afternoon or evening, respectively. Participants were informed that they could train more HRVBF if they wanted to. Additionally, the IG was instructed to do three cycles of resonant breathing without the Qiu throughout the day, with each cycle lasting 10 breaths, trying to emulate the breathing pattern of the biofeedback-guided training. This additional practice aimed at familiarizing participants with the taught breathing techniques in order to facilitate HRVBF training. The HRVBF introduction was supplemented by one guided HRVBF session weekly (i.e., 5 sessions), consisting of approximately 35 min of HRVBF and 25 min for discussing any questions. In order to maintain training quality throughout the study period, the set target GR necessary to achieve a positive feedback was adjusted based on each individual's progression in performance.

Because groups shared the same environment (i.e., clinic) during the study, the IG was instructed not to communicate

any details about the HRVBF with the CG to avoid potential transfer effects. Importantly, participants were stressed not to share their personal HRVBF device, since all training sessions are recorded and supposed to reflect each individual's performance and compliance, respectively.

## Statistical Analyses

Data were analyzed with SPSS 25.0 software. To compare groups regarding demographic, medical, and behavioral variables, chi-square analyses and unpaired *t*-tests were conducted. Shapiro–Wilk tests were performed to analyze distributional characteristics (Shapiro and Wilk, 1965). Accounting for skewed distributions, HRV measures were normalized using natural logarithmic transformation. Separate two-way mixed ANOVAs were performed, with group (IG, CG) as a between-subject factor and time (pre–post; post-follow-up) as a within-subject factor. Correlations were analyzed using Pearson's product–moment correlations. As a measure of effect size, partial eta-squared ($\eta_p^2$) is reported with small, medium, and large effects represented by the values 0.01, 0.06, and 0.14, respectively (Cohen, 2013). Cohen's *d* was reported as effect size for *t*-tests with small, medium, and large effects, represented by the values 0.2, 0.5, and 0.8, respectively (Cohen, 2013).

# RESULTS

## Baseline Sample Characteristics

Regarding demographic and control variables, there were no significant baseline differences between the IG and CG. Both groups showed similar overall antidepressant intake and were slightly overweight with BMI values corresponding to early-stage obesity (World Health Organization, 1998; see, **Table 1**). However, in the IG a tendency for higher SNRI intake, significantly higher use of atypical neuroleptics, and less frequent use of antiepileptics were observed ($ps < 0.05$; **Table 1**).

Inpatients were diagnosed according to ICD-10 (World Health Organization, 1992). The majority was diagnosed with affective disorders (ICD-10: F30–39), followed by neurotic, stress-related, and somatoform disorders and schizophrenia and schizotypal and delusional disorders (ICD-10: F20–29), respectively. However, no information regarding the precise number of episodes in case of recurrent depression was available. Within each group, approximately one third exhibited a comorbid (two or more) disorder with about a fifth exhibiting an additional burnout (Z73.0) diagnosis (**Table 1**).

Importantly, for HRV pre–post analyses, demographic characteristics including both, diagnoses (including specific ICD-10 diagnoses) and control variables, were similar to the depression pre–post analyses. Only the statistical tendency for higher SNRI intake in the IG was significant ($p < 0.05$), while the less frequent use of atypical neuroleptics in the CG was non-significant ($p > 0.05$; **Table 1**).

Of note, groups did not differ in severity of depressive symptoms (including subscales), diagnoses, or HRV variables at baseline ($ps > 0.05$). Both groups showed moderate depression scores at baseline (**Table 2**).

## The Efficacy of HRVBF on Improving Depressive Symptoms

Depressive symptoms decreased over the course of the 5 weeks, as evidenced by a significant main effect of time in the mixed ANOVA with a large effect size [$F(1,66) = 74.510$, $p < 0.001$, $\eta_p^2 = 0.530$]. Further, a moderating effect of group could be shown by a significant group × time interaction of medium effect size [$F(1,66) = 4.60$, $p = 0.036$, $\eta_p^2 = 0.065$; **Figure 2**]. Paired *t*-tests showed significant decreases in the BDI-II score of 12.4 points in the IG [$t(33) = 7.57$, $p < 0.001$, $d = 1.30$] and of 7.5 points in the CG [$t(33) = 4.62$, $p < 0.001$, $d = 0.79$; see **Table 2**]. Main effects for time were also found for the cognitive [$F(1,66) = 43.92$, $p < 0.001$, $\eta_p^2 = 0.400$] and somatic–affective subscales [$F(1,66) = 78.93$, $p < 0.001$, $\eta_p^2 = 0.545$] with comparably large effects. A medium-sized moderating effect of group across time could be found for somatic–affective, [$F(1,66) = 6.23$, $p = 0.015$, $\eta_p^2 = 0.086$], but not for cognitive symptoms ($p = 0.227$, $\eta_p^2 = 0.022$). Paired *t*-tests revealed a significant decrease in the somatic–affective score of 7.9 points in the IG [$t(33) = 7.57$, $p < 0.001$, $d = 1.30$] and 4.4 points in the CG [$t(33) = 4.85$, $p < 0.001$, $d = 0.83$; see **Table 2**], respectively. No group differences in depression (including subscales) were found at post-assessment ($ps > 0.05$).

## The Efficacy of HRVBF on Increasing Resting HRV

For lnLF, a medium-sized main effect for time could be observed [$F(1,67) = 6.10$, $p = 0.016$, $\eta_p^2 = 0.083$], suggesting increasing values from pre- to post-assessment. Albeit no significant interaction for group × time was found ($p = 0.121$, $\eta_p^2 = 0.036$), *post hoc* paired *t*-tests showed significant increases in lnLF for the IG only [$t(33) = -2.64$), $p = 0.013$, $d = 0.45$; see **Figure 3**] and no significant changes in the CG [$t(34) = -0.696$), $p = 0.491$, $d = 0.12$; see **Table 2**].

Regarding lnGR, a large-sized main effect for time [$F(1,67) = 13.42$; $p < 0.001$, $\eta_p^2 = 0.167$] and a significant interaction for group × time of medium effect size were found [$F(1,67) = 4.74$, $p = 0.033$, $\eta_p^2 = 0.066$; see **Figure 4**]. Paired *t*-tests evidenced a significant increase in lnGR for the IG only [$t(33) = -3.55$, $p = 0.001$, $d = 0.61$; **Table 2**]. No significant effects for the other HRV measures, heart rate included, were found ($ps > 0.05$).

## Effects of HRVBF on Resting Breathing Frequency

Baseline breathing rates did not differ significantly between groups ($p = 0.717$, $d = 0.09$) and were within the normal range of human respiration (IG = 13.2 vs. CG = 13.5; Yuan et al., 2013). Overall, resting breathing rate decreased, as evidenced by a large-sized main effect of time in the mixed ANOVA [$F(1,67) = 27.16$, $p < 0.001$, $\eta_p^2 = 0.288$]. A significant medium-sized interaction of time × group illustrated a moderating effect of the HRVBF on resting respiratory rate [$F(1,67) = 6.928$, $p = 0.011$, $\eta_p^2 = 0.094$]. Paired *t*-tests showed large decreases in breathing frequency for the IG, from 13.2 to 9.8 breaths per minute [$t(33) = 5.04$,

**TABLE 2 |** Paired *t*-tests by group for depression and physiological measures at baseline and post-assessment.

| | IG | | | | CG | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***M* (*SD*) Pre** | ***M* (*SD*) Post** | ***t*** | **\|*d*\|** | ***M* (*SD*) Pre** | ***M* (*SD*) Post** | ***t*** | **\|*d*\|** | ***F*** | $\eta_p^2$ |
| BDI-II total | 21.59 (12.51) | 9.18 (10.13) | 7.565** | 1.30 | 19.97 (14.04) | 12.5 (11.11) | 4.621** | 0.79 | 4.602* | 0.065 |
| BDI-II cog | 6.77 (5.85) | 2.88 (3.95) | 5.468** | 0.94 | 7.06 (6.38) | 4.38 (4.56) | 3.882** | 0.67 | 1.485 | 0.022 |
| BDI-II soma | 13.74 (6.96) | 5.82 (6.23) | 7.566** | 1.30 | 11.88 (7.78) | 7.44 (6.56) | 4.846** | 0.83 | 6.230* | 0.086 |
| HR bpm | 66.69 (11.11) | 67.60 (11.60) | −0.593 | 0.10 | 68.50 (11.08) | 69.37 (11.34) | −0.459 | 0.08 | 0.000 | 0.000 |
| BF cpm | 13.15 (3.63) | 9.81 (2.86) | 5.039** | 0.86 | 13.52 (4.77) | 12.42 (3.91) | 2.039* | 0.35 | 6.928* | 0.094 |
| lnSDNN ms | 3.38 (0.45) | 3.42 (0.54) | −0.432 | 0.07 | 3.42 (.44) | 3.42 (.53) | −0.024 | 0.004 | 0.105 | 0.002 |
| rSDNN ms | 32.50 (15.18) | 35.22 (21.55) | | | 33.66 (17.06) | 34.58 (18.33) | | | | |
| lnRMSSD ms | 3.10 (0.63) | 3.00 (0.65) | 1.003 | 0.17 | 2.92 (0.60) | 2.93 (0.68) | −0.148 | 0.025 | 0.377 | 0.012 |
| rRMSSD ms | 27.02 (18.54) | 24.68 (18.96) | | | 22.52 (16.55) | 24.04 (20.50) | | | | |
| lnLF $ms^2$ | 5.25 (1.19) | 5.86 (1.39) | −2.636* | 0.45 | 5.39 (1.29) | 5.52 (1.40) | −0.696 | 0.12 | 2.468 | 0.036 |
| rLF $ms^2$ | 340.77 (347.43) | 1051.14 (2610.37) | | | 563.53 (1252.44) | 549.93 (934.77) | | | | |
| lnHF $ms^2$ | 5.25 (1.37) | 4.85 (1.37) | 1.630 | 0.28 | 4.66 (1.42) | 4.79 (1.56) | −0.662 | 0.11 | 2.860 | 0.041 |
| rHF $ms^2$ | 492.60 (814.66) | 334.96 (587.88) | | | 322.70 (582.84) | 380.42 (696.35) | | | | |
| lnGR | 1.18 (0.73) | 1.72 (0.96) | −3.547* | 0.61 | 1.40 (0.91) | 1.54 (0.88) | −1.291 | 0.22 | 4.736* | 0.066 |
| rGR | 4.09 (2.70) | 9.05 (11.30) | | | 6.08 (6.53) | 6.74 (6.74) | | | | |

*Additional F-statistics for the interaction effects in the mixed ANOVAs. BDI-II total, Beck Depression Inventory II overall score; BDI-II cog, score in the cognitive subscale of the Beck Depression Inventory II; BDI-II soma, score in the somatic-affective subscale of the Beck Depression Inventory II; HR, heart rate; bpm, beats per minute; BF, breathing frequency; cpm, cycles per minute; ms, milliseconds; $ms^2$, milliseconds squared; ln, natural logarithmic normalization of the data; r, raw values; lnSDNN, logarithmized standard deviation of all normal-to-normal RR intervals; lnRMSSD, logarithmized root mean square of successive differences between normal heartbeats; lnLF, logarithmized low-frequency HRV; lnHF, logarithmized high-frequency HRV; lnGR, logarithmized grade of rhythmization; *indicates $p < 0.05$. **indicates $p < 0.001$.*

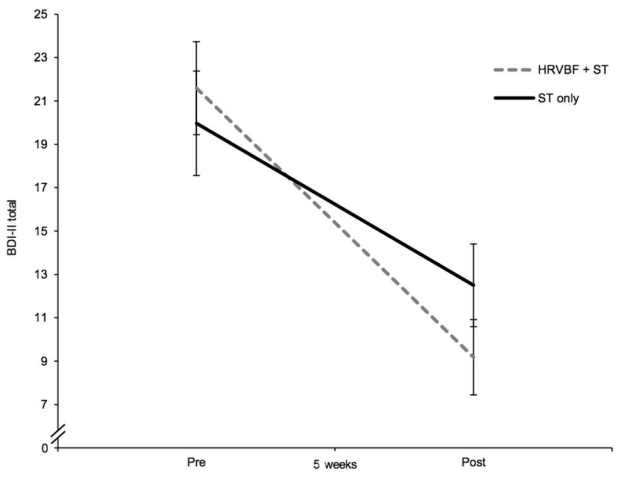



**FIGURE 2 |** The IG, receiving the HRVBF in addition to the ST, showed a significant larger decrease in depressive symptoms over 5 weeks. BDI-II total, Beck Depression Inventory II overall score; HRVBF, heart rate variability biofeedback; ST, standard treatment. Error bars indicate ±1 SE.

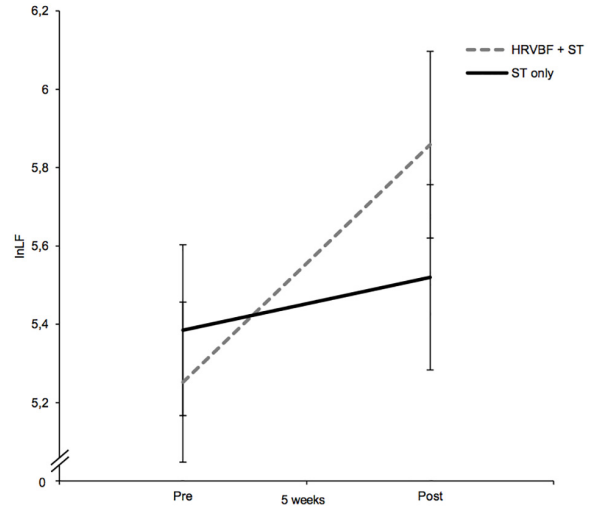



**FIGURE 3 |** While the CL group receiving the ST only did not show any changes in lnLF, the IG (HRVBF + ST) showed an increase in lnLF after the 5-week training period. HRVBF, heart rate variability biofeedback; ST, standard treatment. Error bars indicate ±1 SE.

$p < 0.001$, $d = 0.86$] and a small reduction from 13.5 to 12.4 for the CG [$t(34) = 2.04$, $p = 0.049$, $d = 0.35$; **Table 2**].

## Adherence to HRVBF Training

The mean number of attended group training sessions was 5.5 (*SD* = 0.6; range: 4–6), while the average self-practice frequency was 57 (*SD* = 26.6; range: 14–116) sessions, respectively. This corresponds to reaching a mean of 84.5% (*SD* = 34.5; range: 24.3–164.9) regarding overall target training frequency of 74 sessions (i.e., 68 self-practice sessions and 6 group trainings).

## HRVBF Training Performance

Mean relGR across sessions was 80.0%, which documents that throughout all sessions, participants achieved on average

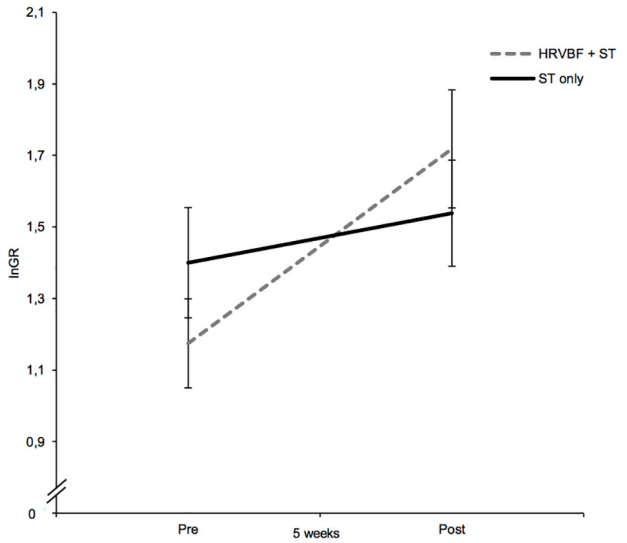



**FIGURE 4 |** While the IG (HRVBF + ST) exhibited significant increases in lnGR, the CG (ST only) did not show any substantial changes. HRVBF, heart rate variability biofeedback; ST, standard treatment. Error bars indicate ±1 SE.

80 percent of their individual maximum HRV peak. This indicates sufficient HRVBF training difficulty to induce potential autonomic adaptations (i.e., HRV increases). The average respiratory rate in the IG, calculated from the biofeedback data, was 5.5 ($SD = 0.46$; range: 4.7–6.8) breaths per minute, which is in line with the findings of previous research using more extensive assessment methods (Vaschillo et al., 2002; Lehrer et al., 2003). Therefore, the GR seems to provide a feasible feedback signal to foster each individual's resonance frequency.

## Exploratory Analyses

Overall depression scores were negatively associated with lnLF ($r = -0.346$, $p = 0.006$) and lnGR ($r = -0.319$, $p = 0.011$) at pre-assessment and at post-assessment (lnLF: $r = -0.286$, $p = 0.020$; lnGR: $r = -0.315$, $p = 0.010$). None of the evaluated compliance (i.e., practice frequency) and performance measures (i.e., relGR) were associated with changes in depression or HRV, respectively ($ps > 0.05$). Also, the observed decreases in depressive symptoms including subscales were not associated with changes in any of the HRV parameters across the whole sample and within each group, respectively ($ps > 0.05$).

## 12-Month Follow-Up of Depression Recovery

From thirty participants (IG = 14; CG = 16), depression follow-up data was available. No group differences in the control variables ($ps > 0.05$) or depression severity ($p = 0.511$, $d = 0.24$) were found at baseline. A mixed ANOVA comparing depression severity between the IG and the CG from post-assessment to follow-up showed neither a significant main effect for time nor a time × group interaction ($ps > 0.05$). However, the IG exhibited significantly lower depression scores of large effect size compared to the CG ($p = 0.042$, $d = 0.79$) at post-assessment. These additional antidepressive benefits due to HRVBF decreased during the 12-month post-discharge, illustrated by slightly smaller depression differences at follow-up ($p = 0.195$, $d = 0.48$). This effect seems to originate from a visible increase in depressive symptoms within the IG during the follow-up period ($p = 0.118$, $d = 0.48$). Importantly, none of the participants from the CG completing the follow-up took part in the brief HRVBF introduction at the end of the rehabilitation.

# DISCUSSION

This study evaluated whether HRVBF could enhance recovery of depressive symptoms and autonomic functioning in inpatients undergoing psychiatric rehabilitation. Moreover, a 12-month follow-up regarding depression trajectories was conducted, assessing the long-term sustainability of potential effects. Within 5 weeks, the IG exhibited a medium-sized, larger recovery in depressive symptoms than the CG, which appeared to be mainly driven by the comparably strong improvements in somatic–affective symptoms. However, these additional benefits gained during the treatment period vanished during the long-term follow-up. Noteworthily, toward the end of the treatment period, the IG showed medium- to large-sized amplification of LF-HRV as well as cardiorespiratory coherence (i.e., grade of rhythmization) at rest and a large reduction in resting breathing frequency, while no significant effects for RMSSD, SDNN, HF-HRV, or HR could be found. In comparison, no significant HRV changes could be observed in the CG, which, however, showed a small decrease in resting breathing rate.

Importantly, the present research complements the hitherto only randomized controlled trial by Caldwell and Steffen (2018), who showed that HRVBF facilitated depression recovery and HRV in psychotherapy patients. These effects were larger as compared to our findings, which might be attributable to differences in sample characteristics. Of note, the comparably young sample in the Caldwell and Steffen study comprised women only, who seem to respond better to depression treatment and show larger autonomic adaptions due to interventions like exercise (Genovesi et al., 2007; Donker et al., 2013). Additionally, young individuals have shown larger HRV increases in response to interventions compared to middle-aged ones (Carter et al., 2003). Noteworthily, age seems to be an important factor regarding the efficacy of HRVBF on HRV, with young samples showing more reliable increases (Lehrer et al., 2006; Alayan et al., 2019). Hence, the sample of the Caldwell and Steffen study could have been more sensitive to treatment effects regarding depression and HRV than those in the present research, who were approximately twice as old and included both sexes. It should also be mentioned that antidepressants seem to reduce HRV, with SNRIs and tricyclics having particularly unfavorable effects on vagal efferent cardiac control (Kemp et al., 2014; Alvares et al., 2016). However, SSRIs seem to attenuate vagal functioning as well, although depending on the SSRI class,

with fluoxetine exhibiting the least adverse effects on HRV (Kemp et al., 2016). In this regard, it is necessary to mention that only four participants included in the HRV analyses (IG = 3; CG = 1) were taking fluoxetine. However, this was supplemented with either antipsychotics, SNRIs, additional SSRI classes, or a combination of these medications. Additionally, antipsychotics have been shown to decrease HRV as well, with atypical neuroleptics seeming especially detrimental to autonomic functioning (Agelink et al., 2001; Iwamoto et al., 2012; Linder et al., 2014). Noteworthily, the IG exhibited a high intake of SNRIs and of atypical neuroleptics, which could explain why no improvements in RMSSD, HF-HRV, or SDNN could be observed. Therefore, we suggest that the advanced age and the density of pharmacological interventions may have attenuated an increase in autonomic functioning in the IG. It is also important to note that small samples tend to exaggerate effects (Button et al., 2013). Thus, our findings may reflect HRVBF's efficacy more accurately than the comparably smaller study of Caldwell and Steffen (2018).

Of note, this study provides first insights regarding the long-term sustainability of HRVBF-induced add-on benefits during inpatient psychiatric rehabilitation. Noteworthily, while groups showed no significant differences regarding the magnitude of depressive symptoms at baseline ($d$ = 0.24), the IG compared to the CG exhibited significantly lower symptom severity of large effect size CG ($d$ = 0.79) at the end of the rehabilitation period. Although these favorable antidepressive gains due to HRVBF became statistically non-significant at the 12-month follow-up assessment, these effects were still visible and of moderate size ($d$ = 0.48). Seemingly, HRVBF generates unique psychophysiological benefits during the training phase, serving as additional resource even after the actual training period, which, however, appears to gradually vanish during a 12-month follow-up. Nevertheless, since we did not assess depressive symptoms and HRV at any time points between post-assessment and follow-up, no conclusions regarding psychophysiological trajectories can be drawn. Furthermore, slow-paced breathing practice during the follow-up period was not assessed, thus limiting the interpretation of the findings. However, it may be assumed that more intense training during stationary rehabilitation and/or continuing HRVBF after discharge may be necessary to maintain the initial benefits. Recently, Lin (2018) reported positive effects of a mobile-based HRVBF on autonomic balance, which could provide a useful tool to secure sustainability of the effects.

Nevertheless, the medium to large favorable effects of HRVBF shown in patients within 5 weeks of inpatient rehabilitation appear compelling and seemingly magnified the already large antidepressant effect of a well-validated, multidimensional treatment program (i.e., 25 h of weekly therapies). Moreover, the amplification of LF-HRV and GR, exclusively observed in the IG, could indicate enhanced autonomic efficacy. Importantly, under controlled resting conditions the LF-HRV seems predominantly influenced by baroreflex and vagal activity, with only minor sympathetic influences (Shaffer et al., 2014). Especially when breathing within the LF frequency range, LF-HRV reflects almost exclusively vagal efference (Kromenacker et al., 2018). Since the IG exhibited a resting breathing rate at the upper end of the LF spectrum at post-assessment, the increases in LF-HRV within the IG could be considered of vagal origin. Regarding the GR, a cautious interpretation of this measure is imperative, as validation studies are lacking. It should be noted though that participants achieved resonance breathing during the Qiu biofeedback, thus indicating the utility of the GR as a marker of cardiorespiratory coherence. Taken together, we tentatively suggest that the IG exhibited improved vagal functioning. Certainly, further studies are needed to verify of falsify this hypothesis, especially since RMSSD, a sensitive marker of vmHRV, was not affected by the resonant breathing intervention (Shaffer et al., 2014). Still, HRVBF may exhibit unique therapeutic benefits independent of concurrent treatments. Hence, these findings emphasize the distinct effect of cultivating physiological coherence through resonance breathing on human psychophysiology. Of note, a study conducted in a similar setting found no additional antidepressive benefit of a mindfulness self-compassion training (Gaiswinkler et al., 2019), despite showing antidepressant effects in a prior study (Neff and Germer, 2013). In general, breathing-based interventions seem to be of merit in improving depressive symptoms, potentially beyond conventional treatments approaches. For example, a study by Sharma et al. (2017) found that Sudarshan Kriya Yoga (SKY), a breathing-based meditation, induced large symptom improvements in depressed individuals resistant to antidepressant medication within 8 weeks. Of note, the largest reduction in depression occurred within the first 4 weeks, with small decreases during the subsequent half of the intervention period. In a further study, practicing SKY, which includes slow-paced breathing, resulted in enhanced vagal functioning in patients suffering from depression and/or anxiety, in addition to symptom reduction (Zope and Zope, 2013; Toschi-Dias et al., 2017).

Recent research indicates that the benefits of paced breathing techniques on psychological well-being could originate from breathing-induced changes in brain activation patterns. During slow-paced breathing, slowly adapting stretch receptors in the lungs are recruited and in response amplify vagal afferent input to the nucleus tractus solitarius (NTS) in the brain stem (Carr and Undem, 2003; Kubin et al., 2006). The NTS projects to cortical and subcortical areas of the brain, including prefrontal areas, the cingulate, the nucleus paraventricularis of the hypothalamus, and the amygdala, which show altered functioning in depressed individuals (Ricardo and Koh, 1978; Petrov et al., 1993; Greicius et al., 2007; Siegle et al., 2007; Bao et al., 2008; Koenigs and Grafman, 2009). Accordingly, cumulative evidence indicates that modulating respiratory patterns could entrain brain activity and, in turn, may generate a neurofunctional signature corresponding to emotional well-being (Zaccaro et al., 2018; Noble and Hochman, 2019). Hence, as hypothesized by Porges (2007), afferent vagal input, including slow-paced breathing, may aid in orchestrating emotional/psychological functioning via the cerebral susceptibility to upstream (i.e., afferent) vagal stimulation.

Indeed, these frequently suggested physiological pathways could be one origin of HRVBF's efficacy. However, since we did not find any associations between HRV changes and

improvements in depressive symptoms, including subscales, psychological mechanisms may also contribute to its potency. For example, successfully modulating Qiu's visual feedback might foster self-efficacy, which seems decreased in depression (Bandura et al., 1999; Maeda et al., 2013). Of note, neither depression nor HRV trajectories were associated with mean relGR. Thus, the degree of participants' exposure to negative (i.e., red), neutral (i.e., orange), or positive (i.e., green) feedback during HRVBF had no distinct effect on the main outcomes. However, the sole experience of intentionally modulating the optical feedback or feelings of relaxation independent of the actual extent may have fostered self-efficacy.

To our knowledge, this study is among the first to objectively assess whether HRVBF self-practice frequency is linked to depression or HRV changes (e.g., Karavidas et al., 2007; Siepmann et al., 2008; Caldwell and Steffen, 2018; Lin et al., 2019). Astoundingly, there were no significant associations. However, it could well be that participants generally engaged in slow-paced breathing practice independent of HRVBF, which unfortunately was not documented. Obviously, more research is necessary, targeting at the psychological and neurobiological mechanisms of the HRVBF effect. Nonetheless, considering no substantial HRV increases due to the standard treatment and the disturbed sympathetic–vagal balance in depression, our results suggest supplementing conventional therapies with HRVBF to specifically target autonomic dys/functioning (e.g., Kim et al., 2009; Koschke et al., 2009; Chien et al., 2015; Schumann et al., 2017). Moreover, the high level of practice adherence observed in the present study further supports the feasibility of HRVBF as adjunctive therapy in depressed individuals.

### Strengths and Limitations

This study has several strengths and limitations that should be mentioned. Overall, the highly standardized environment during inpatient psychiatric rehabilitation allowed us to control for various confounders, thus strengthening the validity of the findings. However, since this and prior studies were not placebo controlled, it has yet to be evaluated whether the promising antidepressive HRVBF effects are independent of a potential placebo effect. In addition, the CG did not receive a control intervention. Therefore, it could be that the additional attention due to the HRVBF group sessions may have fostered increased perceived social support and behavioral activation within the IG, thus contributing to the beneficial effects. Another positive aspect of this study is that we could objectively assess HRVBF training compliance. However, breathing practice independent of HRVBF was not documented, which may have diluted the non-findings regarding training adherence and outcome measures. Noteworthily, assessing depressive symptoms 1 year post-training constitutes a unique feature of this study as compared to previous research, thus elucidating for the first time potential long-term effects of HRVBF. On the contrary, neither HRV data at follow-up nor breathing practice during the 1-year period were obtained, thus limiting the interpretation of our results. Another factor potentially confounding the observed HRV increases is the relatively short ECG recording time of 3 min, as compared to the recommended 5 min (Berntson et al., 1997). However, cumulative research indicates the reliability of ultra-short-term HRV recordings, suggesting the validity of our findings. For example, Shaffer et al. (2016) propose that an ECG recording length of 180 s is sufficient to reliably calculate LF-HRV and HF-HRV, with 3-min recordings yielding almost identical results as 5-min measurements. Further, accurate measures of RMSSD and SDNN may be obtained from 2-min recordings (Munoz et al., 2015).

## CONCLUSION

The present research suggests additional benefits of HRVBF on the recovery of depressive symptoms and autonomic functioning in psychiatric rehabilitation inpatients. Thus, these findings argue for the value of HRVBF as adjunctive therapy during diverse treatment contexts, since it seemingly improves the therapeutic outcome regardless of concurrent treatment diversity and diagnosis. Importantly, the observed incremental effects could serve as resource after the training period and post-discharge, respectively. However, since the observed long-term trends did not reach significance, adequately powered follow-up studies are warranted to confirm this hypothesis and to examine ways to preserve initial therapeutic gains. Nevertheless, our findings support the implementation of HRVBF as additional intervention to foster the recovery of depressive symptoms, especially since it could provide add on-benefits, including enhanced autonomic regulation, as compared to current standard therapies. Further research is needed to examine the robustness of these findings and to control for placebo effects.

## DATA AVAILABILITY STATEMENT

Due to a data policy contract with the clinic we are not allowed to provide any data to third parties. Hence, no data is available.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee University of Graz. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JT, AS, and SH contributed to the conceptualization of the study. JT conducted the data analyses and wrote the initial draft of the manuscript. AS contributed to the manuscript revision. All authors read and approved the submitted manuscript.

## FUNDING

The authors acknowledge the financial support by the University of Graz.

## REFERENCES


Agelink, M. W., Majewski, T., Wurthmann, C. M. D. P., Lukas, K., Ullrich, H., Linka, T., et al. (2001). Effects of newer atypical antipsychotics on autonomic neurocardiac function: a comparison between amisulpride, olanzapine, sertindole, and clozapine. *J. Clin. Psychopharmacol.* 21, 8–13. doi: 10.1097/00004714-200102000-00003

Alayan, N., Eddie, D., Eller, L., Bates, M. E., and Carmody, D. P. (2019). Substance craving changes in university students receiving heart rate variability biofeedback: a longitudinal multilevel modeling approach. *Addict. Behav.* 97, 35–41. doi: 10.1016/j.addbeh.2019.05.005

Alvares, G. A., Quintana, D. S., Hickie, I. B., and Guastella, A. J. (2016). Autonomic nervous system dysfunction in psychiatric disorders and the impact of psychotropic medications: a systematic review and meta-analysis. *J. Psychiatry Neurosci.* 41, 89–104. doi: 10.1503/jpn.140217

Baldessarini, R. J., Lau, W. K., Sim, J., Sum, M. Y., and Sim, K. (2017). Suicidal risks in reports of long-term controlled trials of antidepressants for major depressive disorder II. *Int. J. Neuropsychopharmacol.* 20, 281–284. doi: 10.1093/ijnp/pyw092

Bandura, A., Pastorelli, C., Barbaranelli, C., and Caprara, G. V. (1999). Self-efficacy pathways to childhood depression. *J. Person. Soc. Psychol.* 76, 258–269. doi: 10.1037/0022-3514.76.2.258

Bao, A. M., Meynen, G., and Swaab, D. F. (2008). The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Res. Rev.* 2, 531–553. doi: 10.1016/j.brainresrev.2007.04.005

Beck, A. T., Steer, R. A., and Brown, G. K. (1996). Beck depression inventory-II. *San Antonio* 78, 490–498.

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26. doi: 10.3389/fphys.2013.00026

Bonnemeier, H., Wiegand, U. K., Brandes, A., Kluge, N., Katus, H. A., Richardt, G., et al. (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. *J. Cardiovasc. Electrophysiol.* 14, 791–799. doi: 10.1046/j.1540-8167.2003.03078.x

Burcusa, S. L., and Iacono, W. G. (2007). Risk for recurrence in depression. *Clin. Psychol. Rev.* 27, 959–985. doi: 10.1016/j.cpr.2007.02.005

Button, K. S., Ioannidis, J. P., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S., et al. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nat. Rev. Neurosci.* 14:365. doi: 10.1038/nrn3475

Caldwell, Y. T., and Steffen, P. R. (2018). Adding HRV biofeedback to psychotherapy increases heart rate variability and improves the treatment of major depressive disorder. *Int. J. Psychophysiol.* 131, 96–101. doi: 10.1016/j.ijpsycho.2018.01.001

Carr, M. J., and Undem, B. J. (2003). Bronchopulmonary afferent nerves. *Respirology* 8, 291–301. doi: 10.1046/j.1440-1843.2003.00473.x

Carter, J. B., Banister, E. W., and Blaber, A. P. (2003). The effect of age and gender on heart rate variability after endurance training. *Med. Sci. Sports Exer.* 35, 1333–1340. doi: 10.1249/01.MSS.0000079046.01763.8F

Chan, A. S., Han, Y. M. Y., and Cheung, M.-C. (2008). Electroencephalographic (EEG) measurements of mindfulness-based triarchic body-pathway relaxation technique: a pilot study. *Appl. Psychophysiol. Biofeedb.* 33, 39–47. doi: 10.1007/s10484-008-9050-5

Chien, H. C., Chung, Y. C., Yeh, M. L., and Lee, J. F. (2015). Breathing exercise combined with cognitive behavioural intervention improves sleep quality and heart rate variability in major depression. *J. Clin. Nurs.* 24, 3206–3214. doi: 10.1111/jocn.12972

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., et al. (2018). Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Focus* 16, 420–429. doi: 10.1176/appi.focus.16407

Cohen, J. (2013). *Statistical Power Analysis for the Behavioral Sciences*. New York: Routledge, doi: 10.4324/9780203771587

Cornelissen, V. A., Verheyden, B., Aubert, A. E., and Fagard, R. H. (2010). Effects of aerobic training intensity on resting, exercise and post-exercise blood pressure, heart rate and heart-rate variability. *J. Hum. Hyperten.* 24, 175–182. doi: 10.1038/jhh.2009.51

Davidson, J. R. (2010). Major depressive disorder treatment guidelines in America and Europe. *J. Clin. Psychiatry* 71, e04–e04. doi: 10.4088/JCP.9058se1c.04gry

Donker, T., Batterham, P. J., Warmerdam, L., Bennett, K., Bennett, A., Cuijpers, P., et al. (2013). Predictors and moderators of response to internet-delivered interpersonal psychotherapy and cognitive behavior therapy for depression. *J. Affect. Disord.* 151, 343–351. doi: 10.1016/j.jad.2013.06.020

Druschky, K., and Druschky, A. (2015). Mobile biofeedback of heart rate variability in patients with diabetic polyneuropathy: a preliminary study. *Clin. Physiol. Funct. Imaging* 35, 332–333. doi: 10.1111/cpf.12130

Ewing, D. J., and Clarke, B. F. (1982). Diagnosis and management of diabetic autonomic neuropathy. *Br. Med. J.* 285:916. doi: 10.1136/bmj.285.6346.916

Gaiswinkler, L., Kaufmann, P., Pollheimer, E., Ackermann, A., Holasek, S., Kapfhammer, H. P., et al. (2019). Mindfulness and self-compassion in clinical psychiatric rehabilitation: a clinical trial. *Mindfulness* 11, 374–383. doi: 10.1007/s12671-019-01171-1

Genovesi, S., Zaccaria, D., Rossi, E., Valsecchi, M. G., Stella, A., and Stramba-Badiale, M. (2007). Effects of exercise training on heart rate and QT interval in healthy young individuals: are there gender differences? *Europace* 9, 55–60. doi: 10.1093/europace/eul145

Gerteis, A. K. S., and Schwerdtfeger, A. R. (2016). When rumination counts: perceived social support and heart rate variability in daily life. *Psychophysiology* 53, 1034–1043. doi: 10.1111/psyp.12652

Greicius, M. D., Flores, B. H., Menon, V., Glover, G. H., Solvason, H. B., Kenna, H., et al. (2007). Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biol. Psychiatry* 62, 429–437. doi: 10.1016/j.biopsych.2006.09.020

Haberfellner, E. M., Jungmayr, J., Grausgruber-Berner, R., and Grausgruber, A. (2008). Medical rehabilitation of patients with mental or psychosomatic disorders in austria-findings of a catamnestic study. *Die Rehabil.* 47, 164–171. doi: 10.1055/s-2008-1076707

Hartogs, B. M., Bartels-Velthuis, A. A., Van der Ploeg, K., and Bos, E. H. (2017). Heart rate variability biofeedback stress relief program for depression. *Methods Inform. Med.* 56, 419–426. doi: 10.3414/ME16-02-0033

Hayano, J., Yamada, M., Sakakibara, Y., Fujinami, T., Yokoyama, K., Watanabe, Y., et al. (1990). Short-and long-term effects of cigarette smoking on heart rate variability. *Am. J. Cardiol.* 65, 84–88. doi: 10.1016/0002-9149(90)90030-5

Herrero, J. L., Khuvis, S., Yeagle, E., Cerf, M., and Mehta, A. D. (2018). Breathing above the brain stem: volitional control and attentional modulation in humans. *J. Neurophysiol.* 119, 145–159. doi: 10.1152/jn.00551.2017

Huang, C., and Chen, J. H. (2015). Meta-analysis of the factor structures of the beck depression inventory–II. *Assessment* 22, 459–472. doi: 10.1177/1073191114548873

Iwamoto, Y., Kawanishi, C., Kishida, I., Furuno, T., Fujibayashi, M., Ishii, C., et al. (2012). Dose-dependent effect of antipsychotic drugs on autonomic nervous system activity in schizophrenia. *BMC Psychiatry* 12:199. doi: 10.1186/1471-244X-12-199

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., et al. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet* 392, 1789–1858. doi: 10.1016/S0140-6736(18)32279-7

Karavidas, M. K., Lehrer, P. M., Vaschillo, E., Vaschillo, B., Marin, H., Buyske, S., et al. (2007). Preliminary results of an open label study of heart rate variability biofeedback for the treatment of major depression. *Appl. Psychophysiol. Biofeedb.* 32, 19–30. doi: 10.1007/s10484-006-9029-z

Karlen, W., Brouse, C. J., Cooke, E., Ansermino, J. M., and Dumont, G. A. (2011). "Respiratory rate estimation using respiratory sinus arrhythmia from photoplethysmography," in *Proceedings of the 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, (Boston, MA: IEEE), 1201–1204. doi: 10.1109/IEMBS.2011.6090282

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., Carvalho de Figueiredo, R., et al. (2014). Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil

cohort baseline study. *Am. J. Psychiatry* 171, 1328–1334. doi: 10.1176/appi.ajp.2014.13121605

Kemp, A. H., Fráguas, R., Brunoni, A. R., Bittencourt, M. S., Nunes, M. A., Dantas, E. M., et al. (2016). Differential associations of specific selective serotonin reuptake inhibitors with resting-state heart rate and heart rate variability: implications for health and well-being. *Psychosom. Med.* 78, 810–818. doi: 10.1097/PSY.0000000000000336

Kim, W., Lim, S. K., Chung, E. J., and Woo, J. M. (2009). The effect of cognitive behavior therapy-based psychotherapy applied in a forest environment on physiological changes and remission of major depressive disorder. *Psychiatry Invest.* 6, 245–254. doi: 10.4306/pi.2009.6.4.245

Kingsley, J. D., and Figueroa, A. (2016). Acute and training effects of resistance exercise on heart rate variability. *Clin. Physiol. Funct. Imaging* 36, 179–187. doi: 10.1111/cpf.12223

Koch, C., Wilhelm, M., Salzmann, S., Rief, W., and Euteneuer, F. (2019). A meta-analysis of heart rate variability in major depression. *Psychol. Med.* 49, 1948–1957. doi: 10.1017/S0033291719001351

Koenigs, M., and Grafman, J. (2009). The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav. Brain Res.* 201, 239–243. doi: 10.1016/j.bbr.2009.03.004

Koschke, M., Boettger, M. K., Schulz, S., Berger, S., Terhaar, J., Voss, A., et al. (2009). Autonomy of autonomic dysfunction in major depression. *Psychosom. Med.* 71, 852–860. doi: 10.1097/PSY.0b013e3181b8bb7a

Kromenacker, B. W., Sanova, A. A., Marcus, F. I., Allen, J. J., and Lane, R. D. (2018). Vagal mediation of low-frequency heart rate variability during slow yogic breathing. *Psychosom. Med.* 80, 581–587. doi: 10.1097/PSY.0000000000000603

Kubin, L., Alheid, G. F., Zuperku, E. J., and McCrimmon, D. R. (2006). Central pathways of pulmonary and lower airway vagal afferents. *J. Appl. Physiol.* 101, 618–627. doi: 10.1152/japplphysiol.00252.2006

Lehrer, P., Vaschillo, E., Lu, S. E., Eckberg, D., Vaschillo, B., Scardella, A., et al. (2006). Heart rate variability biofeedback: effects of age on heart rate variability, baroreflex gain, and asthma. *Chest* 129, 278–284. doi: 10.1378/chest.129.2.278

Lehrer, P. M., and Gevirtz, R. (2014). Heart rate variability biofeedback: how and why does it work? *Front. Psychol.* 5:756. doi: 10.3389/fpsyg.2014.00756

Lehrer, P. M., Vaschillo, E., and Vaschillo, B. (2000). Resonant frequency biofeedback training to increase cardiac variability: rationale and manual for training. *Appl. Psychophysiol. Biofeedb.* 25, 177–191. doi: 10.1023/A:1009554825745

Lehrer, P. M., Vaschillo, E., Vaschillo, B., Lu, S. E., Eckberg, D. L., Edelberg, R., et al. (2003). Heart rate variability biofeedback increases baroreflex gain and peak expiratory flow. *Psychosom. Med.* 65, 796–805. doi: 10.1097/01.PSY.0000089200.81962.19

Lehrer, P., Vaschillo, B., Zucker, T., Graves, J., Katsamanis, M., Aviles, M., et al. (2013). Protocol for heart rate variability biofeedback training. *Appl. Psychophysiol. Biofeedback* 41, 98–109. doi: 10.5298/1081-5937-41.3.08

Levy, W. C., Cerqueira, M. D., Harp, G. D., Johannessen, K. A., Abrass, I. B., Schwartz, R. S., et al. (1998). Effect of endurance exercise training on heart rate variability at rest in healthy young and older men. *Am. J. Cardiol.* 82, 1236–1241. doi: 10.1016/s0002-9149(98)00611-0

Lin, I. M. (2018). Effects of a cardiorespiratory synchronization training mobile application on heart rate variability and electroencephalography in healthy adults. *Int. J. Psychophysiol.* 134, 168–177. doi: 10.1016/j.ijpsycho.2018.09.005

Lin, I. M., Fan, S. Y., Yen, C. F., Yeh, Y. C., Tang, T. C., Huang, M. F., et al. (2019). Heart rate variability biofeedback increased autonomic activation and improved symptoms of depression and insomnia among patients with major depression disorder. *Clin. Psychopharmacol. Neurosci.* 17:222. doi: 10.9758/cpn.2019.17.2.222

Linder, J. R., Sodhi, S. K., Haynes, W. G., and Fiedorowicz, J. G. (2014). Effects of antipsychotic drugs on cardiovascular variability in participants with bipolar disorder. *Hum. Psychopharmacol.* 29, 145–151. doi: 10.1002/hup.2380

Lu, C. L., Zou, X., Orr, W. C., and Chen, J. D. Z. (1999). Postprandial changes of sympathovagal balance measured by heart rate variability. *Digest. Dis. Sci.* 44, 857–861. doi: 10.1023/A:1026698800742

Maeda, U., Shen, B. J., Schwarz, E. R., Farrell, K. A., and Mallon, S. (2013). Self-efficacy mediates the associations of social support and depression with treatment adherence in heart failure patients. *Int. J. Behav. Med.* 20, 88–96. doi: 10.1007/s12529-011-9215-0

Maslej, M. M., Bolker, B. M., Russell, M. J., Eaton, K., Durisko, Z., Hollon, S. D., et al. (2017). The mortality and myocardial effects of antidepressants are moderated by preexisting cardiovascular disease: a meta-analysis. *Psychother. Psychosom.* 86, 268–282. doi: 10.1159/000477940

Mather, M., and Thayer, J. F. (2018). How heart rate variability affects emotion regulation brain networks. *Curr. Opin. Behav. Sci.* 19, 98–104. doi: 10.1016/j.cobeha.2017.12.017

Mocking, R. J. T., Harmsen, I., Assies, J., Koeter, M. W. J., Ruhé, H., and Schene, A. H. (2016). Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. *Transl. Psychiatry* 6:e756. doi: 10.1038/tp.2016.29

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., et al. (2015). Validity of (ultra-) short recordings for heart rate variability measurements. *PLoS One* 10:e0138921. doi: 10.1371/journal.pone.0138921

Neff, K. D., and Germer, C. K. (2013). A pilot study and randomized controlled trial of the mindful self−compassion program. *J. Clin. Psychol.* 69, 28–44. doi: 10.1002/jclp.21923

Nikolova, V., Zaidi, S. Y., Young, A. H., Cleare, A. J., and Stone, J. M. (2019). Gut feeling: randomized controlled trials of probiotics for the treatment of clinical depression: systematic review and meta-analysis. *Ther. Adv. Psychopharmacol.* 9:2045125319859963. doi: 10.1177/2045125319859963

Noble, D. J., and Hochman, S. (2019). Hypothesis: pulmonary afferent activity patterns during slow, deep breathing contribute to the neural induction of physiological relaxation. *Front. Physiol.* 10:1176. doi: 10.3389/fphys.2019.01176

Ormel, J., Spinhoven, P., de Vries, Y. A., Cramer, A. O., Siegle, G. J., Bockting, C. L., et al. (2020). The antidepressant standoff: why it continues and how to resolve it. *Psychol. Med.* 50, 177–186. doi: 10.1017/S0033291719003295

Ottaviani, C. (2018). Brain−heart interaction in perseverative cognition. *Psychophysiology* 55:e13082. doi: 10.1111/psyp.13082

Peterson, R. A. (1994). A meta-analysis of Cronbach's coefficient alpha. *J. Consum. Res.* 21, 381–391. doi: 10.1086/209405

Petrov, T., Krukoff, T. L., and Jhamandas, J. H. (1993). Branching projections of catecholaminergic brainstem neurons to the paraventricular hypothalamic nucleus and the central nucleus of the amygdala in the rat. *Brain Res.* 609, 81–92. doi: 10.1016/0006-8993(93)90858-K

Piarulli, A., Zaccaro, A., Laurino, M., Menicucci, D., De Vito, A., Bruschini, L., et al. (2018). Ultra-slow mechanical stimulation of olfactory epithelium modulates consciousness by slowing cerebral rhythms in humans. *Sci. Rep.* 8, 1–17. doi: 10.1038/s41598-018-24924-9

Pieters, N., Plusquin, M., Cox, B., Kicinski, M., Vangronsveld, J., and Nawrot, T. S. (2012). An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. *Heart* 98, 1127–1135. doi: 10.1136/heartjnl-2011-301505

Porges, S. W. (2007). The polyvagal perspective. *Biol. Psychol.* 74, 116–143. doi: 10.1016/j.biopsycho.2006.06.009

Reyes del Paso, G. A., Langewitz, W., Mulder, L. J., Van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Ricardo, J. A., and Koh, E. T. (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res.* 153, 1–26. doi: 10.1016/0006-8993(78)91125-3

Romanowicz, M., Schmidt, J. E., Bostwick, J. M., Mrazek, D. A., and Karpyak, V. M. (2011). Changes in heart rate variability associated with acute alcohol consumption: current knowledge and implications for practice and research. *Alcoholism* 35, 1092–1105. doi: 10.1111/j.1530-0277.2011.01442.x

Schefft, C., Kilarski, L. L., Bschor, T., and Koehler, S. (2017). Efficacy of adding nutritional supplements in unipolar depression: a systematic review and meta-analysis. *Eur. Neuropsychopharmacol.* 27, 1090–1109. doi: 10.1016/j.euroneuro.2017.07.004

Schrumpf, F., Sturm, M., Bausch, G., and Fuchs, M. (2016). Derivation of the respiratory rate from directly and indirectly measured respiratory signals using autocorrelation. *Curr. Dir. Biomed. Eng.* 2, 241–245. doi: 10.1515/cdbme-2016-0054

Schumann, A., Andrack, C., and Baer, K. J. (2017). Differences of sympathetic and parasympathetic modulation in major depression. *Prog. Neuro*

*Psychopharmacol. Biol. Psychiatry* 79, 324–331. doi: 10.1016/j.pnpbp.2017.07.009

Schwerdtfeger, A. R., Schwarz, G., Pfurtscheller, K., Thayer, J. F., Jarczok, M. N., and Pfurtscheller, G. (2019). Heart rate variability (HRV): from brain death to resonance breathing at 6 breaths/minute. *Clin. Neurophysiol.* 131, 676–693. doi: 10.1016/j.clinph.2019.11.013

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shaffer, F., McCraty, R., and Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front. Psychol.* 5:1040. doi: 10.3389/fpsyg.2014.01040

Shaffer, F., Shearman, S., and Meehan, Z. M. (2016). The promise of ultra-short-term (UST) heart rate variability measurements. *Biofeedback* 44, 229–233. doi: 10.5298/1081-5937-44.3.09

Shaffer, J. A., Edmondson, D., Wasson, L. T., Falzon, L., Homma, K., Ezeokoli, N., et al. (2014). Vitamin D supplementation for depressive symptoms: a systematic review and meta-analysis of randomized controlled trials. *Psychosom. Med.* 76, 190–196. doi: 10.1097/PSY.0000000000000044

Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (Complete Samples). *Biometrika* 52, 591–611. doi: 10.2307/2333709

Sharma, A., Barrett, M. S., Cucchiara, A. J., Gooneratne, N. S., and Thase, M. E. (2017). A breathing-based meditation intervention for patients with major depressive disorder following inadequate response to antidepressants: a randomized pilot study. *J. Clin. Psychiatry* 78, e59–e63. doi: 10.4088/JCP.16m10819

Shields, R. W. (2009). Heart rate variability with deep breathing as a clinical test of cardiovagal function. *Clevel. Clin. J. Med.* 76(Suppl 2), S37–S40. doi: 10.3949/ccjm.76.s2.08

Siegle, G. J., Thompson, W., Carter, C. S., Steinhauer, S. R., and Thase, M. E. (2007). Increased amygdala and decreased dorsolateral prefrontal BOLD responses in unipolar depression: related and independent features. *Biol. Psychiatry* 61, 198–209. doi: 10.1016/j.biopsych.2006.05.048

Siepmann, M., Aykac, V., Unterdörfer, J., Petrowski, K., and Mueck-Weymann, M. (2008). A pilot study on the effects of heart rate variability biofeedback in patients with depression and in healthy subjects. *Appl. Psychophysiol. Biofeedb.* 33, 195–201. doi: 10.1007/s10484-008-9064-z

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Steer, R. A., and Clark, D. A. (1997). Psychometric characteristics of the beck depression inventory-ii with college students. *Meas. Eval. Couns. Dev.* 30:128. doi: 10.1080/07481756.1997.12068933

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Claude bernard and the heart–brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Tong, G. M., Zhang, H. C., Guo, J. H., and Han, F. (2014). Detection of sleep apnea-hypopnea syndrome with ECG derived respiration in Chinese population. *Int. J. Clin. Exp. Med.* 7:1269.

Toschi-Dias, E., Tobaldini, E., Solbiati, M., Costantino, G., Sanlorenzo, R., Doria, S., et al. (2017). Sudarshan Kriya Yoga improves cardiac autonomic control in patients with anxiety-depression disorders. *J. Affect. Disord.* 214, 74–80. doi: 10.1016/j.jad.2017.03.017

Umetani, K., Singer, D. H., McCraty, R., and Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. College Cardiol.* 31, 593–601. doi: 10.1016/S0735-1097(97)00554-8

Vaschillo, E., Lehrer, P., Rishe, N., and Konstantinov, M. (2002). Heart rate variability biofeedback as a method for assessing baroreflex function: a preliminary study of resonance in the cardiovascular system. *Appl. Psychophysiol. Biofeedb.* 27, 1–27. doi: 10.1023/A:1014587304314

Vaschillo, E. G., Vaschillo, B., and Lehrer, P. M. (2006). Characteristics of resonance in heart rate variability stimulated by biofeedback. *Appl. Psychophysiol. Biofeedb.* 31, 129–142. doi: 10.1007/s10484-006-9009-3

Wang, Y. P., and Gorenstein, C. (2013). Psychometric properties of the beck depression inventory-II: a comprehensive review. *Braz. J. Psychiatry* 35, 416–431. doi: 10.1590/1516-4446-2012-1048

World Health Organization (1992). *The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines*. Geneva: World Health Organization.

World Health Organization (2017). *Depression and Other Common Mental Disorders: Global Health Estimates*. Geneva: World Health Organization, 1–24.

World Health Organization (1998). *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation on Obesity, Geneva, 3-5 June 1997 (No. WHO/NUT/NCD/98.1)*. Geneva: World Health Organization.

Young, H. A., and Benton, D. (2018). Heart-rate variability: a biomarker to study the influence of nutrition on physiological and psychological health? *Behav. Pharmacol.* 29, 140–151. doi: 10.1097/fbp.0000000000000383

Yuan, G., Drost, N. A., and McIvor, R. A. (2013). Respiratory rate and breathing pattern. *McMaster Univ. Med. J.* 10, 23–25.

Zaccaro, A., Piarulli, A., Laurino, M., Garbella, E., Menicucci, D., Neri, B., et al. (2018). How breath-control can change your life: a systematic review on psycho-physiological correlates of slow breathing. *Front. Hum. Neurosci.* 12:353. doi: 10.3389/fnhum.2018.00353

Zelano, C., Jiang, H., Zhou, G., Arora, N., Schuele, S., Rosenow, J., et al. (2016). Nasal respiration entrains human limbic oscillations and modulates cognitive function. *J. Neurosci.* 36, 12448–12467. doi: 10.1523/JNEUROSCI.2586-16

Zope, S. A., and Zope, R. A. (2013). Sudarshan kriya yoga: breathing for health. *Int. J. Yoga* 6, 4–10. doi: 10.4103/0973-6131.105935

**Conflict of Interest:** During the conduction of the reported study, SH was employed as the medical director of the St. Radegund Betriebs GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 23 July 2020
doi: 10.3389/fphys.2020.00779

# Heart Rate Variability (HRV) and Pulse Rate Variability (PRV) for the Assessment of Autonomic Responses

*Elisa Mejía-Mejía*, Karthik Budidha, Tomas Ysehak Abay, James M. May and Panayiotis A. Kyriacou*

*Research Centre for Biomedical Engineering (RCBE), School of Mathematics, Engineering and Computer Science, University of London, London, United Kingdom*

OPEN ACCESS

**Edited by:**
*Sylvain Laborde, German Sport University Cologne, Germany*

**Reviewed by:**
*Moacir Fernandes Godoy, Faculty of Medicine of São José do Rio Preto, Brazil*
*Luiz Carlos Marques Vanderlei, São Paulo State University, Brazil*

***Correspondence:**
*Elisa Mejía-Mejía*
*elisa.mejia-mejia@city.ac.uk*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

**Received:** *14 February 2020*
**Accepted:** *15 June 2020*
**Published:** *23 July 2020*

**Citation:**
*Mejía-Mejía E, Budidha K, Abay TY, May JM and Kyriacou PA (2020) Heart Rate Variability (HRV) and Pulse Rate Variability (PRV) for the Assessment of Autonomic Responses. Front. Physiol. 11:779. doi: 10.3389/fphys.2020.00779*

**Introduction:** Heart Rate Variability (HRV) and Pulse Rate Variability (PRV), are non-invasive techniques for monitoring changes in the cardiac cycle. Both techniques have been used for assessing the autonomic activity. Although highly correlated in healthy subjects, differences in HRV and PRV have been observed under various physiological conditions. The reasons for their disparities in assessing the degree of autonomic activity remains unknown.

**Methods:** To investigate the differences between HRV and PRV, a whole-body cold exposure (CE) study was conducted on 20 healthy volunteers (11 male and 9 female, 30.3 ± 10.4 years old), where PRV indices were measured from red photoplethysmography signals acquired from central (ear canal, ear lobe) and peripheral sites (finger and toe), and HRV indices from the ECG signal. PRV and HRV indices were used to assess the effects of CE upon the autonomic control in peripheral and core vasculature, and on the relationship between HRV and PRV. The hypotheses underlying the experiment were that PRV from central vasculature is less affected by CE than PRV from the peripheries, and that PRV from peripheral and central vasculature differ with HRV to a different extent, especially during CE.

**Results:** Most of the PRV time-domain and Poincaré plot indices increased during cold exposure. Frequency-domain parameters also showed differences except for relative-power frequency-domain parameters, which remained unchanged. HRV-derived parameters showed a similar behavior but were less affected than PRV. When PRV and HRV parameters were compared, time-domain, absolute-power frequency-domain, and non-linear indices showed differences among stages from most of the locations. Bland-Altman analysis showed that the relationship between HRV and PRV was affected by CE, and that it recovered faster in the core vasculature after CE.

**Conclusion:** PRV responds to cold exposure differently to HRV, especially in peripheral sites such as the finger and the toe, and may have different information not available in

HRV due to its non-localized nature. Hence, multi-site PRV shows promise for assessing the autonomic activity on different body locations and under different circumstances, which could allow for further understanding of the localized responses of the autonomic nervous system.

**Keywords: autonomic nervous system, pulse rate variability, heart rate variability, photoplethysmography, peripheral circulation, cold stress, vasoconstriction**

## 1. INTRODUCTION

Heart rate variability (HRV) is a widely used physiological variable that non-invasively assesses the cardiac autonomic nervous system (ANS) by measuring the changes in the cardiac rhythm through time (Shaffer and Ginsberg, 2017). HRV is considered as a reflection of changes of the cardiac sympathetic and parasympathetic branches of the ANS (Clifford et al., 2006). Several models have been proposed to explain HRV (Laborde et al., 2017), which could describe the relationship between HRV, vagal tone and several physiopathological processes. Low values of HRV indices have been found to relate to cardiac events, such as myocardial infarction; progression of atherosclerosis; and heart failure (Huikuri et al., 1999). Some studies have also associated HRV values with conditions such as coronary artery disease and sudden death (Xhyheri et al., 2012), diabetes mellitus (da Silva et al., 2016), pain (Broucqsault-Dédrie et al., 2016), acute and chronic stress (Murray, 2012; Castaldo et al., 2015), metabolic syndrome (Stuckey et al., 2014), depression (Koenig et al., 2016), and bipolar disorders (Bassett, 2015). Furthermore, HRV has been used as a marker of social interaction (Shahrestani et al., 2015), sports performance (Dong, 2016; Gavrilova, 2016), and emotional states (Choi et al., 2017).

HRV information is usually measured from the electrocardiographic signal (ECG) (Clifford et al., 2006), and standards of measurement have been established in an attempt to align methodologies and allow for the comparison of results presented by different studies (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). However, in the past few years, several studies have reported the use of physiological signals other than the ECG to extract HRV information. Hence, the term Pulse Rate Variability (PRV) has been used to refer to HRV information obtained from pulse wave signals, such as the photoplethysmograms (PPG).

Photoplethysmography (PPG) is a simple, low-cost, non-invasive, optical measurement technique which is used for the detection of blood volume changes in peripheral tissue (Kyriacou, 2006; Allen, 2007). Due to its widespread use in clinical and everyday activities, researches have tried to obtain as much information from this signal as possible. One of the main avenues which has been explored widely by researches is extraction of PRV information from PPG signals (Georgiou et al., 2018). PRV has been derived from PPG for the analysis of ANS changes under different conditions, such as the presence of mental (Dagdanpurev et al., 2018; Can et al., 2019) or somatic diseases (Bolea et al., 2017; Lan et al., 2018), during sleep (Lázaro et al., 2014; Garde et al., 2019), or for evaluating the effects of pharmacological drugs (Sluyter et al., 2016). Nonetheless, although PRV has been treated as a valid surrogate of HRV, their relationship is not entirely clear, and PRV has been found to significantly differ from HRV under certain circumstances (Schäfer and Vagedes, 2013).

It has been hypothesized that factors such as stress (Giardino et al., 2002), respiratory patterns (Jan et al., 2019), exercise (Lin et al., 2014), orthostatic changes (Pernice et al., 2018), and ambient temperature (Shin, 2016) may have different effects on PRV, when compared to HRV, and therefore affecting their relationship. However, the origin of these differences is still not clear, and may be related to changes in hemodynamics, blood pressure or pulse transit time (PTT) (Charlot et al., 2009; Gil et al., 2010; Chen et al., 2015). Since hemodynamics are largely controlled by the ANS (Fox, 2016), PRV might be affected by changes in this regulation in response to external stimuli, such as colder temperature.

Thermal balance in humans exposed to extreme weather is mainly achieved by vasoconstriction or vasodilation of vessels in the skin and peripheral tissues (Daanena and Lichtenbeltd, 2016). Specifically, cold exposure generates changes in the autonomic response of humans by activating the sympathetic nervous system to maintain homeothermy (Gordon, 2009). As explained by Gordon (2009), when exposed to cold temperatures, the thermoreceptors send this information to the hypothalamus, that regulates the rate of heat production and, if necessary, stimulates the efferent nerves in the sympathetic nervous system, which has the primary role of stimulating peripheral vasoconstriction (Mäkinen et al., 2008). This activation generates changes in cardiovascular dynamics, by changing the level of constriction of vessels, generating the involuntary contraction of muscles, and increasing heart rate, cardiac output, and blood pressure (Fox, 2016). Several techniques that reflect autonomic activity have been shown to reflect these changes during cold exposure to a different extent, such as evaluating the changes in amplitude of PPG signals (Budidha and Kyriacou, 2019), the changes in central hemodynamics variables such as augmentation index (King et al., 2013), the changes in microneurography (Sawasaki et al., 2001; Greaney et al., 2017), or the changes in HRV (Mäkinen et al., 2008; Okamoto-Mizuno, 2009; Hintsala et al., 2014).

The strength of the sympathetic vasoconstriction, however, varies between core and peripheral locations, as was verified by Budidha and Kyriacou (2019). Understanding how these changes vary in each body location could improve the comprehension of ANS activity during cold exposure and the relationship between

HRV and PRV under these circumstances. Also, although HRV is mainly a reflection of vagal tone (Laborde et al., 2017), sympathetic activity may influence some of the indices obtained from HRV and PRV, and might affect PRV and HRV in a different manner when subjects are exposed to colder temperatures; and the agreement between PRV and HRV has been shown to be affected by sympathetic shift of the ANS activity (Chen et al., 2015).

To investigate the dependency of PRV on external factors such as the acquisition site and the temperature, a whole-body cold exposure study was performed on healthy volunteers. PRV and HRV information was extracted from simultaneously obtained PPG and ECG signals, respectively. Red (660 nm) PPG signals were recorded from the earlobe, the ear canal, the finger, and the toe. It was hypothesized that (1) PRV information obtained from the earlobe and ear canal might not be equally affected by cold exposure as that of the finger and the toe; and (2) the agreement between HRV and PRV is altered by whole-body cold exposure, maintaining a higher agreement between HRV and PRV measured from central locations such as the earlobe and the ear canal. The results obtained from this study are important for understanding the possible differences between HRV and PRV, and might lead to further research that aims to better understand PRV, how the sympathetic and parasympathetic activity may affect it, and its clinical applications.

# 2. MATERIALS AND METHODS

## 2.1. Experimental Protocol

Twenty healthy volunteers (11 male and 9 female, 30.3 ± 10.4 years old) were recruited to take part in this study. Subjects with any cardiovascular, pulmonary, or metabolic conditions were excluded. All subjects were normotensive, normothermic, and did not take any medication at the time of the study. The study protocol was approved by Senate Research Ethics Committee at City, University of London, and all subjects gave informed consent before taking part in the study.

The measurement protocol is shown in **Figure 1**. Subjects were asked to refrain from ingesting beverages with caffeine and alcohol, not to exercise or smoke at least 2 h before the test, and they were instructed to wear only one layer of clothes during the data acquisition period, in order to maximize the effect of the stimulus on the body. Data from all subjects was collected between 10:00 a.m. and 6:00 p.m., under controlled conditions of temperature and humidity.

Upon arrival, subjects were seated in a room maintained at 24 ± 1°C for at least 10 min, to ensure hemodynamic stabilization. After this period, the sensors for acquiring the signals were attached to the subjects. The measurement started with a 2-min baseline measurement (BM) stage, in which 2 min of signals were recorded from the subjects while the room temperature was 24 ± 1°C. The volunteers were then moved to an adjacent, temperature-controlled room, maintained at 10 ± 1°C (Cold Exposure, CE). This temperature was selected because it reflects a more realistic change in ambient temperature, which can be sustained for longer periods of time by healthy adults, and generates changes in hemodynamics (King et al., 2013). Subjects remained in this room and signals were recorded for 10 min before returning to the original room at 24 ± 1°C, for additional 10 min of signals recording (Cold Recovery, CR). During each phase of the measurement protocol, subjects were seated in a comfortable *swivel chair*, with both hands located on the arm rest.

After each of the recording on the different stages, the measurement was paused and the subject was wheeled to the room for recording the next stage. The recording was resumed as soon as the subject was moved, in order to record the shock response of the autonomic activity on the periphery.

## 2.2. Signal Acquisition and Processing

### 2.2.1. Signal Acquisition

Disposable electrodes were placed on the left and right shoulders, and on the right hip (reference electrode) for obtaining lead I ECG signals, while PPG signals from the left index finger (F), toe (T), ear canal (EC), and earlobe (EL) were obtained from each subject during the three stages of the study. Red light (660 nm) was used for acquiring PPG's. The signal acquisition was paused during the transitions between the two rooms in order to avoid movement artifacts.

All PPG and ECG measurements were acquired using a research PPG acquisition system (*ZenPPG*) developed in the Research Center for Biomedical Engineering, at City, University of London (Budidha, 2016). All signals were acquired at a sampling rate of 1 kHz.

### 2.2.2. PPG Signal Processing

PPG signals were down-sampled to 100 Hz to restrict the bandwidth of the signals and remove any unwanted noise. Afterwards, they were detrended, and the first and last 10 s of each stage of the protocol were removed, to eliminate any non-stationarities of the signal. Signals were then filtered using a fourth-order bandpass Butterworth filter, with cut-off frequencies of 0.1 and 2 Hz. These cutoff frequencies were selected to attenuate any unwanted noise and strengthen the pulsatile component of the PPG signal.

Different fiducial points such as systolic peaks (PKS), onsets of the pulse (ONS), maximum slope point (SLO), and the intersection point between tangent lines from the onset and the maximum slope point were obtained from each PPG signal (TI), applying an algorithm based on Li et al. (2010). Once detected, signal quality indices described in the literature (Karlen et al., 2012; Li and Clifford, 2012; Elgendi, 2016; Calle Uribe, 2018), were applied to identify the quality of the pulses segmented by each fiducial point in each PPG signal during each test stage, and those that better segmented the pulses of each PPG signal were selected and used for measuring PRV.

Using a k-means algorithm, the cardiac cycles were classified as bad and good quality. This was done assuming that during the first stage of the test the quality of the signal was maximal, and the cluster with most of the cardiac cycles of this stage was considered as the good quality (GQ) group. Hence, the cycles classified in this group during the other two stages (CE and CR) were considered as good-quality pulses. The proportion between GQ pulses and the total number of pulses was measured for each

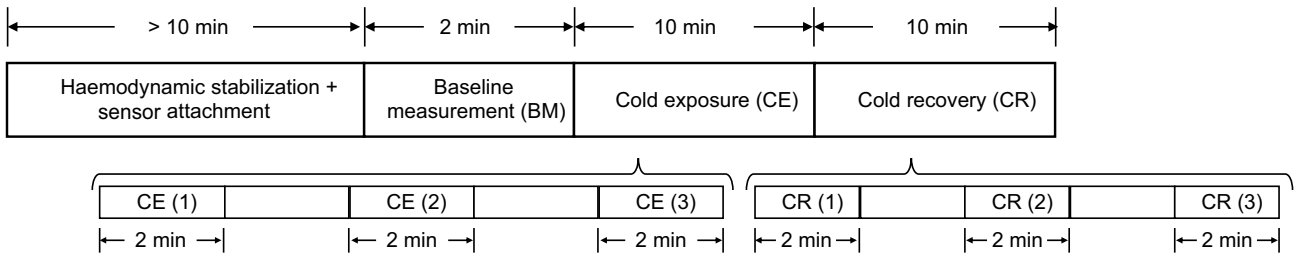



**FIGURE 1 |** Measurement protocol for the whole-body cold exposure study.

fiducial point during each stage and from each body location. Then, the fiducial point that showed the highest proportion of GQ pulses in each case were selected for further analysis. This was performed to diminish the effect of noise in the measurement of PRV, and in an attempt to automatically determine the better fiducial point for each condition, as proposed in Pinheiro et al. (2016).

### 2.2.3. ECG Signal Processing

ECG signals were also down-sampled to 100 Hz and R peaks were detected using an algorithm based on Pan and Tompkins (1985) and Hamilton and Tompkins (1986) algorithms. These processing steps were performed using the 2019a version of MATLAB® (Mathworks, USA). **Figure 2** shows a segment of PPG and ECG signals and the extracted fiducial points from each of these signals.

### 2.2.4. HRV and PRV Analysis

Using the selected fiducial points from PPG and the R peaks obtained from the ECG, interbeat intervals (IBI's), and R-to-R intervals (RRI's) were measured for the extraction of HRV and PRV information, respectively. IBI's and RRIs that were 50% above or below their median value were corrected. Two minutes segments of IBI's and RRI's were obtained from each stage, and time- and frequency-domain indices, as well as Poincaré plot-derived indices, were obtained from these traces, as recommended in the literature (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996; Khandoker et al., 2013; Shaffer and Ginsberg, 2017).

The standard deviation of normal-to-normal intervals (SDNN), the root mean square of successive interval differences (RMSSD) and the percentage of successive intervals that differ by more than 50 ms (pNN50). For frequency-domain analysis, traces were interpolated using cubic-spline interpolation and a sampling rate of 4 Hz, and the power spectra were obtained using fast Fourier transform (FFT). The absolute and relative powers of the low-frequency (0.04–0.14 Hz, LF, and nLF) and high-frequency (0.15–0.4 Hz, HF, and nHF) bands, as well as the total power of the spectra between 0.0033 and 0.4 Hz (TP) and the ratio of the low-frequency and high-frequency powers (LF/HF) were measured. Finally, the standard deviation of data located perpendicular (SD1) and along (SD2) the line of identity of the Poincaré plot and their ratio (SD1/SD2) were obtained (Khandoker et al., 2013).

As explained by Shaffer and Ginsberg (2017), SDNN reflects both the SNS and PNS activity, although it is thought that its main source of variation in short-term recordings is the respiratory sinus arrhythmia (RSA), while RMSSD is the primary time-domain measure to evaluate the vagal activity reflected in HRV; pNN50 has been shown to be closely related to PNS activity and RMSSD measurements. Similarly, these authors explain that LF is mainly produced by both SNS and PNS activity, together with blood pressure regulation performed by baroreceptors (Shaffer and Ginsberg, 2017); during normal respiratory rates, this frequency band is thought to reflect baroreflex activity and not cardiac sympathetic innervation, whereas during slow breathing, LF can be modified by vagal activity (Khandoker et al., 2013; Shaffer and Ginsberg, 2017). HF, on the other hand, is considered as the respiratory band and reflects mainly parasympathetic activity and RSA; it has been observed that total vagal blockade eliminates most of the frequency components in this frequency band (Pomeranz et al., 1985; Malliani et al., 1991). For both LF and HF bands, it is possible to obtain a measure of relative power in normalized units, which emphasizes the behavior of cardiac autonomic activity (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Also, TP is the summation of both LF and HF bands (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). LF/HF is a more controversial measurement, traditionally thought to reflect the sympathovagal balance, especially in studies involving 24-h recordings (Shaffer and Ginsberg, 2017); however, this fact has been questioned due to the important effect that PNS activity has on LF as well as the lack of correlation between increased sympathetic activity and higher values of this ratio (Billman, 2013). Hence, LF/HF should not be considered as a marker of sympathovagal balance but as a reflection of baroreflex activity (Goldstein et al., 2011).

Finally, Poincaré plot-derived indices have also been associated with changes in the SNS and PNS activity. As explained by Khandoker et al. (2013), the dispersion of the points perpendicular to the line of identity, i.e., SD1, reflects the short-term variability of interbeat intervals, and relates to RMSSD; whereas the dispersion of the points along the line

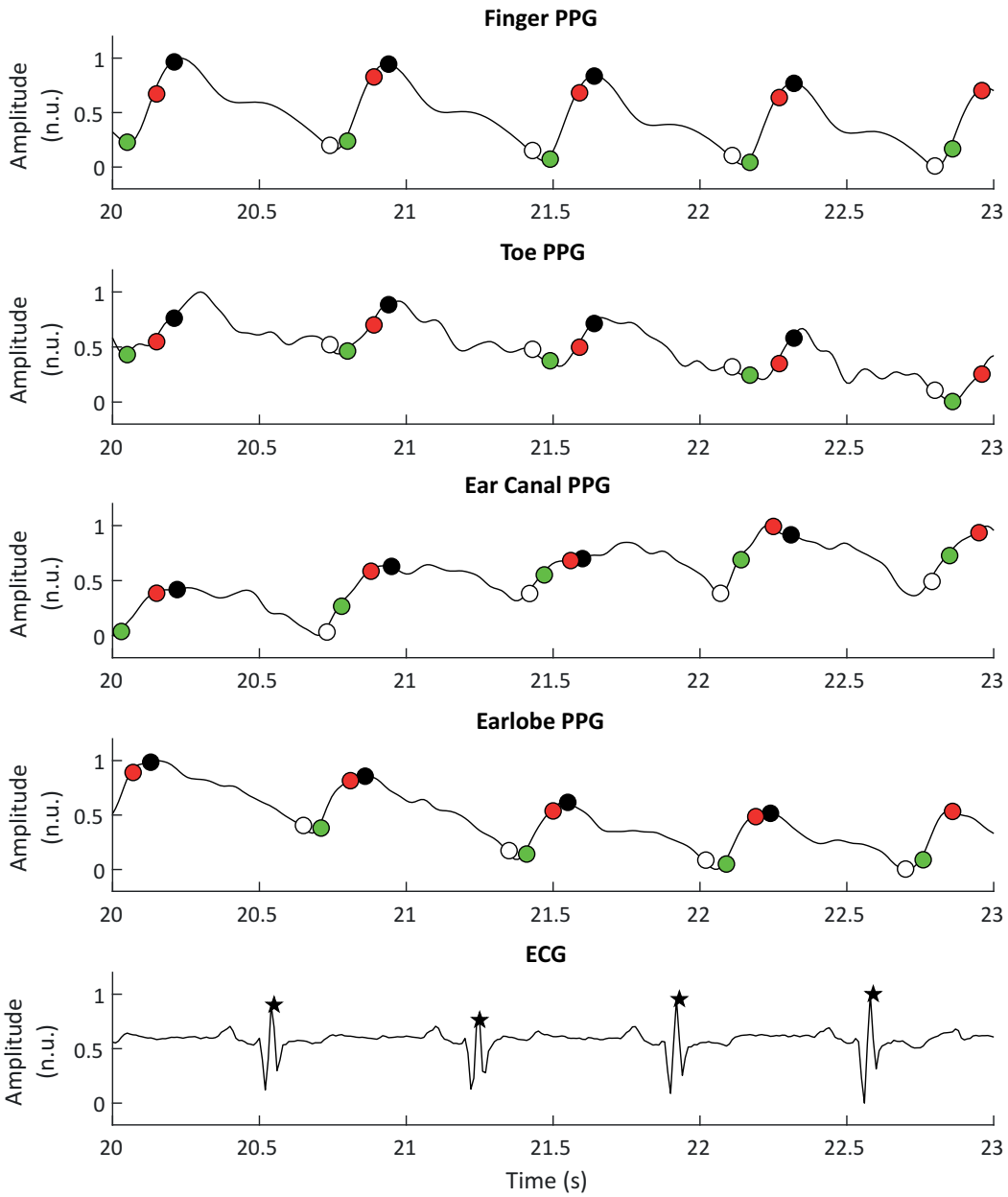



**FIGURE 2 |** Example of photoplethysmographic (PPG) and electrocardiographic (ECG) signals used for the extraction of pulse rate variability and heart rate variability, respectively. From top to bottom, the PPG signals correspond to the signals obtained from the finger, the toe, the ear canal, and the earlobe. The black stars show the R peaks detected from the ECG signal, while the white, black, red, and green circles show the detected onsets, peaks, maximum slope points, and tangent intersection points, respectively.

of identity, i.e., SD2, relates to the standard deviation of the interbeat intervals, the SDNN index.

## 2.3. Statistical Analysis

The aim of this study was to compare the behavior of core and peripheral PRV during cold exposure, and to evaluate if and how PRV differed to HRV during mild whole body cold exposure. Hence, two hypotheses were proposed: (1) PRV from core vasculature (ear canal and earlobe) is less affected by cold exposure than PRV from the periphery (finger and toe); and (2) PRV from core vasculature is more similar to HRV than PRV from peripheral tissue, especially during cold exposure.

To evaluate the first hypothesis, PRV indices obtained during the first 2 min of each stage of the test were compared using repeated-measures analysis of variance (ANOVA), with sphericity corrections. Multiple comparisons with pairwise t-tests and Bonferroni corrections were performed in case the ANOVA showed a statistically significant difference during at least one

stage. Also, the first (min 0–2), middle (min 4–6), and last (min 8–10) segments of cold exposure and cold recovery stages were compared to baseline measurement, to evaluate the behavior of PRV and HRV indices when the ambient temperature was changing and during stabilization in each stage.

The second hypothesis was evaluated using Bland-Altman analysis, to assess the agreement between PRV and HRV indices during the first 2 min of each stage of the test. A Bland-Altman ratio (BAR) was defined as the ratio of half the range of limits of agreement (LoA, Equation 1) to the average of the pairwise measurement means, as proposed by Peng et al. (2015) (Equation 2). Agreements were considered as good (BAR $\leq$ 10%), moderate (10% $\leq$ BAR $\leq$ 20%), or insufficient (BAR $\geq$ 20%). Also, the behavior of the indices extracted from PRV and HRV during each stage of the test was evaluated using a Friedman rank sum test and *post hoc* analyses were performed using Nemenyi's test. Finally, the level of linear relationship between the indices, was assessed using Pearson or Spearman correlation analysis, for normally and non-normally distributed data respectively. Normality of data was determined using a Shapiro-Wilk test and a significance level of 5% ($p$-value $< 0.05$) was considered significant for all analyses.

$$LoA = \overline{(x)} \pm 1.96\sigma_x, x = HRV - PRV \quad (1)$$

$$BAR = \left| \frac{1.96(\sigma_x)}{(HRV + PRV)} \right|, x = HRV - PRV \quad (2)$$

# 3. RESULTS

## 3.1. Selection of Fiducial Points

After applying the proposed algorithm for selecting the best fiducial point in each condition, it was observed that the lower proportion of good quality cardiac cycles was obtained in the finger and toe, i.e., the peripheral tissue. The most accurate results were obtained when cardiac cycles were segmented using the intersection of tangent lines (TI) and the location of the maximum slope (SLO) as fiducial points. The lowest performance was achieved when the systolic peaks (PKS) were used, except for the finger PPG in which the performance of the peak detection algorithm was better than most of the others. A summary of the behavior of the extracted indices from these fiducial points and from HRV is shown in **Table 1**.

## 3.2. Changes in PRV and HRV During Cold Exposure

Results from the repeated-measures ANOVA and its related multiple comparisons for time-domain and Poincaré plot indices are shown in **Table 2**, while **Table 3** shows the results obtained from frequency-domain indices.

### 3.2.1. Time-Domain Indices

SDNN showed statistically significant differences between baseline measurement and cold exposure when measured from any location, except for the ear canal, while RMSSD and pNN50 did not show statistically significant differences between these stages when measured from the finger and the ear canal. All indices behaved similarly when cold exposure and cold recovery were compared, except for pNN50 measured from the earlobe. Ear canal was the only location from which none of the indices measured show any statistically-significant differences among stages.

### 3.2.2. Frequency-Domain Indices

Relative-power indices, i.e., nLF, nHF, and LF/HF, did not show any difference among stages. Regarding absolute-power indices (LF, HF, and TP), and similar to what was observed from time-domain indices, the ear canal-derived PRV indices did not show any differences among stages. HRV-derived LF and HF did not show differences among stages, and finger HF did not show differences in the *post-hoc* analyses. LF, HF, and TP did not differ between cold exposure and cold recovery when measured from any of the locations.

### 3.2.3. Non-linear Indices

Ear canal-derived indices did not differ among stages from any of the Poincaré plot-derived indices. Similarly, SD1 and SD1/SD2 did not show differences among stages when measured from the finger. Most differences were obtained when baseline measurement and cold exposure where compared, while no differences were shown when cold exposure and cold recovery were compared.

## 3.3. Behavior of PRV and HRV Indices

**Figure 3** illustrates the behavior of the extracted indices when 2-min segments of each stage were compared. Most indices showed a similar behavior when measured from PRV and HRV, but with a notorious overestimation of most indices when measured from PRV. LF/HF was the only index that was underestimated when measured from PRV, while nLF was the index with the least overestimation when measured from PRV. The ear canal showed the higher differences in the trends between PRV and HRV. Time-domain and non-linear indices showed that values tended to increase after baseline measurement, and then, during cold recovery, indices tended to recover to the values obtained during baseline. Frequency-domain indices did not show this behavior, probably due to the short segments used for analysis. When indices measured from the different segments were compared using a repeated-measures ANOVA (results not shown), ear canal was found to be the only location with non-statistically significant differences among each 2-min segments. Most differences observed were among baseline measurements and the segments obtained during cold exposure, and among cold exposure and cold recovery segments. On the other hand, baseline measurement and cold recovery were statistically similar, except for SD1, SD2, and RMSSD. Regarding frequency-domain indices, nLF and LF/HF failed to show any difference among stages, probably due to the short time segments used for this analysis.

**TABLE 1 |** Mean value ± standard deviation of indices measured from HRV and PRV data.

| Stage | Index | HRV | PRV | | | |
|---|---|---|---|---|---|---|
| | | | Finger | Toe | Ear canal | Earlobe |
| BM | SDNN (s) | 0.048 ± 0.02 | 0.057 ± 0.02 | 0.095 ± 0.07 | 0.109 ± 0.12 | 0.059 ± 0.02 |
| | RMSSD (s) | 0.045 ± 0.02 | 0.075 ± 0.02 | 0.134 ± 0.10 | 0.156 ± 0.18 | 0.076 ± 0.03 |
| | pNN50 | 0.258 ± 0.20 | 0.339 ± 0.14 | 0.478 ± 0.19 | 0.464 ± 0.19 | 0.343 ± 0.16 |
| | LF ($s^2$) | 527.5 ± 420.1 | 498.4 ± 244.6 | 3209.5 ± 6820.0 | 8910.4 ± 29673.2 | 534.4 ± 251.6 |
| | HF ($s^2$) | 907.5 ± 928.3 | 1195.9 ± 742.0 | 4716.0 ± 6859.3 | 4399.0 ± 6505.8 | 1246.2 ± 835.5 |
| | TP ($s^2$) | 2024.7 ± 1487.0 | 2103.6 ± 1149.5 | 8525.9 ± 13472.2 | 15964.3 ± 43563.8 | 2151.1 ± 1173.5 |
| | nLF (n.u.) | 0.293 ± 0.15 | 0.268 ± 0.11 | 0.276 ± 0.13 | 0.324 ± 0.13 | 0.289 ± 0.13 |
| | nHF (n.u) | 0.398 ± 0.14 | 0.555 ± 0.08 | 0.568 ± 0.12 | 0.527 ± 0.16 | 0.549 ± 0.13 |
| | LF/HF | 0.947 ± 0.85 | 0.506 ± 0.26 | 0.554 ± 0.42 | 0.887 ± 1.22 | 0.604 ± 0.48 |
| | SD1 (s) | 0.032 ± 0.01 | 0.053 ± 0.02 | 0.094 ± 0.07 | 0.111 ± 0.13 | 0.054 ± 0.02 |
| | SD2 (s) | 2.528 ± 0.35 | 2.524 ± 0.36 | 2.782 ± 0.74 | 3.057 ± 1.56 | 2.536 ± 0.35 |
| | SD1/SD2 | 0.013 ± 0.01 | 0.021 ± 0.01 | 0.032 ± 0.02 | 0.031 ± 0.02 | 0.021 ± 0.01 |
| CE | SDNN (s) | 0.066 ± 0.03 | 0.075 ± 0.03 | 0.135 ± 0.07 | 0.134 ± 0.08 | 0.073 ± 0.03 |
| | RMSSD (s) | 0.066 ± 0.04 | 0.099 ± 0.04 | 0.193 ± 0.10 | 0.184 ± 0.11 | 0.095 ± 0.04 |
| | pNN50 | 0.396 ± 0.25 | 0.409 ± 0.16 | 0.623 ± 0.17 | 0.520 ± 0.15 | 0.478 ± 0.16 |
| | LF ($s^2$) | 1228.3 ± 1563.1 | 1450.3 ± 1417.9 | 5258.6 ± 7295.6 | 7957.4 ± 15225.5 | 1074.6 ± 799.8 |
| | HF ($s^2$) | 2380.1 ± 3516.1 | 2543.3 ± 2246.4 | 9538.4 ± 7976.8 | 7727.5 ± 8258.2 | 2592.9 ± 2312.8 |
| | TP ($s^2$) | 4139.3 ± 5155.4 | 4528.8 ± 3885.1 | 16342.6 ± 16589.6 | 18075.5 ± 23059.4 | 4129.6 ± 3175.1 |
| | nLF (n.u.) | 0.307 ± 0.17 | 0.315 ± 0.12 | 0.278 ± 0.12 | 0.320 ± 0.15 | 0.268 ± 0.11 |
| | nHF (n.u.) | 0.498 ± 0.21 | 0.553 ± 0.12 | 0.626 ± 0.16 | 0.571 ± 0.17 | 0.596 ± 0.13 |
| | LF/HF | 0.924 ± 0.93 | 0.651 ± 0.44 | 0.530 ± 0.38 | 1.388 ± 3.73 | 0.506 ± 0.33 |
| | SD1 (s) | 0.047 ± 0.03 | 0.070 ± 0.03 | 0.136 ± 0.07 | 0.130 ± 0.08 | 0.067 ± 0.03 |
| | SD2 (s) | 2.613 ± 0.35 | 2.638 ± 0.38 | 3.050 ± 0.82 | 3.449 ± 1.84 | 2.626 ± 0.36 |
| | SD1/SD2 | 0.018 ± 0.01 | 0.026 ± 0.01 | 0.043 ± 0.02 | 0.037 ± 0.01 | 0.026 ± 0.01 |
| CR | SDNN (s) | 0.057 ± 0.02 | 0.074 ± 0.03 | 0.127 ± 0.06 | 0.110 ± 0.08 | 0.066 ± 0.02 |
| | RMSSD (s) | 0.060 ± 0.03 | 0.100 ± 0.05 | 0.180 ± 0.08 | 0.154 ± 0.10 | 0.086 ± 0.03 |
| | pNN50 | 0.326 ± 0.25 | 0.448 ± 0.19 | 0.588 ± 0.14 | 0.513 ± 0.20 | 0.372 ± 0.15 |
| | LF ($s^2$) | 697.0 ± 603.0 | 1003.7 ± 904.1 | 5003.6 ± 6751.8 | 6446.5 ± 13699.1 | 870.7 ± 687.1 |
| | HF ($s^2$) | 1586.7 ± 2052.1 | 2592.7 ± 2455.7 | 8123.6 ± 6813.6 | 5684.9 ± 6215.7 | 1996.8 ± 1422.7 |
| | TP ($s^2$) | 2938.5 ± 2871.7 | 4182.9 ± 3601.2 | 14634.3 ± 14235.6 | 13936.7 ± 22646.1 | 3376.0 ± 2299.0 |
| | nLF (n.u.) | 0.273 ± 0.15 | 0.250 ± 0.09 | 0.291 ± 0.10 | 0.303 ± 0.14 | 0.250 ± 0.09 |
| | nHF (n.u.) | 0.473 ± 0.23 | 0.583 ± 0.16 | 0.584 ± 0.13 | 0.545 ± 0.19 | 0.587 ± 0.15 |
| | LF/HF | 1.098 ± 1.23 | 0.501 ± 0.30 | 0.547 ± 0.31 | 0.752 ± 0.74 | 0.476 ± 0.26 |
| | SD1 (s) | 0.042 ± 0.02 | 0.071 ± 0.03 | 0.127 ± 0.06 | 0.109 ± 0.07 | 0.061 ± 0.02 |
| | SD2 (s) | 2.632 ± 0.36 | 2.632 ± 0.35 | 3.075 ± 0.86 | 3.145 ± 1.25 | 2.643 ± 0.35 |
| | SD1/SD2 | 0.016 ± 0.01 | 0.027 ± 0.01 | 0.040 ± 0.01 | 0.033 ± 0.01 | 0.023 ± 0.01 |

*BM, baseline measurement; CE, cold exposure; CR, cold recovery.*

## 3.4. Agreement Between HRV and PRV

### 3.4.1. Friedman Rank Sum Tests

Results for the Friedman rank sum tests and its *post hoc* comparisons are presented in **Table 4**. Since the aim was to evaluate the relationship between HRV and PRV, only multiple comparisons between HRV and PRV are shown.

During baseline measurement, nLF and LF/HF did not show differences between HRV and PRV, while LF, TP, and SD2 failed to show differences from *post hoc* analysis. Most of the other indices showed differences between HRV and toe PRV, and between HRV and ear canal PRV, while RMSSD, nHF, SD1, and SD1/SD2 showed differences when PRV was measured from any location.

Similar behavior was observed for nLF and LF/HF durng cold exposure. However, all other indices showed differences from *post-hoc* analyses, mainly between HRV and toe PRV, and HRV and ear canal PRV. None of the indices showed differences from all locations, but RMSSD, SD1, and SD1/SD2 showed statistically significant differences when measured from the earlobe.

Finally, during cold recovery, the same results were obtained for nLF and LF/HF. In this stage, also nHF failed to show any difference among locations, and *post hoc* analyses from SD2 did not show any differences between HRV and any of the PRV data. All differences observed were between HRV and toe PRV, and between HRV and ear canal PRV.

**TABLE 2 |** *P*-values obtained from the repeated-measures ANOVA and its *post-hoc* analyses, when applied to time-domain and Poincaré plot-derived indices of PRV and HRV.

| Index | Source | ANOVA | *post-hoc* comparisons | | |
|---|---|---|---|---|---|
| | | | BM vs. CE | CE vs. CR | BM vs. CR |
| SDNN (s) | HRV | <0.001 | 0.005 | 0.086 | 0.025 |
| | Finger PRV | 0.007 | 0.039 | 1.000 | 0.024 |
| | Toe PRV | <0.001 | <0.001 | 0.295 | 0.003 |
| | Ear canal PRV | 0.118 | – | – | – |
| | Earlobe PRV | 0.006 | 0.021 | 0.199 | 0.310 |
| RMSSD (s) | HRV | 0.001 | 0.004 | 0.174 | 0.004 |
| | Finger PRV | 0.015 | 0.066 | 1.000 | 0.053 |
| | Toe PRV | <0.001 | <0.001 | 0.208 | 0.007 |
| | Ear canal PRV | 0.250 | – | – | – |
| | Earlobe PRV | 0.009 | 0.022 | 0.331 | 0.265 |
| pNN50 | HRV | <0.001 | <0.001 | 0.068 | 0.062 |
| | Finger PRV | 0.006 | 0.123 | 0.633 | 0.018 |
| | Toe PRV | <0.001 | 0.002 | 0.639 | 0.011 |
| | Ear canal PRV | 0.168 | – | – | – |
| | Earlobe PRV | <0.001 | <0.001 | 0.002 | 0.774 |
| SD1 (s) | HRV | 0.001 | 0.004 | 0.170 | 0.005 |
| | Finger PRV | 0.015 | 0.067 | 1.000 | 0.053 |
| | Toe PRV | <0.001 | <0.001 | 0.206 | 0.007 |
| | Ear canal PRV | 0.250 | – | – | – |
| | Earlobe PRV | 0.009 | 0.023 | 0.330 | 0.266 |
| SD2 (s) | HRV | 0.001 | 0.017 | 1.000 | 0.003 |
| | Finger PRV | 0.004 | 0.029 | 1.000 | 0.012 |
| | Toe PRV | 0.002 | 0.003 | 1.000 | 0.013 |
| | Ear canal PRV | 0.076 | – | – | – |
| | Earlobe PRV | 0.001 | 0.036 | 1.000 | <0.001 |
| SD1/SD2 | HRV | 0.001 | 0.005 | 0.131 | 0.015 |
| | Finger PRV | 0.025 | 0.097 | 1.000 | 0.083 |
| | Toe PRV | 0.002 | 0.004 | 0.261 | 0.046 |
| | Ear canal PRV | 0.044 | 0.178 | 0.060 | 1.000 |
| | Earlobe PRV | 0.021 | 0.047 | 0.192 | 0.724 |

*Values in red indicate statistical significance (p-value < 0.05). Sphericity corrections using Greenhouse-Geisser correction were applied when Mauchly's test showed statistically significant results, and p-values shown are after these corrections. BM, baseline measurement; CE, cold exposure; CR, cold recovery.*

### 3.4.2. Correlation Analysis

The results from the correlation analyses between HRV and PRV are shown in **Figure 4**.

During baseline measurement, non-significant correlation were observed from RMSSD, nLF, LF/HF, SD1, and SD1/SD2 when these indices were measured from toe and ear canal PRV. nHF did not show significant correlations when measured from any location, and SDNN and LF had non-significant correlations when measured from the ear canal. pNN50, HF, TP, and SD2 showed statistically significant correlations when measured from all locations.

The correlation between HRV and PRV during cold exposure showed that non-significant correlations were obtained from SDNN, RMSSD, LF, TP, and SD1, when measured from the toe and the ear canal; from HF, nHF, and SD1/SD2, when measured from the toe; from nLF, when measured from the earlobe; and from LF/HF, when measured from the toe and the earlobe. Significant correlations from all locations were only observed from pNN50 and SD2.

Similarly, during cold recovery, pNN50 and SD2 showed significant correlations from all locations. However, non-significant correlations were obtained from SDNN, RMSSD, nLF, nHF, LF/HF, SD1, and SD1/SD2, when measured from the toe; from LF and TP, when measured from the toe and the ear canal; and from HF, when measured from the ear canal.

### 3.4.3. Bland-Altman Analysis

Since a high correlation does not necessarily indicate a strong agreement (Bland and Altman, 1986), Bland-Altman analysis was performed to assess the agreement between PRV and HRV. Bland-Altman ratios (BAR's) are presented in **Figure 5**, and Bland-Altman plots are included as **Supplementary Material**. Agreement between HRV and PRV measured from the earlobe was the highest and most stable during the three stages, while ear

**TABLE 3 |** *P*-values obtained from the repeated-measures ANOVA and its *post-hoc* analyses, when applied to frequency-domain indices of PRV and HRV.

| Index | Source | ANOVA | *post-hoc* comparisons | | |
|---|---|---|---|---|---|
| | | | BM vs. CE | CE vs. CR | BM vs. CR |
| LF ($s^2$) | HRV | 0.057 | – | – | – |
| | Finger PRV | 0.008 | 0.028 | 0.162 | 0.077 |
| | Toe PRV | 0.009 | 0.010 | 1.000 | 0.083 |
| | Ear canal PRV | 0.622 | – | – | – |
| | Earlobe PRV | 0.004 | 0.016 | 0.352 | 0.122 |
| HF ($s^2$) | HRV | 0.024 | 0.070 | 0.134 | 0.059 |
| | Finger PRV | 0.016 | 0.058 | 1.000 | 0.057 |
| | Toe PRV | <0.001 | <0.001 | 0.236 | 0.013 |
| | Ear canal PRV | 0.057 | – | – | – |
| | Earlobe PRV | 0.008 | 0.018 | 0.458 | 0.016 |
| TP ($s^2$) | HRV | 0.031 | 0.085 | 0.230 | 0.061 |
| | Finger PRV | 0.013 | 0.047 | 1.000 | 0.052 |
| | Toe PRV | <0.001 | <0.001 | 0.546 | 0.007 |
| | Ear canal PRV | 0.581 | – | – | – |
| | Earlobe PRV | 0.002 | 0.015 | 0.459 | 0.037 |

*Values in red indicate statistical significance (p-value < 0.05). Sphericity corrections using Greenhouse-Geisser correction were applied when Mauchly's test showed statistically significant results, and p-values shown are after these corrections. Normalized frequency-domain indices did not show statistical differences between stages from any of the signals. BM, baseline measurement; CE, cold exposure; CR, cold recovery.*

canal PRV showed the worst agreement in most of the indices during the three stages.

From time-domain indices, pNN50 showed a relatively stable, moderate agreement when measured from all locations except for the finger. SDNN and RMSSD had the lowest agreement when measured from the ear canal and the toe.

Frequency-domain indices obtained using absolute powers (i.e., LF, HF, and TP) showed the worst agreement, reaching BAR's of up to 120%. Once again, earlobe showed the best agreement during the three stages. Relative-power indices had a different behavior: nHF had good agreement from most locations and stages, and moderate agreement was obtained when PRV was measured from the toe; and LF/HF showed a good agreement from every location and during all stages, and only toe-derived measurements showed a diminished agreement during cold exposure.

From Poincaré plot indices, SD2 showed good agreement in every location except for the ear canal; the earlobe, finger and toe measurements showed a good and stable agreement. SD1 showed a bad agreement from the earcanal, especially during the baseline measurement.

Bland-Altman plots showed something similar. Cold exposure affected agreement in most of the cases, but the measurement was not necessarily recovered during cold recovery. SDNN, nHF, and TP from the earlobe and the ear canal tended to recover the agreement faster during cold recovery, than that measured from the finger. However, most of the indices (i.e., nHF, LF/HF, SD1, SD2, and SD1/SD2) showed that agreement was diminished during cold exposure, but did not recover during the first 2 min of measurement during cold recovery. From these plots, it can be seen that all indices showed an overestimation of the measurement when obtained from PRV, except for LF/HF that tends to be underestimated. Over- and underestimation tend to be larger during cold exposure stage, and toe-derived PRV indices were strongly affected by under- and overestimation.

## 4. DISCUSSION

The main aims of this study were to evaluate if PRV, understood as the time difference in pulse-to-pulse cycles measured from PPG signals, showed any difference between body locations during and after whole-body cold exposure, and if HRV and PRV differed during these thermal changes. The obtained results provide strong evidence for the primary hypotheses regarding the differences between HRV and PRV: Results indicate that cold exposure may affect PRV in different ways when obtained from peripheral and core vasculature, and that PRV may contain different information that is not available in HRV. Although HRV and PRV showed a similar trend during the whole-body cold exposure test, it was evident that PRV overestimated the indices obtained from HRV, usually in a larger scale during the cold exposure. Also, HRV and PRV should not be regarded as the same when different temperature conditions are studied, and PRV may contain different information not available from HRV, although further studies are needed to better understand the contribution of SNS to PRV measurements. These results are further discussed in the following sections.

### 4.1. Effects of Cold Exposure in Peripheral and Core Vasculature

The sympathetic control of the ANS over cutaneous blood vessels is thought to act differently over peripheral and core vasculature during cold exposure, probably caused by modifications of cutaneous blood flow to changes in temperature, which are


**FIGURE 3 |** Behavior of indices measured from HRV (blue line) and PRV from the finger (orange line), toe (gray line), ear canal (yellow line), and earlobe (green line). BM: Baseline measurement; CE (1): Cold exposure between the start of the stage and the second minute of this stage; CE (2): Cold exposure between the 4th and 6th min of this stage; CE (3): Cold exposure between the 8th and 10th min of this stage; CR (1): Cold recovery between the start of the stage and the second minute of this stage; CE (2): Cold recovery between the 4th and 6th min of this stage; CE (3): Cold recovery between the 8th and 10th min of this stage.

intended to maintain thermoregulation and homeostasis (Fox, 2016). The sympathetic nervous system generates vasoconstriction in the cutaneous vessels when the temperature is low, producing a decrease in the cutaneous blood flow, which reduces the rate at which the body losses heat, and the amount of blood that is traveling to peripheral tissues such as the fingertips, the palms of the hands, the toes, and the nose, among others.

Budidha and Kyriacou (2019) and Alian et al. (2011a,b) have reported differences in core and peripheral vasculature response to cardiovascular changes. The former showed that PPG amplitude was differently affected by whole-body cold exposure when PPG was measured from the finger (peripheral tissue) and the earlobe and ear canal (core tissue), and concluded that ANS regulation is highly affected in peripheral tissue, whereas core vasculature remains almost untouched, indicating a prevalence of the body to maintain the conditions in vital organs at the expense of peripheral circulation (Budidha and Kyriacou, 2019). Alian et al. demonstrated that, when low-body negative pressure (LBNP) was used as a model of hemorrhage, both time- (Alian et al., 2011a) and frequency-domain parameters (Alian et al., 2011b) measured from the variability of PPG amplitude from the earlobe (core vasculature) and the finger (peripheral vasculature) showed different behavior after LBNP, and that peripheral vasculature showed larger changes that were not significant from core tissue, probably due to greater changes in vasoconstriction in peripheral tissue controlled by sympathetic activity.

In this study, it was observed that most PRV- and HRV-derived indices increased during cold exposure when measured from any of the locations. However, certain differences were observed. Remarkably, ear canal indices did not show a

**TABLE 4 |** *P*-values obtained from the Friedman rank sum test and the multiple comparison tests performed between HRV and PRV from each location (F, Finger; T, Toe; EC, Ear canal; EL, Earlobe), during each stage (BM, baseline measurement; CE, cold exposure; CR, cold recovery) and with each index.

| Stage | Index | Friedman test | PRV vs. HRV (Nemenyi's test) | | | |
|---|---|---|---|---|---|---|
| | | | F | T | EC | EL |
| BM | SDNN (s) | <0.001 | 0.028 | 0.001 | <0.001 | 0.067 |
| | RMSSD (s) | <0.001 | 0.001 | <0.001 | <0.001 | 0.005 |
| | pNN50 | <0.001 | 0.488 | <0.001 | <0.001 | 0.429 |
| | LF ($s^2$) | <0.001 | 1.000 | 1.000 | 0.610 | 1.000 |
| | HF ($s^2$) | <0.001 | 0.270 | 0.007 | 0.002 | 0.488 |
| | TP ($s^2$) | <0.001 | 0.940 | 0.302 | 0.143 | 0.992 |
| | nLF (n.u.) | 0.527 | – | – | – | – |
| | nHF (n.u.) | <0.001 | 0.013 | 0.001 | 0.007 | 0.016 |
| | LF/HF | 0.107 | – | – | – | – |
| | SD1 (s) | <0.001 | 0.001 | <0.001 | <0.001 | 0.005 |
| | SD2 (s) | <0.001 | 0.954 | 0.650 | 0.372 | 1.000 |
| | SD1/SD2 | <0.001 | 0.001 | <0.001 | <0.001 | 0.007 |
| CE | SDNN (s) | <0.001 | 0.988 | <0.001 | <0.001 | 0.529 |
| | RMSSD (s) | <0.001 | 0.302 | <0.001 | <0.001 | 0.028 |
| | pNN50 | <0.001 | 1.000 | <0.001 | 0.061 | 0.429 |
| | LF ($s^2$) | <0.001 | 0.988 | 0.092 | 0.019 | 1.000 |
| | HF ($s^2$) | <0.001 | 0.954 | <0.001 | <0.001 | 0.569 |
| | TP ($s^2$) | <0.001 | 0.999 | <0.001 | <0.001 | 0.923 |
| | nLF (n.u.) | 0.558 | – | – | – | – |
| | nHF (n.u.) | 0.026 | 0.997 | 0.107 | 0.650 | 0.213 |
| | LF/HF | 0.431 | – | – | – | – |
| | SD1 (s) | <0.001 | 0.302 | <0.001 | <0.001 | 0.028 |
| | SD2 (s) | <0.001 | 0.988 | 0.005 | 0.014 | 0.960 |
| | SD1/SD2 | <0.001 | 0.270 | <0.001 | <0.001 | 0.013 |
| CR | SDNN (s) | <0.001 | 0.372 | <0.001 | <0.001 | 0.336 |
| | RMSSD (s) | <0.001 | 0.164 | <0.001 | <0.001 | 0.092 |
| | pNN50 | <0.001 | 0.762 | 0.001 | 0.003 | 1.000 |
| | LF ($s^2$) | <0.001 | 0.992 | 0.001 | 0.040 | 0.975 |
| | HF ($s^2$) | <0.001 | 0.610 | <0.001 | <0.001 | 0.448 |
| | TP ($s^2$) | <0.001 | 0.940 | <0.001 | 0.008 | 0.855 |
| | nLF (n.u.) | 0.387 | – | – | – | – |
| | nHF (n.u.) | 0.160 | – | – | – | – |
| | LF/HF | 0.390 | – | – | – | – |
| | SD1 (s) | <0.001 | 0.164 | <0.001 | <0.001 | 0.092 |
| | SD2 (s) | 0.001 | 1.000 | 0.143 | 0.187 | 0.827 |
| | SD1/SD2 | <0.001 | 0.057 | <0.001 | <0.001 | 0.107 |

*Values in red indicate statistically significant differences (p-value < 0.05).*

statistically significant difference due to cold exposure when any of the indices were compared among stages, while most of the other locations showed differences between baseline measurement and cold exposure, as well as between baseline measurement and cold recovery. This behavior observed from the ear canal could be a hint of the differences on vascular regulation that is performed by the ANS when the body is exposed to temperature differences (Fox, 2016). Interestingly, HRV failed to show any difference among stages when LF, HF, and TP were measured, while most of the PRV-derived indices showed differences between baseline measurement and the subsequent stages. Nonetheless, these results need to be considered with care due to the short segments used for analysis, that may affect the results obtained from frequency-domain analysis (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

Although the same trend was observed between data obtained from HRV and most PRV locations during the test, it is remarkable how over- and under-estimation are a constant factor in PRV analysis. Moreover, it tended to increase during cold exposure, and was higher when PRV was measured from the toe and the ear canal. These two locations could be considered as the most peripheral and the most core vasculature

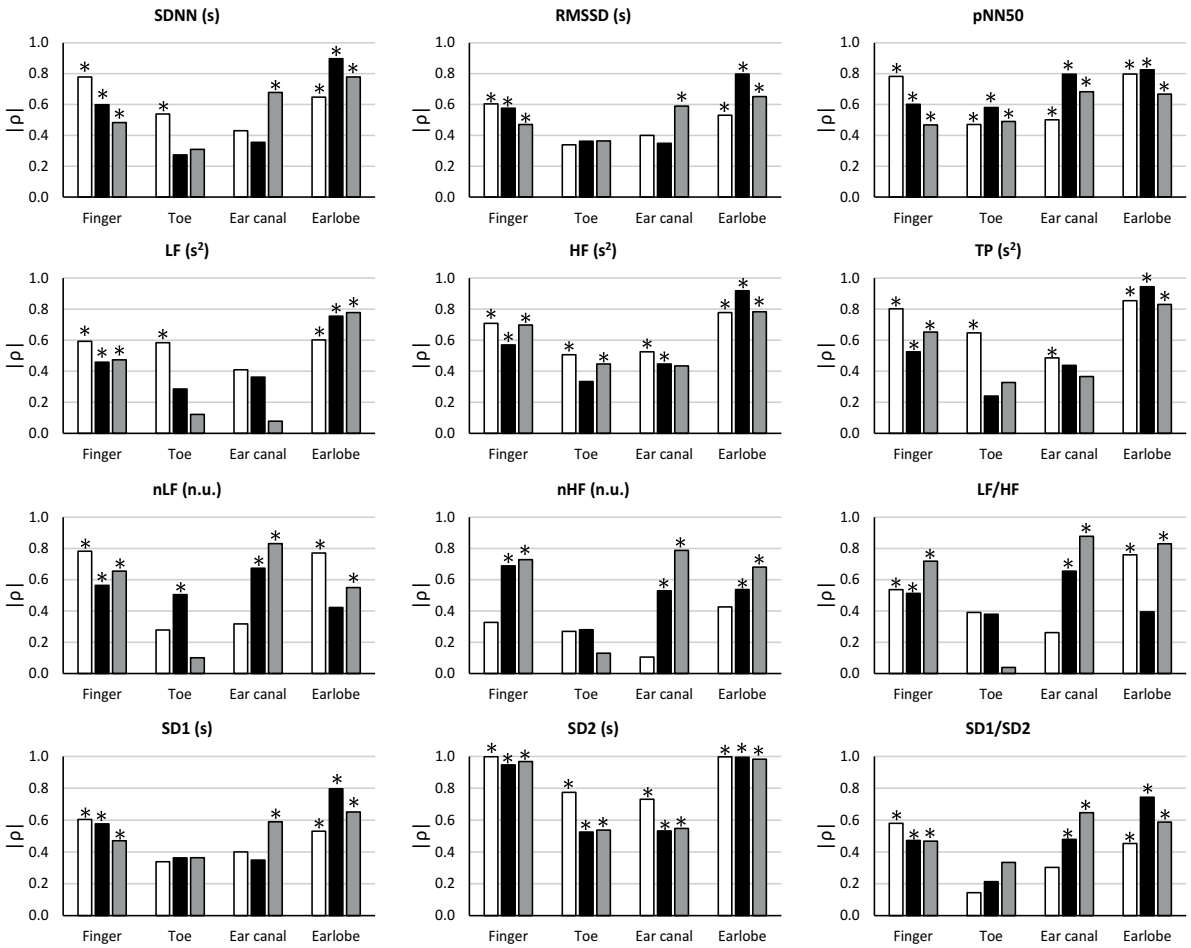



**FIGURE 4 |** Correlation coefficients ($\rho$) between HRV and PRV from each location during each stage (BM: baseline measurement, white bars; CE: cold exposure, black bars; CR: cold recovery, gray bars) and with each index. Stars over bars indicate statistically significant correlations ($p$-value $< 0.05$).

of the four locations used in this study, respectively, and it should be further analyzed how this differences may be influenced by ANS activity in these sites. It is also interesting to observe how the values measured during baseline were achieved from almost all locations after 10 min of recovery from the cold recovery, but how they were affected almost immediately at the beginning of the cold exposure. This could be considered as an example of the behavior of ANS regulation performed over the cardiovascular system during thermal changes.

It is important to remark that only the fiducial points that proved to detect cardiac cycles in a strongly reliable way were used for each subject and each PPG signal, and PRV data was obtained after important pre-processing stages applied to the PPG signals in order to improve their signal-to-noise ratio. Hence, these results can be considered as a strong indication of the differences between PRV and HRV when cardiovascular conditions are modified.

## 4.2. Relationship Between PRV and HRV During Whole-Body Cold Exposure

It was hypothesized that cold exposure affected the relationship between HRV and PRV, implying that PRV may not be a suitable surrogate of HRV under conditions that alter the vasculature and that it may contain different information due to cardiovascular changes.

From the Friedman rank sum test, it was observed that there were statistically significant differences between HRV and PRV, when the latter was measured from different body sites. However, the relationship between PRV and HRV changed during each stage, and from each location. Interestingly, and in line with the results obtained from the other analyses, toe and ear canal PRV consistently showed statistically significant differences to HRV. Also, frequency-domain indices, especially nLF and LF/HF, were not found different between HRV and PRV. This was probably due to the short time of analysis.

In general, the finger and the earlobe were the locations in which less differences were observed, and during all

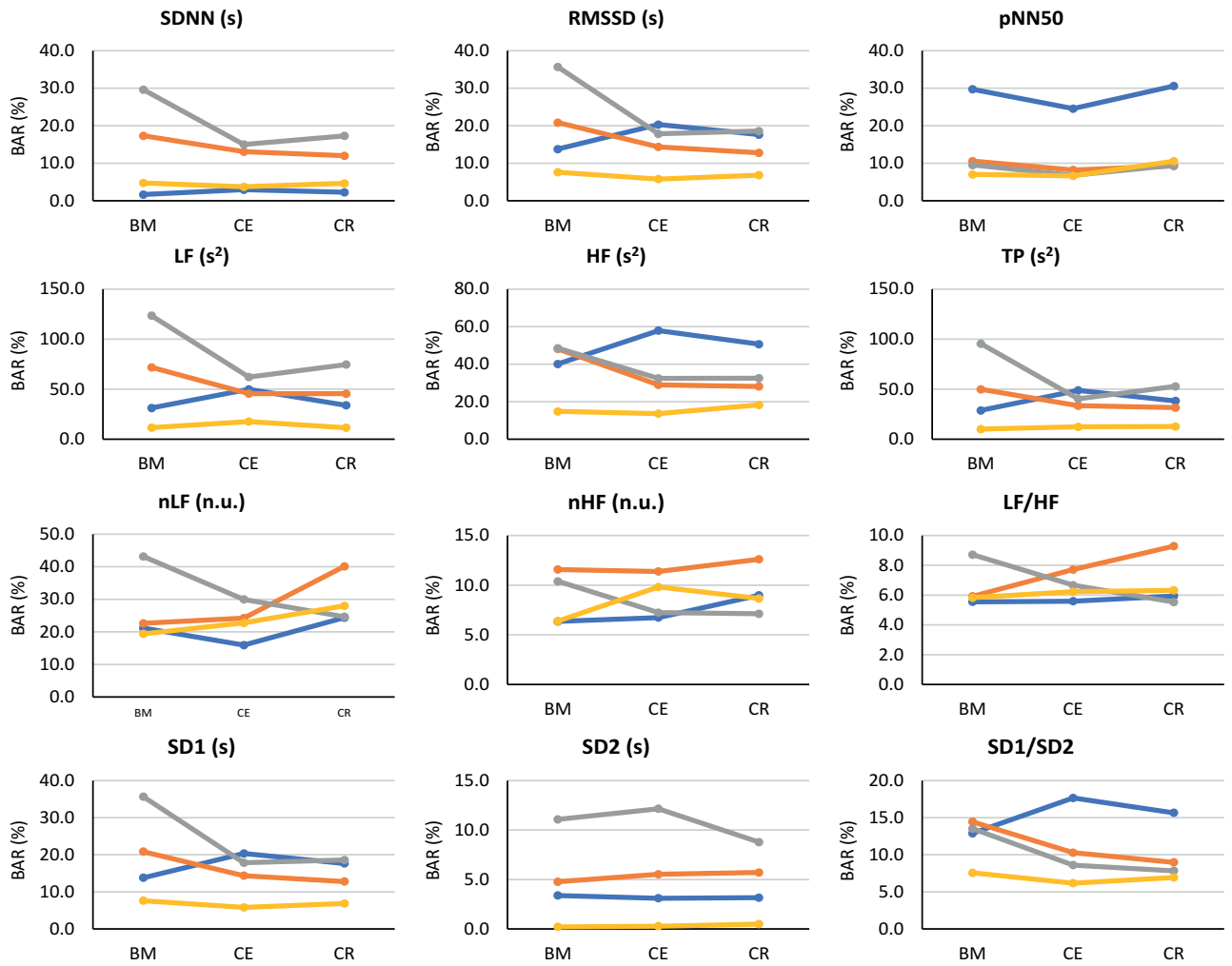



**FIGURE 5 |** Bland-Altman ratios (BAR's) between HRV and PRV from each location (finger: blue line; toe: orange line; ear canal: gray line; earlobe: yellow line), during each stage (BM: baseline measurement; CE: cold exposure; CR: cold recovery) and with each index. Agreements were considered as good (BAR $\leq$ 10%), moderate (10% $\leq$ BAR $\leq$ 20%), or insufficient (BAR $\geq$ 20%).

stages the earlobe proved to be the body site in which the relationship between HRV and PRV was less affected by the changes in temperature. Especially during the baseline measurement, it was observed that HRV and PRV differed especially when RMSSD, nHF, SD1, and SD1/SD2 parameters were measured. All these parameters, except for SD1/SD2, reflect the short-term HRV and PRV. Hence, PRV and HRV tend to differ more in short-term indices. In this same line, some indices showed no difference among locations. These parameters, which include LF, TP, nLF, LF/HF, and SD2, are expected to be a measurement of long-term variability (Khandoker et al., 2013). Hence, the lack of differences may be explained by the short measurement and due to the changes that are induced with short exposure to cold temperatures, that may not be reflected in long-term variability changes.

SD1, RMSSD, and HF reflect parasympathetic activity in HRV (Shaffer and Ginsberg, 2017), which usually leads to diminished heart rate and lowered force of atrial contraction, among other effects (Drew and Sinoway, 2012). In vessels, most of the ANS activity is controlled by the sympathetic nervous system, which is in charge of vasoconstriction and vasodilation in response to environmental changes (Lombard and Cowley, 2012). In this study, it was observed that a diminished temperature induced a higher similarity between these indices from HRV and PRV in different body sites, which might be explained by a lower parasympathetic activity and an increased sympathetic activity. It is not clear how sympathetic and parasympathetic changes may be affecting PRV-derived indices, and it might be possible that PRV may be affected by these changes in a different manner when compared to HRV, which is mainly a reflection of vagal activity (Laborde et al., 2017), and that sympathetic changes in

vascular autonomic activity are observable from PRV indices. SD1/SD2, on the other hand, is supposed to be an index of short-term and long-term changes of ANS activity (Khandoker et al., 2013). Hence, a change in either parasympathetic or sympathetic activity should be reflected in this index, as was observed in the results obtained in this study.

However, further studies are needed to better understand and characterize PRV changes, and to evaluate how sympathetic changes may be affecting PRV-extracted indices. This could be done by using blockade techniques for assessing the contribution of each branch of the ANS to PRV indices, or by comparing PRV results to more specific measurements such as microneurography. To the knowledge of the authors, the only blockade study that has been performed to evaluate changes in PRV was done by Pellegrino et al. (2014). They showed that cardiovagal blockade induced an overestimation of HF measured from PRV; cardiac sympathetic blockade implied a moderate to high agreement between HRV and PRV in time- and frequency-domain indices; and dual blockade implied a poor accuracy and precision for normalized measures and LF/HF indices. Also, non-linear indices obtained from HRV and PRV were largely affected by both sympathetic and parasympathetic blockade. Hence, PRV and HRV can be supposed to act differently under different ANS conditions.

The correlation analysis was performed to further compare HRV and PRV. The main result was the stronger correlations observed from the earlobe and the finger, in all indices, compared to those measured from the ear canal and the toe. SD2 showed an interesting behavior: Significant correlations tended to show a lower correlation coefficient when PRV was measured from all locations during cold exposure; during baseline measurement and cold recovery, the correlation is slightly higher, indicating that the correlation of SD2 from HRV and PRV was more affected during the induced hypothermia response. Several studies have used correlation analysis to assess the relationship between HRV and PRV, some of them finding results similar to those reported in this paper. When PRV and HRV correlation was assessed in subjects at rest, earlobe and finger PRV have a good correlation to HRV indices (Shi et al., 2008; Lu et al., 2009; Bulte et al., 2011; Okkesim et al., 2016); however, certain changes in cardiovascular conditions have been found to alter the correlation between HRV and PRV, including changes due to mental stress (Giardino et al., 2002), changes in the position of the subjects (Lu et al., 2008; Gil et al., 2010), and changes in cardiovascular dynamics (Charlot et al., 2009).

Finally, from the Bland-Altman analysis, SD2, and pNN50, to a lower extent, showed the better agreement between HRV and PRV in all stages and from all body sites. This is in line with the results obtained from the correlation analysis and the Friedman's test results. However, LF/HF showed a good agreement as well, which is not reflected in the other analyses. This could be due to the short recordings which highly affect frequency-domain indices. Interestingly, some of the Bland-Altman plots derived from HRV and PRV data showed a behavior similar to what was hypothesized: The agreement is affected during cold exposure in all locations, but during cold recovery, the agreement tends to recover. Although Shin (2016) does not explain the location from which PPG signals were obtained, these results are in line with those shown by in his study, in which differences in the relationship between PRV and HRV were observed when ambient temperature increased.

Frequency-domain indices reflecting absolute powers, i.e., LF, HF, and TP, showed higher values of BAR's than any other indices, reaching BAR's above 100%. This might be an indication of the effect of short-term recordings on these indices, but further analyses should be performed to better understand how these indices may relate when extracted from PRV and HRV. Also, the toe and ear canal measurements were the ones that showed the higher differences between HRV and PRV, in all three stages. It is hard to conclude regarding the origin of these differences. Regarding the toe measurements, although the quality of the signals was the lowest, it is plausible that the higher differences were due to the measurement site: PRV has been shown to be affected by pulse transit time (PTT) variability (Gil et al., 2010), and the distance between the heart and the toe is larger than the others, implying a longer time for the pulse wave to arrive to the site of measurement and increasing the chances of cardiovascular changes that may affect PTT variability. And regarding the ear canal, these differences in agreement might be explained by the hypothesis that core vasculature is less affected by environmental changes than the other locations. Although HRV is measured directly from the heart, it could be considered as a measurement of the summation of the changes in ANS activity in the cardiovascular system as a whole, whereas the ear canal might be a reflection of more localized changes. Nevertheless, the measurement on this body site is relatively new (Budidha, 2016), and further analyses should be performed.

## 4.3. Limitations of the Study

One of the main limitations for the analysis of PRV under the exposed circumstances is the fact that PPG signals are highly affected by changes in vasculature derived from cold exposure. This represents an increased difficulty for obtaining high quality PPG signals and, therefore, for extracting reliable fiducial points from the signals. To overcome this difficulty, different signal quality indices were extracted from PPG cardiac cycles delimited by several fiducial points, i.e., systolic peaks, diastolic onsets, maximum slope points, and the point of intersection between tangent lines from the diastolic onset and the maximum slope point. It was found that the best quality of cardiac cycles was when cycles were delimited by the intersection point between the tangent lines, whereas the worst quality was obtained from the cardiac cycles delimited by systolic peaks. These results are in line with those obtained by Peng et al. (2015) and Hemon and Phillips (2016). However, the fiducial point selected for each case was different, according to the results of each signal, as recommended in the literature (Pinheiro et al., 2016). With this methodology, the probabilities of having a low-quality PRV time-series was reduced. Also, the IBI's and RRI's were manually corrected, to avoid outliers and mistakes that could affect the results.

It is important to consider as well that the sample size of this study was relatively small, and composed mainly of young and healthy subjects, that do not represent the population as a whole. Finally, a note should be made on the the short segments of signals used for the analyses performed in this study, which might affect the results, especially those obtained from frequency-domain parameters. These short recordings were selected in order to be able to compare the three stages, and to observe the differences along time. Although longer recordings are recommended, several studies have shown that short recordings of less than 10 min can be used reliably for the analysis of time-domain and nonlinear indices from HRV and PRV (Shaffer and Ginsberg, 2017).

## 5. CONCLUSION

From these results, it can be concluded that PRV and HRV should not be regarded as equal under all circumstances, and that hypothermia affects PRV in a different manner, not only when compared to HRV but also when compared among different body sites. PRV generally overestimates HRV indices, especially under cold exposure. Moreover, there seems to be a tendency to maintain the autonomic balance more properly in core vasculature. Although further investigation is needed, the results shown in this study serve as an indication of the effects of changes in vessel characteristics that can be observed in PRV, but are not reflected in HRV, and are promising for future research, which may aim to understand the contribution of parasympathetic and sympathetic activity in the measurement of these indices from PRV. Nonetheless, further research that aims to clarify the contribution of SNS and PNS on PRV, by using methodological considerations such as using blockade studies, are needed to better understand the results obtained in this study.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Senate Research Ethics Committee at City, University of London. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

EM-M, PK, and JM have identified the gaps in the literature and formed a hypothesis for using PRV as a tool for identifying differences in ANS activity in core and peripheral tissue. KB and TA recruited the volunteers, carried out the experiments, and collected the data. EM-M carried out the relevant data analysis as described in the manuscript. All authors have participated in discussing the obtained results and in writing this manuscript.

## ACKNOWLEDGMENTS

The manuscript presented here is entirely original, it has not been copyrighted, published, submitted or accepted for publication elsewhere.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00779/full#supplementary-material

## REFERENCES

Alian, A., Galante, N., Stachenfeld, N., Silverman, D., and Shelley, K. (2011a). Impact of central hypovolemia on photoplethysmographic waveform parameters in healthy volunteers. Part 1: time domain analysis. *J. Clin. Monit. Comput.* 25, 377–385. doi: 10.1007/s10877-011-9316-y

Alian, A., Galante, N., Stachenfeld, N., Silverman, D., and Shelley, K. (2011b). Impact of central hypovolemia on photoplethysmographic waveform parameters in healthy volunteers part 2: frequency domain analysis. *J. Clin. Monit. Comput.* 25, 387–396. doi: 10.1007/s10877-011-9317-x

Allen, J. (2007). Photoplethysmography and its application in clinical physiological measurement. *Physiol. Meas.* 28, R1–R39. doi: 10.1088/0967-3334/28/3/R01

Bassett, D. (2015). A literature review of heart rate variability in depressive and bipolar disorders. *Austral. N. Z. J. Psychiatry* 50, 1–9. doi: 10.1177/0004867415622689

Billman, G. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26. doi: 10.3389/fphys.2013.00026

Bland, J., and Altman, D. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1, 307–310. doi: 10.1016/S0140-6736(86)90837-8

Bolea, J., Làzaro, J., Gil, E., Rovira, E., Remartínez, J., Laguna, P., et al. (2017). Pulse rate and transit time analysis to predict hypotension events after spinal anesthesia during programmed cesarean labor. *Ann. Biomed. Eng.* 45, 2253–2263. doi: 10.1007/s10439-017-1864-y

Broucqsault-Dédrie, C., Jonckheere, J. D., Jeanne, M., and Nseir, S. (2016). Measurement of heart rate variability to assess pain in sedated critically ill patients: a prospective observational study. *PLoS ONE* 11:e0147720. doi: 10.1371/journal.pone.0147720

Budidha, K. (2016). *In vivo investigations of photoplethysmograms and arterial oxygen saturation from the auditory canal in conditions of compromised peripheral perfusion* (Ph.D. thesis). University of London, London, United Kingdom.

Budidha, K., and Kyriacou, P. A. (2019). Photoplethysmography for quantitative assessment of sympathetic nerve activity (SNA) during cold stress. *Front. Physiol.* 9:1863. doi: 10.3389/fphys.2018.01863

Bulte, C., Keet, S., and Bouwman, R. (2011). Level of agreement between heart rate variability and pulse rate variability in healthy individuals. *Eur. J. Anaesthesiol.* 28, 34–38. doi: 10.1097/EJA.0b013e32834088c4

Calle Uribe, A. (2018). *Sistema de medición ambulatorio para la estimación del tiempo de tránsito de pulso con potencial uso en estudios de hipertensión arterial* (Master's thesis). Universidad EIA, Envigado, Colombia.

Can, Y., Chalabianloo, N., Ekiz, D., and Ersoy, C. (2019). Continuous stress detection using wearable sensors in real life: algorithmic programming contest case study. *Sensors* 19:E1849. doi: 10.3390/s19081849

Castaldo, R., Melillo, P., Bracale, U., Caserta, M., Triassi, M., and Pecchia, L. (2015). Acute mental stress assessment via short term HRV analysis in healthy adults: a systematic review with meta-analysis. *Biomed. Signal Process. Control* 18, 370–377. doi: 10.1016/j.bspc.2015.02.012

Charlot, K., Cornolo, J., Brugniaux, J., Richalet, J., and Pichon, A. (2009). Interchangeability between heart rate and photoplethysmography variabilities during sympathetic stimulations. *Physiol. Meas*. 30, 1357–1369. doi: 10.1088/0967-3334/30/12/005

Chen, X., Huang, Y.-Y., Yun, F., Chen, T.-J., and Li, J. (2015). Effect of changes in sympathovagal balance on the accuracy of heart rate variability obtained from photoplethysmography. *Exp. Ther. Med*. 10, 2311–2318. doi: 10.3892/etm.2015.2784

Choi, K.-H., Kim, J., Kwon, O., Kim, M. J., Ryu, Y. H., and Park, J.-E. (2017). Is heart rate variability (HRV) an adequate tool for evaluating human emotions? A focus on the use of the International Affective Picture System (IAPS). *Psychiatry Res*. 251, 192–196. doi: 10.1016/j.psychres.2017.02.025

Clifford, G., Azuaje, F., and McSharry, P. E. (2006). *Advanced Methods and Tools for ECG Data Analysis*. Norwood, MA: Artech House.

da Silva, A. F., da Costa de Rezende Barbosa, M. P., Vanderlei, F. M., Christofaro, D. D., and Vanderlei, L. M. (2016). Application of heart rate variability in diagnosis and prognosis of individuals with diabetes mellitus: systematic review. *Ann. Noninvasive Electrocardiol*. 21, 223–235. doi: 10.1111/anec.12372

Daanena, H., and Lichtenbeltd, W. V. M. (2016). Human whole body cold adaptation. *Temperature* 3, 104–118. doi: 10.1080/23328940.2015.1135688

Dagdanpurev, S., Sun, G., Shinba, T., Kobayashi, M., Kariya, N., Choimaa, L., et al. (2018). Development and clinical application of a novel autonomic transient response-based screening system for major depressive disorder using a fingertip photoplethysmographic sensor. *Front. Bioeng. Biotechnol*. 6:64. doi: 10.3389/fbioe.2018.00064

Dong, J. (2016). The role of heart rate variability in sports physiology. *Exp. Ther. Med*. 11, 1531–1536. doi: 10.3892/etm.2016.3104

Drew, R., and Sinoway, L. (2012). "Chapter 36: Autonomic control of the heart," in *Primer on the Autonomic Nervous System, 3rd Edn*. eds D. Robertson, P. Low, and R. Polinsky (Elsevier), 177–180. doi: 10.1016/B978-0-12-386525-0.00036-6. Available online at: https://www.sciencedirect.com/book/9780123865250/primer-on-the-autonomic-nervous-system#book-info

Elgendi, M. (2016). Optimal signal quality index for photoplethysmogram signals. *Bioengineering* 3:21. doi: 10.3390/bioengineering3040021

Fox, S. (2016). *Human Physiology*. New York, NY: McGraw Hill.

Garde, A., Hoppenbrouwer, X., Dehkordi, P., Zhou, G., Rollinson, A., Wensley, D., et al. (2019). Pediatric pulse oximetry-based OSA screening at different thresholds of the apnea-hypopnea index with an expression of uncertainty for inconclusive classifications. *Sleep Med*. 60, 45–52. doi: 10.1016/j.sleep.2018.08.027

Gavrilova, E. (2016). Heart rate variability and sports. *Hum. Physiol*. 42, 571–578. doi: 10.1134/S036211971605008X

Georgiou, K., Larentzakis, A., Khamis, N., Alsuhaibani, G., Alaska, Y., and Giallafos, E. (2018). Can wearable devices accurately measure heart rate variability? A systematic review. *Folia Med*. 60, 7–20. doi: 10.2478/folmed-2018-0012

Giardino, N., Lehrer, P., and Edelberg, R. (2002). Comparison of finger plethysmograph to ECG in the measurement of heart rate variability. *Psychophysiology* 39, 246–253. doi: 10.1111/1469-8986.3920246

Gil, E., Orini, M., Bailòn, R., Vergara, J., Mainardi, L., and Laguna, P. (2010). Photoplethysmography pulse rate variability as a surrogate measurement of heart rate variability during non-stationary conditions. *Physiol. Meas*. 31, 1271–1290. doi: 10.1088/0967-3334/31/9/015

Goldstein, D., Bentho, O., Park, M., and Sharabi, Y. (2011). LF power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol*. 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Gordon, C. (2009). "Autonomic nervous system: central thermoregulatory control," in *Encyclopedia of Neuroscience*, ed L. Squire (Oxford: Academic Press), 891–898. doi: 10.1016/B978-008045046-9.00650-1

Greaney, J., Kenney, W., and Alexander, L. (2017). Sympathetic function during whole body cooling is altered in hypertensive adults. *J. Appl. Physiol*. 123, 1617–1624. doi: 10.1152/japplphysiol.00613.2017

Hamilton, P., and Tompkins, W. (1986). Quantitative investigation of QRS detection rules using the MIT/BIH arrythmia database. *IEEE Trans. Biomed. Eng*. 33, 1157–1165. doi: 10.1109/TBME.1986.325695

Hemon, M., and Phillips, J. (2016). Comparison of foot finding methods for deriving instantaneous pulse rates from photoplethysmographic signals. *J. Clin. Monit. Comput*. 30, 157–168. doi: 10.1007/s10877-015-9695-6

Hintsala, H., Kenttä, T., Tulppo, M., Kiviniemi, A., Huikuri, H., Mäntysaari, M., et al. (2014). Cardiac repolarization and autonomic regulation during short-term cold exposure in hypertensive men: an experimental study. *PLoS ONE* 9:e99973. doi: 10.1371/journal.pone.0099973

Huikuri, H., Mäkikallio, T., Airaksinen, K., Mitrani, R., Castellanos, A., and Myerburg, R. (1999). Measurement of heart rate variability: a clinical tool or a research toy? *J. Am. Coll. Cardiol*. 34, 1878–1883. doi: 10.1016/S0735-1097(99)00468-4

Jan, H.-Y., Chen, M.-F., Fu, T.-C., Lin, W.-C., Tsai, C.-L., and Lin, K.-P. (2019). Evaluation of coherence between ECG and PPG derived parameters on heart rate variability and respiration in healthy volunteers with/without controlled breathing. *J. Med. Biol. Eng*. 39, 783–795. doi: 10.1007/s40846-019-00468-9

Karlen, W., Kobayashi, K., Ansermino, J., and Dumont, G. (2012). Photoplethysmogram signal quality estimation using repeated Gaussian filters and cross-correlation. *Physiol. Meas*. 33, 1617–1629. doi: 10.1088/0967-3334/33/10/1617

Khandoker, A., Karmakar, C., Brennan, M., Voss, A., and Palaniswami, M. (2013). *Poincar? Plot Methods for Heart Rate Variability Analysis*. New York, NY: Springer. doi: 10.1007/978-1-4614-7375-6

King, S., Ahuja, K., Wass, J., Shing, C., Adams, M., Davies, J., et al. (2013). Effect of whole-body mild-cold exposure on arterial stiffness and central haemodynamics: a randomised, cross-over trial in healthy men and women. *Eur. J. Appl. Physiol*. 113, 1257–1269. doi: 10.1007/s00421-012-2543-1

Koenig, J., Kemp, A., Beauchaine, T., Thayer, J., and Kaess, M. (2016). Depression and resting state heart rate variability in children and adolescents - a systematic review and metaanalysis. *Clin. Psychol. Rev*. 46, 136–150. doi: 10.1016/j.cpr.2016.04.013

Kyriacou, P. (2006). Pulse oximetry in the oesophagus. *Physiol. Meas*. 27, R1–R35. doi: 10.1088/0967-3334/27/1/R01

Laborde, S., Mosley, E., and Thayer, J. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Physiol*. 8:213. doi: 10.3389/fpsyg.2017.00213

Lan, K.-C., Raknim, P., Kao, W.-F., and Huang, J.-H. (2018). Toward hypertension prediction based on PPG-derived HRV signals: a feasibility study. *J. Med. Syst*. 42:103. doi: 10.1007/s10916-018-0942-5

Lázaro, J., Gil, E., Vergara, J., and Laguna, P. (2014). Pulse rate variability analysis for discrimination of sleep-apnea-related decreases in the amplitude fluctuations of pulse photoplethysmographic signal in children. *IEEE J. Biomed. Heal. Informatics* 18, 240–246. doi: 10.1109/JBHI.2013.2267096

Li, B., Dong, M., and Vai, M. (2010). On an automatic delineator for arterial blood pressure waveforms. *Biomed. Signal Process Control* 5, 76–81. doi: 10.1016/j.bspc.2009.06.002

Li, Q., and Clifford, G. (2012). Dynamic time warping and machine learning for signal quality assessment of pulsatile signals. *Physiol. Meas*. 33, 1491–1501. doi: 10.1088/0967-3334/33/9/1491

Lin, W.-H., Wu, D., Li, C., Zhang, H., and Zhang, Y.-T. (2014). "Comparison of heart rate variability from PPG with that from ECG," in *The International Conference on Health Informatics, IFMBE Proceedings*, ed Y. T. Zhang (Cham: Springer), 213–215. doi: 10.1007/978-3-319-03005-0_54

Lombard, J., and Cowley, A. (2012). "Chapter 38: Neural control of blood vessels," in *Primer on the Autonomic Nervous System, 3rd Edn*, eds D. Robertson, P. Low, and R. Polinsky (Elsevier), 187–191. doi: 10.1016/B978-0-12-386525-0.00038-X. Available online at: https://www.sciencedirect.com/book/9780123865250/primer-on-the-autonomic-nervous-system#book-info

Lu, G., Yang, F., Taylor, J., and Stein, J. (2009). A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J. Med. Eng. Technol*. 33, 634–641. doi: 10.3109/03091900903150998

Lu, S., Zhao, H., Ju, K., Shin, K., Lee, M., Shelley, K., et al. (2008). Can photoplethysmography variability serve as an alternative approach to obtain heart rate variability information? *J. Clin. Monit. Comput*. 22, 23–29. doi: 10.1007/s10877-007-9103-y

Mäkinen, T., Mäntysaari, M., Pääkkönen, T., Jokelainen, J., Palinkas, L., Hassi, J., et al. (2008). Autonomic nervous function during whole-body cold exposure before and after cold acclimation. *Aviat. Space Environ. Med.* 79, 875–882. doi: 10.3357/ASEM.2235.2008

Malliani, A., Pagani, M., Lombardi, F., and Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84, 482–492. doi: 10.1161/01.CIR.84.2.482

Murray, A. (2012). *Examining heart rate variability and alpha-amylase levels in predicting PTSD in combat-experienced marines* (Ph.D. thesis). Alliant International University, Alhambra, CA, United States.

Okamoto-Mizuno, K. (2009). Effects of low ambient temperature on heart rate variability during sleep in humans. *Eur. J. Appl. Physiol.* 105, 191–197. doi: 10.1007/s00421-008-0889-1

Okkesim, S., Celik, G., Yildirim, M., Ilhan, M., Karaman, O., Tasan, E., et al. (2016). Comparison of pulse rate variability and heart rate variability for hypoglycemia syndrome. *Methods Inf. Med.* 55, 250–257. doi: 10.3414/ME15-01-0088

Pan, J., and Tompkins, W. (1985). A real-time QRS detection algorithm. *IEEE Trans Biomed Eng.* 32, 230–6. doi: 10.1109/TBME.1985.325532

Pellegrino, P., Schiller, A., and Zucker, I. (2014). Validation of pulse rate variability as a surrogate for heart rate variability in chronically instrumented rabbits. *Am. J. Physiol. Heart Circ. Physiol.* 307, H97–H109. doi: 10.1152/ajpheart.00898.2013

Peng, R.-C., Zhou, X.-L., Lin, W.-H., and Zhang, Y.-T. (2015). Extraction of heart rate variability from smartphone photoplethysmograms. *Comput. Math. Methods Med.* 2015:516826. doi: 10.1155/2015/516826

Pernice, R., Javorka, M., Krohova, J., Czippelova, B., Turianikova, Z., Busacca, A., et al. (2018). Reliability of short-term heart rate variability indexes assessed through photoplethysmography. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2018, 5610–5513. doi: 10.1109/EMBC.2018.8513634

Pinheiro, N., Couceiro, R., Henriques, J., Muehlsteff, J., Quintal, I., Goncalves, L., et al. (2016). Can PPG be used for HRV analysis? *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2016, 2945–2949. doi: 10.1109/EMBC.2016.7591347

Pomeranz, B., Macaulay, R., Caudill, M., Kutz, I., Adam, D., Gordon, D., et al. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *Am. J. Physiol.* 248(1 Pt 2), H151–H153. doi: 10.1152/ajpheart.1985.248.1.H151

Sawasaki, N., Iwase, S., and Mano, T. (2001). Effect of skin sympathetic response to local or systemic cold exposure on thermoregulatory functions in humans. *Auton. Neurosci.* 87, 274–281. doi: 10.1016/S1566-0702(00)00253-8

Schäfer, A., and Vagedes, J. (2013). How accurate is pulse rate variability as an estimate of heart rate variability? A review on studies comparing photoplethysmographic technology with an electrocardiogram. *Int. J. Cardiol.* 166, 15–29. doi: 10.1016/j.ijcard.2012.03.119

Shaffer, F., and Ginsberg, J. (2017). An overview of heart rate variability metrics and norms. *Front. Public Heal.* 5:258. doi: 10.3389/fpubh.2017.00258

Shahrestani, S., Stewart, E., Quintana, D., Hickie, I., and Guastella, A. (2015). Heart rate variability during adolescent and adult social interactions: a meta-analysis. *Biol. Psychol.* 105, 43–50. doi: 10.1016/j.biopsycho.2014.12.012

Shi, P., Hu, S., and Zhu, Y. (2008). A preliminary attempt to understand compatibility of photoplethysmographic pulse rate variability with electrocardiogramic heart rate variability. *J. Med. Biol. Eng.* 28, 173–180.

Shin, H. (2016). Ambient temperature effect on pulse rate variability as an alternative to heart rate variability in young adult. *J. Clin. Monit. Comput.* 30, 939–948. doi: 10.1007/s10877-015-9798-0

Sluyter, J., Hughes, A., Lowe, A. C. C. Jr, and Scragg, R. (2016). Statin utilisation in a real-world setting: a retrospective analysis in relation to arterial and cardiovascular autonomic function. *Pharm. Res. Percept.* 4:e00276. doi: 10.1002/prp2.276

Stuckey, M., Tulppo, M., Kiviniemi, A., and Petrella, R. (2014). Heart rate variability and the metabolic syndrome: a systematic review of the literature. *Diabetes. Metab. Res. Rev.* 30, 784–793. doi: 10.1002/dmrr.2555

Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381.

Xhyheri, B., Manfrini, O., Mazzolini, M., Pizzi, C., and Bugiardini, R. (2012). Heart rate variability today. *Prog. Cardiovasc. Dis.* 55, 321–331. doi: 10.1016/j.pcad.2012.09.001

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 14 August 2020
doi: 10.3389/fneur.2020.00842

# Interictal Heart Rate Variability Analysis Reveals Lateralization of Cardiac Autonomic Control in Temporal Lobe Epilepsy

*Fedele Dono[1†], Giacomo Evangelista[1†], Valerio Frazzini[2,3], Catello Vollono[4*], Claudia Carrarini[1], Mirella Russo[1], Camilla Ferrante[1], Vincenzo Di Stefano[1,5], Luciano P. Marchionno[6], Maria V. De Angelis[6], Massimiliano Faustino[7], Laura Bonanni[1,8], Marco Onofrj[1,8], Stefano L. Sensi[1,8*‡] and Francesca Anzellotti[6‡]*

[1] Department of Neuroscience, Imaging and Clinical Science, "G. D'Annunzio" University of Chieti-Pescara, Chieti, Italy, [2] AP-HP, Epilepsy Unit, Pitié-Salpêtrière Hospital, and Sorbonne University, Paris, France, [3] Brain and Spine Institute (INSERM UMRS1127, CNRS UMR7225, Sorbonne Université), Pitié-Salpêtrière Hospital, Paris, France, [4] Unit of Neurophysiopathology and Sleep Medicine, Department of Geriatrics, Neurosciences and Orthopedics, IRCCS Policlinico Universitario Agostino Gemelli, Catholic University, Rome, Italy, [5] Department of Biomedicine, Neuroscience and Advanced Diagnostic (BIND), University of Palermo, Palermo, Italy, [6] Department of Neurology, "SS Annunziata" Hospital, Chieti, Italy, [7] Department of Cardiology, "SS Annunziata" Hospital, Chieti, Italy, [8] Center for Advanced Studies and Technology - CAST, "G. D'Annunzio" University of Chieti-Pescara, Chieti, Italy

**Purpose:** The temporal lobe, a critical hub for cognition, also plays a central role in the regulation of autonomic cardiovascular functions. Lesions in this area are usually associated with abnormalities in the regulation of heart rate (HR) and blood pressure (BP). The analysis of the heart rate variability (HRV) is useful to evaluate the cardiac parasympathetic nervous system activity. This study aims at comparing HRV changes occurring in two groups of patients suffering from Temporal Lobe Epilepsy (TLE). To that aim, we evaluated patients differentiated by the right or left location of the epileptic foci.

**Materials and Methods:** Fifty-two adult patients with a diagnosis of TLE were enrolled. Each patient underwent a 20-min EEG + EKG recording in resting state. According to the localization of epileptic focus, patients were divided into two subgroups: right TLE (R-TLE) and left TLE (L-TLE). HRV parameters were calculated with a short-lasting analysis of EKG recordings. Time-domain and frequency domain-related, as well as non-linear analysis, parameters, were compared between the two groups.

**Results:** Compared to the R-TLE group, L-TLE subjects showed a significant decrease in low frequency (LF) ($p < 0.01$) and low frequency/high-frequency ratio (LF/HF) ($p < 0.001$) as well as increased HF values ($p < 0.01$), a parameter indicative of the presence of an increased cardiac vagal tone. These results were also confirmed in the subgroup analysis that took into account the seizure types, responses to antiepileptic drugs, seizure frequencies, and etiology.

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Karin Schiecke, Friedrich Schiller University Jena, Germany*
*Emma Mosley, Southampton Solent University, United Kingdom*

***Correspondence:**
*Catello Vollono*
*catello.vollono@policlinicogemelli.it*
*Stefano L. Sensi*
*ssensi@uci.edu*

[†]*These authors have contributed equally to this work*

[‡]*These authors share senior authorship*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neurology*

***Received:*** *02 December 2019*
***Accepted:*** *07 July 2020*
***Published:*** *14 August 2020*

***Citation:***
*Dono F, Evangelista G, Frazzini V, Vollono C, Carrarini C, Russo M, Ferrante C, Di Stefano V, Marchionno LP, De Angelis MV, Faustino M, Bonanni L, Onofrj M, Sensi SL and Anzellotti F (2020) Interictal Heart Rate Variability Analysis Reveals Lateralization of Cardiac Autonomic Control in Temporal Lobe Epilepsy. Front. Neurol. 11:842. doi: 10.3389/fneur.2020.00842*

**Conclusions:** The main finding of the study is that, compared to R-TLE, L-TLE is associated with increased cardiac vagal tone. These results indicate that patients with TLE exhibit a lateralized cardiac autonomic control. L-TLE patients may have a lower risk of developing cardiac dysfunctions and less susceptible to develop Sudden Death for Epilepsy (SUDEP).

**Keywords: temporal lobe epilepsy, interictal epileptic discharges, heart rate variability, autonomic nervous system, cardiovascular risk**

## INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy in adults and accounts for 60% of all epilepsy forms (1). TLE is associated with a large variety of clinical manifestations. A less studied phenomenon associated with TLE and other forms of epilepsy is the simultaneous presence of autonomic imbalance (2–7). However, experimental data indicate that ictal and interictal epileptogenic activity can spread from the temporal lobe and interfere with autonomic functions. Moreover, the temporal lobe plays a central role in the activity of the "Central Autonomic Network" (CAN), a complex system that includes cortical, midbrain, and brainstem regions that are in control of the autonomic cardiovascular functions (8, 9).

Additional areas like the central nucleus of the amygdala (CeA) and some hypothalamic regions are also involved in the CAN (10–16). The activation of these structures contributes to the dysregulation of cardiovascular activity as well as the production of arrhythmic and blood pressure changes that are often observed in TLE patients. The two regions are strongly connected to other cortical regions, like the insular cortex (I.C.), the prefrontal cortex (PFC), and the anterior cingulate cortex (ACC), that are also part of the CAN [(8, 9); **Figure 1**].

Although the mechanisms leading to the development of epilepsy-related autonomic changes are not entirely understood, a current hypothesis postulates that these phenomena result from the progressive alterations, occurring in autonomic centers, that are triggered by repetitive seizure discharges (22). In line with this hypothesis, experimental data indicate that autonomic alterations depend on the pharmacological modulations exerted by specific anti-seizure (ASD) treatments [like carbamazepine (3, 23) and phenytoin (24)] or by the length of the patient epileptic history (25). Furthermore, in drug naïve patients, the autonomic imbalance has been found to be less present in the early stages of the disease but becomes more prominent with the progression of the epileptic process (25). Moreover, chronic TLE can induce structural lesions characterized by the presence of significant neuronal loss and sclerosis in two regions that are involved in the CAN, like the amygdala and the hippocampus (26–28). These structural alterations play a role in the pathophysiology and development of the Sudden Death for Epilepsy (SUDEP) (27–31).

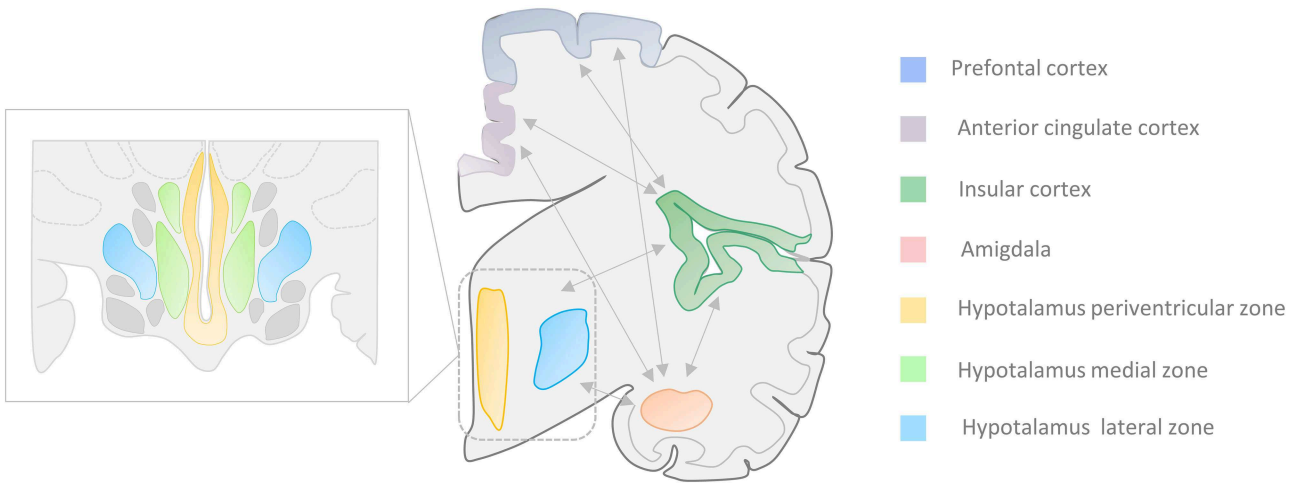



**FIGURE 1 |** Central Autonomic Network (CAN). Autonomic cardiovascular functions (ACF) are regulated by a complex system called "Central Autonomic Network" that encompasses cortical, midbrain, and brainstem areas. Several portions of the temporal lobe, along with the central nucleus of the amygdala (CeA) and the hypothalamic nuclei, are involved in the ACF regulation. The insular and medial prefrontal cortex, as well as the amygdala, are involved in high-order processing of viscerosensory information and the initiation of integrated autonomic responses. These areas are intimately interconnected with each other as well as with the hypothalamus (periventricular and lateral zone), the anterior cingulate cortex, and brainstem regions. Seizures that arise from the amygdala-hippocampal, cingulate, opercular, anterior frontopolar, and orbitofrontal regions can produce autonomic manifestations that include cardiac arrhythmias, viscerosensory phenomena, vomiting, genitourinary symptoms, and sexual arousal (17–21). *Zoom rectangle*: Functional organization of the hippocampus in the periventricular, medial, and lateral zones.

Several studies have suggested the presence of hemispheric lateralization of the autonomic control of the cardiovascular functions. Bradycardic effects have been associated with the stimulation of the left temporal lobe, while tachycardic responses are linked with the right stimulation (32, 33). However, it is still unclear whether transient modifications of the brain activity—like the ones occurring upon interictal epileptic discharges (IED)—influence cardiac functioning in a hemispheric-specific fashion (34). Previous studies have demonstrated that IED affect the occurrence of Heart Rate Variability (HRV) modifications that are observed in focal epilepsy (35). However, little is known about the specific autonomic changes associated with TLE.

The analysis of HRV is a useful tool to evaluate the impairment of cardiac autonomic control (36). The HRV represents the change in the time interval between successive heartbeats (37, 38). HRV provides an index of the parasympathetic nervous system activity (36, 39), whereas possible inferences on sympathetic components have been revised and rejected (20, 36, 40). Some authors (21, 41) have indicated that HRV cannot be employed to infer activities of the sympathetic nervous system as some HRV parameters mostly relate to baroreflex functions. The relationship between HRV and parasympathetic activity has been extensively described (41).

The analysis of HRV has been extensively employed to study changes of the sympathovagal balance that occurs upon physiological responses of healthy subjects as well as in patients affected by cardiac or neurological diseases. It is now well-established that the HRV is reduced in individuals who have epilepsy. The phenomenon is present in newly diagnosed and drug naïve patients (42) and a relevant marker of cardiovascular risks like, for instance, the predisposition to generate ventricular arrhythmias (43–46).

The present study aimed at comparing the interictal changes of HRV that occur in TLE patients differentiated by the right or left location of the epileptic foci. In these two groups, the relationship between interictal epileptiform discharges and modifications of the autonomic cardiac control assessed.

# METHODS

## Patient Demographics and Clinical Features

Fifty-two adult patients (24 men and 28 women, mean age 42.9 ± 16.4 years, age range = 20–73 years) affected by TLE with a clear EEG interictal discharges lateralization (right or left) were retrospectively selected from the database of patients who underwent a 21-channel video-electroencephalogram (video-EEG) recording at the Epilepsy Center of the University "G. D'Annunzio" of Chieti-Pescara and the Epilepsy Center of the Catholic University of the Sacred Heart of Rome. Video-EEGs were recorded using a sampling frequency of 256 Hz. During the recording sessions, patients were supine and relaxed. Two neurologists subspecialized in epilepsy made a qualitative evaluation of EEG recordings, highlighted IED, and their specific localization. All the investigated patients exhibited clear lateralization of EEG interictal discharges. Patients with bilateral IED were excluded from the analysis. No ictal events were recorded.

All patients received a diagnosis of TLE based on clinical (43), neurophysiological, and interictal video-EEG, as well as by brain magnetic resonance (MRI) scans performed to determine the epilepsy etiology. The mean disease duration time was 14.9 ± 15.2 (duration range = 1–61 years). All patients were right-handed.

The group was equally divided into a subset of 26 patients presenting with left temporal IED and 26 patients exhibiting right temporal IED. Patients did not exhibit psychiatric comorbidity as assessed by the Symptom checklist-90 (SCL-90) (47). None of the patients were treated with drugs that interfere with the functioning of the Autonomic Nervous System (ANS), including oral contraceptives. None of the investigated patients had a history of heart diseases, endocrine disorders, metabolic deficits, uremia, or any other known disease that could have affected autonomic functions, including sleep-related apnea. Selected patients were no-smoker and had no history of alcohol or drug abuse. No coffee, tea or other energizing drinks, as well as meals, were ingested in the 2 h before EEG recordings. No intense physical activity was reported in the day before EEG. All patients reported regular sleep routines in the 7 days before the recording. Patient responses to pharmacotherapy were analyzed according to the ILAE diagnostic criteria for pharmaco-resistant epilepsy (48). Patients treated with carbamazepine or phenytoin were excluded from the analysis. The clinical and demographic features of the study cohort are summarized in **Table 1**.

## EKG Samples and Heart Rate Variability (HRV) Analysis

Bipolar electrocardiogram (EKG) recordings from lead I of a 12-lead EKG were carried out utilizing the EKG channel of the EBN-Neuro EEGNet System (EBN Neuro–Florence Italy). According to guidelines for HRV measurement in epileptic patients (34), we took in consideration EKGs only in patients who were recorded (1) at least 8 h after the last tonic-clonic seizure, (2) at least 1 h after the last known clinical, subclinical electroencephalographic seizure, and (3) at least 1 h before the next seizure. We excluded from the analysis patients who presented a respiration rate above 12 cycles/min (0.2 Hz) during the EKG registration to rule out biases due to individual differences in respiration. EKG data were sampled at a frequency of 256 Hz and exported from the EBN system (EEGNET, Florence, Italy) in the European Data Format (EDF). All data were subsequently processed using dedicated software for HRV analysis (Kubios, HRV software version 2.1, University of Eastern Finland, Kuopio, Finland). The software identified QRS complexes and R peaks using a multiscale wavelet-based peak detection algorithm. Before proceeding with the HRV analysis, all the RRI samples were visually inspected by two trained neurologists to remove any artifacts, extrasystoles, and erroneously detected R waves or insertions of missed R beats. The rate of artifacts that were detected and removed was 5% of all RRI in the EKG recordings.

**TABLE 1 |** Demographics and clinical data.

| | Right TLE ($n = 26$) | Left TLE ($n = 26$) | |
|---|---|---|---|
| **Age (years)** | 40.32 ± 14.32 | 45.35 ± 18.13 | $p = 0.301$ |
| **Disease duration (years)** | 16.6 ± 16.072 | 12.91 ± 14.32 | $p = 0.399$ |
| **Seizure types** | | | $p = 0.095$ |
| Focal | 9 (35%) | 15 (56%) | |
| - Focal cognitive seizures | 5 | 11 | |
| - Focal automatism seizures | 4 | 5 | |
| - Focal sensory seizures | 0 | 1 | |
| Focal to bilateral | 17 (65%) | 11 (44%) | |
| **Seizure control** | | | $p = 0.569$ |
| Seizure-free | 15 (64%) | 17 (65%) | |
| No seizure-free | 11 (44%) | 9 (35%) | |
| **Response to ASD** | | | $p = 0.792$ |
| Pharmacoresistance | 5 (20%) | 6 (23%) | |
| Non-pharmacoresistance | 18 (79%) | 18 (69%) | |
| Undefined | 3 (11%) | 2 (8%) | |
| **Etiology** | | | $p = 0.780$ |
| Unknown etiology | 11 (44%) | 12 (46%) | |
| Known etiology | 15 (60%) | 14 (54%) | |
| *Brain tumor* | 3 | 3 | |
| *Cortical malformation* | 7 | 6 | |
| - Post-traumatic | 1 | 3 | |
| - Ischemic Stroke | 2 | 0 | |
| - Vascular malformation | 1 | 1 | |
| - Multiple Sclerosis | 1 | 0 | |
| - Infectious encephalitis | 0 | 1 | |

*Demographics, clinical data, and statistical comparisons performed in the right and left TLE patients.*
*TLE, temporal lobe epilepsy; ASD, anti-seizure drugs.*

A short-term recording analysis (49) (time-series length = 5 min) was performed to assess heart rate variations in time and frequency domains as well as non-linear analysis.

The time-domain methods are derived from the beat-to-beat R.R. interval values in the time domain. HRV parameters that measure the variability within the R.R. time intervals in the time-domain assessed in terms of (1) mean R.R. (the mean heart rate in a precise R.R. sequence); (2) SDNN (standard deviation of all R.R. intervals); (3) RMSSD (root mean square of the difference of adjacent R.R. intervals); (4) pNN50 (the percentage of successive R.R. intervals differing more than 50 ms); (5) HRV triangular index (integral of the density of the R.R. interval histogram divided by its height), and (6) TINN (baseline width of the R.R. interval histogram). According to the current literature (50), short-term analysis of SDNN and RMSSD is the most reliable HRV time-domain parameters. SDNN assesses sympathetic and parasympathetically-mediated HRV variations. It should be pointed out that SDNN appears to be more accurate when calculated over 24 h compared to shorter periods, thereby representing the "gold standard" for the medical stratification of cardiac risk. The RMSSD is the primary time-domain measure used to estimate the vagus-mediated changes of HRV (51). Lower RMSSD values correlate with higher scores on a risk inventory for SUDEP (52).

Frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands. The power spectral density (PSD) of the R.R. series was calculated using parametric methods (based on self-regressive models, AR). PSD was analyzed by calculating the frequency of waves for the different frequency bands. According to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) (36), H.R. oscillations should be analyzed taking in consideration selected frequencies: Very Low Frequency (VLF, 0–0.04 Hz), Low Frequency (LF, 0.04–0.15 Hz), and High Frequency (HF, 0.15–0.4 Hz). The most common frequency domain parameters include the powers of the bands VLF, LF, HF expressed in absolute (VLF [$ms^2$], LF [$ms^2$], and HF [$ms^2$]) and relative values (VLF%, LF%, and HF%), the normalized power of the LF and HF bands (LF n.u. = LF [$ms^2$]/(total power [$ms^2$] – VLF [$ms^2$]; HF n.u. = HF [$ms^2$]/(total power [$ms^2$] – VLF [$ms^2$]), and the LF/HF ratio.

Spectral analysis allows the discrete analysis of different autonomic components. HF band reports the parasympathetic components, whereas the interpretation of the LF band is controversial (21, 53–59). VLF, LF, and HF bands can be expressed in absolute ($ms^2$) or relative (expressed in % or n.u.) units, but absolute values are preferred (59). A recent study (60) investigated the general pattern and timeframe of cardiac autonomic changes that occur upon aging. The study showed that HF as well as LF expressed in absolute units, are decreased by 30–35% with aging. On the contrary, normalized values were affected less (0.8–1.2%).

In line with recent recommendations (17) we present and discuss results of spectral analysis expressed in absolute and normalized units. As recommended by the European Society of Cardiology (ESC) guidelines (36), VLF assessment in a short-term EKG analysis is of dubious value, and its interpretation should be avoided. The LF/HF ratio has been considered as an index of sympathovagal balance. However, this view has been criticized (50). The consensus is now that the precise physiological underpinning of LF/HF is unclear, thereby questioning its predictive value to assess autonomic balance. However, several studies have reported that the index has prognostic value as far as the mortality risk due to cardiovascular or non-cardiovascular causes (61–65).

The non-linear analysis is another alternative way to characterize the variability of heart rate by measuring complex fluctuations of cardiac rhythms. This method allows the definition of the unpredictability of time series resulting from the complexity of the mechanisms that regulate HRV. SD1 and SD2 non-linear parameters can be extrapolated from the Poincaré plot (obtained by plotting every R-R interval against the prior interval and thereby creating a scatter plot). SD1 assesses short-term HRV, correlates with the HF power, and is directly related to RMSSD (66), whereas SD2 investigated short- and long-term HRV and correlates with the LF power. Another useful non-linear parameter is approximate entropy (ApEn), which has been developed to measure the complexity of relatively short time-series. Applied to HRV data, large ApEn values indicate low

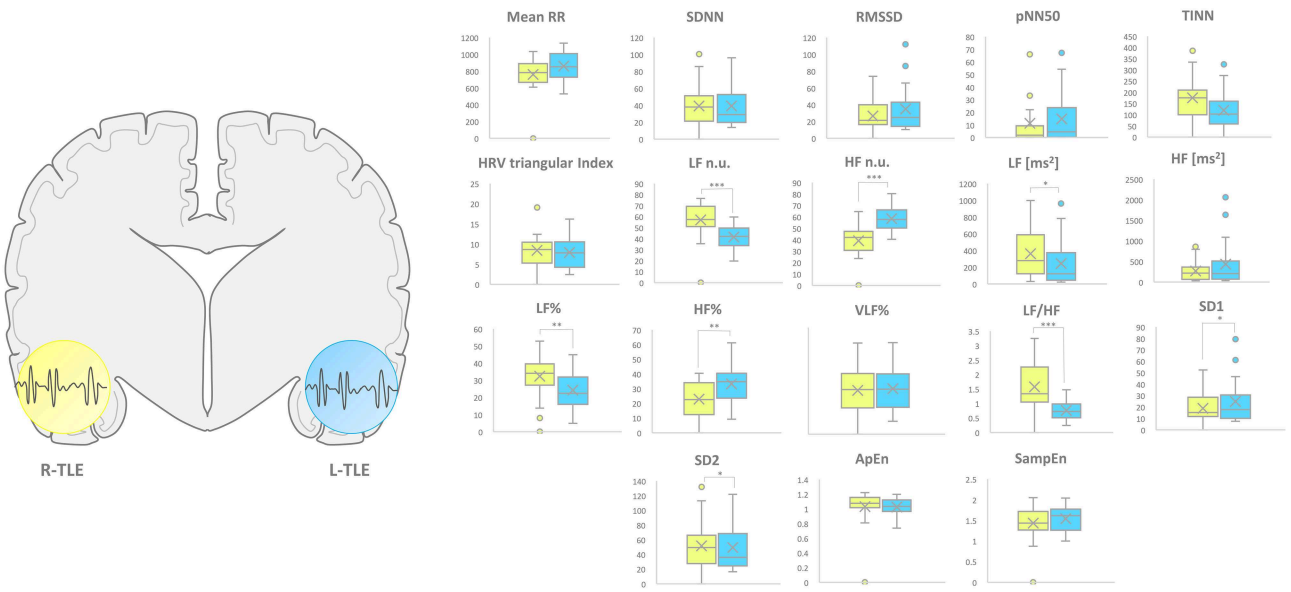



**FIGURE 2 |** HRV-related parameter comparison in patients with R-TLE and L-TLE. Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.01$), HF n.u. ($p < 0.01$), LF [ms$^2$] ($p < 0.05$), LF% ($p < 0.0001$), HF% ($p < 0.001$), and LF/HF ratio ($p < 0.0001$), SD1 ($p < 0.05$) as well as SD2 ($p < 0.05$) in the two study groups. Mann-Whitney test was employed for statistical analysis. $p < 0.05$ was considered statistically significant. *$p < 0.05$; **$p < 0.001$; and ***$p < 0.0001$.

predictability of fluctuations in successive R.R. intervals (67), whereas small ApEn values indicate signals that are regular and predictable (68). A modified version of ApEn is sample entropy (SampEn), used to assess the complexity of physiological time-series signals (69). SampEn values are interpreted and used like ApEn and can be calculated from shorter time-series (<5 min). Other non-linear parameters like detrended fluctuation analysis (DFA) are designed to analyze time series that span over several hours of data; therefore, their significance in short term analysis is not relevant.

## Statistics

Before comparing the two groups (left vs. right TLE), the normality of distribution of all metric data was tested with the Shapiro-Wilk test. Significance was set at $p < 0.05$. The analysis revealed a non-normal distribution of the data. Metric variables (age, disease duration, MeanRR, SDNN, RMSSD, pNN50, R.R. triangular index, TINN, SD1, SD2, ApEN, SampEN, HF, and LF absolute power, HF and LF normalized unit, HF and LF percentage, LF/HF ratio) were compared employing the one-way analysis of variance with the Mann-Whitney *U*-test. Nominal variables (seizure type, seizure control, response to ASD, etiology) were analyzed and compared between left and right TLE with 2 × 2 contingency tables using the chi-square or Fisher's exact test. Correlations between disease duration and HRV frequency parameters were performed using the Sperman correlation test.

The level of significance was set at $p < 0.05$. Statistical analyses were performed using SYSTAT 12 software (SYSTAT® Software Inc., 2007).

# RESULTS

L-TLE and R-TLE groups did not show any significant difference as far as age ($p = 0.301$), disease duration ($p = 0.399$), seizure control ($p = 0.569$), etiology ($p = 0.780$), and seizure types ($p = 0.095$). The two groups also showed no differences in time-domain parameters (MeanRR, SDNN, RMSSD, pNN50, R.R. triangular index, and TINN). The HRV spectral component analysis indicated a significantly decreased LF/HF ratio in the L-TLE patients when compared to the R-TLE individuals ($p < 0.0001$). Compared to the R-TLE group, L-TLE showed significantly increased HF n.u. ($p < 0.0001$) and HF% ($p < 0.0001$) with decreased LF [ms$^2$] ($p < 0.05$), LF n.u. ($p < 0.001$), and LF% ($p < 0.001$). The non-linear analysis showed significantly increased SD1 ($p < 0.01$) and decreased SD2 ($p < 0.01$) in the L-TLE group. No significant differences were observed when comparing ApEN ($p = 0.133$) and SampEN ($p = 0.570$) between the two groups. Time-domain, frequency domain, and non-linear analysis features are summarized in **Figure 2**. No correlations between disease duration and LF, HF, or LF/HF ratio values were observed.

## Subgroups Analysis

We analyzed and compared HRV parameters in the R-TLE and L-TLE groups taking into account (1) the seizure types (focal seizures vs. focal-to-bilateral tonic-clonic seizures); (2) the response to ASDs (pharmacoresistant vs. no pharmacoresistant); (3) seizure frequency (seizure-free vs. non-seizure-free patients), and (4) the etiology (unknown etiology vs. epilepsy of known etiology).

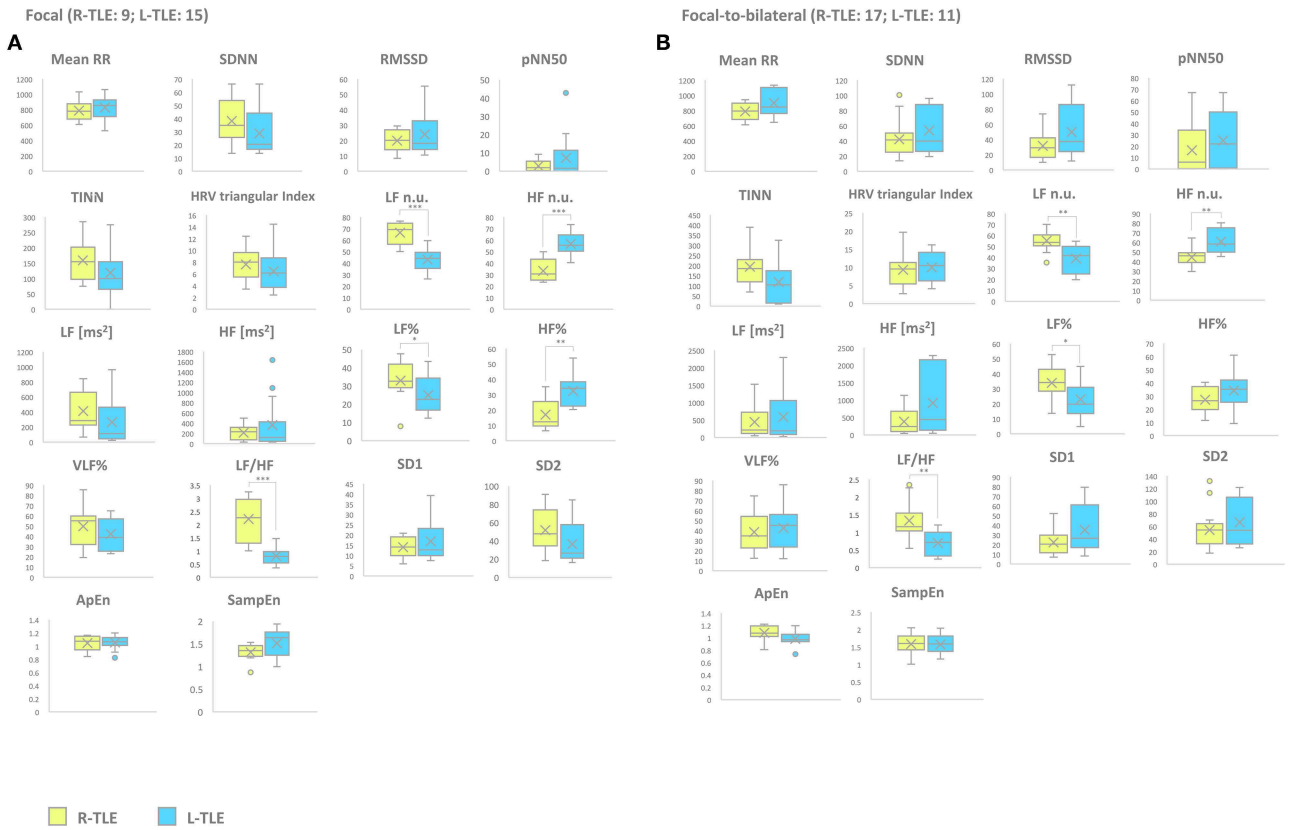



**FIGURE 3 |** HRV-related parameter comparison in patients with focal or focal-to-bilateral seizures. **(A)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.0001$), HF n.u. ($p < 0.0001$), LF% ($p < 0.01$), HF% ($p < 0.001$), and LF/HF ratio ($p < 0.0001$) of the two study groups of patients suffering from focal seizures. **(B)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.001$), HF n.u. ($p < 0.001$), LF% ($p < 0.01$), and LF/HF ratio ($p < 0.001$) of the two study groups of patients suffering from focal-to-bilateral seizures. Mann-Whitney test was employed for statistical analysis. $p < 0.05$ was considered statistically significant. *$p < 0.05$; **$p < 0.001$; and ***$p < 0.0001$.

## Seizure Type (Focal Seizures vs. Focal-to-bilateral Tonic-Clonic Seizures)

Patients with focal seizures in the L-TLE group showed significantly increased HF n.u. ($p < 0.0001$) and HF% ($p < 0.01$) as well as decreased LF n.u. ($p < 0.0001$), LF% ($p < 0.01$), and LF/HF ratio ($p < 0.001$) when compared to homologous R-TLE patients. Patients with focal-to-bilateral tonic-clonic seizures in the L-TLE group showed significantly increased HF n.u. ($p < 0.001$), decreased LF n.u. ($p < 0.001$), LF % ($p < 0.01$), and LF/HF ratio ($p < 0.001$) compared to homologous R-TLE patients. No statistically significant differences were observed between the two groups as far as time-domain parameters (MeanRR, SDNN, RMSSD, pNN50, R.R. triangular index, and TINN) and non-linear analysis (SD1, SD2, ApEn, and SampEn). Time-domain, frequency domain, and non-linear analysis features of the subgroups are summarized in **Figure 3**.

## Response to ASDs (Pharmacoresistant vs. Non-pharmacoresistant)

Patients with pharmacoresistant epilepsy in the L-TLE group showed significantly increased HF n.u. ($p < 0.001$), HF% ($p < 0.01$) as well as decreased LF n.u. ($p < 0.001$), and LF/HF ratio ($p < 0.001$) compared to homologous R-TLE patients. Patients with non-pharmacoresistant epilepsy in the L-TLE group showed significantly increased HF n.u. ($p < 0.0001$), HF% ($p < 0.01$), decreased LF n.u. ($p < 0.0001$), LF% ($p < 0.01$), and LF/HF ratio ($p < 0.0001$) when compared to matched R-TLE patients. No statistically significant differences were observed between the two subgroups as far as time-domain parameters (MeanRR, SDNN, RMSSD, pNN50, R.R. triangular index, and TINN) and non-linear analysis (SD1, SD2, ApEn, and SampEn). Time-domain, frequency domain, and non-linear analysis features are summarized in **Figure 4**.

## Seizure Frequency (Seizure-Free vs. No Seizure-Free Patients)

Seizure-free patients in the L-TLE group showed significantly increased HF n.u. ($p < 0.0001$), decreased LF n.u. ($p < 0.0001$), LF% ($p < 0.01$), and LF/HF ratio ($p < 0.0001$) compared to homologous R-TLE patients. No seizure-free patients in the L-TLE group showed significantly increased HF n.u. ($p < 0.001$), HF% ($p < 0.001$), decreased LF n.u. ($p < 0.001$), and LF/HF ratio ($p < 0.001$) when compared to homologous R-TLE patients. No statistically significant differences were observed between

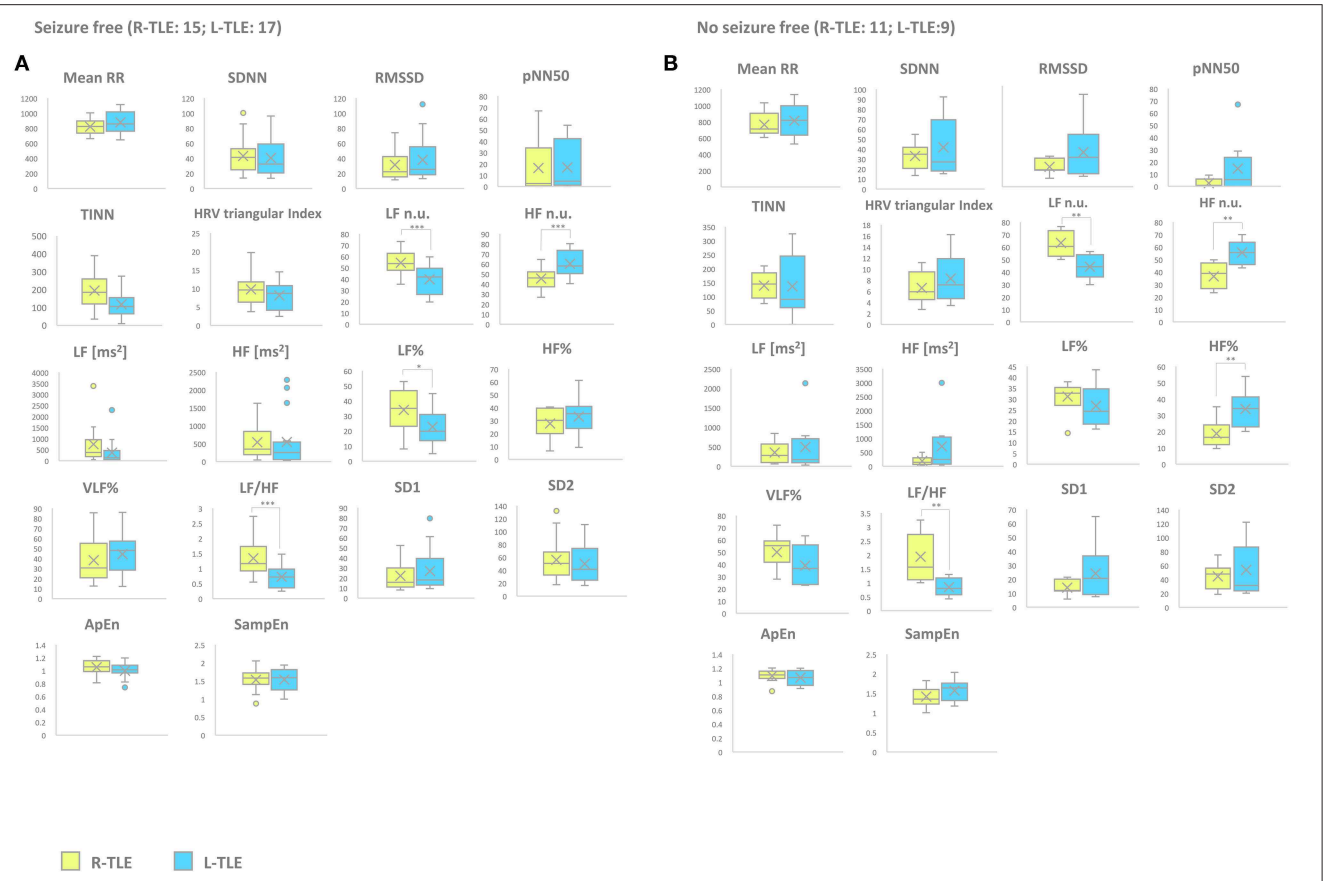



**FIGURE 4 |** HRV-related parameter comparison in *seizure-free* or *no seizure-free* patients. **(A)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.0001$), HF n.u. ($p < 0.0001$), and LF/HF ratio ($p < 0.0001$) of *seizure-free* TLE patients. **(B)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.001$), HF n.u. ($p < 0.001$), HF% ($p < 0.001$), and LF/HF ratio ($p < 0.001$) *of no seizure-free* TLE patients. Mann-Whitney test was employed for statistical analysis. $p < 0.05$ was considered statistically significant. *$p < 0.05$; **$p < 0.001$; and ***$p < 0.0001$.

the two subgroups as far as time-domain parameters (MeanRR, SDNN, RMSSD, pNN50, R.R. triangular index, and TINN) and non-linear analysis (SD1, SD2, ApEn, and SampEn). Time-domain, frequency domain, and non-linear analysis features are summarized in **Figure 5**.

### Etiology (Unknown Etiology vs. Known Etiology)

Patients with epilepsy with unknown etiology in the L-TLE group showed significantly increased HF. n.u. ($p < 0.001$), decreased LF n.u. ($p < 0.001$), LF% ($p < 0.01$), and LF/HF ratio ($p < 0.001$) when compared to matched R-TLE patients. Patients with epilepsy with known etiology in the L-TLE group showed significantly increased HF n.u. ($p < 0.0001$) and HF% ($p < 0.01$), decreased LF n.u. ($p < 0.0001$), LF% ($p < 0.01$), and LF/HF ratio ($p < 0.0001$) when compared to homologous R-TLE patients. No statistically significant differences were observed in the two subgroups as far as time-domain parameters (MeanRR, SDNN, RMSSD, pNN50, R.R. triangular index, and TINN) and non-linear analysis (SD1, SD2, ApEn, and SampEn). Time-domain, frequency domain, and non-linear analysis features are summarized in **Figure 6**.

## DISCUSSION

The present study aimed at comparing the interictal changes of HRV in TLE patients who were differentiated by the right or left location of the epileptic foci. R-TLE and L-TLE subjects were investigated to assess the relationship between interictal epileptiform discharges and modifications of the autonomic cardiac control. Our data show that R-TLE patients exhibit a reduced parasympathetic tone, as indicated by the presence of HF reductions. These patients also exhibit a higher risk of mortality as assessed by the LF/HF parameter.

Autonomic dysregulation and HRV variations are important cardiovascular risk factors as these alterations underlie a potentially harmful decrease of the parasympathetic tone.

The reduced parasympathetic tone may predispose to a "pro-arrhythmic" condition (44, 46, 70), which, in turn, may lead to an increased risk of SUDEP. The quantification of HRV is an established method to assess the parasympathetic activity of

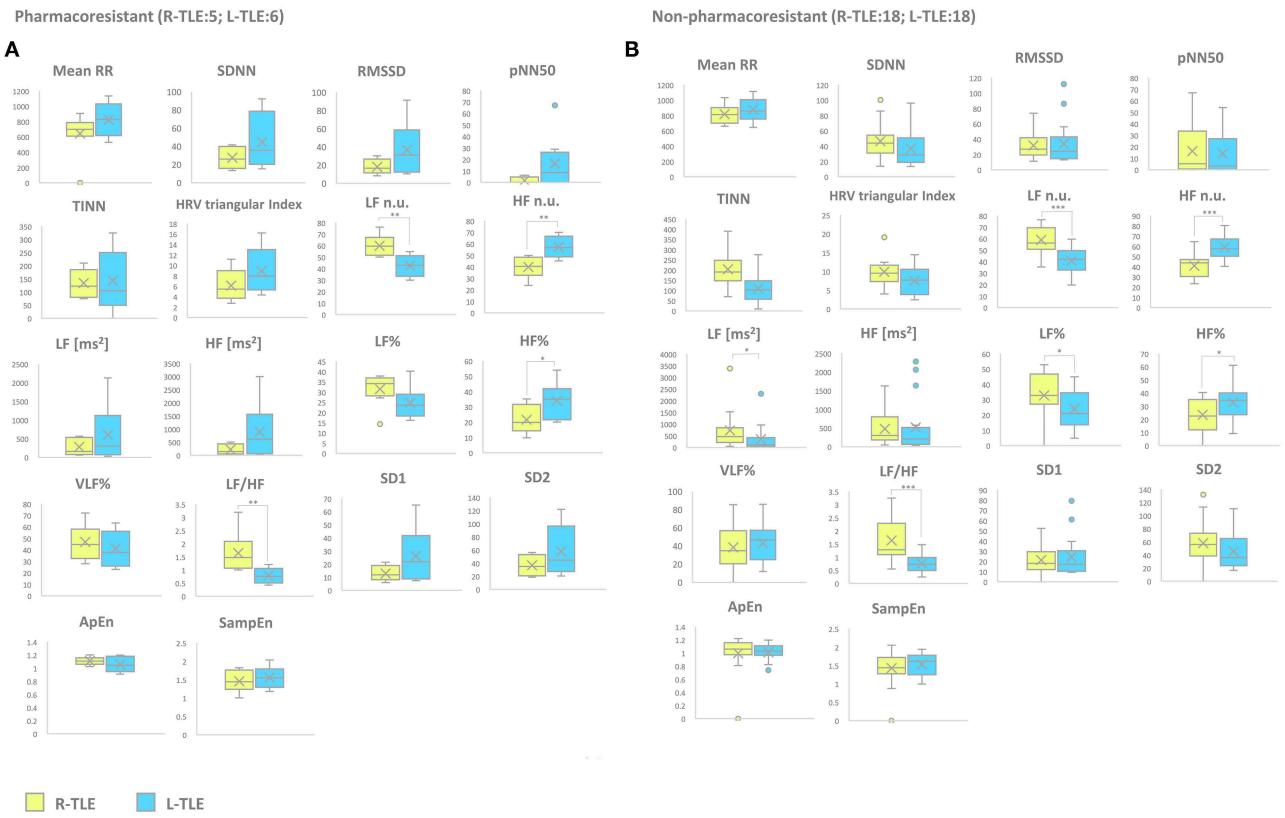



**FIGURE 5 |** HRV-related parameter comparison in patients with pharmacoresistant or non-pharmacoresistant epilepsy. **(A)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.001$), HF n.u. ($p < 0.001$), HF% ($p < 0.05$), and LF/HF ratio ($p < 0.001$) of pharmacoresistant TLE patients. **(B)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.0001$), HF n.u. ($p < 0.0001$), LF [ms$^2$] ($p < 0.05$), LF% ($p < 0.01$), HF% ($p < 0.01$), and LF/HF ratio ($p < 0.001$) of non-pharmacoresistant TLE patients. Mann-Whitney test was employed for statistical analysis. $p < 0.05$ was considered statistically significant. *$p < 0.05$; **$p < 0.001$; and ***$p < 0.0001$.

the ANS. The standard recording period of ≤5 min (short-term analysis) is now recommended by the Taskforce of the European Society of Cardiology as a valid procedure for the assessment of HRV components (36).

Clinical data concerning the possibility of a lateralized control of autonomic functions in epileptic patients are controversial. Although HRV features associated with ictal events have been extensively described (70–72), little is known about the role of IED changes in the cardiac autonomic regulation of patients with epilepsy. By definition, interictal epileptic discharges are brief spikes or sharp waves that are not associated with clinical symptoms. Despite their brief nature, IED play an essential role in the development of some epilepsy-related comorbidities, including cognitive decline (73, 74) and hormonal changes (75). However, the role of IED in the development of cardiac alterations is not well-understood. Compared to healthy controls, patients with epilepsy exhibit a higher incidence of subtle abnormalities in HRV as well as abnormalities in the cardiac response to physiological stimuli (3, 4, 6, 76).

In patients affected by generalized seizures, the presence of HRV alterations that are related to IED were initially described by Faustmann and Ganz (76). These authors have indicated that patients with normal interictal EEG exhibit HRV that are similar to healthy controls. HRV modifications may be driven by ASD. Carbamazepine (3, 23) and phenytoin (24), in particular, have been shown to promote HRV modifications even though the role of carbamazepine is controversial (77). Other studies, especially those investigating drug-free, naïve, patients (42), reported that the role of ASD in the modification of HRV is marginal, thereby stressing the importance of the central control exerted by the cortex on the activity of the ANS.

Our results are in line with data indicating the presence of an asymmetrical autonomic innervation of the heart as well as the lateralization of the cardiac autonomic output in the brainstem. These data are thereby suggesting a different contribution of the two hemispheres in the control of the heart rate. In that context, preclinical and clinical data have demonstrated that lesions of the right hemisphere produce an increased sympathetic tone (33, 78). Furthermore, the stimulation of the left insula has been shown to induce a bradycardic response, whereas tachycardia and pressor responses are more elicited from the stimulation of the right insula (32). Studies based on the pharmacological inactivation of both hemispheres, obtained through the intracarotid injection of amobarbital, have produced conflicting results (79–81). One

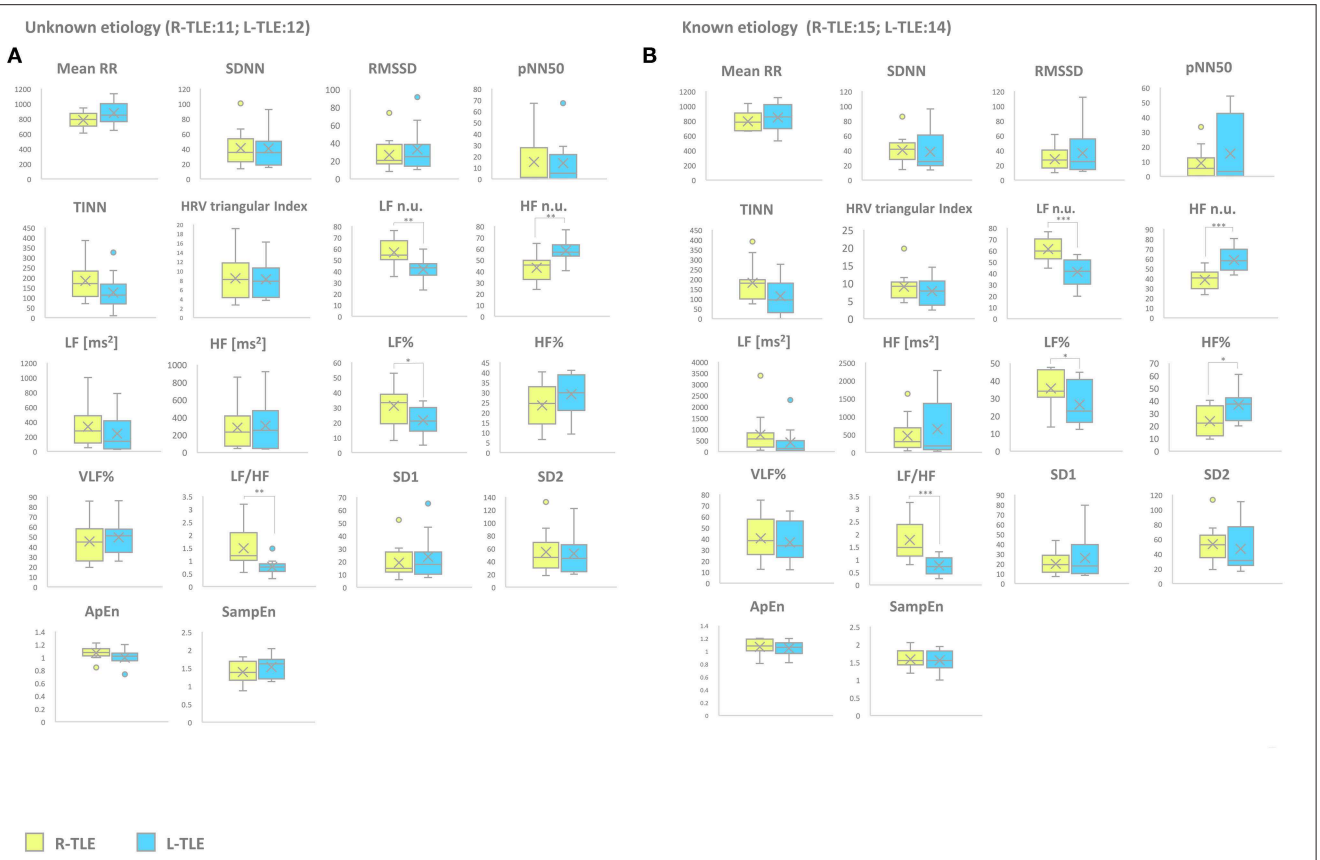



**FIGURE 6 |** HRV-related parameter comparison in patients with epilepsy with unknown or known etiology. **(A)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.001$), HF n.u. ($p < 0.001$), LF% ($p < 0.05$), and LF/HF ratio ($p < 0.001$) of unknown etiology TLE patients. **(B)** Right (yellow) and left (light blue) graph boxplots depict statistically significant differences in LF n.u. ($p < 0.001$), HF n.u. ($p < 0.0001$), LF% ($p < 0.05$), HF% ($p < 0.05$), and LF/HF ratio ($p < 0.0001$) of known etiology TLE patients. Mann-Whitney test was employed for statistical analysis. $p < 0.05$ was considered statistically significant. *$p < 0.05$; **$p < 0.001$; and ***$p < 0.0001$.

study (79), albeit robust in terms of the size of the enrolled patients, suffers from methodological issues related to the length of the HRV evaluation (i.e., ultra-short lasting analysis) and the presence of anticonvulsant treatment as 65 of the 73 enrolled patients have been treated with carbamazepine or phenytoin.

Additional evidence supporting the notion of lateralization of the control of the cardio-autonomic functions comes from studies employing functional Magnetic Resonance Imaging (fMRI). fMRI data related to the cortical control of spontaneous and arousal-induced fluctuations in the amplitude of skin conductance responses (SCR) support the presence of a sympathetic activity that is mainly controlled by the right hemisphere (82). These findings are also confirmed by electrophysiological studies performed on patients with side-specific hemispheric lesions that indicated a decreased galvanic skin response in patients with right-hemisphere lesions. In contrast, increased responses were found in subjects with lesions of the left hemisphere (83, 84).

Studies employing positron emission tomography to investigate hemispheric-specific increases of blood flow showed increased perfusion in the right insula of healthy patients undergoing physical activity or exposure to mental stress. In contrast, increased blood flow occurred in the left insula of subjects performing non-strenuous tasks (85).

## CONCLUSIONS

Our data provide experimental evidence to support the notion of lateralized cortical control of the cardiac autonomic functions in TLE patients. In particular, our findings suggest that epileptic patients with an L-TLE focus exhibit a lower risk of developing cardiac dysfunctions independently of the disease duration. Given the well-known correlations between the presence of HRV modifications and the occurrence of SUDEP, it can be inferred that patients with L-TLE may be less susceptible to develop SUDEP. A recent study (86) attempted to define the incidence of clinically relevant arrhythmias in refractory focal epilepsy and assessed the predicting value of postictal arrhythmias as risk markers for SUDEP. The study investigated people with refractory epilepsy (both TLE and non-TLE) who were implanted with a loop recorder and followed for 2 years. The study found no clinically relevant arrhythmias during the follow-up. It is conceivable that the inclusion of non-TLE patients in the study could

have blurred the results, thereby open the possibility that findings may be different when taking into consideration only TLE patients and when employing HRV alterations as selection criteria.

We acknowledge some limitations of our study. Given the small number of patients in the subgroup analysis, we were not able to investigate the impact of every specific ASDs in the modification of HRV features. However, although the role of the most recent ASDs in the modulation of HRV remains to be defined, our data indicate that, independently of specific ASDs, R-TLE patients exhibit a reduced vagal tone. Further studies will be needed to understand the impact that specific ASDs may have on HRV modifications as well as to assess the role of pharmaco-resistance in producing worse cardioautonomic balance. Finally, our results indicate that it would be important to investigate, with long-term EKG monitoring, whether R-TLE patients show a higher risk of developing arrhythmias.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

FD, GE, MO, and CV contributed to the conception and design of the study. GE organized the database. CV performed the statistical analysis. FD, VF, GE, and SS wrote the manuscript and supervised all the data. MF evaluated EKG data. All authors contributed to manuscript revisions, read, and approved the submitted version.

## ACKNOWLEDGMENTS

We thank Daniela Virdis, Stefania Nanni, and Antonio Saracino for performing the EEG recordings.

## REFERENCES

1. Wiebe S. Epidemiology of temporal lobe epilepsy. *Can J Neurol Sci.* (2000) 27(Suppl. 1):S6-10; discussion: S20–1. doi: 10.1017/S0317167100000561
2. Frysinger RC, Engel J, Harper RM. Interictal heart rate patterns in partial seizure disorders. *Neurology.* (1993) 43:2136–9. doi: 10.1212/WNL.43.10.2136
3. Devinsky O, Perrine K, Theodore WH. Interictal autonomic nervous system function in patients with epilepsy. *Epilepsia.* (1994) 35:199–204. doi: 10.1111/j.1528-1157.1994.tb02933.x
4. Massetani R, Strata G, Galli R, Gori S, Gneri C, Limbruno U, et al. Alteration of cardiac function in patients with temporal lobe epilepsy: different roles of EEG-ECG monitoring and spectral analysis of RR variability. *Epilepsia.* (1997) 38:363–9. doi: 10.1111/j.1528-1157.1997.tb01129.x
5. Tomson T, Ericson M, Ihrman C, Lindblad LE. Heart rate variability in patients with epilepsy. *Epilepsy Res.* (1998) 30:77–83. doi: 10.1016/S0920-1211(97)00094-6
6. Ansakorpi H, Korpelainen JT, Suominen K, Tolonen U, Myllylä VV, Isojärvi JI. Interictal cardiovascular autonomic responses in patients with temporal lobe epilepsy. *Epilepsia.* (2000) 41:42–7. doi: 10.1111/j.1528-1157.2000.tb01503.x
7. Wannamaker BB. Autonomic nervous system and epilepsy. *[Review]. Epilepsia.* (1985) 26(Suppl. 1): S31–9. doi: 10.1111/j.1528-1157.1985.tb05722.x
8. Palma JA, Benarroch EE. Neural control of the heart: recent concepts and clinical correlations. *Neurology.* (2014) 83:261–71. doi: 10.1212/WNL.0000000000000605
9. Benarroch EE. The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin Proc.* (1993) 68:988–1001. doi: 10.1016/S0025-6196(12)62272-1
10. Saper CB. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu Rev Neurosci.* (2002) 25:433–69. doi: 10.1146/annurev.neuro.25.032502.111311
11. Cechetto DF. Central representation of visceral function. *Fed Proc.* (1987) 46:17–23.
12. Barbas H. Flow of information for emotions through temporal and orbitofrontal pathways. *J Anat.* (2007) 211:237–49. doi: 10.1111/j.1469-7580.2007.00777.x
13. Ter Horst GJ, Postema F. Forebrain parasympathetic control of heart activity: retrograde transneuronal viral labeling in rats. *Am J Physiol.* (1997) 273:H2926–30. doi: 10.1152/ajpheart.1997.273.6.H2926
14. Spyer KM. Annual review prize lecture. Central nervous mechanisms contributing to cardiovascular control. *J Physiol.* (1994) 474:1–19. doi: 10.1113/jphysiol.1994.sp019997
15. Barbas H, Saha S, Rempel-Clower N, Ghashghaei T. Serial pathways from primate prefrontal cortex to autonomic areas may influence emotional expression. *BMC Neurosci.* (2003) 4:25. doi: 10.1186/1471-2202-4-25
16. Verberne AJ, Owens NC. Cortical modulation of the cardiovascular system. *Prog Neurobiol.* (1998) 54:149–68. doi: 10.1016/S0301-0082(97)00056-7
17. Laborde S, Mosley E, Thayer JF. Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front Psychol.* (2017) 8:213. doi: 10.3389/fpsyg.2017.00213
18. Pumprla J, Howorka K, Groves D, Chester M, Nolan J. Functional assessment of heart rate variability: physiological basis and practical applications. *Int J Cardiol.* (2002) 84:1–14. doi: 10.1016/S0167-5273(02)00057-8
19. Berntson GG, Norman GJ, Hawkley LC, Cacioppo JT. Cardiac autonomic balance versus cardiac regulatory capacity. *Psychophysiology.* (2008) 45:643–52. doi: 10.1111/j.1469-8986.2008.00652.x
20. Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol.* (1985) 248:H151–3. doi: 10.1152/ajpheart.1985.248.1.H151
21. Rahman F, Pechnik S, Gross D, Sewell L, Goldstein DS. Low frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Clin Auton Res.* (2011) 21:133–41. doi: 10.1007/s10286-010-0098-y
22. Sevcencu C, Struijk JJ. Autonomic alterations and cardiac changes in epilepsy. *Epilepsia.* (2010) 51:725–37. doi: 10.1111/j.1528-1167.2009.02479.x
23. Persson H, Ericson M, Tomson T. Carbamazepine affects autonomic cardiac control in patients with newly diagnosed epilepsy. *Epilepsy Res.* (2003) 57:69–75. doi: 10.1016/j.eplepsyres.2003.10.012
24. Lathers CM, Schraeder PL. Autonomic dysfunction in epilepsy: characterization of autonomic cardiac neural discharge associated with pentylenetertazol-induced epileptogenic activity. *Epilepsia.* (1982) 23:633–47. doi: 10.1111/j.1528-1157.1982.tb05079.x
25. Persson H, Ericson M, Tomson T. Heart rate variability in patients with untreated epilepsy. *Seizure.* (2007) 16:504–8. doi: 10.1016/j.seizure.2007.03.010

26. Margerison JH, Corsellis JA. Epilepsy and the temporal lobes: a clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain.* (1966) 89:499–530. doi: 10.1093/brain/89.3.499
27. Babb TL, Brown WJ, Pretorius J, Davenport C, Lieb JP, Crandall PH. Temporal lobe volumetric cell densities in temporal lobe epilepsy. *Epilepsia.* (1984) 25:729–40. doi: 10.1111/j.1528-1157.1984.tb03484.x
28. Frysinger RC, Harper RM. Cardiac and respiratory correlations with unit discharge in epileptic human temporal lobe. *Epilepsia.* (1990) 31:162–71. doi: 10.1111/j.1528-1167.1990.tb06301.x
29. Jeppesen J, Fuglsang-Frederiksen A, Brugada R, Pedersen B, Rubboli G, Johansen P, et al. Heart rate variability analysis indicates preictal parasympathetic overdrive preceding seizure-induced cardiac dysrhythmias leading to sudden unexpected death in a patient with epilepsy. *Epilepsia.* (2014) 55:e67–71. doi: 10.1111/epi.12614
30. Myers KA, Bello-Espinosa LE, Symonds JD, Zuberi SM, Clegg R, Sadleir L.G., et al. Heart rate variability in epilepsy: a potential biomarker of sudden unexpected death in epilepsy risk. *Epilepsia.* (2018) 59:1372–80. doi: 10.1111/epi.14438
31. Nayak CS, Sinha S, Nagappa M, Thennarasu K, Taly AB. Lack of heart rate variability during sleep-related apnea in patients with temporal lobe epilepsy (TLE)-an indirect marker of SUDEP? *Sleep Breath.* (2017) 21:163–72. doi: 10.1007/s11325-016-1453-6
32. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. *Neurology.* (1992) 42:1727–32. doi: 10.1212/WNL.42.9.1727
33. Zamrini EY, Meador KJ, Loring DW, Nichols FT, Lee GP, Figueroa RE, et al. Unilateral cerebral inactivation produces differential left/right heart rate responses. *Neurology.* (1990) 40:1408–11. doi: 10.1212/WNL.40.9.1408
34. Myers AK, Sivathamboo S, Perrucca P. Heart rate variability measurement in epilepsy: how can we move from research to clinical practice? *Epilepsia.* (2018) 59:2169–78. doi: 10.1111/epi.14587
35. Zaatreh MM, Quint SR, Tennison MB, D'Cruz O, Vaughn BB. Heart rate variability during interictal epileptiform discharges. *Epilepsy Res.* (2003)54:85–90. doi: 10.1016/S0920-1211(03)00059-7
36. Malik M, Bigger JT, Breithardt G, Cerutti S, Cohen RJ, Coumel P, et al. Heart rate variability Standards of measurement, physiological interpretation, and clinical use. Task force of the European society of cardiology and the North American society of pacing and electrophysiology (Membership of the Task Force listed in the Appendix). *Eur Heart J.* (1996) 17:354–81.
37. Camm JA, Malik, M, Bigger JT, Breithardt G, Cerutti S, Cohen RJ, et al. Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation.* (1996) 93:1043–65.
38. Akselrod S, Gordon D, Ubel AF, Shannon DC, Barger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science.* (1981) 213:220–2. doi: 10.1126/science.6166045
39. Chapleau MW, Sabharwal R. Methods of assessing vagus nerve activity and reflexes. *Heart Fail Rev.* (2011) 16:109–27. doi: 10.1007/s10741-010-9174-6
40. Billman GE. The effect of heart rate on the heart rate variability response to autonomic interventions. *Front Physiol.* (2013) 4:222. doi: 10.3389/fphys.2013.00222
41. Goldstein DS, Bentho O, Park MY, Sharabi Y. LF power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp Physiol.* (2011) 96:1255–61. doi: 10.1113/expphysiol.2010.056259
42. Romigi A, Albanese M, Placidi F, Izzi F, Mercuri NB, Marchi A, et al. Heart rate variability in untreated newly diagnosed temporal lobe epilepsy: evidence for ictal sympathetic dysregulation. *Epilepsia.* (2016) 57:418–26. doi: 10.1111/epi.13309
43. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE commission for classification and terminology. *Epilepsia.* (2017) 58:512–21. doi: 10.1111/epi.13709
44. Gould PA, Yii M, McLean C, Finch S, Marshall T, Lambert GW, et al. Evidence for increased atrial sympathetic innervation in persistent human atrial fibrillation. *Pacing Clin Electrophysiol.* (2006) 29:821–9. doi: 10.1111/j.1540-8159.2006.00447.x
45. Schwartz PJ. Cardiac sympathetic denervation to prevent life-threatening arrhythmias. *Nat Rev Cardiol.* (2014) 11:346–53. doi: 10.1038/nrcardio.2014.19
46. Hou Y, Zhou Q, Po SS. Neuromodulation for cardiac arrhythmia. *Heart Rhythm.* (2016) 13:584–92. doi: 10.1016/j.hrthm.2015.10.001
47. Derogatis LR, Lipman RS, Covi L. Symptom checklist-90. *Corsini Encyclopedia Psychol.* (2010) 1:1–2. doi: 10.1002/9780470479216.corpsy0970
48. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the *ad hoc* task force of the ILAE commission on therapeutic strategies. *Epilepsia.* (2010) 51:1069–77. doi: 10.1111/j.1528-1167.2009.02397.x
49. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. *Front Public Health.* (2017) 5:258. doi: 10.3389/fpubh.2017.00258
50. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol.* (2013) 4:26. doi: 10.3389/fphys.2013.00026
51. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front Psychol.* (2014) 5:1040. doi: 10.3389/fpsyg.2014.01040
52. deGiorgio CM, Miller P, Meymandi S, Chin A, Epps J, Gordon S, et al. RMSSD, a measure of vagus-mediated heart rate variability, is associated with risk factors for SUDEP: the SUDEP-7 inventory. *Epilepsy Behav.* (2010) 19:78–81. doi: 10.1016/j.yebeh.2010.06.011
53. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation.* (1991) 84:482–92. doi: 10.1161/01.CIR.84.2.482
54. Kamath MV, Fallen EL. Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. *Crit Rev Biomed Eng.* (1993) 21:245–311.
55. Rimoldi O, Pierini S, Ferrari A, Cerutti S, Pagani M, Malliani A. Analysis of short-term oscillations of R-R and arterial pressure in conscious dogs. *Am J Physiol.* (1990) 258(4 Pt 2):H967–76. doi: 10.1152/ajpheart.1990.258.4.H967
56. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation.* (1994) 90:1826–31. doi: 10.1161/01.CIR.90.4.1826
57. Malliani A, Lombardi F, Pagani M. Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms. *Br Heart J.* (1994) 71:1–2. doi: 10.1136/hrt.71.1.1
58. Appel ML, Berger RD, Saul JP, Smith JM, Cohen RJ. Beat to beat variability in cardiovascular variables: noise or music? *J Am Coll Cardiol.* (1989) 14:1139–48. doi: 10.1016/0735-1097(89)90408-7
59. Heathers JA. Everything Hertz: methodological issues in short-term frequency-domain HRV. *Front Physiol.* (2014) 5:177. doi: 10.3389/fphys.2014.00177
60. Jandackova VK, Scholes S, Britton A, Steptoe A. Are changes in heart rate variability in middle aged and older people normative or caused by pathological conditions? Findings from a large population-based longitudinal cohort study. *J Am Heart Assoc.* (2016) 5:e002365. doi: 10.1161/JAHA.115.002365
61. de Castilho FM, Ribeiro ALP, Nobre V, Barros G, de Sousa MR. Heart rate variability as predictor of mortality in sepsis: a systematic review. *PLoS ONE.* (2018) 13:e0203487. doi: 10.1371/journal.pone.0203487
62. Chang Y-M, Huang Y-T, Chen I-L, Yang C-L, Leu S-C, Su H-L, et al. Heart rate variability as an independent predictor for 8-year mortality among chronic hemodialysis patients. *Sci Rep.* (2020) 10:881. doi: 10.1038/s41598-020-57792-3
63. Tsuji H, Venditti FJ Jr., Manders ES, Evans JC, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham heart study. *Circulation.* (1994) 90:878–83. doi: 10.1161/01.CIR.90.2.878
64. Chandra P, Sands RL, Gillespie BW, Levin NW, Kotanko P, Kiser M, et al. Predictors of heart rate variability and its prognostic significance in chronic kidney disease. *Nephrol Dial Transplant.* (2012) 27:700–9. doi: 10.1093/ndt/gfr340
65. Kuo G, Chen SW, Huang JY, Wu C-Y, Fu C-M, Chang C-H, et al. Short-term heart rate variability as a predictor of long-term survival in patients with chronic hemodialysis: a prospective cohort study. *J Formos Med Assoc.* (2018) 117:1058–64. doi: 10.1016/j.jfma.2018.09.006

66. Ciccone AB, Siedlik JA, Wecht JM, Deckert JA, Nguyen ND, Weir JP. Reminder: RMSSD and SD1 are identical heart rate variability metrics. *Muscle Nerve.* (2017) 56:674–8. doi: 10.1002/mus.25573
67. Beckers F, Ramaekers D, Aubert AE. Approximate entropy of heart rate variability: validation of methods and application in heart failure. *Cardiovasc Eng.* (2001) 1:177–82. doi: 10.1590/s0100-879x2012007500025
68. Tarvainen MP, Lipponen J, Niskanen JP, Ranta-Aho P. *Kubios HRV Version 3 – User's Guide*. Kuopio: University of Eastern Finland (2017).
69. Richman JS, Moorman JR. Physiological time-series analysis using approximate entropy and sample entropy. *Am J Physiol Heart Circ Physiol.* (2000) 278:H2039–49. doi: 10.1152/ajpheart.2000.278.6.H2039
70. Marshall DW, Westmoreland BF, Sharbrough FW. Ictal tachycardia during temporal lobe seizures. *Mayo Clin Proc.* (1983) 58:443-446.
71. Blumhardt LD, Smith PE, Owen L. Electrocardiographic accompaniments of temporal lobe epileptic seizures. *Lancet.* (1986) 8489:1051–6. doi: 10.1016/S0140-6736(86)91328-0
72. Epstein MA, Sperling MR, O'Connor MJ. Cardiac rhythm during temporal lobe seizures. *Neurology.* (1992) 42:50–3. doi: 10.1212/WNL.42.1.50
73. Aarts JH, Binnie CD, Smit AM, Wilkins AJ. Selective cognitive impairment during focal and generalized epileptiform EEG activity. *Brain.* (1984) 107(Pt 1):293–308. doi: 10.1093/brain/107.1.293
74. Binnie CD, Channon S, Marston DL. Behavioral correlates of interictal spikes. *Adv Neurol.* (1991) 55:113–26.
75. Herzog AG, Coleman AE, Jacobs AR. Acute hypothalamopituitary dysfunction following interictal unilateral temporal epileptiform discharges in women with epilepsy. *Epilepsia.* (2000) 40(Suppl. 3):124.
76. Faustmann PM, Ganz RE. Central cardio-autonomic disorganization in interictal states of epilepsy detected by phase space analysis. *Int J Neurosci.* (1994) 78:43–7. doi: 10.3109/00207459408986044
77. Sathyaprabha TN, Koot LAM, Hermans BHM, Adoor M, Sinha S, Kramer BW, et al. Effects of chronic carbamazepine treatment on the ECG in patients with focal seizures. *Clin Drug Investig.* (2018) 38:845–51. doi: 10.1007/s40261-018-0677-6
78. Hachinski VC, Oppenheimer SM, Wilson JX, Guiraudon C, Cechetto DF. Asymmetry of sympathetic consequences of experimental stroke. *Arch Neurol.* (1992) 49:697–702. doi: 10.1001/archneur.1992.00530310039010
79. Ahern GL, Sollers JJ, Lane RD, Labiner DM, Herring AM, Weinand ME, et al. Heart rate and heart rate variability changes in the intracarotid sodium amobarbital test. *Epilepsia.* (2001) 42:912–21. doi: 10.1046/j.1528-1157.2001.042007912.x
80. Yoon B-W, Morillo CA, Cechetto DF, Hachinski V. Cerebral hemispheric lateralization in cardiac autonomic control. *Arch Neurol.* (1997) 54:741–4. doi: 10.1001/archneur.1997.00550180055012
81. Hilz MJ, Du tsch M, Perrine K, Nelson PK, Rauhut U, Devinsky O. Hemispheric influence on autonomic modulation and baroreflex sensitivity. *Ann Neurol.* (2001) 49:575–84. doi: 10.1002/ana.1006
82. Critchley HD, Elliott R, Mathias CJ, Dolan RJ. Neural activity relating to generation and representation of galvanic skin conductance responses: a functional magnetic resonance imaging study. *J Neurosci.* (2000) 20:3033–40. doi: 10.1523/JNEUROSCI.20-08-03033.2000
83. Zoccolotti P, Caltagirone C, Benedetti N, Gainotti G. Disorders of autonomic responses to emotional stimuli in patients with unilateral hemispherical lesions. *Encephale.* (1986) 12:263–8.
84. Heilman KM, Schwartz HD, Watson RT. Hypoarousal in patients with the neglectsyndromeandemotional indifference. *Neurology.* (1978) 28229–23.
85. Critchley HD, Taggart P, Sutton PM, Holdright DR, Batchvarov V, Hnatkova K, et al. Mental stress and sudden cardiac death: asymmetric midbrain activity as a linking mechanism. *Brain.* (2005) 128:75–85. doi: 10.1093/brain/awh324
86. van der Lende M, Arends JB, Lamberts RJ, Tan HL, de Lange FJ, Sander JW, et al. The yield of long-term electrocardiographic recordings in refractory focal epilepsy. *Epilepsia.* (2019) 60:2215–23. doi: 10.1111/epi.16373


**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 31 August 2020
doi: 10.3389/fnins.2020.00855

# Not All Competitions Come to Harm! Competitive Biofeedback to Increase Respiratory Sinus Arrhythmia in Managers

*Elisabetta Patron[1]*, Marianna Munafò[1], Simone Messerotti Benvenuti[1,2], Luciano Stegagno[1] and Daniela Palomba[1,2]*

[1] Department of General Psychology, University of Padua, Padua, Italy, [2] Padova Neuroscience Center, University of Padua, Padua, Italy

Despite the positive impact on achievement, competition has been associated with elevated psychophysiological activation, potentially leading to a greater risk of cardiovascular diseases. Competitive biofeedback (BF) can be used to highlight the effects of competition on the same physiological responses that are going to be controlled through BF. However, it is still unknown whether competition could enhance the effects of respiratory sinus arrhythmia (RSA)-BF training in improving cardiac vagal control. The present study explored whether competitive RSA-BF could be more effective than non-competitive RSA-BF in increasing RSA in executive managers, who are at higher cardiovascular risk of being commonly exposed to highly competitive conditions. Thirty managers leading outstanding private or public companies were randomly assigned to either a Competition ($n$ = 14) or a Control ($n$ = 16) RSA-BF training lasting five weekly sessions. Managers in the Competition group underwent the RSA-BF in couples and each participant was requested to produce a *better* performance (i.e., higher RSA) than the paired challenger. After the training, results showed that managers in the Competition group succeeded in increasing cardiac vagal control, as supported by the specific increase in RSA ($p < 0.001$), the standard deviation of R-R wave intervals (SDNN; $p < 0.001$), and root mean square of the successive differences between adjacent heartbeats (rMSSD; $p < 0.001$). A significant increase in the percentage of successive normal sinus beat to beat intervals more than 50 ms (pNN50; $p = 0.023$; $\eta^2_p = 0.17$), low frequency ($p \leq 0.001$; $\eta^2_p = 0.44$), and high frequency power ($p = 0.005$; $\eta^2_p = 0.25$) emerged independently from the competitive condition. Intriguingly, managers who compete showed the same reduction in resting heart rate (HR; $p = 0.003$, $\eta^2_p = 0.28$), systolic blood pressure (SBP; $p = 0.013$, $\eta^2_p = 0.20$), respiration rate ($p < 0.001$; $\eta^2_p = 0.46$), and skin conductance level (SCL; $p = 0.001$, $\eta^2_p = 0.32$) as non-competitive participants. Also, the same reduction in social anxiety ($p = 0.005$; $\eta^2_p = 0.25$), state ($p = 0.038$, $\eta^2_p = 0.14$) and trait anxiety ($p = 0.001$, $\eta^2_p = 0.31$), and depressive symptoms ($p = 0.023$, $\eta^2_p = 0.17$) emerged in the two groups. The present results showed that managers competing for increasing RSA showed a greater improvement in their parasympathetic modulation

OPEN ACCESS

**Edited by:**
*Sylvain Laborde, German Sport University Cologne, Germany*

**Reviewed by:**
*Alessandro Tonacci, Institute of Clinical Physiology (CNR), Italy*
*Renata Maria Lataro, Federal University of Santa Catarina, Brazil*

***Correspondence:**
*Elisabetta Patron*
*elisabetta.patron@unipd.it*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

**Received:** *13 May 2020*
**Accepted:** *22 July 2020*
**Published:** *31 August 2020*

**Citation:**
*Patron E, Munafò M, Messerotti Benvenuti S, Stegagno L and Palomba D (2020) Not All Competitions Come to Harm! Competitive Biofeedback to Increase Respiratory Sinus Arrhythmia in Managers. Front. Neurosci. 14:855. doi: 10.3389/fnins.2020.00855*

than non-competing managers. Most importantly, competition did not lead to the classic pattern of increased psychophysiological activation under competitive RSA-BF. Therefore, competition could facilitate the use of self-regulation strategies, especially in highly competitive individuals, to promote adaptive responses to psychological stress.

**Keywords: competition, biofeedback, managers, respiratory sinus arrhythmia, autonomic nervous system**

## INTRODUCTION

Competition has been consistently referred to as a type of social motivation, and, it has been addressed in many fields, including sports, job-related productivity, and academic achievement. Indeed, under challenging conditions, competition can motivate individual behavior more than cooperation. Certainly, competition contributes strongly to achievement-oriented behavior (Lam et al., 2004) by enhancing both competitor's intrinsic motivation (Tauer and Harackiewicz, 2004), creativity (Baer et al., 2010), and by fostering the mastery of a skill (Cooke et al., 2011). In modern work environments, especially among high-level managers and leaders, competition is embraced to reach a high work pace and top efficiency (Zhu and Zhou, 2014).

On the other side of the coin, competition represents a considerable source of social pressure, leading to aversive emotional states (Baumeister and Showers, 1986; Cerin et al., 2000; Martinent et al., 2012) and promoting psychophysiological activation. Under competitive conditions, increased psychophysiological activation, supported by large sympathetic nervous system responses, especially involving the cardiovascular system, have been commonly reported (Harrison et al., 2001; van Zanten et al., 2002). Faster heart rate (HR) and a shortening of the pre-ejection period (an index of increased myocardial contractility), both markers of beta-adrenergic activation and reduced parasympathetic cardiac modulation (as measured by the root mean square of the successive differences between adjacent heartbeats; rMSSD), have been reported during competitive conditions independently of individuals competitiveness trait (van Zanten et al., 2002). Nonetheless, individuals with high competitive traits were found to show higher blood pressure (BP) reactions and greater shortening of the pre-ejection period during a competitive condition compared to non-competitive individuals (Harrison et al., 2001). More recently, Cooke et al. (2011) reported that competition elicited cardiac beta-adrenergic activation (as measured by a shortening of the R-wave to pulse interval), alpha-adrenergic activation of the vasculature (as measured by decreased pulse amplitude), and decreased total heart rate variability (HRV) as measured by the standard deviation of R-R intervals (SDNN). Intriguingly, the authors reported that a decrease in SDNN mediated the improvement in endurance performance during competition (Cooke et al., 2011). Altogether, these results suggest that competitive conditions induce a psychophysiological activation that seems to be supported by a cardiac parasympathetic withdrawal co-occurring along with sympathetic activation (van Zanten et al., 2002; Cooke et al., 2011).

Such an important psychophysiological activation during competition, while being a powerful factor in achievement motivation, is also a strong stimulus condition for enhancing sympathetic arousal and triggering cardiovascular responses. Importantly, excessive cardiovascular response (i.e., high HR and BP increase) are related to a heightened risk of developing cardiovascular diseases, and high competitiveness trait might be a mechanism enhancing this relation (Glass et al., 1980; Sherwood et al., 1989; Shahidi et al., 1991; Harrison et al., 2001; Ricarte et al., 2001; Matsumura et al., 2011). Indeed, competitiveness is a core feature of the "Type A behavior" pattern (also called "Type A coronary-prone behavior"), a set of behavioral dispositions characterized by time urgency, impatience, restlessness, hostility, hyperalertness, and job involvement (Friedman and Rosenman, 1974) that has been associated with increased risk for coronary heart disease (Haynes and Feinleib, 1982; Orth-Gomer and Unden, 1990). Under competitive conditions, individuals classified as Type A have been reported to display larger cardiovascular responses (i.e., elevated HR and BP reactions) to laboratory and environmental challenges (Van Egeren et al., 1978; Dembroski et al., 1979; Glass et al., 1980; Chida and Hamer, 2008). In the majority of the studies focusing on the relation between competitiveness and increased cardiovascular risk, competition has been manipulated using psychosocial tasks, the most common being playing a game while competing against another person to win a prize (e.g., money). During such tasks, the participants are induced to compete against each other (or with a stooge opponent), while cardiovascular responses are monitored (Adam et al., 2015).

Intriguingly, competition during biofeedback (BF) can be used to highlight the effects of competition on the same physiological response that is going to be changed (Stegagno and Vaitl, 1979). BF is a well-known autoregulation procedure that allows the individual to control, through feedback, his/her own physiological functions, including the cardiovascular functions (Obrist et al., 1975; Williamson and Blanchard, 1979; Schwartz and Andrasik, 2017). BF can be used as a procedure to exploit competition as a motivational factor for challenging individuals to change their physiological activity (Stegagno and Vaitl, 1979; Stern and Elder, 1982; Shahidi and Salmon, 1992; Palomba and Stegagno, 1993). In an early study, a competitive BF procedure directly aimed at controlling HR was developed: participants were required to increase their HR to a greater extent than the challenger (in fact an experimental manipulation). Each participant received information on his/her HR, plus visual feedback (a red light) indicating when and for how long his/her own HR was higher

compared to the challenger's HR. The competitive situation resulted in a higher HR increase compared to the non-competitive one. Also, the respiration rate, muscle tension, and systolic blood pressure (SBP) increased significantly during the competition, reflecting general physiological arousal. It could be argued that the positive results were sustained by the synergism between the task requests (i.e., increase HR) and the motivational disposition induced by the competitive condition (Stegagno and Vaitl, 1979).

Competitive BF has also been applied to obtain a decrease in HR. Specifically, participants were rewarded for producing a physiological directional change (i.e., HR reduction) incompatible with the general psychophysiological activation induced by competition. Results showed that participants could reduce their HR under competition, although this reduction was smaller compared to the non-competitive one. Remarkably, respiration rate, muscle tension, and SBP showed no modifications during the competitive HR-BF, suggesting the idea that BF could counteract the psychophysiological activation usually elicited by competition (Palomba and Stegagno, 1993).

Competing to achieve deep relaxation was also tested. Participants received an HR-BF and were told that they would earn a monetary reward based on their ability to relax. Individuals with high competitive traits were able to reduce their HR even more in the competitive condition compared to not competitive ones. When striving to excel, individuals high in competitiveness could be more motivated to produce the expected performance, even when the performance implies a reduction in physiological activation, a response presumably incompatible with the effects of competition (Shahidi and Salmon, 1992). This is intriguing given that competitive situations are usually associated with a greater psychophysiological activation, which, in turn, has been consistently implicated in the risk to develop cardiovascular diseases, such as hypertension, coronary heart disease, heart failure, and myocardial infarction (Treiber et al., 2003; Kupper et al., 2015).

In health settings, BF is generally employed as an intervention to *decrease* psychophysiological activation, for example, through reducing HR (Hauri, 1975; Sharpley, 1989; Huang and Luk, 2015; Brown and Bray, 2019). In the early 80s, BF to improve respiratory sinus arrhythmia (RSA-BF), also called HRV-BF, was developed to target specifically the parasympathetic nervous system (Lehrer, 2013). During RSA-BF, individuals learn to synchronize the respiratory rate with variations in HR, in order to maximize RSA and the cardiac vagal control (Lehrer et al., 2000; Schwartz and Andrasik, 2017). The beneficial effects of RSA-BF have been hypothesized to be underlain different mechanisms. First, RSA-BF is linked to an increase in parasympathetic autonomic modulation (Lehrer and Gevirtz, 2014). It has been proposed that the mechanical effects of slow breathing stimulate the vagal nerve both phasically and tonically (Gerritsen and Band, 2018). Also, the synchronized oscillation in respiratory rate and HR stimulates the baroreflex (Vaschillo et al., 2006, 2011). Furthermore, positive effects have been sown on the respiratory system, and specifically an increase in gas exchange efficiency (Grossman and Taylor, 2007). More recently, some indirect anti-inflammatory effects of RSA-BF have been suggested (Gevirtz, 2013; Noble and Hochman, 2019; Lehrer et al., 2020).

RSA-BF is of particular relevance given that reduced cardiac vagal control (measured as low HRV or RSA) has been linked to several medical (Patron et al., 2012; Zhou et al., 2016; Benichou et al., 2018; Carvalho et al., 2018) and psychopathological conditions (Clamor et al., 2016; Cheng et al., 2019; Koch et al., 2019). Indeed, RSA-BF has been shown to effectively improve cardiac vagal control and, in turn, lower anxious and depressive symptoms (Karavidas et al., 2007; Gevirtz, 2013; Patron et al., 2013; Goessl et al., 2017; Caldwell and Steffen, 2018) and improve athletic performance, sleep, and quality of life (Zaccaro et al., 2018; Lehrer et al., 2020). Furthermore, RSA-BF was found to be effective in increasing cardiac vagal control and reducing SBP in a group of high-status-position managers (Munafò et al., 2016).

In addition to the mechanisms previously cited, the positive effects of RSA-BF on psychophysiological flexibility and emotions could involve improved functional connectivity between cortical brain areas. According to the neurovisceral integration model (Thayer and Lane, 2000, 2009), the heart is bidirectionally linked to areas in the prefrontal cortex through the vagus nerve and subcortical areas included in the central autonomic network (Benarroch, 1993, 1997). Mather and Thayer (2018) proposed that increasing RSA through RSA-BF could promote functional connectivity between brain regions involved in emotion regulation, such as the medial prefrontal cortex and the amygdala. Supporting this hypothesis, higher HRV has been reported to correlate with higher prefrontal cortex activity (Thayer et al., 2012; Chang et al., 2013; Patron et al., 2019), greater functional connectivity between the medial prefrontal cortex and the amygdala (Jennings et al., 2016) and with improved emotional regulation and psychological health (Hansen et al., 2003; O'Connor et al., 2007; Lane et al., 2013; Gillie et al., 2014).

Despite the positive effects of RSA-BF on cardiac vagal control, no study, to date, has applied competition to RSA-BF to examine whether competing to increase RSA could boost the motivation, leading to a greater reduction in psychophysiological activation. It could be argued that BF aiming at competing to increase RSA could improve parasympathetic modulation on the heart to a greater extent than non-competitive RSA-BF. This, in turn, could counteract the psychophysiological activation usually linked to competition. In the present study, managers in highly competitive job contexts and characterized by high competitiveness traits were randomly assigned to five sessions of competitive or non-competitive RSA-BF training. Participants in the competition group underwent BF in couples and were requested to achieve a better RSA than their competitors, while participants in the non-competition group were asked to enhance their RSA as much as they could. First, it was hypothesized that participants in the competition group would be able to enhance their RSA (i.e., increase cardiac vagal control) to a greater extent than participants in the non-competition (control) condition. Second, it was hypothesized that competing to improve RSA would counteract the psychophysiological activation usually linked to competition, leading to a reduction of HR, SBP, and skin conductance level (SCL).

# MATERIALS AND METHODS

## Participants

The present study enrolled 30 managers from private (banking group, manufacturing industries, and media) and public (health service, education system, local government, and military) companies in the northeastern region of Italy. A power analysis was conducted to determine the sample size for repeated measure of analysis of variance (ANOVA) with an effect size $F = 0.30$, a correlation among repeated measures of $r = 0.62$ and a power = 0.95. Participants were recruited through advertisements in the newsletter of the association of the General Confederation of Italian Industry (Confindustria) and voluntarily participated in this study. Participants were in charge either of the whole company (manager) or departments in organization managing (middle manager), subject to a highly competitive work environment. Part of the sample from a previously published report (Munafò et al., 2016) was included in the present study. All participants were males, aged 35–67 years (mean ± SD age = 49.30 ± 8.15), with a high-level education (mean ± SD education years = 17.60 ± 2.50), and they were all actively employed, with no precedent heart problems or other chronic mental or neurological diseases. None of the participants were taking medications influencing HR (e.g., beta-blockers), tranquilizers, or antidepressants.

Participants were instructed about the study procedure and gave written informed consent. After the assessment evaluation, they were randomly assigned to the Competition ($n = 14$) or Non-Competition (Control; $n = 16$) group. The study was carried out in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethical Committee of the Psychology section of the University of Padova (prot. No. 1159).

## Measurements and Apparatus

A semi-structured interview was conducted to collect sociodemographic (age and education) and health behavior data, including weight, height, physical activity, sleep, family history of hypertension, and cardiovascular diseases as well as medication intake (including medications influencing cardiac activity and psychotropic drugs).

The Jenkins Activity Survey (JAS; Jenkins et al., 1979) was administered to assess competitiveness traits. The JAS is a self-report measure containing 54 items investigating the way of responding to situations that should elicit Type A behavior in the susceptible individual (e.g., having to wait in long lines or to work with a slow partner). The JAS has four major components: Type A scale, factor S (speed impatience), factor J (job involvement), and factor H (hard-driving and competitive).

The Social Interaction Anxiety Scale (SIAS; Mattick and Clarke, 1998) was administered to assess the fear of general social interaction. The SIAS is a self-report questionnaire that includes 20 items describing the typical cognitive, affective, or behavioral reaction to different situations requiring interaction with other persons (one or more). Each item is rated on a scale from 0 to 4, which indicates to what extent the statements reflect the respondent characteristics. Total scores range from 0 to 80, higher scores reflect higher levels of social interactional anxiety.

The State and Trait Anxiety Inventory (STAI Y1 and STAI Y2) (Spielberger et al., 1983; Pedrabissi and Santinello, 1989) was administered to assess self-reported state (Y1) and trait (Y2) anxiety symptoms. The scores range between 20 and 80; higher scores represent higher long-lasting and persistent anxiety.

The Center for Epidemiological Study of Depression scale (CES-D) (Radloff, 1977; Fava, 1982) is a 20-item self-report questionnaire designed to measure the presence of common symptoms of depression over the previous week. Each item is rated on a four-point Likert scale and scores range from 0 to 60, higher scores indicating greater depressive symptoms.

## Physiological Measures

Blood volume pulse (BVP) was recorded by a photoplethysmographic detection sensor (BVP-Flex/Pro) attached to the right ring finger. Photoplethysmography (PPG) is a more convenient and less invasive alternative to the gold standard electrocardiogram. Several studies have reported that HRV indexes calculated from PPG signal and gold standard electrocardiographic recording are highly correlated (Lu et al., 2008, 2009; Gil et al., 2010; Jeyhani et al., 2015; Pinheiro et al., 2016; Menghini et al., 2019). PPG recordings have satisfactory accuracy in healthy individuals (Pinheiro et al., 2016) during resting conditions in the absence of motion (Schäfer and Vagedes, 2013; Menghini et al., 2019).

After recording the raw BVP, the signal was visually inspected and corrected for movement artifacts, and ectopic beats were detected and eliminated. Then to obtain the interbeat intervals (IBIs) series, heartbeats were automatically identified by an algorithm based on the detection of the point of maximum deviation in the BVP signal. Then IBIs series were exported in the Kubios-HRV 2.0 (Kuopio, Finland) software where an additional artifacts correction was run applying a piecewise cubic spline interpolation method that generates missing or corrupted values into the IBIs series.

Respiration rate was recorded employing a respiration belt with strain gauges/tube filled with conduction fluid (Respiration-Flex/Pro sensor) worn around the participant's abdomen. The software calculated the respiration rate from differences in the abdomen expansion in the raw signal waveform. The specific respiration range for each participant was calculated (i.e., maximum respiration rate *minus* minimum respiration rate, expressed in cycles/min), and converted in Hz (i.e., from cycles/min to cycles/s).

RSA was calculated through HRV analysis. Specifically, HRV is the physiological variation in the intervals between heartbeats, and most importantly, some indexes of HRV [e.g., rMSSD and the power in the high-frequency (HF) band)] have been shown to be reliable measures of the modulation of the parasympathetic branch of the autonomic nervous system on the heart in response to both internal and external challenges. In line with current recommendations (Laborde et al., 2017), the most common time- and frequency-domain HRV indexes were calculated and analyzed. Specifically, SDNN was calculated, which displays the cyclic components responsible for the total HRV. rMSSD was

also computed, which is highly sensitive to the fluctuation of high-frequency HRV and is considered an index of vagal control on the heart. Moreover, rMSSD has been shown as relatively independent of respiration rate influences (Hill et al., 2009). The percentage of successive normal sinus beat to beat intervals more than 50 ms (pNN50) was computed, as it indicates cardiac vagal control (Berntson et al., 1993; Malik et al., 1996; Hill et al., 2009; Laborde et al., 2017). In the frequency domain, the power spectrum in the very low-frequency (VLF; from 0 to 0.04 Hz), in the low-frequency (LF; from 0.04 to 0.15 Hz), and in the high-frequency band (HF; 0.15–0.40 Hz) were obtained and logarithmically transformed to normalize their distribution (Malik et al., 1996).

Since BF training was specifically focused on slow breathing and RSA, which is a cardiorespiratory phenomenon characterized by inter-beat intervals fluctuations occurring in phase with respiration (Grossman and Taylor, 2007), RSA was also computed. RSA is specifically considered to display the rhythmic increase and decrease of cardiac vagal efferent effects upon the sinoatrial node that are linked to respiratory frequency (Eckberg, 2003; Yasuma and Hayano, 2004; Laborde et al., 2018). Since RSA is modulated by physiological mechanisms that comprehend the interaction between cardiac and respiratory responses (Grossman, 1983), respiration can confound the relation between cardiac vagal control and RSA (Gevirtz and Lehrer, 2003; Grossman and Taylor, 2007). Therefore, to acquire a more reliable measure of cardiac vagal control, RSA was obtained, controlling for the respiration rate of each participant. RSA was calculated as the power spectrum of the IBIs series occurring within the specific respiration rate range for each participant. Specifically, a Fast Fourier Transformation was applied to the variation of IBIs occurring within the specific respiration rate range for each participant (Aysin and Aysin, 2006; Grossman and Taylor, 2007). RSA values were expressed in $ms^2$.

SBP and diastolic blood pressure (DBP) were recorded on the left arm. Three readings were taken at rest after adaptation to the laboratory at intervals of 1 min, and averaged, according to the recommendations for BP measurement of the American Heart Association (Pickering et al., 2005).

SCL was recorded employing two Ag/AgCl surface electrodes applied on the first and middle fingers of the right hand (Skin conductance-Flex/Pro sensor) (Fowles et al., 1981). The probe signal was constant voltage (0.5 V), and no conductive paste was applied on the skin. SCL, which is a measure of tonic electrodermal activity, has been widely used as an index of sympathetic nervous system activation that also reflects the level of psychophysiological activation (Boucsein, 2012; Boucsein et al., 2012). Specifically, the SCL signal recorded was visually examined for the occurrence of artifacts and non-specific skin conductance responses and manually corrected. Then, SCL was computed as the mean of SCL measurements across the non-artifactual recording.

BVP, respiration, and SCL were continuously recorded using a FlexComp Infiniti$^{TM}$ encoder, which is a computerized recording system approved by the US Food and Drug Administration and visualized through the BioGraph Infiniti software (Thought Technology Ltd., Montreal, QC, Canada). Data were processed via a 14-bit analog-to-digital converter with a sampling rate of 256 Hz (bandwidth DC – 64Hz) and stored for analysis in a personal computer (DELL VOSTRO notebook, Intel Core$^{TM}$ 2). SBP and DBP were recorded by a validated automatic wrist device (NAIS EW272, Matsushita Electrics Works Italia S.r.l.).

## Assessment

All managers underwent the same assessment protocol before the first RSA-BF session (i.e., pre-training) and approximately 2 weeks after the end of the fifth RSA-BF session (i.e., post-training), in a laboratory purposely set up at participant's worksite. Before each session, participants were asked to abstain from alcohol, caffeinated beverages, and smoking for the 3 h preceding psychophysiological recordings. Self-report questionnaires (SIAS, STAI Y1, STAI Y2, and CES-D) were administered individually by a trained psychologist blind to the participant's group assignment (Competition or Control group). Then, participants were invited to sit on a comfortable armchair, in a quiet, dimly lit room at a constant temperature (about 21°C). No support for the legs was employed to avoid the possible confounding effect of body position on cardiac activity. Before starting the physiological assessment, all the participants were informed of the sensors attached and the respective physiological measures being monitored (i.e., BVP, respiration rate, SCL, and BP). BVP signal was then analyzed to calculate HRV and RSA indexes. After the sensors' placement and adaptation to the laboratory (10 min), SBP and DBP were measured. Then the recording of BVP, respiration, and SCL was carried on over 4 min at rest, and SBP and DBP were measured again at the end of the physiological recording. To note, all the physiological measures analyzed and included in the study were recorded in resting conditions during the pre-training assessment (before the first RSA-BF session) and the post-training assessment (about 2 weeks after the fifth RSA-BF session). Participants were asked to breathe normally. After the pre-training assessment, participants were randomly assigned to either the Competition or the Control group.

## Training

The training consisted of five weekly sessions, each lasting about 40 min, performed in the same laboratory of assessment. All participants were asked to abstain from alcohol, caffeinated beverages, and smoking for the 3 h preceding each BF session. RSA-BF was aimed at increasing RSA and, therefore, at opposing autonomic dysregulation, especially vagal inhibition associated with stress (Lehrer et al., 2000). Before starting the first RSA-BF session, all the managers were informed about the feedback system, and they were told that augmenting the amplitude of HR changes in phase with breathing would increase RSA. Then, instructions similar to those proposed in Lehrer's et al. protocol (2000) were given to all participants. Specifically, they were told to try to breathe in phase with their HR, such that when the HR accelerate, they had to start inhaling, and when the HR decelerate, they had to exhale. Also, they were instructed to breathe so that their abdomen expanded during inhalation and contracted during exhalation and, more importantly, to

breathe out slower than they breathed in. Finally, they were asked to breathe in through their nose and breathe out through pursed lips. After the sensors' placement, the BF session started with a resting period of 3 min followed by two 6 min BF trials, spaced out by 1 min at rest. The BF session ended with 3 min at rest. Feedback was provided to all participants using the same instruments used for psychophysiological assessment, on a 15-inch PC display positioned in front of them at a distance of 50 cm. RSA feedback consisted of an HR beat-to-beat tachogram (i.e., beats/min) superimposed over the abdominal respiration signal on the same axis. Participants were required to synchronize HR and abdominal respiration until the two signals covaried in phase, thus leading to the maximal amplitude of RSA. The online moving feedback display (the graph representing the tachogram and abdominal respiration curves) was updated at successive 30 s periods. During each RSA-BF session, participants were reminded not to breathe too deeply to avoid hyperventilation. No pacing stimulus was provided during the training sessions.

Participants in the Competition group underwent the RSA-BF training in couples [paired for age, body mass index (BMI), and physical activity level) and were requested to have a *better* performance compared to the paired challenger (i.e., increase RSA *more than* the competitor). Participants in the Competition group were presented two stepped bars increasing from left to right: one bar represented their own performance and the other reflected the competitor's one (see **Figure 1**). Each step increase on the bar corresponded to 10 s RSA above the mean level of RSA as recorded during the baseline (i.e., during the first 3 min at rest). Participants competing to increase RSA were asked to increase the number of steps displayed on the bar more than the competitor.

Participants in the Control group were also trained in couples, but they had no competitive feedback: one stepped bar increasing from left to right was displayed on the screen to represent feedback for participant's performance. Participants in the Control group were asked to increase the stepped bar as much as they could.

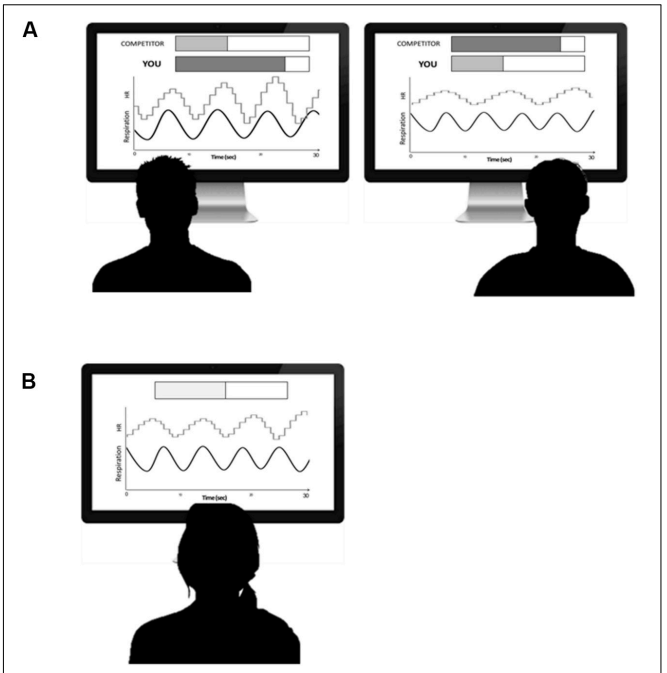



**FIGURE 1 | (A)** Schematic representation of one session of competition biofeedback. On the screens (duration 30 s) are representations of abdominal breathing (black line) and heart rate tachogram (gray line). Also, two bars were included on the screen to represent feedback for the subject's ("YOU") and competitor's ("COMPETITOR") performance. The participant was asked to synchronize heart rate with abdominal breathing better than his/her competitor. **(B)** Schematic representation of one session of Control Biofeedback. On the screen (duration 30 s) are representations of abdominal breathing (black line) and heart rate tachogram (gray line). Also, one bar was included on the screen to represent feedback for the subject's performance. The participant was asked to synchronize heart rate with abdominal breathing as much as he/she could.

## Data Reduction and Analysis

Data reduction and analyses were performed on questionnaire scores (SIAS, STAI Y1, STAI Y2, and CES-D) and physiological signals (i.e., RSA, HR, SDNN, rMSSD, pNN50, VLF, LF, HF, SBP, and DBP, respiration rate, and SCL) recorded over a 4 min period at rest during pre- (i.e., before the first RSA-BF session) and post-training (about 2 weeks after the end of the fifth RSA-BF session) sessions.

Whether a variable resulted not normally distributed from the Shapiro-Wilk test, a log transformation was applied for data normalization. For this reason, pre- and post-training RSA and SCL were log-transformed. The mean respiration rate was calculated over 4 min at rest during pre- and post-training assessment sessions. SBP and DPB were separately averaged across the three recordings during pre- and post-training sessions.

Student's *t*-tests for independent groups were performed to compare age, education, BMI, sleep time, and scores on JAS scales in the two groups (Competition and Control). $\chi^2$s were calculated to test differences between groups in sleep disorders, smoking, physical activity, family history of hypertension, and cardiovascular disease.

A series of repeated measure ANOVA, with Group (Competition and Control) as a between-subjects factor, and Time (pre- and post-training), as a within-subjects factor were performed on questionnaires scores (SIAS, STAI Y1, STAI Y2, and CES-D) and all physiological measures (RSA, HR, SDNN, rMSSD, pNN50, VLF, LF, HF, SBP, DBP, respiration rate, and SCL). Moreover, to evaluate whether the modification in RSA after RSA-BF training was clinically relevant, percent improvement index was calculated with the following formula [(log(RSA)post-training – log(RSA)pre-training)/log(RSA) pre-training *100] (Blanchard and Andrasik, 1987). Then a Mann-Whitney *U*-test was run to compare the RSA percent improvement index in the two groups (Competition and Control). Partial eta-squared ($\eta_p^2$) was reported as a measure of the effect size. Significant interactions ($p < 0.05$) were followed by Tukey *post hoc* comparisons to identify specific differences. All analyses were performed using

Jamovi version 0.9 (Şahin and Aybek, 2019). A $p < 0.05$ was considered statistically significant.

# RESULTS

## Sociodemographic and Health Behavior Data

Student's *t*-tests for independent groups and chi-square analyses revealed no group differences for age, education, BMI, family history of hypertension, cardiovascular disease, physical activity, reported sleep time, sleep disorders, and competitiveness traits (JAS scores; all *p's* > 0.296; see **Table 1**).

## Questionnaires Scores

Repeated measures ANOVAs on questionnaires scores revealed a significant reduction in fear of social interaction, state and trait anxiety, and depressive symptoms from pre- to post-training in both groups, as shown by the significant Time main effects [SIAS: $F_{(1,\ 28)} = 9.13$; $p = 0.005$; $\eta^2_p = 0.25$; STAI Y1: $F_{(1,\ 28)} = 4.73$; $p = 0.038$; $\eta^2_p = 0.14$; STAI Y2: $F_{(1,\ 28)} = 12.41$; $p = 0.001$; $\eta^2_p = 0.31$; CES-D: $F_{(1,\ 28)} = 5.82$; $p = 0.023$; $\eta^2_p = 0.17$; see **Tables 2**, **3**]. No significant main Group effect nor Group × Time interaction emerged for these measures (all *p*'s > 0.136).

## Physiological Data

The ANOVA on RSA at rest showed a Group × Time interaction [$F_{(1,\ 27)} = 8.78$; $p = 0.006$; $\eta^2_p = 0.24$; see **Tables 2**, **3** and **Figure 2A**). *Post hoc* comparisons yielded a significant RSA increase from pre- to post-training in the Competition group ($p < 0.001$), whereas in the Control group pre- to post-training comparison did not reach significance ($p = 0.066$). Post-training comparison between the Competition and Control group was not significant ($p = 0.479$). A significant main effect of Time emerged [$F_{(1,\ 27)} = 42.47$; $p < 0.001$.; $\eta^2_p = 0.60$], revealing

**TABLE 1 |** Sociodemographic characteristics, health behaviors, and JAS scores of participants assigned to Competition and Control groups.

| Participants' characteristics | Competition (*n* = 14) | Control (*n* = 16) | *p* |
|---|---|---|---|
| Age (year) | 48.86 (7.12) | 49.69 (9.17) | 0.786 |
| Education (years) | 17.50 (2.59) | 17.69 (2.50) | 0.842 |
| BMI (Kg/m$^2$) | 26.26 (2.88) | 26.73 (3.72) | 0.706 |
| Physical activity (none/occasional/regular) | 3 (21)/7 (50)/4 (29) | 5 (31)/4 (25)/7 (44) | 0.366 |
| Sleep time (hours) | 6.82 (0.54) | 6.78 (0.98) | 0.893 |
| Sleep disorders (N, %) | 7 (50) | 11 (69) | 0.296 |
| Family history of hypertension (N, %) | 6 (43) | 7 (44) | 0.961 |
| Family history of cardiovascular disease (N, %) | 7 (50) | 9 (56) | 0.732 |
| JAS – Speed Impatience | 216.86 (80.12) | 243.63 (60.54) | 0.307 |
| JAS – Job Involvement | 260.79 (29.97) | 252.06 (29.24) | 0.427 |
| JAS – Hard-driving and Competitive | 128.21 (32.68) | 127.44 (35.83) | 0.951 |

*Data are M (SD) of continuous and N of categorical variables. BMI, Body Mass Index; JAS, Jenkins Activity Survey.*

**TABLE 2 |** Psychophysiological and psychological indexes from pre- to post-training in participants who underwent competition RSA-BF and controls.

| Variable | Pre-training | Post-training |
|---|---|---|
| **SIAS** | | |
| Competition | 19.07 (8.69) | 16.00 (7.75) |
| Control | 14.06 (8.39) | 12.38 (7.11) |
| **STAI Y1** | | |
| Competition | 36.07 (6.57) | 33.07 (7.21) |
| Control | 33.00 (4.89) | 30.25 (8.38) |
| **STAI Y2** | | |
| Competition | 34.93 (5.64) | 32.64 (4.63) |
| Control | 37.56 (8.02) | 34.50 (7.56) |
| **CES-D** | | |
| Competition | 9.14 (3.74) | 7.14 (2.32) |
| Control | 11.19 (5.14) | 8.69 (5.59) |
| **RSA (log[ms$^2$])** | | |
| Competition | 1.90 (0.63) | 2.87 (0.75) |
| Control | 2.15 (0.70) | 2.51 (0.58) |
| **HR (bpm)** | | |
| Competition | 71.09 (10.20) | 65.84 (8.61) |
| Control | 73.22 (11.74) | 67.07 (8.26) |
| **SDNN (ms)** | | |
| Competition | 30.06 (17.21) | 55.98 (25.73) |
| Control | 28.55 (20.46) | 39.18 (25.46) |
| **rMSSD (ms)** | | |
| Competition | 25.29 (16.46) | 49.31 (23.12) |
| Control | 24.38 (20.25) | 31.94 (21.59) |
| **pNN50** | | |
| Competition | 6.89 (9.23) | 13.07 (12.00) |
| Control | 5.45 (12.62) | 7.15 (12.69) |
| **VLF (log[ms$^2$])** | | |
| Competition | 3.48 (0.93) | 4.31 (0.97) |
| Control | 3.16 (1.25) | 3.54 (1.77) |
| **LF (log[ms$^2$])** | | |
| Competition | 5.95 (0.95) | 7.20 (1.30) |
| Control | 5.63 (1.40) | 6.22 (1.60) |
| **HF (log[ms$^2$])** | | |
| Competition | 4.92 (1.20) | 5.97 (1.29) |
| Control | 4.99 (1.29) | 5.28 (1.24) |
| **SBP (mmHg)** | | |
| Competition | 125.18 (11.21) | 123.14 (14.93) |
| Control | 130.66 (14.06) | 121.44 (9.07) |
| **DBP (mmHg)** | | |
| Competition | 79.32 (8.37) | 79.54 (9.02) |
| Control | 82.75 (9.11) | 80.59 (7.83) |
| **Respiration rate (breath/min)** | | |
| Competition | 15.60 (2.99) | 11.28 (4.15) |
| Control | 13.93 (2.64) | 12.01 (3.64) |
| **SCL (log[μMho])** | | |
| Competition | 0.41 (0.24) | 0.36 (0.18) |
| Control | 0.43 (0.17) | 0.27 (0.13) |

*Data are M (SD). SIAS, Social Interaction Anxiety Scale; STAI Y1, State and Trait Anxiety Inventory form Y1; STAI Y2, State and Trait Anxiety Inventory form Y2; CES-D, Center for Epidemiological Study of Depression scale; RSA, respiratory sinus arrhythmia; HR, heart rate; SDNN, standard deviation of normal sinus beat to beat intervals; rMSSD, root mean square of the successive differences between adjacent heartbeats; pNN50, percentage of successive normal sinus beat to beat intervals more than 50 ms; VLF, power in the very low frequency; LF, power in the low frequency; HF, power in the high frequency; SBP, systolic blood pressure; DBP, diastolic blood pressure; SCL, skin conductance level.*

**TABLE 3 |** Results of ANOVAs on questionnaires scores and physiological data from pre- to post-training in participants in the competition and control groups.

| | Time main effects | | | Group main effects | | | Time × Group interactions | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variable** | $F_{(1, 27)}$ | $P$ | $\eta^2_p$ | $F_{(1, 27)}$ | $p$ | $\eta^2_p$ | $F_{(1, 27)}$ | $p$ | $\eta^2_p$ |
| SIAS | 9.13 | 0.005 | 0.25 | 2.35 | 0.136 | 0.08 | 0.77 | 0.387 | 0.03 |
| STAY1 | 4.73 | 0.038 | 0.14 | 1.89 | 0.180 | 0.06 | 0.01 | 0.925 | 0.001 |
| STAI Y2 | 12.41 | 0.001 | 0.31 | 0.93 | 0.344 | 0.03 | 0.26 | 0.613 | 0.01 |
| CES-D | 5.82 | 0.023 | 0.17 | 1.79 | 0.192 | 0.06 | 0.07 | 0.791 | 0.001 |
| RSA (log[ms$^2$]) | 42.47 | < 0.001 | 0.60 | 0.05 | 0.822 | 0.001 | 8.78 | 0.006 | 0.24 |
| HR (bpm) | 10.91 | 0.003 | 0.28 | 0.28 | 0.599 | 0.01 | 0.07 | 0.796 | 0.002 |
| SDNN (ms) | 37.82 | < 0.001 | 0.19 | 1.42 | 0.244 | 0.05 | 6.62 | 0.016 | 0.19 |
| rMSSD (ms) | 28.23 | < 0.001 | 0.50 | 1.76 | 0.196 | 0.06 | 7.67 | 0.010 | 0.22 |
| pNN50 | 5.75 | 0.023 | 0.17 | 0.85 | 0.365 | 0.03 | 1.86 | 0.184 | 0.06 |
| VLF (log[ms$^2$]) | 3.92 | 0.058 | 0.12 | 2.28 | 0.142 | 0.08 | 0.51 | 0.481 | 0.02 |
| LF (log[ms$^2$]) | 22.31 | < 0.001 | 0.44 | 2.07 | 0.161 | 0.07 | 2.85 | 0.103 | 0.09 |
| HF (log[ms$^2$]) | 9.50 | 0.005 | 0.25 | 0.60 | 0.444 | 0.02 | 3.05 | 0.092 | 0.10 |
| SBP (mmHg) | 7.08 | 0.013 | 0.20 | 0.22 | 0.645 | 0.01 | 2.88 | 0.101 | 0.09 |
| DBP (mmHg) | 0.27 | 0.605 | 0.01 | 0.78 | 0.384 | 0.03 | 0.41 | 0.528 | 0.01 |
| Respiration rate(breath/min) | 24.38 | < 0.001 | 0.47 | 0.19 | 0.664 | 0.01 | 3.62 | 0.067 | 0.11 |
| SCL (log[μMho]) | 13.24 | 0.001 | 0.32 | 0.39 | 0.540 | 0.01 | 3.16 | 0.086 | 0.10 |

*SIAS, Social Interaction Anxiety Scale; STAI Y2, State and Trait Anxiety Inventory form Y2; CES-D, Center for Epidemiological Study of Depression scale; RSA, respiratory sinus arrhythmia; HR, heart rate; SDNN, standard deviation of normal sinus beat to beat intervals; rMSSD, root mean square of the successive differences between adjacent heartbeats; pNN50, percentage of successive normal sinus beat to beat intervals more than 50 ms; VLF, power in the very low frequency; LF, power in the low frequency; HF, power in the high frequency; SBP, systolic blood pressure; DBP, diastolic blood pressure; SCL, skin conductance level.*

higher resting RSA during post-training assessment compared to pre-training. No significant main effect of Group emerged ($p = 0.822$)[1].

The Mann-Whitney U test on percent improvement index revealed that managers in the Competition group after RSA-BF had a greater percent improvement index (57%), than the Control group (27%) (Mann-Whitney $U = 51.00$; $p = 0.010$).

The ANOVA on SDNN revealed a significant Group × Time interaction [$F_{(1, 27)} = 6.62$; $p = 0.016$; $\eta^2_p = 0.19$; see **Tables 2**, **3** and **Figure 2B**). Tukey *post hoc* comparisons displayed a significant SDNN increase from pre- to post-training in the Competition group ($p < 0.001$), whereas the comparison between pre- and post-training in the Control group did not reach statistical significance ($p = 0.064$). Post-training comparison between the Competition and Control group was not significant ($p = 0.194$). Main Time effect yielded a significant increase in SDNN from pre- to post-training [$F_{(1, 28)} = 37.82$; $p \leq 0.001$; $\eta^2_p = 0.19$]. No significant main effect of Group emerged ($p = 0.244$).

The ANOVA on rMSSD showed a significant Group × Time interaction [$F_{(1, 27)} = 7.67$; $p = 0.010$; $\eta^2_p = 0.22$; see **Tables 2**, **3** and **Figure 2C**]. *Post hoc* comparisons yielded a significant rMSSD increase from pre- to post-training in the Competition group ($p < 0.001$), whereas the comparison between pre- and post-training in the Control group was not significant ($p = 0.267$). Post-training comparison between the Competition and Control group was not significant ($p = 0.113$). Main Time effect yielded a significant increase in rMSSD from pre- to post-training [$F_{(1, 28)} = 28.23$; $p \leq 0.001$; $\eta^2_p = 0.50$]. No significant main effect of Group emerged ($p = 0.196$).

Also, a significant Time main effect emerged showing an increase in pNN50, LF and HF [pNN50: $F_{(1, 28)} = 5.75$; $p = 0.023$; $\eta^2_p = 0.17$; see **Figure 2D**; LF: $F_{(1, 28)} = 22.31$; $p = 0.001$; $\eta^2_p = 0.44$; see **Figure 2E**; HF: $F_{(1, 28)} = 9.50$; $p = 0.005$; $\eta^2_p = 0.25$; see **Tables 2**, **3** and **Figure 2F**).

A significant reduction in HR, SBP, respiration rate, and SCL occurred from pre- to post-training for both groups, as shown by the significant Time main effects [HR: $F_{(1, 28)} = 10.91$; $p = 0.003$; $\eta^2_p = 0.28$; see **Figure 2G**; SBP: $F_{(1, 28)} = 7.08$; $p = 0.013$; $\eta^2_p = 0.20$; see **Figure 2H**; respiration rate: $F_{(1, 28)} = 24.38$; $p < 0.001$; $\eta^2_p = 0.47$;, see **Figure 2I**; SCL: $F_{(1, 28)} = 13.24$; $p = 0.001$; $\eta^2_p = 0.32$;, see **Tables 2**, **3** and **Figure 2J**]. No other significant effects emerged (all $p$'s $> 0.058$; see **Figures 2K,L**).

## DISCUSSION

The present study examined whether managers characterized by high competitiveness traits who were asked to compete to enhance their own cardiac vagal control through BF would achieve a greater improvement in RSA in comparison to managers undergoing a traditional (non-competitive) RSA-BF. Moreover, competing to improve RSA was expected to

[1]A repeated measure analysis of covariance (ANCOVA) on RSA controlling for change from pre- to post-training in respiration rate as a covariate was computed. Results showed a significant Group × Time interaction [$F_{(1, 27)} = 4.46$; $p = 0.044$; $\eta^2_p = 0.14$]. *Post hoc* comparisons yielded a significant RSA increase from pre- to post-training in both the Competition group ($p < 0.001$) and the Control group ($p = 0.003$). Post-training comparison between the Competition and Control group was not significant ($p = 0.958$). A significant main effect of Time emerged [$F_{(1, 27)} = 10.42$; $p = 0.003$; $\eta^2_p = 0.28$], revealing higher resting RSA during post-training assessment compared to pre-training. No significant main effect of Group emerged ($p = 0.776$).

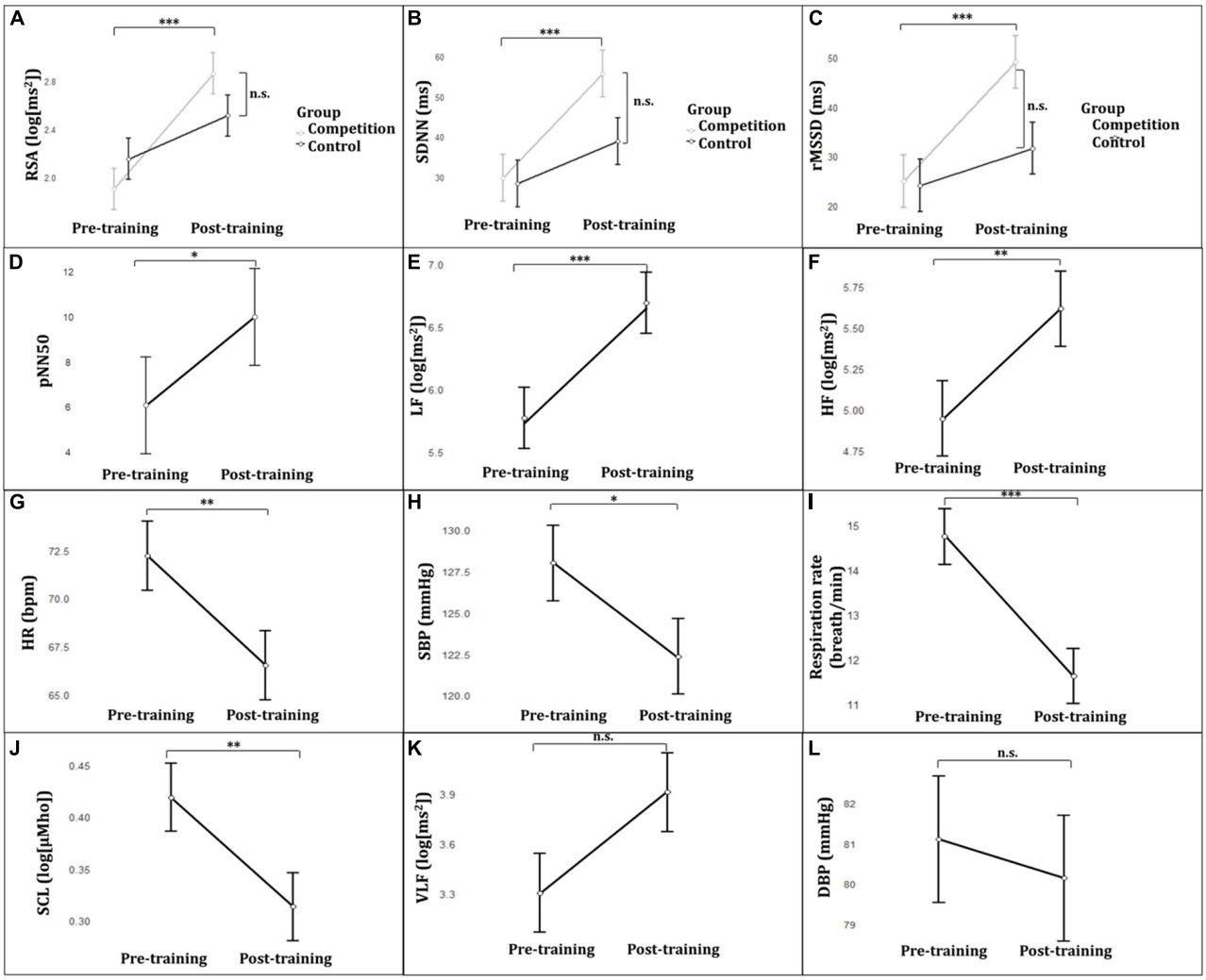



**FIGURE 2 | (A)** RSA in the Competition and Control group from pre-to post-training. **(B)** SDNN in the Competition and Control group from pre-to post-training. **(C)** rMSSD in the Competition and Control group from pre-to post-training. **(D)** pNN50 from pre- to post-training. **(E)** LF from pre- to post-training. **(F)** HF from pre- to post-training. **(G)** HR from pre- to post-training. **(H)** SBP from pre- to post-training. **(I)** Respiration rate from pre- to post-training. **(J)** SCL from pre- to post-training. **(K)** VLF from preto post-training. **(L)** DBP from pre- to post-training. Error bars represent the standard error of the mean. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. RSA, respiratory sinus arrhythmia; SDNN, standard deviation of normal sinus beat to beat intervals; rMSSD, root mean square successive difference of normal sinus beat to beat intervals; pNN50, percentage of successive normal sinus beat to beat intervals more than 50 ms; LF, power in the low frequency; HF, power in the high frequency; HR, heart rate; SBP, systolic blood pressure; SCL, skin conductance level; VLF, power in the very low frequency; DBP, diastolic blood pressure.

counteract the psychophysiological activation commonly linked to competition, leading to a reduction in HR, SBP, and SCL.

One major result of the present study was that managers in the Competition group significantly increased RSA from pre- to post-training. Importantly, managers in the Competition group specifically showed a consistent increase in indexes reflecting total HRV (i.e., SDNN) and greater cardiac vagal control (i.e., RSA and rMSSD). Additionally, to evaluate whether the modification in RSA after RSA-BF training was clinically relevant, percent improvement index was calculated. According to Blanchard and Andrasik (1987), a percent improvement index is clinically relevant when higher than 50%. In the present study, RSA percent improvement was clinically relevant only in the Competition group (57%), whereas the Control group showed a significantly lower RSA percent improvement (27%). Taken together these results support the idea that competitive BF was effective in improving cardiac vagal control to a greater extent than traditional non-competitive RSA-BF. This supports the idea that competition could have increased participants' motivation for success. Specifically, the feedback may become more relevant for the participants when they can use it in a competitive situation to achieve better results.

Intriguingly, the literature commonly reports the link between competition and excessive sympathetic activation (i.e., increased pre-ejection period) and general psychophysiological activation (i.e., increased HR and BP) (Harrison et al., 2001;

van Zanten et al., 2002). In contrast, the present results suggest a different perspective, showing that competition can be associated with an increase in parasympathetic cardiac modulation (i.e., higher RSA and rMSSD). In line with the idea that managers competing to improve cardiac vagal control were able to counteract the psychophysiological activation commonly associated with competition, managers in the competition group showed, after the training, a reduction in resting HR, SBP, and SCL comparable to that found in managers who did not compete. A previous study showed that competition was effective in improving performance during autoregulation, especially when the physiological modification requested by BF was compatible with the competition condition (i.e., accelerating HR) (Stegagno and Vaitl, 1979). In that case, a synergy between the physiological modification (i.e., to increase HR) and competition emerged together with a generalization of the effect to other cardiovascular responses possibly linked to increased psychophysiological activation (i.e., increased BP). On the contrary, when the direction of the physiological modification requested is incompatible with competition activation (e.g., compete to reduce the physiological activation), a mutual inhibition is expected between competition and autocontrol. However, results from previous studies suggest that it is possible for individuals to control an activating situation (i.e., competition) that is incompatible with the task requested by the feedback (i.e., physiological deactivation) (Shahidi and Salmon, 1992; Palomba and Stegagno, 1993).

The present results suggest that both competitive and non-competitive conditions were associated with increased HRV indexes (i.e., higher pNN50, LF, and HF from pre- to post-training) and lower psychophysiological activation (i.e., lower HR, SBP, and SCL from pre- to post-training). These findings are consistent with a previous study reporting the positive effects of RSA-BF in enhancing cardiac vagal control in highly competitive managers (Munafò et al., 2016). Also, the present results are in line with previous studies reporting reduced overall physiological arousal after RSA-BF training (Gevirtz and Lehrer, 2003; Wheat and Larkin, 2010). These results also suggest the possible contribution of RSA-BF to reducing the harmful effects of cardiovascular activation (Lehrer et al., 1997; Sherlin et al., 2009). This is noteworthy given that epidemiological studies in the general population have consistently shown that elevated levels of resting HR and SBP (even if not clinically relevant) are associated with increased risk for cardiovascular mortality (Kannel, 1996; Palatini et al., 2006; Reil and Böhm, 2007; Gu et al., 2008; Palatini, 2009).

Managers in a highly competitive job context and characterized by high competitiveness due to the elevated levels of involvement, competition, and responsibility have been shown to have a higher risk of cardiovascular disease (Kivimäki et al., 2002; Belkic et al., 2004; Backé et al., 2012). To decrease cardiovascular risk by reducing physiological arousal, occupational stress, and job strain, a wide variety of interventions including stress-management, relaxation, meditation techniques, and diaphragmatic deep breathing have been suggested (Lazarus and Folkman, 1984; Ivancevich et al., 1990; Giga et al., 2003). Whereas outcome evaluation of these interventions relied mainly on self-reporting measures (Kushnir et al., 1998) – with no objective measurement of the effectiveness in the reduction of psychophysiological activation – in the present study, physiological measures have been specifically targeted. From the present results, it could be argued that competing to increase RSA allowed participants to enhance their cardiac vagal control (i.e., RSA and rMSSD) to a greater extent than participants in the non-competition group. This, in turn, might have contributed in counteracting the psychophysiological cardiovascular activation usually found during competition, which have been proposed as one of the factors increasing cardiovascular risk.

An improvement in RSA has been linked to greater physiological flexibility and adaptive regulation to environmental challenges, as well as to psychological well-being, including anxiety and depressive symptoms reduction (Karavidas et al., 2007; Patron et al., 2013; Goessl et al., 2017; Caldwell and Steffen, 2018). The present study showed that managers competing to increase RSA reported a reduction in social anxiety, state and trait anxiety, and depressive symptoms corresponding to the reduction showed by managers undergoing traditional RSA-BF. This is in line with previous studies showing the effectiveness of RSA-BF in reducing anxiety symptoms and improving mood and psychological health (Karavidas et al., 2007; Gevirtz, 2013; Patron et al., 2013; Goessl et al., 2017; Caldwell and Steffen, 2018; Zaccaro et al., 2018; Lehrer et al., 2020). Recently it has been suggested that increasing RSA through RSA-BF could promote functional connectivity between certain brain regions involved in emotion regulation (Mather and Thayer, 2018). Future studies are warranted to verify whether the positive effects on mood and emotion regulation after RSA-BF are associated with greater brain functional connectivity.

It should be considered that in the present study participants were specifically recruited for their exposition to a competitive environment and their high competitiveness traits. The literature reports that individual high in trait competitiveness is characterized by more pronounced physiological activation to challenges (Harrison et al., 2001). Since highly competitive individuals strive to excel (e.g., Stern and Elder, 1982; Shahidi and Salmon, 1992), competitiveness could be manipulated as a motivational factor to enhance the performance, even when a reduction in physiological activation is demanded. Moreover, it has been reported that stronger motivation and better performances are observed only under appropriate competition conditions (Stanne et al., 1999). Indeed, the competition must be appropriately balanced (e.g., avoiding an excessive emphasis on winning, unequal participants matching), and participants should be able to estimate their progress relative to their opponent. In the present study, great attention was directed on setting an appropriate competition condition: the BF protocol created a fair challenging competition, providing each participant with a realistic and equal chance of winning; competitors were paired based on their age, BMI, and physical activity levels; the rules were clear and straightforward. Finally, participants could constantly assess their progress, relative to their opponent, through the feedback. It could be argued that individuals characterized by high competitiveness traits might take the most advantage from competition to motivate better

self-regulation. Future studies are warranted to investigate whether competitiveness traits modulate the effectiveness of competitive BF in increasing cardiac vagal control.

The current findings should be interpreted considering some possible methodological limitations. First, this study used a relatively small sample size; therefore, the results need to be replicated to fully understand the effects of competition BF in the acquisition of autonomic regulation in individuals with high levels of competitiveness. Nonetheless, the sample was determined through a power analysis based on previous studies on RSA-BF using the same protocol (Patron et al., 2013; Munafò et al., 2016). Second, although the current study showed that RSA is modifiable through competitive BF in a short time frame, the long-term effects of competitive RSA-BF were not assessed. Future research is warranted to replicate and extend the present findings by conducting long-term follow-up studies to demonstrate first the longevity of the improvements in RSA and whether the positive effects of RSA-BF could be linked to reduced cardiovascular risk. Third, although the present study focused on the effects of RSA-BF on cardiac vagal control, and secondly on the effects of RSA-BF on psychophysiological activation as measured by HR, SBP, and SCL, no specific index of the sympathetic nervous system influence on the heart was included. Future studies including measures of cardiac sympathetic nervous system influence and specific measures of cardiac output are warranted to directly compare the possible effects of RSA-BF on both the parasympathetic and sympathetic cardiac influence.

To summarize, managers competing to improve their cardiac vagal control showed a greater increase in RSA and rMSSD than managers in the non-competitive condition. Despite competition have been consistently associated with increased psychophysiological activation, the present results yield that managers competing for improving their cardiac vagal control (by increasing RSA) were able to reduce psychophysiological activation (i.e., lower HR, SBP, and SCL) and decrease anxiety and depressive symptoms to the same extent as managers in a non-competitive condition. In conclusion, the present study suggests that individuals with high competitiveness traits may benefit from competitive conditions during BF to increase cardiac vagal control. In turn, increased cardiac vagal control may counteract the psychophysiological activation linked to competition possibly leading to better autonomic regulation and psychophysiological well-being.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethic Committee of the Department of General Psychology, University of Padua. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

DP, LS, and MM contributed conception and design of the study. MM and EP gathered the data, organized the dataset, and wrote the manuscript. SM and EP performed the statistical analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

## FUNDING

The study was supported by a grant from MIUR (Dipartimenti di Eccellenza DM 11/05/2017 n. 262) to the Department of General Psychology, University of Padua.

## REFERENCES

Adam, M. T. P., Krämer, J., and Müller, M. B. (2015). Auction fever! how time pressure and social competition affect bidders' arousal and bids in retail auctions. *J. Retail.* 91, 468–485. doi: 10.1016/j.jretai.2015.01.003

Aysin, B., and Aysin, E. (2006). Effect of respiration in heart rate variability (HRV) analysis. *IEEE Eng. Med. Biol. Soc. Annu. Conf.* 1, 1776–1779. doi: 10.1109/IEMBS.2006.260773

Backé, E. M., Seidler, A., Latza, U., Rossnagel, K., and Schumann, B. (2012). The role of psychosocial stress at work for the development of cardiovascular diseases: a systematic review. *Int. Arch. Occup. Environ. Health* 85, 67–79. doi: 10.1007/s00420-011-0643-6

Baer, M., Leenders, R., Oldham, G. R., and Vadera, A. K. (2010). Win or lose the battle for creativity: the power and perils of intergroup competition. *Acad. Manag. J.* 53, 827–845. doi: 10.5465/AMJ.2010.52814611

Baumeister, R. F., and Showers, C. J. (1986). A review of paradoxical performance effects: choking under pressure in sports and mental tests. *Eur. J. Soc. Psychol.* 16, 361–383. doi: 10.1002/ejsp.2420160405

Belkic, K., Landsbergis, P., Schnall, P. L., and Baker, D. (2004). Is job strain a major source of cardiovascular disease risk? *Scand. J. Work. Environ. Health* 30, 85–128. doi: 10.5271/sjweh.769

Benarroch, E. (1997). *Central Autonomic Network: Functional Organization and Clinical Correlations*. London: Futura Publishing Company.

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin. Proc.* 68, 988–1001. doi: 10.1016/S0025-6196(12)62272-1

Benichou, T., Pereira, B., Mermillod, M., Tauveron, I., Pfabigan, D., Maqdasy, S., et al. (2018). Heart rate variability in type 2 diabetes mellitus: a systematic review and meta-analysis. *PLoS One* 13:e0195166. doi: 10.1371/journal.pone.0195166

Berntson, G., Cacioppo, J., and Quigley, K. (1993). Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology* 30, 183–196. doi: 10.1111/j.1469-8986.1993.tb01731.x

Blanchard, E. B., and Andrasik, F. (1987). *"Biofeedback Treatment of Vascular Headache," in Biofeedback and Self-Regulation*. Boston, MA: Springer, 1–79.

Boucsein, W. (2012). *Electrodermal Activity: Second Edition*. New York, NY: Springer.

Boucsein, W., Fowles, D. C., Grimnes, S., Ben-Shakhar, G., Roth, W. T., Dawson, M. E., et al. (2012). Publication recommendations for electrodermal measurements. *Psychophysiology* 49, 1017–1034. doi: doi:10.1111/j.1469-8986.2012.01384.x

Brown, D. M. Y., and Bray, S. R. (2019). Heart rate biofeedback attenuates effects of mental fatigue on exercise performance. *Psychol. Sport Exerc.* 41, 70–79. doi: 10.1016/j.psychsport.2018.12.001

Caldwell, Y. T., and Steffen, P. R. (2018). Adding HRV biofeedback to psychotherapy increases heart rate variability and improves the treatment of major depressive disorder. *Int. J. Psychophysiol.* 131, 96–101. doi: 10.1016/j.ijpsycho.2018.01.001

Carvalho, T. D., Massetti, T., Silva, T. D., Crocetta, T. B., Guarnieri, R., Vanderlei, L. C. M., et al. (2018). Heart rate variability in individuals with Down syndrome – A systematic review and meta-analysis. *Auton. Neurosci. Basic Clin.* 213, 23–33. doi: 10.1016/j.autneu.2018.05.006

Cerin, E., Szabo, A., Hunt, N., and Williams, C. (2000). Temporal patterning of competitive emotions: a critical review. *J. Sports Sci.* 18, 605–626. doi: 10.1080/02640410050082314

Chang, C., Metzger, C. D., Glover, G. H., Duyn, J. H., Heinze, H. J., and Walter, M. (2013). Association between heart rate variability and fluctuations in resting-state functional connectivity. *Neuroimage* 68, 93–104. doi: 10.1016/j.neuroimage.2012.11.038

Cheng, Y. C., Huang, Y. C., and Huang, W. L. (2019). Heart rate variability as a potential biomarker for alcohol use disorders: a systematic review and meta-analysis. *Drug Alcohol Depend.* 204:107502. doi: 10.1016/j.drugalcdep.2019.05.030

Chida, Y., and Hamer, M. (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. *Psychol. Bull.* 134, 829–885. doi: 10.1037/a0013342

Clamor, A., Lincoln, T. M., Thayer, J. F., and Koenig, J. (2016). Resting vagal activity in schizophrenia: meta-analysis of heart rate variability as a potential endophenotype. *Br. J. Psychiatry* 208, 9–16. doi: 10.1192/bjp.bp.114.160762

Cooke, A., Kavussanu, M., McIntyre, D., and Ring, C. (2011). Effects of competition on endurance performance and the underlying psychological and physiological mechanisms. *Biol. Psychol.* 86, 370–378. doi: 10.1016/j.biopsycho.2011.01.009

Dembroski, T., MacDougall, J., Herd, J. A., and Shields, J. L. (1979). Effect of level of challenge on pressor and heart rate responses in type A and B subjects. *J. Appl. Soc. Psychol.* 9, 209–228. doi: 10.1111/j.1469-8986.1981.tb01532.x

Eckberg, D. L. (2003). The human respiratory gate. *J. Physiol.* 548, 339–352. doi: 10.1113/jphysiol.2002.037192

Fava, G. (1982). *Versione Italiana del CES-D Per La Valutazione Degli Stati Depressivi [Italian Version of the CES-D for the Assessment of Depressive States]*. Firenze: Giunti OS.

Fowles, D., Christie, M., and Edelberg, R. (1981). Committee report. Publication recommendations for electrodermal measurements. *Psychophysiology* 18, 232–239. doi: 10.1111/j.1469-8986.1981.tb03024.x

Friedman, M., and Rosenman, R. H. (1974). *Type A Behavior and Your Heart*. New York, NY: Fawcett Crest.

Gerritsen, R. J. S., and Band, G. P. H. (2018). Breath of life: the respiratory vagal stimulation model of contemplative activity. *Front. Hum. Neurosci.* 12:397. doi: 10.3389/fnhum.2018.00397

Gevirtz, R. (2013). The promise of heart rate variability biofeedback: evidence-based applications. *Biofeedback* 41, 110–120. doi: 10.5298/1081-5937-41.3.01

Gevirtz, R., and Lehrer, P. M. (2003). "Resonance frequency heart rate biofeedback," in *Biofeedback: A Practitioner's Guide*, eds M. Schwartz and F. Andrasik (New York, NY: Guilford Press), 245–264.

Giga, S. I., Cooper, C. L., and Faragher, B. (2003). The development of a framework for a comprehensive approach to stress management interventions at work. *Int. J. Stress Manag.* 10, 280–296. doi: 10.1037/1072-5245.10.4.280

Gil, E., Orini, M., Bailón, R., Vergara, J. M., Mainardi, L., and Laguna, P. (2010). "Time-varying spectral analysis for comparison of HRV and PPG variability during tilt table test," in *Proceedings of the 2010 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBC'10 (Conf Proc IEEE Eng Med Biol Soc)* (Piscataway, NJ: IEEE), 3579–3582.

Gillie, B. L., Vasey, M. W., and Thayer, J. F. (2014). Heart rate variability predicts control over memory retrieval. *Psychol. Sci.* 25, 458–465. doi: 10.1177/0956797613508789

Glass, D., Krakoff, L., Contrada, R., Hilton, W., Kehoe, K., Mannucci, E., et al. (1980). Effect of harassment and competition upon cardiovascular and plasma catecholamine responses in type A and type B individuals. *Psychophysiology* 17, 453–463. doi: 10.1111/j.1469-8986.1980.tb00183.x

Goessl, V. C., Curtiss, J. E., and Hofmann, S. G. (2017). The effect of heart rate variability biofeedback training on stress and anxiety: a meta-analysis. *Psychol. Med.* 47, 2578–2586. doi: 10.1017/S0033291717001003

Grossman, P. (1983). Respiration, stress, and cardiovascular function. *Psychophysiology* 20, 284–300. doi: 10.1111/j.1469-8986.1983.tb02156.x

Grossman, P., and Taylor, E. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsycho.2005.11.014

Gu, Q., Burt, V., Paulose-Ram, R., Yoon, S., and Gillum, R. (2008). High blood pressure and cardiovascular disease mortality risk among U.S. adults: the third National Health and Nutrition Examination Survey mortality follow-up study. *Ann. Epidemiol.* 18, 302–309. doi: 10.1016/j.annepidem.2007.11.013

Hansen, A. L., Johnsen, B. H., and Thayer, J. F. (2003). Vagal influence on working memory and attention. *Int. J. Psychophysiol.* 48, 263–274. doi: 10.1016/S0167-8760(03)00073-4

Harrison, L., Denning, S., Easton, H., Hall, J., Burns, V., Ring, C., et al. (2001). The effects of competition and competitiveness on cardiovascular activity. *Psychophysiology* 38, 601–606. doi: 10.1111/1469-8986.3840601

Hauri, P. P. (1975). Biofeedback and self control of physiological functions: clinical applications. *Psychiatry Med.* 6, 255–265. doi: 10.2190/l766-9gfr-hxfd-wffx

Haynes, S. G., and Feinleib, M. (1982). Type A behavior and the incidence of coronary heart disease in the framingham heart study. *Adv. Cardiol.* 29, 85–94. doi: 10.1159/000406201

Hill, L. B. K., Siebenbrock, A., Sollers, J. J., and Thayer, J. F. (2009). Are all measures created equal? Heart rate variability and respiration. *Biomed. Sci. Instrum.* 45, 71–76.

Huang, Y. C., and Luk, C. H. (2015). "Heartbeat Jenga: a biofeedback board game to improve coordination and emotional control," in *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, ed. A. Marcus (Cham: Springer), 263–270. doi: 10.1007/978-3-319-20889-3_25

Ivancevich, J. M., Matteson, M. T., Freedman, S. M., and Phillips, J. S. (1990). worksite stress management interventions. *Am. Psychol.* 45, 252–261. doi: 10.1037//0003-066x.45.2.252

Jenkins, C. D., Zyzanski, S. J., and Rosenman, R. H. (1979). *Manual for the Jenkins Activity Survey*. New York, NY: The Psychological Corporation.

Jennings, J. R., Sheu, L. K., Kuan, D. C.-H., Manuck, S. B., and Gianaros, P. J. (2016). Resting state connectivity of the medial prefrontal cortex covaries with individual differences in high-frequency heart rate variability. *Psychophysiology* 53, 444–454. doi: 10.1111/psyp.12586

Jeyhani, V., Mahdiani, S., Peltokangas, M., and Vehkaoja, A. (2015). "Comparison of HRV parameters derived from photoplethysmography and electrocardiography signals," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS* (Piscataway, NJ: Institute of Electrical and Electronics Engineers Inc), 5952–5955.

Kannel, W. B. (1996). Blood pressure as a cardiovascular risk factor: prevention and treatment. *JAMA* 275, 1571–1576. doi: 10.1001/jama.1996.03530440051036

Karavidas, M. K., Lehrer, P. M., Vaschillo, E., Vaschillo, B., Marin, H., Buyske, S., et al. (2007). Preliminary results of an open label study of heart rate variability biofeedback for the treatment of major depression. *Appl. Psychophysiol. Biofeedback* 32, 19–30. doi: 10.1007/s10484-006-9029-z

Kivimäki, M., Leino-Arjas, P., Luukkonen, R., Riihimäki, H., Vahtera, J., and Kirjonen, J. (2002). Work stress and risk of cardiovascular mortality: prospective cohort study of industrial employees. *Br. Med. J.* 325:857. doi: 10.1136/bmj.325.7369.857

Koch, C., Wilhelm, M., Salzmann, S., Rief, W., and Euteneuer, F. (2019). A meta-Analysis of heart rate variability in major depression. *Psychol. Med.* 49, 1948–1957. doi: 10.1017/S0033291719001351

Kupper, N., Denollet, J., Widdershoven, J., and Kop, W. J. (2015). Cardiovascular reactivity to mental stress and mortality in patients with heart failure. *JACC Hear. Fail.* 3, 373–382. doi: 10.1016/j.jchf.2014.12.016

Kushnir, T., Malkinson, R., and Ribak, J. (1998). Rational thinking and stress management in health workers: a psychoeducational program. *Int. J. Stress Manag.* 5, 169–178. doi: 10.1023/A:1022941031900

Laborde, S., Mosley, E., and Mertgen, A. (2018). Vagal tank theory: the three rs of cardiac vagal control functioning - resting, reactivity, and recovery. *Front. Neurosci.* 12:458. doi: 10.3389/fnins.2018.00458

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - Recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lam, S., Yim, P., Law, J., and Cheung, R. (2004). The effects of competition on achievement motivation in Chinese classrooms. *Br. J. Educ. Psychol.* 74, 281–296. doi: 10.1348/000709904773839888

Lane, R. D., Weidenbacher, H., Smith, R., Fort, C., Thayer, J. F., and Allen, J. J. B. (2013). Subgenual anterior cingulate cortex activity covariation with cardiac vagal control is altered in depression. *J. Affect. Disord.* 150, 565–570. doi: 10.1016/j.jad.2013.02.005

Lazarus, R. S., and Folkman, S. (1984). *Stress, Appraisal and Coping*. Cham: Springer.

Lehrer, P. M. (2013). How does heart rate variability biofeedback work? Resonance, the baroreflex, and other mechanisms. *Biofeedback* 41, 26–31. doi: 10.5298/1081-5937-41.1.02

Lehrer, P. M., Carr, R. E., Smetankine, A., Vaschillo, E., Peper, E., Porges, S., et al. (1997). Respiratory sinus arrhythmia versus neck/trapezius EMG and incentive inspirometry biofeedback for asthma: a pilot study. *Appl. Psychophysiol. Biofeedback* 22, 95–109. doi: 10.1023/a:1026224211993

Lehrer, P. M., and Gevirtz, R. (2014). Heart rate variability biofeedback: how and why does it work? *Front. Psychol.* 5:756. doi: 10.3389/fpsyg.2014.00756

Lehrer, P. M., Kaur, K., Sharma, A., Shah, K., Huseby, R., Bhavsar, J., et al. (2020). Heart rate variability biofeedback improves emotional and physical health and performance: a systematic review and meta analysis. *Appl. Psychophysiol. Biofeedback* 45, 109–129. doi: 10.1007/s10484-020-09466-z

Lehrer, P. M., Vaschillo, E., and Vaschillo, B. (2000). Resonant frequency biofeedback training to increase cardiac variability: rationale and manual for training. *Appl. Psychophysiol. Biofeedback* 25, 177–191. doi: 10.1023/a:1009554825745

Lu, G., Yang, F., Taylor, J. A., and Stein, J. F. (2009). A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J. Med. Eng. Technol.* 33, 634–641. doi: 10.3109/03091900903150998

Lu, S., Zhao, H., Ju, K., Shin, K., Lee, M., Shelley, K., et al. (2008). Can photoplethysmography variability serve as an alternative approach to obtain heart rate variability information? *J. Clin. Monit. Comput.* 22, 23–29. doi: 10.1007/s10877-007-9103-y

Malik, M., Bigger, J. T., Camm, A. J., Kleiger, R. E., Malliani, A., Moss, A. J., et al. (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1161/01.CIR.93.5.1043

Martinent, G., Campo, M., and Ferrand, C. (2012). A descriptive study of emotional process during competition: nature, frequency, direction, duration and co-occurrence of discrete emotions. *Psychol. Sport Exerc.* 13, 142–151. doi: 10.1016/j.psychsport.2011.10.006

Mather, M., and Thayer, J. F. (2018). How heart rate variability affects emotion regulation brain networks. *Curr. Opin. Behav. Sci.* 19, 98–104. doi: 10.1016/j.cobeha.2017.12.017

Matsumura, K., Yamakoshi, T., Yamakoshi, Y., and Rolfe, P. (2011). The effect of competition on heart rate during kart driving: a field study. *BMC Res. Notes* 4:342. doi: 10.1186/1756-0500-4-342

Mattick, R. P., and Clarke, J. C. (1998). Development and validation of measures of social phobia scrutiny fear and social interaction anxiety. *Behav. Res. Ther.* 36, 455–470. doi: 10.1016/s0005-7967(97)10031-6

Menghini, L., Gianfranchi, E., Cellini, N., Patron, E., Tagliabue, M., and Sarlo, M. (2019). Stressing the accuracy: wrist-worn wearable sensor validation over different conditions. *Psychophysiology* 56:e13441. doi: 10.1111/psyp.13441

Munafò, M., Patron, E., and Palomba, D. (2016). Improving managers' psychophysical well-being: effectiveness of respiratory sinus arrhythmia biofeedback. *Appl. Psychophysiol. Biofeedback* 41, 129–139. doi: 10.1007/s10484-015-9320-y

Noble, D. J., and Hochman, S. (2019). Hypothesis: pulmonary afferent activity patterns during slow, deep breathing contribute to the neural induction of physiological relaxation. *Front. Physiol.* 10:1176. doi: 10.3389/fphys.2019.01176

Obrist, P. A., Galosy, R. A., Lawler, J. E., Gaebelein, C. J., Howard, J. L., and Shanks, E. M. (1975). Operant conditioning of heart rate: somatic correlates. *Psychophysiology* 12, 445–455. doi: 10.1111/j.1469-8986.1975.tb00022.x

O'Connor, M. F., Gündel, H., McRae, K., and Lane, R. D. (2007). Baseline vagal tone predicts BOLD response during elicitation of grief. *Neuropsychopharmacology* 32, 2184–2189. doi: 10.1038/sj.npp.1301342

Orth-Gomer, K., and Unden, A. L. (1990). Type A behavior, social support, and coronary risk: interaction and significance for mortality in cardiac patients. *Psychosom. Med.* 52, 59–72. doi: 10.1097/00006842-199001000-00005

Palatini, P. (2009). Elevated heart rate: a "new" cardiovascular risk factor?. *Prog. Cardiovasc. Dis.* 52, 1–5. doi: 10.1016/j.pcad.2009.06.001

Palatini, P., Dorigatti, F., Zaetta, V., Mormino, P., Mazzer, A., Bortolazzi, A., et al. (2006). Heart rate as a predictor of development of sustained hypertension in subjects screened for stage 1 hypertension: the HARVEST study. *J. Hypertens.* 24, 1873–1880. doi: 10.1097/01.hjh.0000242413.96277.5b

Palomba, D., and Stegagno, L. (1993). "Competizione, biofeedback e variazioni della frequenza cardiaca," in *Psicologia Della Motivazione: Indicazioni di Ricerca*, ed. A. Negri Dell'antonio (Padova: CLEUP).

Patron, E., Mennella, R., Messerotti Benvenuti, S., and Thayer, J. F. (2019). The frontal cortex is a heart-brake: reduction in delta oscillations is associated with heart rate deceleration. *Neuroimage* 188, 403–410. doi: 10.1016/j.neuroimage.2018.12.035

Patron, E., Messerotti Benvenuti, S., Favretto, G., Valfrè, C., Bonfà, C., Gasparotto, R., et al. (2012). Association between depression and heart rate variability in patients after cardiac surgery: a pilot study. *J. Psychosom. Res.* 73, 42–46. doi: 10.1016/j.jpsychores.2012.04.013

Patron, E., Messerotti Benvenuti, S., Favretto, G., Valfrè, C., Bonfà, C., Gasparotto, R., et al. (2013). Biofeedback assisted control of respiratory sinus arrhythmia as a biobehavioral intervention for depressive symptoms in patients after cardiac surgery: a preliminary study. *Appl. Psychophysiol. Biofeedback* 38, 1–9. doi: 10.1007/s10484-012-9202-5

Pedrabissi, L., and Santinello, M. (1989). *State-Trait Anxiety Inventory*. Firenze: Giunti OS.

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., et al. (2005). Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the subcommittee of professional and public education of the American heart association cou. *Circulation* 111, 697–716. doi: 10.1161/01.CIR.0000154900.76284.F6

Pinheiro, N., Couceiro, R., Henriques, J., Muehlsteff, J., Quintal, I., Goncalves, L., et al. (2016). "Can PPG be used for HRV analysis?," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS* (Piscataway, NJ: Institute of Electrical and Electronics Engineers Inc), 2945–2949.

Radloff, L. S. (1977). The CES-D scale: a self-report depression scale for research in the general population. *Appl. Psychol. Meas.* 1, 385–401. doi: 10.1177/014662167700100306

Reil, J. C., and Böhm, M. (2007). The role of heart rate in the development of cardiovascular disease. *Clin. Res. Cardiol.* 96, 585–592. doi: 10.1007/s00392-007-0537-5

Ricarte, J., Salvador, A., Costa, R., Torres, M. J., and Subirats, M. (2001). Heart rate and blood pressure responses to a competitive role-playing game. *Aggress. Behav. Off. J. Int. Soc. Res. Aggress.* 57, 351–359. doi: 10.1002/ab.1020

Şahin, M. D., and Aybek, E. C. (2019). Jamovi: an easy to use statistical software for the social scientists. *Int. J. Assess. Tools Educ.* 6, 670–692. doi: 10.21449/ijate.661803

Schäfer, A., and Vagedes, J. (2013). How accurate is pulse rate variability as an estimate of heart rate variability?: a review on studies comparing photoplethysmographic technology with an electrocardiogram. *Int. J. Cardiol.* 166, 15–29. doi: 10.1016/j.ijcard.2012.03.119

Schwartz, M. S., and Andrasik, F. E. (2017). *Biofeedback: A Practitioner's Guide*. New York, NY: Guilford Press.

Shahidi, S., Henley, S., Willows, J., and Furnham, A. (1991). Type A behaviour pattern: the effect of competition on heart rate and performance on a

driving game. *Pers. Individ. Dif.* 12, 1277–1282. doi: 10.1016/0191-8869(91)90201-L

Shahidi, S., and Salmon, P. (1992). Contingent and non-contingent biofeedback training for Type A and B healthy adults: can type as relax by competing? *J. Psychosom. Res.* 36, 477–483. doi: 10.1016/0022-3999(92)90008-P

Sharpley, C. F. (1989). Biofeedback training versus simple instructions to reduce heart rate reactivity to a psychological stressor. *J. Behav. Med.* 12, 435–447. doi: 10.1007/BF00844877

Sherlin, L., Gevirtz, R., Wyckoff, S., and Muench, F. (2009). Effects of respiratory sinus arrhythmia biofeedback versus passive biofeedback control. *Int. J. Stress Manag.* 16:233. doi: 10.1037/a0016047

Sherwood, A., Light, K. C., and Blumenthal, J. A. (1989). Effects of aerobic exercise training on hemodynamic responses during psychosocial stress in normotensive and borderline hypertensive type A men: a preliminary report. *Psychosom. Med.* 51, 123–136. doi: 10.1097/00006842-198903000-00002

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., and Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stanne, M. B., Johnson, D. W., and Johnson, R. T. (1999). Does competition enhance or inhibit motor performance: a meta-analysis. *Psychol. Bull.* 125, 133–154. doi: 10.1037/0033-2909.125.1.133

Stegagno, L., and Vaitl, D. (1979). "Voluntary heart rate acceleration under conditions of binary feedback and social competition," in *Biofeedback and Self-Regulation*, eds N. Birbaumer and H. Kimmel (Hillsdale, NJ: Lawrence Erlbaum), 197–204.

Stern, G. S., and Elder, R. D. (1982). The role of challenging incentives in feedback-assisted heart rate reduction for coronary-prone adult males. *Biofeedback Self. Regul.* 7, 53–69. doi: 10.1007/BF00999055

Tauer, J., and Harackiewicz, J. (2004). The effects of cooperation and competition on intrinsic motivation and performance. *J. Pers. Soc. Psychol.* 86, 849–861. doi: 10.1037/0022-3514.86.6.849

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., Wager, T. D., Åhs, F., et al. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Neuroscience and Biobehavioral Reviews Claude Bernard and the heart – brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Treiber, F. A., Kamarck, T., Schneiderman, N., Sheffield, D., Kapuku, G., and Taylor, T. (2003). Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosom. Med.* 65, 46–62. doi: 10.1097/00006842-200301000-00007

Van Egeren, L. F., Abelson, J. L., and Thornton, D. W. (1978). Cardiovascular consequences of expressing anger in a mutually-dependent relationship. *J. Psychosom. Res.* 22, 537–548. doi: 10.1016/0022-3999(78)90012-0

van Zanten, J. J., De Boer, D., Harrison, L., Ring, C., Carroll, D., Willemsen, G., et al. (2002). Competitiveness and hemodynamic reactions to competition. *Psychophysiology* 39, 759–766. doi: 10.1111/1469-8986.3960759

Vaschillo, E. G., Vaschillo, B., Buckman, J. F., Pandina, R. J., and Bates, M. E. (2011). "The investigation and clinical significance of resonance in the heart rate and vascular tone baroreflexes," in *Communications in Computer and Information Science*, eds A. Fred, J. Filipe, and H. Gamboa (Cham: Springer), 224–237. doi: 10.1007/978-3-642-18472-7_18

Vaschillo, E. G., Vaschillo, B., and Lehrer, P. M. (2006). Characteristics of resonance in heart rate variability stimulated by biofeedback. *Appl. Psychophysiol. Biofeedback* 31, 129–142. doi: 10.1007/s10484-006-9009-3

Wheat, A. L., and Larkin, K. T. (2010). Biofeedback of heart rate variability and related physiology: a critical review. *Appl. Psychophysiol. Biofeedback* 35, 229–242. doi: 10.1007/s10484-010-9133-y

Williamson, D. A., and Blanchard, E. B. (1979). Heart rate and blood pressure biofeedback: I. A review of the recent experimental literature. *Biofeedback Self. Regul.* 4, 1–34. doi: 10.1007/bf00998947

Yasuma, F., and Hayano, J. I. (2004). Respiratory sinus arrhythmia: why does the heartbeat synchronize with respiratory rhythm? *Chest* 125, 683–690. doi: 10.1378/chest.125.2.683

Zaccaro, A., Piarulli, A., Laurino, M., Garbella, E., Menicucci, D., Neri, B., et al. (2018). How breath-control can change your life: a systematic review on psycho-physiological correlates of slow breathing. *Front. Hum. Neurosci.* 12:353. doi: 10.3389/fnhum.2018.00353

Zhou, X., Ma, Z., Zhang, L., Zhou, S., Wang, J., Wang, B., et al. (2016). Heart rate variability in the prediction of survival in patients with cancer: a systematic review and meta-analysis. *J. Psychosom. Res.* 89, 20–25. doi: 10.1016/j.jpsychores.2016.08.004

Zhu, J., and Zhou, M. (2014). How does a servant leader fuel the service fire? A multilevel model of servant leadership, individual self identity, group competition climate, and customer service performance. *J. Appl. Psychol.* 100, 511–521. doi: 10.1037/a0038036

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 17 September 2020
doi: 10.3389/fphys.2020.566399

# Heart Rate Variability and Exceptional Longevity

*Adrián Hernández-Vicente[1,2]*, David Hernando[3,4], Alejandro Santos-Lozano[5,6], Gabriel Rodríguez-Romo[7,8], Germán Vicente-Rodríguez[1,2,9,10], Esther Pueyo[3,4], Raquel Bailón[3,4] and Nuria Garatachea[1,2,9,10]*

[1]GENUD (Growth, Exercise, NUtrition and Development) Research Group, University of Zaragoza, Zaragoza, Spain, [2]Department of Physiatry and Nursing, Faculty of Health and Sport Sciences (FCSD), University of Zaragoza, Huesca, Spain, [3]BSICoS Group, Aragón Institute for Engineering Research (I3A), IIS Aragón, University of Zaragoza, Zaragoza, Spain, [4]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain, [5]i+HeALTH, European University Miguel de Cervantes, Valladolid, Spain, [6]Research Institute of Hospital 12 de Octubre ("i+12"), Madrid, Spain, [7]Faculty of Physical Activity and Sports Sciences, INEF, Universidad Politécnica de Madrid, Madrid, Spain, [8]CIBERFES, Madrid, Spain, [9]Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBER-Obn), Madrid, Spain, [10]Instituto Agroalimentario de Aragón -IA2- (CITA-Universidad de Zaragoza), Zaragoza, Spain

Centenarians are the paradigm of human extreme longevity and healthy aging, because they have postponed, if not avoided, mayor age-related diseases. The purpose of this study was to investigate potential differences in resting heart rate variability (HRV) between young adults, octogenarians, and centenarians and assess whether HRV variables are predictors of all-cause mortality in centenarians. To this end, three groups of participants: young adults ($N$ = 20; 20.6 ± 2.3 years), octogenarians ($N$ = 18; 84.1 ± 2.6 years), and centenarians ($N$ = 17; 101.9 ± 1.9 years) were monitored for 15 min at rest (seated, without moving or talking) to measure RR intervals, from which HRV was evaluated. Our results showed a clear decrease with age in the main parasympathetic HRV variables, as well as in the standard deviation (SD) of the RR series [SD of normal-to-normal interval (SDNN)] and in low frequency (LF) heart rate (HR) oscillations, although differences between octogenarians and centenarians did not reach statistical significance. In 14 centenarians followed until death, only SDNN showed significant correlation ($\rho$ = 0.536; $p$ = 0.048) with survival prognosis. Additionally, SDNN <19 ms was associated with early mortality (≤1 year) in centenarians (Hazard Ratio = 5.72). In conclusion, HRV indices reflecting parasympathetic outflow as well as SDNN and LF all present an age-related reduction, which could be representative of a natural exhaustion of allostatic systems related to age. Moreover, low SDNN values (<19 ms) could be associated with early mortality in centenarians. HRV seems to play a role in exceptional longevity, which could be accounted for by centenarians' exposome.

**Keywords: electrocardiography, autonomic nervous system, parasympathetic nervous system, heart rate, heart rate variability, mortality, centenarians, aging**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Luca Carnevali,*
*University of Parma, Italy*
*Dirk Cysarz,*
*Witten/Herdecke University, Germany*

****Correspondence:***
*Adrián Hernández-Vicente*
*ahernandez@unizar.es*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

***Received:*** *27 May 2020*
***Accepted:*** *21 August 2020*
***Published:*** *17 September 2020*

***Citation:***
*Hernández-Vicente A, Hernando D, Santos-Lozano A, Rodríguez-Romo G, Vicente-Rodríguez G, Pueyo E, Bailón R and Garatachea N (2020) Heart Rate Variability and Exceptional Longevity. Front. Physiol. 11:566399. doi: 10.3389/fphys.2020.566399*

## INTRODUCTION

Heart rate variability (HRV) is defined as "the oscillation in the interval between consecutive heart beats" (Malik et al., 1996). HRV is the result of the interaction of multiple regulatory mechanisms that operate at different time scales, including long-term mechanisms like circadian rhythms, core body temperature, or metabolism and short-term mechanisms involving the autonomic, cardiovascular, and respiratory systems (Shaffer and Venner, 2013). Short-term spectral analysis of HRV usually reveals at least two frequency components, a low frequency (LF) component (0.04–0.15 Hz) and a high frequency (HF) component (>0.15 Hz; Malik et al., 1996). These components have been widely used to measure sympathetic and parasympathetic nervous systems, although their underlying physiological mechanisms are still unclear and a matter of debate (Billman, 2013).

In the last decades, several studies have reported that HRV decreases with age, suggesting an age-dependent decline in autonomic nervous system (ANS) activity in geriatric patients (Craft and Schwartz, 1995; Piccirillo et al., 1995). The majority of these studies have been mainly performed in older adults up to 80–85 years old, whereas older adults over age 85 have not received much attention. Centenarians represent the survival tail of the population (with a lifespan at least 15–20 years longer than the average westerner) and a model of healthy aging (Christensen et al., 2008). Indeed, centenarians escaped the diseases of the pre-antibiotic era and have postponed/avoided aging-related diseases as well as their fatal consequences (Salvioli et al., 2008).

The study of centenarians constitutes a fascinating research into the characteristics that allow individuals to attain an exceptionally long lifespan. Few works have studied HRV in centenarians. Piccirillo et al. (1998) and Paolisso et al. (1999) found that centenarians present higher power in HF heart rate (HR) oscillations and lower power in LF than old adults (75–100 years old in Paolisso et al. and 81–100 years old in Piccirillo et al.), suggesting age-related increase in parasympathetic activity and reduction in sympathetic activity. These results are in line with those obtained by Zulfiqar (2010) who enrolled subjects up to 99 years old and demonstrated that parasympathetic time-domain HRV measures decrease with age, reaching a nadir in the 7th–8th decade. From the 8th decade, these HRV measures are shown to rise, with the authors proposing this reversal of the decrease in parasympathetic function as a key determinant of longevity. In contrast, another study conducted in centenarians linked HRV with mortality during 4-year follow-up, showing that among all frequency-domain variables only higher LF/HF ratio was associated with survival (Shimizu et al., 2002).

Eight out of 10 centenarians are women (Teixeira et al., 2017). In 2016, Koenig and Thayer (2016) published a meta-analysis with 63,612 participants (31,970 females), revealing that: although adult women showed greater mean HR (MHR) than adult male, the female heart is characterized by a dominance of vagal activity (greater HF) and lower standard deviation (SD) of normal-to-normal intervals (SDNN). However, these sex differences may disappear in older adults (Voss et al., 2015; Koenig and Thayer, 2016), as a consequence of a variety of age-related changes such as: endocrine, brain structure, brain perfusion, or behavioral differences.

Due to the lack of current evidence and the discrepancies in the reported outcomes, the present study aimed at investigating potential differences in women's HRV between young adults, octogenarians, and centenarians and assess whether HRV variables can predict all-cause mortality in centenarians followed up until the time of death.

## MATERIALS AND METHODS

### Participants

Women aged 18–26 years in the group of young adults, 80–90 years in the group of octogenarians, and ≥100 years in the case of centenarians were included in the study. Due to the low number of centenarians, four men were additionally included in this group. In total, the young adults, octogenarians, and centenarians groups contained 20, 18, and 17 subjects, respectively. Exclusion criteria included the following: subjects going through an acute disease, suffering from heart diseases (e.g., heart failure or atrial fibrillation), or being on cardiac medication. Subjects who had a stroke or were suffering from chronic diseases such as diabetes, hypertension, chronic obstructive pulmonary disease, osteoarthritis, dementia, Parkinson's, or thyroid diseases were included in the study because of their high prevalence in the last decades of life. The study was approved by the Clinical Research Ethics Committee of the University Hospital of Alcorcón (ID of the approval: 16/50) and was conducted adhering to the Declaration of Helsinki. After a clear explanation of the potential risks of the study, all volunteers (or their legally responsible for older adults with cognitive problems) provided written informed consent to participate in the study.

### Experimental Design

All the subjects completed one test session. Prior to the test session, subjects were asked to adhere to the following instructions: (1) avoid exercise or strenuous physical activity the day before the test; (2) drink plenty of fluids over the 24 h period preceding the test; (3) get an adequate amount of sleep (6–8 h) the night before the test; (4) avoid substances such as tobacco, alcohol, or stimulants (caffeine, theine, taurine, etc.) in the 8 h before the test; (5) avoid food for 3 h prior to taking the test; and (6) wear comfortable, loose-fitting clothing. All the subjects were tested in an environmentally controlled room (22–23°C) between 9:00 and 13:00 h. They were monitored for 15 min at rest (seated, without any movement or talking) to measure RR intervals. RR intervals were recorded on a beat-to-beat basis by using an HR monitor (RS800, Polar Electro Oy, Kempele, Finland) with a sampling frequency of 1,000 Hz, thus providing an accuracy of 1 ms for each RR period. This device has been recently validated, showing to provide comparable performance with respect to the electrocardiogram when analyzing HRV at rest (de Rezende Barbosa et al., 2016; Hernando et al., 2016).

## HRV Variables

HRV variables have commonly used to assess sympathetic and parasympathetic nervous systems. The LF component of HRV is assumed to provide information on cardiac sympathetic and parasympathetic neural activity, together with other regulatory mechanisms and baroreflex (Eckberg, 1997). The HF component, on the other hand, is assumed to be vagally mediated and driven by respiration, measuring the so-called respiratory sinus arrhythmia (RSA; Berntson et al., 1993). Based on these assumptions, the ratio of LF to HF (LF/HF) has been proposed to quantify the relationship between sympathetic and parasympathetic activities (i.e., the sympatho-vagal balance; Malik et al., 1996).

However, although these spectral indices are well-standardized, their physiological interpretation has been criticized. This especially applies to the relationship between LF power and cardiac sympathetic regulation (Eckberg, 1997; Billman, 2013; Reyes del Paso et al., 2013), with LF power decreasing during situations expected to increase sympathetic activity, such as exercise or myocardial ischemia, and lack of correlation between direct recording of sympathetic nerve activity and LF power in either healthy subjects or patients with heart failure. The interpretation of HF power has been also challenged, especially when the respiratory rate does not fall within the HF band (0.15–0.4 Hz; Laborde et al., 2017). Different approaches have been proposed to overcome this limitation by redefining the HF band (Bailón et al., 2007; Varon et al., 2018). It has also been suggested that sympathetic neural activity may modulate the HF component (Billman, 2013). Therefore, the physiological interpretation of the LF/HF ratio is unclear and likely underestimates the complex interactions between the sympathetic and parasympathetic regulation of HR (Billman, 2013).

Normalized LF power (LFn) represents the proportional contribution of sympathetic modulation (Malik et al., 1996), in the same way and with the same limitations as LF/HF ratio represents sympatho-vagal balance. A mathematical relationship exists between LFn and LF/HF ratio: $LFn = (1 + (LF/HF) - 1) - 1$, so individual LFn values contain no more information than individual LF/HF ratio values (Heathers, 2014). However, statistical results on them might differ due to the volatility of the LF/HF ratio when HF power approaches zero (Billman, 2013; Heathers, 2014).

Regarding time-domain variables, SDNN reflects all the cyclic components responsible for HRV (Laborde et al., 2017). Lastly, the root mean square of successive differences (RMSSD), the percentage of RR intervals which exceed 50 ms from the previous one (pNN50), and the SD of successive differences (SDSD) are correlated with the HF band, so vagal activity is considered to be in the physiological origin of these three variables. Of these, RMSSD is normally preferred since it is less influenced by respiration (Laborde et al., 2017). Despite the former caveats in their interpretation, the study of HRV indices is an area of great interest as they provide a low-cost and non-invasive window into ANS regulation of the heart.

## Data Acquisition and Processing

HRV analysis was performed on 3-min running windows taken every 30 s. In each window, outlier RR intervals were identified by imposing a limit on the derivative of the instantaneous HR, which cannot exceed a time-varying threshold based on the median of its previous values (Mateo and Laguna, 2003). Only those windows with less than 10 outliers (always below 5% in this study) were considered for further analysis. Two different HRV representations were used for time and frequency domain HRV indices estimation.

For time domain indices, the RR series was used, after correction of identified outlier RR values using the interpolation proposed in Mateo and Laguna (2003). The following indices were computed: MHR, RMSSD, pNN50, SDSD, and SDNN (Malik et al., 1996).

For frequency domain indices, the HRV representation used is the modulating signal, based on the heart timing signal, since it was shown to outperform other HRV representations for frequency domain indices estimation (Mateo and Laguna, 2000). The modulating signal, assumed to carry information from the ANS, was estimated from the beat occurrence time series, derived from the recorded RR intervals, based on the time-varying integral pulse frequency modulation model (Bailón et al., 2011). First, the instantaneous HR signal was estimated, sampled at a sampling frequency $Fs$ = 4 Hz, and denoted by $d_{HR}(n)$. Subsequently, the time-varying MHR signal, $d_{MHR}(n)$, was estimated by low-pass filtering $d_{HR}(n)$ with a cutoff frequency of 0.03 Hz. The modulating signal, $m(n)$, was estimated by normalizing the HRV signal, $d_{HRV}(n) = d_{HR}(n) - d_{MHR}(n)$, by the time-varying MHR, i.e., $m(n) = d_{HRV}(n)/d_{MHR}(n)$. Note that the modulating signal $m(n)$ is adimensional. The purpose of this normalization is to alleviate the effect that changes in MHR have on HRV (Bailón et al., 2011). Then, the power spectral density (PSD) of the modulating signal $m(n)$ was estimated using Welch periodogram with internal window of 2 min and 50% overlap. The power in the following bands was estimated: (i) LF, from 0.04 to 0.15 Hz; (ii) HF, from 0.15 to 0.40 Hz; (iii) extended HF (HFext), from 0.15 to half the MHR, to avoid misestimation of the HF component when respiratory rate is above 0.4 Hz (24 breaths per minute; Bailón et al., 2007). The LFn power was computed by dividing LF power by the sum of LF and HF powers (LFn), and the extended LFn was determined by dividing LF by the sum of LF and HFext (LFn_ext). Finally, the ratio between the LF and HF powers (LF/HF) and the ratio between LF and HFext powers (LF/HFext) were calculated.

As HRV analysis was performed on running 3-min windows taken every 30 s, the mean of each HRV variable in all running windows with less than 10 outlier RR values was computed to characterize each subject.

## Statistical Analysis

Descriptive values are presented as mean ± SD and HRV values are reported as median and (1st quartile–3rd quartile). The normality of data was checked with the Shapiro-Wilk test. Since the data distribution violated the assumption of normality required by parametric tests and could not be corrected by common transformations, a non-parametric analysis was used. To assess differences between the three age groups, Kruskal-Wallis test (non-parametric equivalent of one-way ANOVA) with Bonferroni correction was performed.

The Dunn-Bonferroni *post hoc* method was used for pairwise comparisons. A multiple linear regression was performed to control for potential confounding effects like the body mass index (BMI). To evaluate the magnitude of the difference, effect size (ES) was calculated as: ES = $chi^2/(k - 1)$, where $k$ = total number of subjects. The difference was considered as small when ES < 0.2, small to medium when ES = 0.2–0.5, medium to large when ES = 0.5–0.8, and large when ES > 0.8 (Cohen, 1992).

A sub-analysis was carried out by following centenarians until their death and calculating Spearman's correlation coefficient ($\rho$) between HRV variables and "time to death." For the HRV variables showing significant correlation to "time to death," centenarians were divided into two groups by setting a cut point that defined a high risk group containing one third of the centenarian population and a low risk group containing the remaining two thirds. The Mann-Whitney U test was used to compare differences between the statistical distributions of the two groups. Kaplan-Meier survival analysis was performed and the log-rank test was used to test survival differences between the two groups. Additionally, the value of HRV variables in predicting survival was determined by Cox proportional hazards analyses. Statistical analyses were performed using IBM SPSS (version 25; Chicago, IL, United States). The significance level was set at $p < 0.05$.

## RESULTS

**Table 1** shows the descriptive characteristics of the groups.

An illustrative example of the RR interval series for each group is shown in **Figure 1**. Results obtained for the HRV variables in each age group are shown in **Table 2**. Differences between groups were only observed when analyzing the parasympathetic variables: RMSSD, pNN50, HF, HFext, and SDSD as well as LF and SDNN. All these variables decreased significantly with age (main effect: $p < 0.05$) but no statistically significant differences were found between octogenarians and centenarians.

We were able to follow up 14 centenarians until death. The three subjects with incomplete data were females but no significant differences with the 14 subjects included in the sub-analysis were found either in the descriptive variables or in the HRV variables. The only HRV variable that presented significant correlation with survival prognosis in centenarians (**Table 3**) was SDNN ($\rho$ = 0.536, $p$ = 0.048).

The 14 centenarians were divided according to the SDNN variable into two groups based on a cut point of 19 ms: Group 1 presenting low SDNN values ($N$ = 4; 15 ± 4 ms, range: 10–18) and Group 2 presenting high SDNN values ($N$ = 10; 49 ± 25 ms, range: 27–110). The difference between the statistical distributions of the two groups was statistically significant. Group 2 was associated with greater survival (1.6 ± 0.9 years, range: 0.3–3.5) than Group 1 (0.6 ± 0.3 years, range: 0.3–1.0) in Kaplan-Meier analysis (*Log-rank test* = 0.010, **Figure 2**). Mortality risk in Group 1 was five times higher ($p$ = 0.028; Hazard Ratio = 5.72) than in Group 2.

**TABLE 1** | Descriptive characteristics of the groups.

| Variable | Young adults ($N$ = 20) | Octogenarians ($N$ = 18) | Centenarians ($N$ = 17) |
|---|---|---|---|
| Age (years) | 20.6 ± 2.3 (18–26) | 84.1 ± 2.6 (80–88) | 101.9 ± 1.9 (100–105) |
| Women (%) | 100 | 100 | 76.5 |
| BMI (kg/m$^2$) | 20.7 ± 1.9 (17–24) | 27.0 ± 2.8 (22–31) | 23.1 ± 3.4 (17–28) |
| Chronic diseases (%)* | Osteoarthritis | 50 | 59 |
| | CVD | 44 | 53 |
| | Dementia | 11 | 47 |
| | Diabetes | 33 | 18 |
| | AHT | 61 | 53 |
| | COPD | 6 | 6 |
| | Others | 61 | 65 |
| | Total number | 3.3 ± 1.7 (0–5) | 3.3 ± 1.0 (2–6) |

*All young adults were healthy.*
*Scale values are mean ± standard deviation (SD) and min-max; BMI, body mass index; CVD, cardiovascular disease; AHT, arterial hypertension; COPD, chronic obstructive pulmonary disease.*

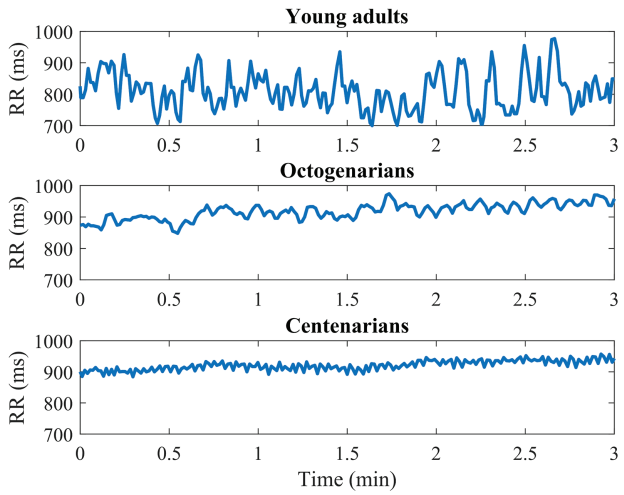



**FIGURE 1** | Example of the RR interval series for one subject from each age group.

No relation was found between subjects' age at RR recording time and time to death ($p$ = 0.477).

## DISCUSSION

The present study shows that parasympathetic time-domain HRV measures as well as SDNN and LF all decrease with age; moreover, other variables such as LFn or LF/HF ratio do not indicate differences between age groups. In relation to survival prognosis, SDNN was the only HRV measure showing moderate correlation ($\rho$ = 0.5–0.7) with time to death in centenarians, with SDNN values below 19 ms being associated with early mortality (≤1 year) in centenarians (Hazard Ratio = 5.72).

**TABLE 2** | Differences between groups using Kruskal-Wallis non parametric test.

| Variable | Young adults (*N* = 20) | Octogenarians (*N* = 18) | Centenarians (*N* = 17) | Main effect | |
|---|---|---|---|---|---|
| | Median ($Q_1$–$Q_3$) | Median ($Q_1$–$Q_3$) | Median ($Q_1$–$Q_3$) | *p* | ES |
| LF | 0.0015 (0.0012–0.0031)†‡ | 0.0004 (0.0001–0.0017) | 0.0002 (0.0001–0.0004) | <0.001* | 0.441 |
| LFn | 0.5708 (0.5204–0.6266) | 0.6131 (0.4608–0.7031) | 0.5366 (0.3867–0.6456) | 0.504 | 0.025 |
| LFn_ext | 0.5517 (0.4969–0.5997) | 0.5701 (0.4286–0.6202) | 0.4426 (0.2840–0.5887) | 0.168 | 0.066 |
| HF | 0.0016 (0.0007–0.0032)†‡ | 0.0002 (0.0000–0.0009) | 0.0001 (0.0001–0.0003) | <0.001* | 0.414 |
| HFext | 0.0018 (0.0008–0.0035)†‡ | 0.0002 (0.0001–0.0010) | 0.0002 (0.0001–0.0005) | <0.001* | 0.367 |
| RMSSD | 57.97 (41.83–109.35)†‡ | 24.37 (10.14–67.29) | 36.37 (18.71–50.55) | 0.005* | 0.194 |
| pNN50 | 24.31 (12.61–41.24)†‡ | 2.62 (0.26–22.00) | 6.88 (0.85–9.29) | <0.001* | 0.283 |
| SDSD | 58.02 (41.91–109.59)†‡ | 24.41 (10.16–67.44) | 36.44 (18.74–50.57) | 0.005* | 0.194 |
| SDNN | 68.13 (52.55–130.75)†‡ | 41.38 (17.41–82.03) | 36.77 (21.25–45.90) | 0.001* | 0.276 |
| LF/HF | 1.44 (1.12–1.91) | 1.78 (0.91–2.39) | 1.36 (0.68–2.19) | 0.650 | 0.016 |
| LF/HFext | 1.32 (1.03–1.61) | 1.41 (0.80–1.75) | 0.85 (0.43–1.60) | 0.303 | 0.044 |
| MHR | 72.49 (65.87–79.05) | 70.50 (64.44–76.62) | 72.61 (62.23–85.50) | 0.687 | 0.014 |

*$p < 0.05$.
†Different to octogenarian.
‡Different to centenarian.
*Values are expressed as median and (1st quartile–3rd quartile). LF, low frequency; LFn, normalized LF; LFn_ext, extended LFn; HF, high frequency; HFext, extended HF; RMSSD, root mean square of successive differences; pNN50, percentage of RR intervals which exceed 50 ms from the previous one; SDSD, SD of successive differences; SDNN, SD of the RR series; LF/HF, ratio between LF and HF; LF/HFext, ratio between LF and HFext; MHR, mean heart rate; p, p-value for Kruskal-Wallis test; ES, effect size.*

**TABLE 3** | Survival prognosis in centenarians (*N* = 14).

| | LF | LFn | LFn_ext | HF | HFext | RMSSD | pNN50 | SDSD | SDNN | LF/HF | LF/HFext | MHR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\rho$ | 0.423 | 0.172 | 0.295 | 0.190 | 0.232 | 0.304 | 0.247 | 0.304 | 0.536 | 0.214 | 0.251 | –0.082 |
| *p* | 0.131 | 0.557 | 0.305 | 0.516 | 0.426 | 0.290 | 0.395 | 0.290 | 0.048* | 0.463 | 0.386 | 0.782 |

*$p < 0.05$. *LF, low frequency; LFn, normalized LF; LFn_ext, extended LFn; HF, high frequency; HFext, extended HF; RMSSD, root mean square of successive differences; pNN50, percentage of RR intervals which exceed 50 ms from the previous one; SDSD, SD of successive differences; SDNN, SD of the RR series; LF/HF, ratio between LF and HF; LF/HFext, ratio between LF and HFext; MHR, mean heart rate; $\rho$, Spearman's correlation coefficient with "time to death"; p, p-value for Pearson's correlation coefficient.*

## HRV Measures

When analyzing the main parasympathetic HRV variables (RMSSD, pNN50, HF, and SDSD; Malik et al., 1996; Laborde et al., 2017), a clear decrease with age is observed in all of them, with very remarkable differences between young adults and older adults but without significant differences between octogenarians and centenarians. This age-related decrease can be appreciated in the illustrative examples of **Figure 1** and has already been reported by other authors (Umetani et al., 1998; Bonnemeier et al., 2003; Abhishekh et al., 2013). Also, to the best of our knowledge, this is the first time that it has been described in centenarians. It should be emphasized that our results differ from those of Paolisso et al., Zulfiqar et al., and Almeida-Santos et al. since they establish a parasympathetic nadir at 75–80, 70–79, and 60–69 years, respectively, and our study indicates that parasympathetic HRV variables continue to decrease in centenarians (Piccirillo et al., 1998; Zulfiqar et al., 2010; Almeida-Santos et al., 2016). There are several possible explanations for our sample of centenarians not showing a reversal of the decrease in parasympathetic function. First, erratic rhythms may have a confounding effect on age-related changes in parasympathetic HRV indices (Nicolini et al., 2012), which is why our study only analyzes RR segments free of erratic patterns, allowing the presence of no more than 10 outlier values in each 3-min window of analysis. Second, although the BMI of the octogenarian sample was significantly higher, BMI had no effect as a confounding variable ($p > 0.05$ in the multiple linear regression). Some studies have found reduced HRV in underweight and overweight adult women (Triggiani et al., 2017; Gerardo et al., 2019), but studies in the literature investigating the oldest old are scarce. The lower BMI in the centenarian sample could be an indicator of healthy body composition but also a simple consequence of age-associated sarcopenia or osteoporosis. On the other hand, previous studies in centenarians have been very restrictive in the selection of subjects, including only very healthy and independent subjects (Piccirillo et al., 1998; Paolisso et al., 1999; Zulfiqar et al., 2010), which may involve a selection bias (Tan et al., 2019). The parasympathetic decrease in centenarians found in our study could, thus, be more representative of a natural exhaustion of allostatic systems related to age.

As already mentioned, the interpretation of the standard HF band (0.15–0.4 Hz) is compromised when respiratory rate does not fall within this band (9–24 bpm). Since breathing was not monitored, power in the extended HF band (0.15-half MHR) was computed to account for respiratory rates that

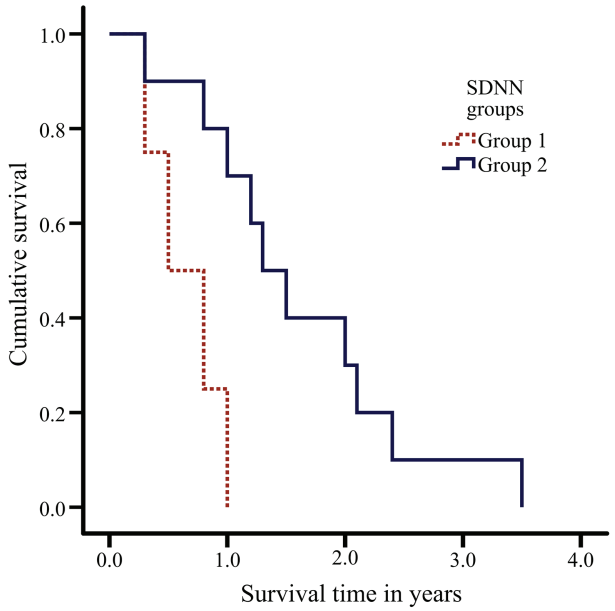



**FIGURE 2 |** Kaplan-Meier curves depicting association between survival in centenarians ($N$ = 14) and SDNN groups based on a cut point of 19 ms.

might exceed 24 bpm, as suggested in Bailón et al., 2007. As it can be seen in **Table 2**, results of the standard HF band were parallel to those of the extended HF band, suggesting that in this database, respiratory rates were in the standard HF band (0.15–0.40 Hz). Therefore, HF could be considered as a measure of the vagal tone (Laborde et al., 2017).

SDNN can be considered as an indicator of global autonomic regulation, although it has been claimed that in short-term recordings, the primary source of its variations is parasympathetically-mediated RSA (Shaffer and Ginsberg, 2017). In agreement with previous studies (Zulfiqar et al., 2010; Almeida-Santos et al., 2016; Sammito and Böckelmann, 2016), SDNN values decline with age, further reflecting an age-dependent decline in ANS activity. LF results were in the same line as the parasympathetic HRV variables and SDNN, probably because the recording was made while sitting upright during resting and under these conditions the primary contributors to HRV have been suggested to be related to parasympathetic and baroreflex activity rather than to sympathetic activity (Shaffer and Ginsberg, 2017).

Other HRV variables, LFn and LF/HF, whose physiological interpretation is usually controversial, have been additionally investigated in our study, neither of them showing statistically significant differences between groups.

## HRV and Survival Prognosis in Centenarians

In recent decades, HRV has been confirmed as a strong, independent predictor of morbidity and all-cause mortality (Billman, 2011; Kemp et al., 2017). To investigate HRV variables that may be associated with survival prognosis in centenarians, we followed up subjects until death. Only SDNN showed significant correlation ($\rho$ = 0.536, $p$ = 0.048) with survival prognosis in centenarians. The group of centenarians with low SDNN values presented five times greater mortality risk than centenarians with high SDNN values. In the framework of the research topic "Horizon 2030: Innovative Applications of Heart Rate Variability," we discuss about HRV and exceptional longevity. However these results should be read with perspective, as the sample of centenarians followed until death is heterogeneous in gender, including 4 men and 10 women.

Since Kleiger et al. set the basis for the use of HRV in post-acute myocardial infarction risk stratification in 1987, SDNN is considered as a "gold standard" when recorded over a 24-h period. SDNN values below 50 ms are classified as unhealthy, 50–100 ms as compromised health, and above 100 ms as healthy (Kleiger et al., 1987). According to Bilchick et al. (2002), each 10-ms increase in SDNN confers a 20% decrease in risk of mortality. SDNN is the only variable presenting significant correlation with time to death in our cohort of centenarians. In particular, SDNN <19 ms turns out to be indicative of early mortality (≤1 year). Of note, one subject presented a value of SDNN of 110 ms and was the one who lived the longest time (3.5 years) calculated from the time point when RR was recorded.

It should be noted that there are other RR-derived variables that have been related to increased mortality risk in the literature. The fact that they have not been found to be associated with time to death in our study could be due to the small sample of our cohort or to the particular characteristics of the studied centenarians. A classic example is high resting HR (Zhang et al., 2015). Additionally, a recent meta-analysis has established LF/HF ratio and SDNN as two of the variables with greater potential as predictors of mortality (Sen and McGill, 2018). Shimizu et al. (2002) have also observed the relevance of LF/HF ratio in a cohort of 27 centenarians. Finally, LF is one of the most controversial HRV indices in the literature. Some studies, such as the Framingham Heart Study, have associated a 1-SD decrement in LF with 1.70 times greater hazard for all-cause mortality (Tsuji et al., 1994). On the other hand, cross-sectional studies in healthy centenarians have reported that high LF values are associated with increased mortality risk (Piccirillo et al., 1998; Paolisso et al., 1999).

## Centenarians and the "Neurovisceral Integration Across a Continuum of Time" Framework

Centenarians are considered to be a model of healthy and successful aging. It is well known that exceptional longevity is a partially inheritable phenotype that could be explained in 20–35% by the genetic load (Rea et al., 2016). Consequently, it could be that the ANS of the centenarians had a greater and innate adaptation level, and therefore they will take 20 years more than the general population to reach a level of depletion of the allostatic systems related to mortality. On the other hand, another feasible explanation would be that centenarians

have healthy behaviors that allow them to experience a less marked decrease in the function of the ANS. Non-genetic factors, including diet, physical activity, health habits, and psychosocial factors contribute approximately 50% of the variability in human lifespan (Rea et al., 2016).

Recently, Kemp et al. (2017) published a theoretical framework called "Neurovisceral Integration Across a Continuum of Time (NIACT)" where they propose that the function of the vagus nerve, indexed by resting-state HRV, plays a regulatory role on a variety of allostatic systems, therefore contributing to an increase or decrease in the risk of future morbidity and mortality. NIACT proposes that while age decreases vagal function, there are many interventions that may be applied to contend such decreases including health behavior, meditation, and positive psychological interventions (Kemp et al., 2017). Health behaviors related to improvements in HRV are similar to those that characterize the lifestyle of centenarians in different populations: regular physical activity, dietary habits, no drinking, and no smoking (Ozaki et al., 2007; Kim et al., 2012; Wu et al., 2017). But psychological moments are also a key element in the NIACT framework, and in the same way, active engagement in community activities, high levels of self-perceived well-being, and satisfaction with life are defining elements of the centenarian population (Ozaki et al., 2007; Kim et al., 2012; Wu et al., 2017; Hitchcott et al., 2018; Yorgason et al., 2018). Therefore, the characteristic lifestyle of centenarians would imply a greater resilience, indexed by greater variability of the HR and, as described above, higher SDNN values would mean better survival prognosis in centenarians.

### Strengths and Limitations

The main strength of the present study is the exceptionality of the sample, considering than being centenarian is a rare phenotype (17.3 centenarians per 100.000 inhabitants; Teixeira et al., 2017). Secondly, centenarians were followed up to death and our study proposes SDNN <19 ms as a cutoff point to define a marker of early mortality (≤1 year), which is obtained from short-term measurements, thus more suitable for ambulatory care and patient monitoring. Given its ease of recording, short-term variability allow measurements under homogeneous conditions, enabling the control of confounding factors and the reproducibility of the study (Li et al., 2019). Moreover, RR measurements were acquired using a validated device and processed with methods that allow better identification of the erratic patterns.

On the other hand, one of the main limitations was gender heterogeneity in the centenarian group, with 76.5% of our centenarians being women. A meta-analysis has highlighted that women show greater vagal activity compared to men, noting the following possible etiological factors: estrogen, oxytocin, and neural control (Koenig and Thayer, 2016). Our sample, however, is very similar to the overall centenarian population in Europe (83.5% women; Teixeira et al., 2017), and sex differences have been reported to disappear in the last age decades, especially in short-term HRV, presumably by the hormonal restructuring especially caused by the menopause in women (Bonnemeier et al., 2003; Voss et al., 2015; Koenig and Thayer, 2016). Indeed, when the statistical analysis of our study was performed by excluding men ($N$ = 4) from the sample of centenarians, results were similar to those reported in **Table 2**. Finally, recording conditions should be taken into account before generalizing the results. For example, paced breathing was not considered in our work. Under resting conditions, the respiratory rate (9–24 bpm) is expected to be in the 0.15–0.40 Hz band (Shaffer and Ginsberg, 2017), but processing should account for the possibility that the respiratory rate goes outside this frequency band, as performed in the present work. A within-subject repeated measure design would have contributed to assess the reproducibility of our evaluations. In future research, more representative samples of centenarians would allow to confirm the results obtained by this study.

In conclusion, HRV indices reflecting parasympathetic outflow (RMSSD, pNN50, HF, and SDSD) as well as SDNN and LF all present an age-related reduction, which could be representative of a natural exhaustion of allostatic systems related to age. Moreover, low SDNN values (<19 ms) are indicative of early mortality (≤1 year) in centenarians. HRV seems to play a role in exceptional longevity, which could be accounted for by centenarians' exposome.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of the University Hospital of Alcorcón (ID of the approval: 16/50). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

NG designed the overall study. AS-L, GR-R, and AH-V collected the data. All authors contributed equally in the interpretation and analysis of the data, revision of manuscript for important intellectual content and have read and approved the final version.

## FUNDING

This work was supported by the European Research Council under grant agreement ERC-StG 638284, by Ministerio de Ciencia e Innovación (Spain) through projects PID2019-105674RB-I00 and RTI2018-097723-B-I00, and by European Social Fund (EU) and Aragón Government through BSICoS group (T39_20R) and projects LMP124-18, LMP24-18 and LMP44-18: Programa Operativo Fondo Europeo de Desarrollo Regional Aragón

2014-2020 "Construyendo Europa desde Aragón". Computations were performed by the ICTS NANBIOSIS (HPC Unit at University of Zaragoza). AH-V is supported by Ministerio de Educación Cultura y Deporte (grant number FPU16/05879).

## ACKNOWLEDGMENTS

We would like to thank the collaboration of all the subjects, families, and caregivers who participated in the study.

## REFERENCES

Abhishekh, H. A., Nisarga, P., Kisan, R., Meghana, A., Chandran, S., Raju, T., et al. (2013). Influence of age and gender on autonomic regulation of heart. *J. Clin. Monit. Comput.* 27, 259–264. doi: 10.1007/s10877-012-9424-3

Almeida-Santos, M. A., Barreto-Filho, J. A., Oliveira, J. L. M., Reis, F. P., da Cunha Oliveira, C. C., and Sousa, A. C. S. (2016). Aging, heart rate variability and patterns of autonomic regulation of the heart. *Arch. Gerontol. Geriatr.* 63, 1–8. doi: 10.1016/j.archger.2015.11.011

Bailón, R., Laguna, P., Mainardi, L., and Sörnmo, L. (2007). "Analysis of heart rate variability using time-varying frequency bands based on respiratory frequency." in *Annual International Conference of the IEEE Engineering in Medicine and Biology – Proceedings*. August 22-26, 2007; 6675–6678.

Bailón, R., Laouini, G., Grao, C., Orini, M., Laguna, P., and Meste, O. (2011). The integral pulse frequency modulation model with time-varying threshold: application to heart rate variability analysis during exercise stress testing. *IEEE Trans. Biomed. Eng.* 58, 642–652. doi: 10.1109/TBME.2010.2095011

Berntson, G. G., Cacioppo, J. T., and Quigley, K. S. (1993). Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology* 30, 183–196. doi: 10.1111/j.1469-8986.1993.tb01731.x

Bilchick, K. C., Fetics, B., Djoukeng, R., Gross Fisher, S., Fletcher, R. D., Singh, S. N., et al. (2002). Prognostic value of heart rate variability in chronic congestive heart failure (Veterans affairs' survival trial of antiarrhythmic therapy in congestive heart failure). *Am. J. Cardiol.* 90, 24–28. doi: 10.1016/S0002-9149(02)02380-9

Billman, G. E. (2011). Heart rate variability – a historical perspective. *Front. Physiol.* 2:86. doi: 10.3389/fphys.2011.00086

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26. doi: 10.3389/fphys.2013.00026

Bonnemeier, H., Wiegand, U. K. H., Brandes, A., Kluge, N., Katus, H. A., Richardt, G., et al. (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects. *J. Cardiovasc. Electrophysiol.* 14, 791–799. doi: 10.1046/j.1540-8167.2003.03078.x

Christensen, K., McGue, M., Petersen, I., Jeune, B., and Vaupel, J. W. (2008). Exceptional longevity does not result in excessive levels of disability. *Proc. Natl. Acad. Sci. U. S. A.* 105, 13274–13279. doi: 10.1073/pnas.0804931105

Cohen, J. (1992). A power primer. *Psychol. Bull.* 112, 155–159. doi: 10.1037/0033-2909.112.1.155

Craft, N., and Schwartz, J. B. (1995). Effects of age on intrinsic heart rate, heart rate variability, and AV conduction in healthy humans. *Am. J. Physiol. Heart Circ. Physiol.* 268, 1441–1452. doi: 10.1152/ajpheart.1995.268.4.H1441

de Rezende Barbosa, M. P. C., da Silva, N. T., de Azevedo, F. M., Pastre, C. M., and Vanderlei, L. C. M. (2016). Comparison of Polar® RS800G3™ heart rate monitor with Polar® S810i™ and electrocardiogram to obtain the series of RR intervals and analysis of heart rate variability at rest. *Clin. Physiol. Funct. Imaging* 36, 112–117. doi: 10.1111/cpf.12203

Eckberg, D. L. (1997). Sympathovagal balance: a critical appraisal. *Circulation* 96, 3224–3232. doi: 10.1161/01.CIR.96.9.3224

Gerardo, G. M., Williams, D. W. P., Kessler, M., Spangler, D. P., Hillecke, T. K., Thayer, J. F., et al. (2019). Body mass index and parasympathetic nervous system reactivity and recovery following graded exercise. *Am. J. Hum. Biol.* 31:e23208. doi: 10.1002/ajhb.23208

Heathers, J. A. J. (2014). Everything Hertz: methodological issues in short-term frequency-domain HRV. *Front. Physiol.* 5:177. doi: 10.3389/fphys.2014.00177

Hernando, D., Garatachea, N., Almeida, R., Casajús, J. A., and Bailón, R. (2016). Validation of heart rate monitor Polar RS800 for heart rate variability analysis during exercise. *J. Strength Cond. Res.* 32, 716–725. doi: 10.1519/JSC.0000000000001662

Hitchcott, P. K., Fastame, M. C., and Penna, M. P. (2018). More to Blue Zones than long life: positive psychological characteristics. *Health Risk Soc.* 20, 163–181. doi: 10.1080/13698575.2018.1496233

Kemp, A. H., Koenig, J., and Thayer, J. F. (2017). From psychological moments to mortality: a multidisciplinary synthesis on heart rate variability spanning the continuum of time. *Neurosci. Biobehav. Rev.* 83, 547–567. doi: 10.1016/j.neubiorev.2017.09.006

Kim, H., Lee, T., Lee, S., Kim, K., Lee, S., Kam, S., et al. (2012). Factors associated with ADL and IADL dependency among Korean centenarians: reaching the 100-year-old life transition. *Int. J. Aging Hum. Dev.* 74, 243–264. doi: 10.2190/AG.74.3.e

Kleiger, R. E., Miller, J. P., Bigger, J. T., and Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 59, 258–282. doi: 10.1016/0002-9149(87)90795-8

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Li, K., Rüdiger, H., and Ziemssen, T. (2019). Spectral analysis of heart rate variability: time window matters. *Front. Neurol.* 10:545. doi: 10.3389/fneur.2019.00545

Malik, M., Camm, A. J., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., et al. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Mateo, J., and Laguna, P. (2000). Improved heart rate variability signal analysis from the beat occurrence times according to the IPFM model. *IEEE Trans. Biomed. Eng.* 47, 985–996. doi: 10.1109/10.855925

Mateo, J., and Laguna, P. (2003). Analysis of heart rate variability in the presence of ectopic beats using the heart timing signal. *IEEE Trans. Biomed. Eng.* 50, 334–343. doi: 10.1109/TBME.2003.808831

Nicolini, P., Ciulla, M. M., Asmundis, C. D. E., Magrini, F., and Brugada, P. (2012). The prognostic value of heart rate variability in the elderly, changing the perspective: from sympathovagal balance to chaos theory. *Pacing Clin. Electrophysiol.* 35, 621–637. doi: 10.1111/j.1540-8159.2012.03335.x

Ozaki, A., Uchiyama, M., Tagaya, H., Ohida, T., and Ogihara, R. (2007). The Japanese centenarian study: autonomy was associated with health practices as well as physical status. *J. Am. Geriatr. Soc.* 55, 95–101. doi: 10.1111/j.1532-5415.2006.01019.x

Paolisso, G., Manzella, D., Barbieri, M., Rizzo, M. R., Gambardella, A., and Varricchio, M. (1999). Baseline heart rate variability in healthy centenarians: differences compared with aged subjects (>75 years old). *Clin. Sci.* 97, 579–584. doi: 10.1042/cs0970579

Piccirillo, G., Bauco, C., Cinti, A. M., Michele, D., Fimognari, F. L., Cacciafesta, M., et al. (1998). Power spectral analysis of heart rate in subjects over a hundred years old. *Int. J. Cardiol.* 63, 53–61. doi: 10.1016/S0167-5273(97)00282-9

Piccirillo, G., Fimognari, F. L., Viola, E., and Marigliano, V. (1995). Age-adjusted normal confidence intervals for heart rate variability in healthy subjects during head-up tilt. *Int. J. Cardiol.* 50, 117–124. doi: 10.1016/0167-5273(95)93680-Q

Rea, I. M., Dellet, M., and Mills, K. I. (2016). Living long and ageing well: is epigenomics the missing link between nature and nurture? *Biogerontology* 17, 33–54. doi: 10.1007/s10522-015-9589-5

Reyes del Paso, G. A., Langewitz, W., Mulder, L. J. M., van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Salvioli, S., Capri, M., Santoro, A., Raule, N., Sevini, F., Lukas, S., et al. (2008). The impact of mitochondrial DNA on human lifespan: a view from studies on centenarians. *Biotechnol. J.* 3, 740–749. doi: 10.1002/biot.200800046

Sammito, S., and Böckelmann, I. (2016). Reference values for time- and frequency-domain heart rate variability measures. *Heart Rhythm.* 13, 1309–1316. doi: 10.1016/j.hrthm.2016.02.006

Sen, J., and McGill, D. (2018). Fractal analysis of heart rate variability as a predictor of mortality: a systematic review and meta-analysis. *Chaos* 28:072101. doi: 10.1063/1.5038818

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shaffer, F., and Venner, J. (2013). Heart rate variability anatomy and physiology. *Biofeedback* 41, 13–25. doi: 10.5298/1081-5937-41.1.05

Shimizu, K., Arai, Y., Hirose, N., Yonemoto, T., and Wakida, Y. (2002). Prognostic significance of heart rate variability in centenarians. *Clin. Exp. Hypertens.* 24, 91–97. doi: 10.1081/CEH-100108719

Tan, J. P. H., Beilharz, J. E., Vollmer-Conna, U., and Cvejic, E. (2019). Heart rate variability as a marker of healthy ageing. *Int. J. Cardiol.* 275, 101–103. doi: 10.1016/j.ijcard.2018.08.005

Teixeira, L., Araújo, L., Jopp, D., and Ribeiro, O. (2017). Centenarians in Europe. *Maturitas* 104, 90–95. doi: 10.1016/j.maturitas.2017.08.005

Triggiani, A. I., Valenzano, A., Ciliberti, M. A. P., Moscatelli, F., Villani, S., Monda, M., et al. (2017). Heart rate variability is reduced in underweight and overweight healthy adult women. *Clin. Physiol. Funct. Imaging* 37, 162–167. doi: 10.1111/cpf.12281

Tsuji, H., Venditti, F. J., Manders, E. S., Evans, J. C., Larson, M. G., Feldman, C. L., et al. (1994). Reduced heart rate variability and mortalit risk in an elderly cohort. The Framingham Heart Study. *Circulation* 90, 878–883. doi: 10.1161/01.CIR.90.2.878

Umetani, K., Singer, D. H., McCraty, R., and Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. Coll. Cardiol.* 31, 593–601. doi: 10.1016/S0735-1097(97)00554-8

Varon, C., Lazaro, J., Bolea, J., Hernando, A., Aguilo, J., Gil, E., et al. (2018). Unconstrained estimation of HRV indices after removing respiratory influences from heart rate. *IEEE J. Biomed. Health Inform.* 23, 2386–2397. doi: 10.1109/JBHI.2018.2884644

Voss, A., Schroeder, R., Heitmann, A., Peters, A., and Perz, S. (2015). Short-term heart rate variability – influence of gender and age in healthy subjects. *PLoS One* 10:e0118308. doi: 10.1371/journal.pone.0118308

Wu, T., Lu, L., Luo, L., Guo, Y., Ying, L., Tao, Q., et al. (2017). Factors associated with activities of daily life disability among centenarians in rural Chongqing, China: a cross-sectional study. *Int. J. Environ. Res. Public Health* 14:1364. doi: 10.3390/ijerph14111364

Yorgason, J. B., Draper, T. W., Bronson, H., Nielson, M., Babcock, K., Jones, K., et al. (2018). Biological, psychological, and social predictors of longevity among Utah centenarians. *Int. J. Aging Hum. Dev.* 87, 225–243. doi: 10.1177/0091415018757211

Zhang, D., Shen, X., and Qi, X. (2015). Resting heart rate and all-cause and cardiovascular mortality in the general population: a meta-analysis. *CMAJ* 188, E53–E63. doi: 10.1503/cmaj.150535

Zulfiqar, U., Jurivich, D. A., Gao, W., and Singer, D. H. (2010). Relation of high heart rate variability to healthy longevity. *Am. J. Cardiol.* 8, 1181–1185. doi: 10.1016/j.amjcard.2009.12.022

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 25 September 2020
doi: 10.3389/fnins.2020.561186

# Heart Rate Variability in the Perinatal Period: A Critical and Conceptual Review

*Marco Chiera[1,2†], Francesco Cerritelli[1*†], Alessandro Casini[1], Nicola Barsotti[1,2], Dario Boschiero[3], Francesco Cavigioli[4], Carla G. Corti[5] and Andrea Manzotti[1,4,6]*

[1] Research and Assistance for Infants to Support Experience Lab, Foundation Center for Osteopathic Medicine Collaboration, Pescara, Italy, [2] Research Commission on Manual Therapies and Mind-Body Disciplines, Società Italiana di Psico Neuro Endocrino Immunologia, Rome, Italy, [3] BioTekna – Biomedical Technologies, Venice, Italy, [4] Neonatal Intensive Care Unit, "V. Buzzi" Children's Hospital, Azienda Socio Sanitaria Territoriale Fatebenefratelli-Sacco, Milan, Italy, [5] Pediatric Cardiology Unit-Pediatric Department, Azienda Socio Sanitaria Territoriale Fatebenefratelli-Sacco, Milan, Italy, [6] Research Department, SOMA, Istituto Osteopatia Milano, Milan, Italy

Neonatal intensive care units (NICUs) greatly expand the use of technology. There is a need to accurately diagnose discomfort, pain, and complications, such as sepsis, mainly before they occur. While specific treatments are possible, they are often time-consuming, invasive, or painful, with detrimental effects for the development of the infant. In the last 40 years, heart rate variability (HRV) has emerged as a non-invasive measurement to monitor newborns and infants, but it still is underused. Hence, the present paper aims to review the utility of HRV in neonatology and the instruments available to assess it, showing how HRV could be an innovative tool in the years to come. When continuously monitored, HRV could help assess the baby's overall wellbeing and neurological development to detect stress-/pain-related behaviors or pathological conditions, such as respiratory distress syndrome and hyperbilirubinemia, to address when to perform procedures to reduce the baby's stress/pain and interventions, such as therapeutic hypothermia, and to avoid severe complications, such as sepsis and necrotizing enterocolitis, thus reducing mortality. Based on literature and previous experiences, the first step to efficiently introduce HRV in the NICUs could consist in a monitoring system that uses photoplethysmography, which is low-cost and non-invasive, and displays one or a few metrics with good clinical utility. However, to fully harness HRV clinical potential and to greatly improve neonatal care, the monitoring systems will have to rely on modern bioinformatics (machine learning and artificial intelligence algorithms), which could easily integrate infant's HRV metrics, vital signs, and especially past history, thus elaborating models capable to efficiently monitor and predict the infant's clinical conditions. For this reason, hospitals and institutions will have to establish tight collaborations between the obstetric, neonatal, and pediatric departments: this way, healthcare would truly improve in every stage of the perinatal period (from conception to the first years of life), since information about patients' health would flow freely among different professionals, and high-quality research could be performed integrating the data recorded in those departments.

**Keywords: autonomic nervous system, vagus, newborns, preterm infants, neonatology, NICU, photoplethysmography, HRV**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Martin Gerbert Frasch,*
*University of Washington,*
*United States*
*Karin Schiecke,*
*Friedrich Schiller University Jena,*
*Germany*

****Correspondence:***
*Francesco Cerritelli*
*francesco.cerritelli@gmail.com*

*† These authors have contributed equally to this work*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *11 May 2020*
***Accepted:*** *28 August 2020*
***Published:*** *25 September 2020*

***Citation:***
*Chiera M, Cerritelli F, Casini A, Barsotti N, Boschiero D, Cavigioli F, Corti CG and Manzotti A (2020) Heart Rate Variability in the Perinatal Period: A Critical and Conceptual Review. Front. Neurosci. 14:561186. doi: 10.3389/fnins.2020.561186*

## INTRODUCTION

The neonatology field is growing in complexity (Biban, 2010). In essence, newborns can show many comorbidities associated with prematurity (WHO, 2016), labor complications (Tribe et al., 2018), and maternal and perinatal stress (Frasch et al., 2007; Babenko et al., 2015; Lobmaier et al., 2020), whereas the neonatal intensive care units (NICUs) are increasing the use of technology to better take care of fetuses, newborns, and infants (Biban, 2010; Chock et al., 2015).

However, many obstacles need to be overcome to efficiently assess and manage infants' conditions: distress, pain, and sepsis need valid and reliable gauges to detect them before they happen (Cremillieux et al., 2018; Rashwan et al., 2019), but several procedures may be time-consuming (Jeng et al., 2000; Als et al., 2005; Cremillieux et al., 2018), invasive, and painful with short- and long-term negative consequences (Holsti et al., 2006; Pillai Riddell et al., 2015).

In the last 40 years, heart rate variability (HRV) has emerged as a reliable and non-invasive measure to monitor preterm and term newborns (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). HRV evaluates the heart rate (HR) fluctuation—the variability of the time intervals between successive heartbeats—, and during the years, more and more techniques have appeared to improve its analysis, from which several metrics can be extracted (**Table 1**; Bravi et al., 2011; Thiriez et al., 2015; Javorka et al., 2017; Oliveira et al., 2019b; Patural et al., 2019).

Several studies showed that HRV correlates with the newborn's stress and stress-related behaviors (Gardner et al., 2018; Hashiguchi et al., 2020), and that it could predict the baby's overall wellbeing and future neurological development. HRV could also accurately identify short- and long-term complications, such as the risk of sepsis (Javorka et al., 2017; Oliveira et al., 2019b; Kumar et al., 2020). HRV was also able to reveal the impact of prenatal stress on fetal brain development (Frasch et al., 2007; Lobmaier et al., 2020).

Despite these results, HRV is still underused in NICUs. Although in the 1960s, one of the first evidences published was about the alteration of HRV metrics preceding fetal distress (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996); to date, some authors argued that there is a lack of understanding of the meaning of HRV metrics in infants: in fact, HRV has been studied especially in adults, and the autonomic nervous system (ANS) behaves differently in newborns, especially in preterm infants (Joshi et al., 2019b). Notwithstanding these contradictions, the only successful example of integration of HRV in NICUs is the Heart Rate Observation (HeRO) monitor developed by J. Randall Moorman's team. The HeRO analyzes bedside electrocardiogram (ECG) in real-time and integrates various HRV metrics to calculate the "HRC index," which can predict the risk of sepsis within 24 h in both preterms and very low birth weight infants (Andersen et al., 2019; Kumar et al., 2020).

However, based on the available literature and on the potential research to be developed, there is a need to further explore the use of HRV in neonatology.

HRV may provide such useful insights since it correlates with the ANS development and functioning. The ANS regulates organic development and connects with the organism's ability to cope with stressors, as well as with cognitive and emotional development (Thayer et al., 2012; Jennings et al., 2015; Schneider et al., 2018; Oliveira et al., 2019b).

To be an innovative tool useful for neonatologists, HRV measurement should rely on a technology that gives reliable metrics with a clear clinical meaning. Harnessing the positive experiences, such as the use of the HeRO monitor, it is paramount to create a system that continuously records HRV and expresses scores that could correlate with the baby's clinical condition and help monitor its evolution (Zhao et al., 2016; Hayano and Yuda, 2019; Pernice et al., 2019b; Kumar et al., 2020).

For this purpose, modern machine learning (ML) and artificial intelligence (AI) algorithms could play a crucial role: through their computational power, they could define models capable of managing the complex physiological interactions between HRV, ANS, and the whole organism, thus boosting our ability to predict the infant's prognosis. Indeed, we already have experiences about the clinical usefulness of ML in both neonatology (Semenova et al., 2018; Ostojic et al., 2020) and HRV analysis (Chiew et al., 2019; Lin et al., 2020).

ML/AI algorithms could also integrate clinical data of different hospital departments, i.e., obstetric, neonatal, and pediatric. Indeed, free clinical data and medical devices sharing among the departments involved in the perinatal care (from conception to the first years of life) would allow clinicians to better understand the prenatal and developmental factors underlying adverse neonatal outcomes (e.g., brain injury) and to better treat them.

Therefore, the present paper aims to address the HRV usefulness in neonatology to prospect it as an innovative tool in the years to come. This focused review is divided into three sections: (1) the first section describes briefly the HRV metrics and examines the relationship between ANS and HRV in fetuses and newborns; (2) the second section examines the technology available in the NICU, how to monitor HRV efficiently, and the usefulness of real-time HRV; and (3) the third and final section will summarize the main findings and outline future perspectives for the clinical use of real-time HRV in the neonatal field, with a brief subsection about its usefulness in low-income countries.

**Abbreviations:** AI, artificial intelligence; AMP, amplitude fluctuations; ANS, autonomic nervous system; AS, active sleep; BSG, ballistography; CAN, central autonomic network; CAP, cholinergic anti-inflammatory pathway; CIMVA, continuous individual multiorgan variability analysis; CNS, central nervous system; COMP, complexity; CPAP, continuous positive airway pressure; CTG, cardiotocography; ECG, electrocardiogram; fABAS, fetal autonomic brain age scale; fECG, fetal electrocardiogram; fHRV, fetal heart rate variability; fMCG, fetal magnetocardiography; FSE, fetal scalp electrode; GA, gestational age; HeRO, Heart Rate Observation system; HIE, hypoxic–ischemic encephalopathy; HR, heart rate; HRC, heart rate characteristics; HRV, heart rate variability; IUGR, intrauterine growth restriction; ML, machine learning; NEC, necrotizing enterocolitis; NIPE, newborn infant parasympathetic evaluation; PNS, parasympathetic nervous system; PPG, photoplethysmography; QS, quiet sleep; PNS, parasympathetic nervous system; PRSA, phase rectified signal averaging; PRV, pulse rate variability; RSA, respiratory sinus arrhythmia; SIDS, sudden infant death syndrome; SNS, sympathetic nervous system; $SpO_2$, partial oxygen saturation; vPPG, non-contact video-photoplethysmography.

**TABLE 1 |** The most common HRV metrics (Hoyer et al., 2013, 2019; Uhrikova et al., 2015; Pichot et al., 2016; Massaro et al., 2017; Shaffer and Ginsberg, 2017; Herry et al., 2019; Oliveira et al., 2019b; Patural et al., 2019; Frasch et al., 2020).

| Metric | Unit | Definition |
|---|---|---|
| HR | bpm | HR (number of heart beats per minute) |
| **Time-domain** | | |
| SDNN | ms | Standard deviation of NN intervals |
| SDRR | ms | Standard deviation of RR intervals |
| SDANN | ms | Standard deviation of the average NN intervals for each 5 min segment of a 24 h HRV recording |
| SDNN Index | ms | Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording |
| NNx | | Number of adjacent NN intervals that differ from each other by more than x ms (e.g., 5, 20, 25, and 50 ms) |
| pNNx | % | Percentage of successive NN intervals that differ by more than x ms (e.g., 5, 20, 25, and 50 ms) |
| RMSSD | ms | Root mean square of consecutive RR interval differences |
| SDSD | ms | Standard deviation of consecutive RR differences |
| **Frequency-domain** | | |
| ULF Power | $ms^2$ | Absolute power of the ultra-low-frequency band ($\leq$0.003 Hz) |
| VLF Power | $ms^2$ | Absolute power of the very-low-frequency band (0.0033–0.04 Hz) |
| LF Peak | Hz | Peak frequency of the low-frequency band (0.04–0.2 Hz for newborns and 0.04–0.15 Hz for infants) |
| LF Power | $ms^2$ | Absolute power of the low-frequency band (0.04–0.2 Hz for newborns and 0.04–0.15 Hz for infants) |
| | Nu | Relative power of the low-frequency band (0.04–0.2 Hz for newborns and 0.04–0.15 Hz for infants) in normal units |
| | % | Relative power of the low-frequency band (0.04–0.2 Hz for newborns and 0.04–0.15 Hz for infants) |
| HF Peak | Hz | Peak frequency of the high-frequency band (0.20–2.00 Hz for newborns and 0.20–1.40 Hz for infants) |
| HF Power | $ms^2$ | Absolute power of the high-frequency band (0.20–2.00 Hz for newborns and 0.20–1.40 Hz for infants) |
| | Nu | Relative power of the high-frequency band (0.20–2.00 Hz for newborns and 0.20–1.40 Hz for infants) in normal units |
| | % | Relative power of the high-frequency band (0.20–2.00 Hz for newborns and 0.20–1.40 Hz for infants) |
| LF/HF | % | Ratio of LF-to-HF power |
| VLF/LF | % | Ratio between very-low (0.02–0.08 Hz) and low (0.08–0.2 Hz) frequency band power |
| LFn | | Normalized power in the low-frequency band of the ECG spectrogram (0.04–0.2 Hz for newborns and 0.04–0.15 Hz for infants), i.e., low-frequency power in relation to total power |
| HFn | | Normalized power in the high-frequency band of the ECG spectrogram (0.20–2.00 Hz for newborns and 0.04–0.15 Hz for infants), i.e., high-frequency power in relation to total power |
| Total power (TP) | $ms^2$ | Total power of the ECG spectrogram |
| **Non-linear** | | |
| S | ms | Area of the ellipse that represents total HRV |
| SD1 | ms | Poincaré plot standard deviation perpendicular the line of identity |
| SD2 | ms | Poincaré plot standard deviation along the line of identity |
| CSI | % | Cardiac Sympathetic Index—SD1/SD2 |
| CVI | | Cardiac Vagal Index—log (SD1*SD2) |
| SVT | | Short-term variability of consecutive beat-to-beat data obtained through Poincaré analysis |
| LTV | | Long-term variation of consecutive beat-to-beat data obtained through Poincaré analysis |
| HRV Triangular Index | | Integral of the density of the RR interval histogram divided by its height |
| HRV Index | | Number of all RR intervals divided by the number of RR intervals at the highest point of the RR histogram |
| TINN | ms | Triangular Interpolation of the NN Interval Histogram—the length of the basis of the minimum square difference of the triangular interpolation for the highest value of the RR histogram or the normalized width of the base of the RR histogram |
| Parseval Index | | Ratio between the square root of the sum of LF and HF powers and the value of SDNN |
| %DET | | Percentage of determinism of a time series from recurrence quantification analysis (RQA): it detects the predictability of dynamical systems |
| ShanEn | | Shannon Entropy—uncertainty of a random variable |
| ApEn | | Approximate entropy, which measures the regularity and complexity of a time series |
| SampEn | | Sample entropy, which measures the regularity and complexity of a time series |
| MSEx | | Multiscale entropy at coarse graining level x |
| gMSE(x) | $bit_{norm}$ | Generalized multiscale entropy at coarse graining level x of NN interval series |
| QSE | | Quadratic Sample Entropy |
| KLPE | | Kullback–Leibler permutation entropy |
| AC | | Acceleration capacity (detection of sequences of two successive RR beats that decrease) obtained through PRSA |
| DC | | Deceleration capacity (detection of sequences of two successive RR beats that increase) obtained through PRSA |

*(Continued)*

**TABLE 1** | Continued

| Metric | Unit | Definition |
|---|---|---|
| ACstx | | Acceleration capacity, slope, and step value at coarse graining level x |
| DFA α1 | | Detrended fluctuation analysis, which describes short-term fluctuations |
| DFA α2 | | Detrended fluctuation analysis, which describes long-term fluctuations |
| DFA $\alpha_S$ | | Detrended fluctuation analysis, which describes short-term fluctuations |
| DFA $\alpha_L$ | | Detrended fluctuation analysis, which describes long-term fluctuations |
| $RMS_S$ | | Root mean square from detrended fluctuation analysis, which describes short-term fluctuations |
| $RMS_L$ | | Root mean square from detrended fluctuation analysis, which describes long-term fluctuations |
| SDLE α | | Scale-dependent Lyapunov exponent slope |
| Skewness | a.u. | Skewness of NN interval series |
| AsymI | | Multiscale time irreversibility asymmetry index: it is the degree of temporal asymmetry and lack of invariance of the statistical properties of a signal |
| $D_2$ | | Correlation dimension, which estimates the minimum number of variables required to construct a model of system dynamics |

# THE ANS PHYSIOLOGY UNDERLYING HRV AND ITS USE IN THE NEONATAL FIELD

## A Brief Introduction on HRV Metrics

From the work of the Task Force for HRV analysis in 1996, which constitutes the foundation for the majority of the papers on HRV, many metrics are developed to describe HRV, and they can be classified into three categories: time-domain, frequency-domain, and non-linear metrics (**Table 1**). These metrics can be obtained from short-term (less than 10 min) or long-term ECG recording (24 h) (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996).

The time-domain metrics are considered to be the simplest methods to extract, since they are directly based on the normal-to-normal (NN) intervals or on the instantaneous HR extracted from the ECG. The time-domain metrics are then calculated through statistical or geometric methods. The statistical methods consist in applying operations, such as mean, standard deviation, or square root, on: the direct NN interval measurements, thus obtaining metrics, such as standard deviation of RR (SDRR), standard deviation of NN (SDNN), standard deviation of the average NN (SDANN), and standard deviation of NN (SDNN) Index, and the difference between NN intervals, thus obtaining metrics, such as root mean square of consecutive RR interval differences (RMSSD), NN50, pNN50, and pNN20 (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Oliveira et al., 2019b; Patural et al., 2019).

The geometric methods consist in transforming the NN interval measurements into a geometric pattern, such as the sample density distribution of the NN intervals (or their difference), from which metrics, such as the triangular interpolation of the NN (TINN), the HRV Triangular Index, and the HRV Index, can be obtained. A Poincaré plot can also be used to plot each NN interval in relation to the previous NN interval and to calculate the standard deviation of the main cluster of data-points, either crosswise (SD1) or lengthwise (SD2) (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Oliveira et al., 2019b; Patural et al., 2019). From the SD1 and SD2 metrics, other metrics, such as Cardiac Sympathetic Index (CSI) and Cardiac Vagal Index (CVI), could be obtained (Oliveira et al., 2019b).

The frequency-domain metrics derive from the analysis of the ECG power spectrum. The power of several frequency bands, in particular ultra-low-frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF), are then extracted, as well as other metrics, such as LF and HF normalized indices (LFn and HFn) and the LF/HF ratio. Another frequency index used is the total power (TP), which represents the global variability (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Patural et al., 2019).

Time- and frequency-domain metrics are, however, limited: they could fail in discriminating among different signals that display similar characteristics, such as the same mean or standard deviation. Moreover, ECG recording can manifest several irregularities in the RR series or complex oscillatory phenomena that linear metrics cannot properly describe. Therefore, non-linear analyses were developed to better grasp the complexity behind the brain and ANS influences on HRV (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Bravi et al., 2011). Actually, the geometric methods can be considered a kind of non-linear analysis (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Javorka et al., 2017; Patural et al., 2019).

Several studies showed the usefulness of non-linear metrics over linear ones in evaluating the autonomic development (Uhrikova et al., 2015; de Souza Filho et al., 2019; Oliveira et al., 2019b), the interactions between ANS, brain, and stress axis (Thayer and Lane, 2000), and the infant's adaptation capacity (Gonçalves et al., 2017; Shaffer and Ginsberg, 2017; Urfer-Maurer et al., 2018).

Many non-linear metrics can be calculated, and every non-linear analysis can be performed at different levels of complexity. Examples are: detrended fluctuation analysis (DFA), sample entropy (SampEn), approximate entropy (ApEn), multiscale entropy (MSE), symbolics dynamics, coarse graining spectral

analysis, fractal analysis, and deceleration and acceleration analyses (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Javorka et al., 2017; Patural et al., 2019).

While linear metrics seem more useful when the HR follows periodic oscillations, non-linear metrics, such as SampEn, ApEn, MSE, DFA, and symbolic dynamics metrics, seem more robust to cardiac recording artifacts (Javorka et al., 2017; Stapelberg et al., 2017). Linear and non-linear metrics can, however, detect complementary physiological behaviors (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Bravi et al., 2011): the HRC index, which contains both, represents the best available evidence of their usefulness (Fairchild and O'Shea, 2010).

Actually, many more different techniques of variability analysis are available. To encompass all of them and to refine the original Task Force classification, further classifications have been proposed. Bravi and colleagues, for example, regrouped the various techniques into five domains based on the information they extrapolate: statistical, geometric, energetic, informational, and invariant (Bravi et al., 2011). The present paper, however, will follow the Task Force classification since it still represents the most used.

Although several HRV metrics have been proved useful, few metrics have a precise physiological and clinical interpretation (**Table 2**). However, we have to consider two crucial limitations: (1) too often HRV is viewed as influenced by only the ANS, with the tendency of reducing a metric to as simply sympathetic or parasympathetic, and (2) although many studies investigate several metrics, the attention is focused mainly on univariate approaches that try to reduce the variables considered at a minimum. This approach could substantially limit the usefulness of the models applied and, thus, the understanding of HRV physiological nature and clinical usefulness (Bravi et al., 2011; Frasch, 2018).

To overcome these limitations, multivariate analyses were introduced—e.g., continuous individual multiorgan variability analysis (CIMVA) (Seely et al., 2011; Frasch et al., 2014)—, and many researchers started to rely on ML and AI algorithms (Semenova et al., 2018; Vassar et al., 2020). As a consequence, research begun to integrate various metrics—as shown by the HeRO monitor, whose HRC index derives from a logistic regression applied on the standard deviation of the RR intervals, sample asymmetry, and SampEn and aims to detect pathological heart decelerations to predict the risk of sepsis within 24 h (Fairchild and O'Shea, 2010)—, to combine measurements from different organs for predicting clinical conditions (Kumar et al., 2020), and to define models able to describe HRV as a phenomenon influenced by the whole organism (Frasch, 2020).

## The HRV as a Window on the ANS

The ANS plays a central role in homeostasis maintenance and allostatic adaptation, allowing the organism to change its behavior based on the circumstances and stressors, whether internal or external (Thayer and Lane, 2000).

The two ANS branches, the sympathetic (SNS) and parasympathetic system (PNS), continuously modify their balance to finely regulate the organic systems, including the cardiovascular, respiratory, gastroenteric, metabolic, and immune-inflammatory ones (Rees, 2014; Mulkey and du Plessis, 2019). The two ANS branches connect with several brain circuits known as the central autonomic network (CAN), which influences both the ANS and the higher cortical functions, such as cognition control, emotional regulation, and behavior (Benarroch, 1993; Thayer and Lane, 2000).

To cope with the environmental stimuli, the CAN regulates the complex non-linear interaction between SNS and PNS. In the sinoatrial node, this interaction modulates the HRV (Billman, 2011; Shaffer and Ginsberg, 2017). Tools, such as ECG or photoplethysmography (PPG), which monitor cardiovascular activity, can thus evaluate autonomic brain–heart interactions and give information regarding the ANS through HRV metrics (**Table 2**).

These metrics are usually viewed as revealing different facets of the SNS and PNS activities, with some metrics more related to one of the two branches and the other reflecting more complex ANS, cardiocirculatory, and respiratory activities (Shaffer and Ginsberg, 2017; Oliveira et al., 2019b). However, HRV may reflect much more than the autonomic regulation of cardiac activity. On the one hand, it has been recently discovered, in ovine models, that the fetal heart has already an intrinsic sinoatrial node activity that can affect HRV and that can be affected by adverse conditions (e.g., chronic hypoxia) in the last trimester of pregnancy (Frasch et al., 2020). On the other hand, HRV seems to be greatly influenced by information coming from the whole organism (e.g., the gut or the immune system) through systemic afferent pathways, such as the vagus nerve (Frasch, 2020).

Moreover, HRV may represent an index of the adaptive regulation processes performed by the CAN (Thayer et al., 2009, 2012). Indeed, HRV correlates with CAN activity measured by functional magnetic resonance imaging (Thayer et al., 2012) and several measures of cognitive, emotional, and behavioral regulation (Holzman and Bridgett, 2017; Forte and Casagrande, 2019). Since various HRV metrics showed to be correlated with stress and inflammation, HRV analysis has the potential to give information about the subject's health (Kim et al., 2018; Williams et al., 2019).

During fetal and neonatal life, the ANS undergoes a prolonged process of development and maturation, during which it remains vulnerable to developmental disruption from a variety of stressful physiological and environmental stimuli. Such stimuli—which can occur during pregnancy (e.g., congenital disease, fetal growth restriction, maternal stress/nutritional deficiency), labor (e.g., premature, complicated, or prolonged birth), and even in the NICU environment (e.g., invasive procedures, loud noise, and bright light)—can significantly influence the developmental trajectory of both the ANS and the CAN. They can reduce the newborn's capacity to efficiently adapt to a continually changing and challenging environment (Mulkey and du Plessis, 2019).

**TABLE 2 |** Interpretations and/or usefulness of some HRV metrics (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Shaffer et al., 2014; Shaffer and Ginsberg, 2017; Oliveira et al., 2019b).

| Metric | Interpretation |
|---|---|
| **Most common metrics** | |
| RMSSD | Main time-domain measure to assess the HRV modulation due to vagal activity |
| SDNN | Standard metric of overall HRV, influenced by both SNS and PNS. Gold standard for medical stratification of cardiac risk in adults when recorded over a 24 h period |
| ULF | No consensus regarding the mechanisms underlying ULF power. Very slow-acting biological processes, such as circadian rhythms, are implicated |
| VLF | Related to the heart's intrinsic nervous system, which generates VLF rhythm when afferent sensory cardiac neurons are stimulated. SNS activity due to physical and stress responses influences its oscillations amplitude and frequency |
| LF | Non-specific index that reflects baroreceptor activity, it contains contributions of both the sympathetic and parasympathetic influences |
| HF | Expression of parasympathetic activity, it corresponds to the HR variations related to the respiratory cycle known as RSA. It changes according to vagal modulation but does not reflect vagal tone |
| LF/HF | Used to estimate SNS and PNS balance, although LF does not purely represent SNS, and PNS and SNS interact in a complex non-linear manner |
| **Complex metrics or groups of metrics** | |
| fABAS | Scale used for evaluating fetal ANS maturation. It derives from the integration of Hoyer et al. (2013):<br>• amplitude (ACTAMP20), evaluating the fluctuation range of heart beat intervals above an approximated baseline increasing complexity;<br>• skewness, evaluating the complexity of heart rate patterns essentially modulated by complex sympatho-vagal rhythms;<br>• gMSE(3), evaluating the asymmetry, contribution of vagal and sympathetic activity with their different time constants, decline of decelerations, and formation of acceleration patterns;<br>• pNN5, evaluating the formation of vagal rhythms;<br>• VLF/LF, evaluating the baseline fluctuation in relation to sympatho-vagal modulations. |
| DFA $\alpha$1 – Asyml – KLPE – SDLE $\alpha$ | Used for assessing vagal modulation in fetuses (Herry et al., 2019) |
| HRC index | Displayed by the HeRO monitor to estimate the risk of sepsis within 24 h. It derives from a logistic regression calculated on standard deviation of the RR intervals, sample asymmetry, and SampEn to detect irregularities and transient decelerations in HR. HRC index is higher in preterm infants than in full term ones (they show less variability), and it decreases as postmenstrual age increases. HRC index can also rise due to acute inflammation, respiratory deterioration, intraventricular hemorrhage, brain injury, NEC, surgery, ventilation, and drugs, such as anticholinergics, anesthetics, and dexamethasone (in this last case, HRC decreases) (Fairchild and O'Shea, 2010; Fairchild, 2013; Kumar et al., 2020) |
| $RMS_S$ – $RMS_L$ – DFA $\alpha_S$ – LF – HF | Used to estimate outcomes in case of HIE, especially during hypothermia treatment. Low values of $RMS_S$, $RMS_L$, DFA $\alpha_S$, and LF and a high value of HF may predict an adverse outcome and the need of adjuvant neuroprotective therapies. These metrics could discriminate among different types of brain injury. All these metrics decreased also proportionally to NEC severity (Metzler et al., 2017; Al-Shargabi et al., 2018; Campbell et al., 2018) |

It is thus essential to know how ANS develops during pregnancy and after birth, and how HRV changes as a result, in order to make efficient use of HRV as an assessment tool.

## The ANS Development and Its Relationship With HRV

Fetal cardiac activity is usually measured through cardiotocography (CTG), which can monitor fetal HR via ultrasound waves (Ayres-de-Campos and Bernardes, 2010). Although able to detect HR accelerations and decelerations, this tool poorly discriminates QRS complexes and is unsuitable to measure fetal HRV (fHRV), especially short-term HRV (Van Leeuwen et al., 2014). Therefore, in the last 20 years, researchers have relied on fetal magnetocardiography (fMCG) and ECG (fECG) to monitor fHRV and assess its correlation with ANS development (Hoyer et al., 2013, 2015).

fECG and fMCG use electrophysiological mechanisms to monitor cardiac activity and better discriminate QRS complexes (Hoyer et al., 2017). fMCG, which is obtained through the spatiotemporal measurement of the heart-related magnetic field, allows a stable and precise HRV assessment during the second and third pregnancy trimesters. However, fMCG is available in a few centers worldwide due to its high cost (Hoyer et al., 2017). fECG can be obtained through electrodes placed on the mother's abdomen (Hoyer et al., 2017) or directly on the fetal cranium during labor (Warmerdam et al., 2016). fECG performs less accurate measurement and, between 28 and 32 weeks gestational age (GA), is less reliable since the fetus is almost entirely covered by the vernix caseosa, which isolates the fetus hindering the recording (Hoyer et al., 2017). Besides, fECG shows a considerable signal loss (30 $\pm$ 24%) with 3.6 $\pm$ 1.7 gaps/min, which limits short-term and entropy-related HRV metrics measurements. As a result, fECG shows higher values for these metrics than fMCG (Van Leeuwen et al., 2014).

Using fMCG, Hoyer and colleagues defined the fetal autonomic brain age score (fABAS) (**Table 2**), which derives from a multivariate analysis of five different metrics—amplitude, gMSE(3), skewness, pNN5, and VLF/LF—and managed to assess ANS development from the 22nd week GA (Hoyer et al., 2013, 2015). Other authors have then proposed to add other metrics to fABAS to define more complete and precise models (Schmidt et al., 2018).

Applying regression models on metrics grouped in short-term and long-term amplitude fluctuations (AMP), complexity (COMP) and pattern categories, Hoyer et al. found that ACst1, STV, MSE4, and skewness were the metrics that best correlated with fetal maturation (Hoyer et al., 2019). During the third trimester, AMP, COMP, and pattern metrics increase, although with different timing (Hoyer et al., 2019), together with sympathetic and parasympathetic modulations. The baseline HR also becomes more regular and with a more stable rhythm (Schneider et al., 2018). As GA increases, even linear metrics—RMSSD, SDNN, LF, and HF—rise as well. These changes indicate the ANS development and better fetal regulatory capacities (Hoyer et al., 2009; Cardoso et al., 2017; Aye et al., 2018).

To achieve an optimal maturation, the ANS, in particular the PNS, needs 37 weeks GA (Patural et al., 2019): indeed, preterm newborns show a sympathetic predominance (Yiallourou et al., 2012). The PNS begins to develop during the first trimester, when the lateral hypothalamus differentiates and the vagus nerve myelination increases (Koutcherov et al., 2003; Cheng et al., 2004). From the 32nd week GA, the vagus nerve regulatory activity increases with the appearance of the baroreflex mechanisms and the respiratory sinus arrhythmia (RSA)—the capacity of HR regulation based on respiratory rate. Meanwhile, fetal movements and HR increase indicate a rise in the SNS activity (DiPietro et al., 1996). The ANS regulatory function, especially the parasympathetic, develops until birth when the ANS has to react to the new environmental conditions by adapting the cardiovascular and respiratory systems during the fetal–neonatal transition (Mulkey and du Plessis, 2018).

At birth, the ANS shows a slight sympathetic predominance—high LF and low HF—, but in the first 24 h after birth, several metrics (e.g., HR, SDNN, CSI, HFn, LFn, TP, SD2, HRV Index, and Parseval Index) change significantly (Oliveira et al., 2019b). In particular, during the first 12 h, the PNS gradually begins to take over, as shown by the LF/HF ratio reduction (Patural et al., 2019).

During the first days and weeks of life, both preterm and term newborns show an increase in RMSSD, SDNN, and HF, although preterm newborns show lower HRV than term ones at the same postmenstrual age: they display lower values of RMSSD, LF, and HF and a higher LF/HF ratio, which likely reflect an ANS that needs more time to mature (Lucchini et al., 2016; Cardoso et al., 2017; Aye et al., 2018).

The ANS, in particular the PNS, continues to develop during the first 2 years of life considerably: indeed, every time, frequency, geometric, and non-linear metric tend to increase (Patural et al., 2019).

Since many HRV metrics change during the ANS development, several authors investigated if specific correlations could be established between those changes and the fetal health status.

According to Hoyer et al. (2019), some AMP and COMP metrics correlate with maternal lifestyle (smoking and physical activity), intrauterine growth restriction (IUGR), and gestational diabetes. Despite the maternal and fetal circulation being distinct systems, HRV fluctuations could represent a coupling mechanism between the two: indeed, maternal HR, TP, and HF correlate with some fHRV metrics, especially during active sleep and when GA > 32 weeks. In particular, maternal HR correlates positively with fHR and negatively with fHRV (Zöllkau et al., 2019).

A different approach based on coupling maternal and fetal HR through phase rectified signal averaging (PRSA) analysis found a correlation between the two HRs, but only for stressed mothers (Lobmaier et al., 2020). A correlation between some fHRV metrics and maternal TP and RMSSD was also found in normotensive mothers, whereas in preeclamptic pregnancies, there was no correlation despite substantial reduction in both maternal HRV (SDNN, RMSSD, pNN50, TP, VLF, LF, HF) and fHRV metrics (SDNN, RMSSD, pNN50, TP, VLF, LF, HF, STV, LTV, N° of accelerations) (Lakhno, 2017).

During labor, although uterine contractions may alter fHRV recording, fetal scalp electrode (FSE) can measure fHRV with adequate precision and give more accurate information about fetal distress than traditional CTG, thus better discriminating between healthy fetuses and those with acidosis (Gonçalves et al., 2006; van Laar et al., 2010, 2011; Spilka et al., 2012; Abry et al., 2013; Georgieva et al., 2013). pH levels during labor were correlated with both linear (Gonçalves et al., 2006; van Laar et al., 2010) and non-linear fHRV metrics (Li et al., 2005; Ferrario et al., 2006; Abry et al., 2013; Chudacek et al., 2014; Signorini et al., 2014), and the combination of linear and non-linear metrics seemed to detect acidosis more precisely than the two types of metrics alone (Spilka et al., 2012; Warmerdam et al., 2018). In addition to FSE, trans-abdominal fECG with a sampling frequency of 900 Hz—way higher than the frequency of 4 Hz used for CTG—may efficiently measure fHRV during labor and predict neonatal pH level and acid–base balance, especially through CIMVA (Frasch et al., 2014; Li et al., 2015). These results are of great significance since acid–base imbalance can be correlated to brain neuroinflammation and, therefore, to the risk of developing brain injury (Xu et al., 2014).

Together with continuous fHRV assessment, the selective measurement and comparison among the fHRV metrics obtained during labor contractions and the ones obtained between successive contractions managed to better detect fetal distress (Warmerdam et al., 2018). Several fHRV metrics increased during contractions and decreased during rest periods (Romano et al., 2006; Cesarelli et al., 2010; Warmerdam et al., 2016), and this variability was lower in fetuses with acidosis than in healthy ones, pointing at a lower cardiovascular adaptation capacity (Warmerdam et al., 2016). Entropy metrics, such as SampEn and ApEn, could also detect fetal distress from the 30th week GA (Ferrario et al., 2006). These two metrics, together with time-domain, complexity (e.g., Lempel Ziv complexity), and PRSA-derived ones, could also distinguish between fetuses with and without IUGR (Signorini et al., 2014).

Non-linear metrics could explain why male fetuses show higher HRV than female but are at higher risk of comorbidity

(DiPietro and Voegtline, 2017): while linear metrics are higher in male newborns, non-linear metrics that evaluate entropy are higher in female newborns (Gonçalves et al., 2017; Spyridou et al., 2018). Moreover, non-linear metrics, such as ApEn or MSE, could describe better than linear metrics the ANS development that happens around the last weeks of gestation, when the fetus prepares for birth (Spyridou et al., 2018).

Interesting results came also from animal models of fetal inflammation. Studies on models of hypoxic–ischemic events in ovine fetuses showed that many fHRV metrics changed in the hours after the event: VLF decreased, whereas SampEn, HF, and RMSSD increased (Frasch et al., 2016; Kasai et al., 2019). Besides, the rise in RMSSD correlated positively with interleukin (IL)-1β levels (Frasch et al., 2016), whereas lipopolysaccharides administration showed that multidimensional fHRV metrics (i.e., both linear and non-linear) could predict the fetal inflammatory response (Durosier et al., 2015; Herry et al., 2016).

In an ovine model of repetitive umbilical cord occlusions, PRSA applied to fHRV detected hypoxic events and distinguished between mild, moderate, and severe hypoxia–acidemia (Rivolta et al., 2014). This result was deepened in another study where an ML algorithm based on RMSSD time series sampled at 1,000 Hz was developed: after 2 h of training with the individual fHRV during labor, the algorithm predicted fetal cardiovascular decompensation with 92% sensitivity, 86% accuracy, and 92% precision. When using 4 Hz CTG, the algorithm showed a 67% sensitivity, 14% accuracy, and 18% precision—that is, sample frequency really matters (Gold et al., 2019).

fHRV assessment could thus give precious information both on the physiological fetal maturation and on the occurrence of pathologies that, whenever left unresolved, are able to induce lifelong complications (Hoyer et al., 2017; Frasch, 2018). Assessing HRV in newborns and infants could, thus, greatly improve neonatal care (Kumar et al., 2020). Therefore, a discussion is required about the technology used to measure HRV in newborns and infants and about the physiological or environmental factors that could influence HRV.

# A TECHNOLOGICAL ANALYSIS TO EFFICIENTLY MONITOR AND USE HRV

## The Current NICU Monitoring Instruments

The NICU incubators are closed devices equipped with advanced devices to monitor the newborn's conditions. In essence, they guarantee a clean and controlled environment concerning temperature, humidity, and even mechanical noise and light. They monitor vital signs through sensors and electrodes placed on the babies (Rajalakshmi et al., 2019).

The foremost vital signs—temperature, blood pressure, pulse frequency, HR, and $SpO_2$—are measured through monitors for the control of pressure, cardiorespiratory system, skin temperature, and carbon dioxide and oxygen concentrations; pulse oximetry; and mechanical ventilator. In the case of anomalies, an alarm alerts the NICU staff to intervene promptly (Rajalakshmi et al., 2019).

ECG represents the gold standard to monitor HR calculated through the RR interval (Phillipos et al., 2016; Alonzo et al., 2018). Pulse oximetry can instead measure HR through the pulse rate, but its usefulness is debated: evaluating pulse rate is difficult when peripheral tissue perfusion is reduced (Mizumoto et al., 2012), and the measurement is sensitive to motion artifacts (Sahni et al., 2003).

Pulse oximetry performs a simple, non-invasive, and accurate $SpO_2$ measurement to evaluate the risk of hypoxia and/or hyperoxemia, the appropriateness of the oxygen administered to the newborn, and the presence of ductal dependent congenital cardiac diseases (Stenson, 2016; Kumar et al., 2020). Pulse oximetry, however, struggles to detect hyperoxemia when $SpO_2 > 94\%$ (Hay, 1987).

Blood pressure (influenced by cardiac output and peripheral vascular resistance) can be measured through an external cuff, which, however, can underestimate it in hypotensive or pathological preterm newborns (Cunningham et al., 1999). A peripheral arterial catheter can continuously monitor blood pressure, although it can induce severe cardiovascular complications (Werther et al., 2018).

The respiratory rate is calculated through two electrodes placed on the thorax and the abdomen that measure the thoracic impedance changes. Plethysmography gives a more reliable measure but requires a higher number of thoracic electrodes (Weese-Mayer et al., 2000).

Temperature is continuously monitored because preterm newborns struggle to regulate it and can suffer from hypothermia. Incubators can assess the newborn's temperature through skin sensors and probes or infrared thermography, which has the advantage of being contactless and non-invasive, and may adapt the environmental temperature to the newborn's skin temperature (Verklan and Walden, 2014). Due to the sensitive skin of newborns, cutaneous sensors could create stress and even damage the skin (Topalidou et al., 2019).

Despite continuous monitoring to assure high care standards, much of this collected information remains unused. This situation is worth noting since integrating these vital signs could improve the predictive power of the NICU monitors and better help reduce infants' mortality (Sullivan et al., 2018; Joshi et al., 2020; Kumar et al., 2020).

Besides monitoring the infant's clinical condition, incorporating the infant's history would also be of the utmost importance. Information about the baby's fetal health, labor, and past adverse events (e.g., brain injuries) could augment the interpretation of the aforementioned vital signs. In fact, different pathologies in different babies could induce similar alterations in vital signs, and the knowledge of the infant's history could help recognize what is happening (Kumar et al., 2020).

In this difficult task, NICU monitors based on ML algorithms could be useful for clinicians: integrating the infant's past and present data, they could discriminate among the several pathologies that could affect the infant, thus pointing at the best preventive and therapeutic strategy. ML algorithms have

already helped in predicting the risk of various conditions (e.g., cerebral hemorrhage and hyperbilirubinemia) and in reducing false alarms, thus improving neonatal care (Daunhawer et al., 2019; Malacova et al., 2020; Ostojic et al., 2020; Turova et al., 2020; Vassar et al., 2020).

## The Current State of HRV Assessment and Limitations

The gold standard to evaluate HRV consists of specific calculations on the electrocardiographic HR registrations (time elapsed between two adjacent R peaks) in term and preterm newborns (Kevat et al., 2017).

However, ECG does not seem to be always used properly, and as a consequence, HRV measurement may suffer from methodological issues: for instance, the NICU tools used to monitor HR may have a low sampling frequency (<200 Hz), making the HRV analysis unreliable under several circumstances (e.g., patients with low RR variability due to heart failure), in particular for frequency and non-linear metrics. Some authors indeed advised to use a sampling frequency of at least 500 Hz (Laborde et al., 2017; Shaffer and Ginsberg, 2017). Moreover, in the research field, there is the tendency to use proprietary software without specifying the exact algorithms used to extract the HRV parameters and, thus, limiting the usefulness of the results (Heathers, 2012; Pagani et al., 2012).

Particularly interesting is the case of the frequency-domain metrics, LF and HF. Despite being widely used, the frequency threshold of 0.15 Hz is used to discriminate between them in adults derived from animals, and it seems that it has never been validated in humans (Hayano and Yuda, 2019). Therefore, the frequency-domain metrics could be biased from the beginning: this could be a reason why LF and HF do not reflect a specific physiologic function, but the interaction between PNS, SNS, and respiratory activity (Hayano and Yuda, 2019). Moreover, not all studies on newborns use the same thresholds for discriminating between LF and HF (Andersen et al., 2019).

Among the several HRV metrics (**Table 1**), few of them have been validated (**Table 2**; Shaffer and Ginsberg, 2017; Hayano and Yuda, 2019), especially in newborns and infants (Cardoso et al., 2017; Oliveira et al., 2019b). For several metrics, their significance can also change according to the measurement duration: short (about 5 min) or long term (until 24 h) (Pernice et al., 2019b).

Therefore, standardizing how researchers and clinicians should monitor HRV and calculate their metrics is essential. A uniform methodology could also help in defining normative HRV values for infants (stratified for their age and other fetal development characteristics)—during the years, several studies attempted to fill this gap (Clairambault et al., 1992; Eiselt et al., 1993; Spassov et al., 1994; Cardoso et al., 2017; Schneider et al., 2018; Oliveira et al., 2019b; Patural et al., 2019; Shuffrey et al., 2019)—to promptly recognize even deadly complications, but results are still controversial.

As with the integration of the baby's vital signs and history, clinicians could rely on ML and AI to overcome this difficulty. ML could both define normative HRV values for single or complex metrics and even create models able to predict how the newborn's condition will evolve based on big-data analysis (Fairchild and Aschner, 2012; Kumar et al., 2020). Indeed, in several research fields including neonatology, neurology, and cardiology, advanced ML is being successfully used to detect specific pathophysiological characteristics (Fraser, 2017; Pinaya et al., 2019; Tu et al., 2020; Turova et al., 2020; Vassar et al., 2020).

## The Use of PPG to Measure HRV as an Alternative to ECG

In the last years, a new methodology that uses PPG has been applied to measure the peripheral pulse rate variability (PRV) through sensors placed on the fingers (Blackford et al., 2016; Choi and Shin, 2017). The growing interest toward PPG rests on its simplicity, low-cost, safety, and minimal invasiveness and on its capacity to assess signs, such as oxygen saturation, and to extract cardiorespiratory parameters (Pernice et al., 2018, 2019a; Singh et al., 2018).

PPG is an optical measurement technology used to detect blood volume changes in peripheral capillaries. It needs a light source and a photodetector that can measure the light intensity variations related to the capillary perfusion changes. PPG is used in physiological and clinical research to measure blood pressure variability (McDuff et al., 2018; Pernice et al., 2018, 2019b).

The absence of electrodes helps reduce the newborn's stress and pain. It may also decrease the risk of limb ischemia and skin lesions due to electrode-related irritation and, thus, the risk of skin infections with subsequent use of antibiotics and disinfectants, which can alter the cerebral pressure and perfusion when inhaled (Blanik et al., 2016; Zhao et al., 2016; Cobos-Torres et al., 2018). ECG alternatives to monitor HRV are paramount since, due to altered evaporation of body water, electrodes on large skin areas may influence the balance between heat and water in preterm newborns who weigh less than 1,000 g (Blanik et al., 2016). Avoiding electrodes would also reduce the workload of NICU professionals since they might avoid controlling the correct positioning of patches (Cobos-Torres et al., 2018).

From a PPG signal, a PRV time series can be registered and analyzed similarly to HRV (Pernice et al., 2018). However, HRV and PRV are different: HRV is calculated from the cardiac electrical activity, whereas PRV from the mechanics of pulsatile blood. PPG and ECG also show different waveforms: they are differently influenced by the sampling frequency (Choi and Shin, 2017).

PPG recording is affected by physiological factors related to the pulse wave transmission along the vascular bed and by measurement errors, such as artifacts due to movements. In the last years, several authors have attempted to reduce or eliminate these errors that can make PRV and HRV disagree (Pernice et al., 2019b). The PRV analysis should also use a correct sampling frequency to obtain measurements concordant with ECG (Choi and Shin, 2017; Hejjel, 2017).

Some authors found that, for several metrics (SDNN, SDSD, RMSSD, NN50, pNN50, TP, HF, LF/HF, LFn, and HFn), a frequency as low as 25 Hz could give comparable results to those obtained with a 10 kHz-sampled ECG in healthy subjects (Choi and Shin, 2017). However, very low sample frequency

could not correctly detect the effect of vagal modulation on HRV/PRV (as it is associated with high frequency), making frequency and non-linear metrics unreliable—time-domain metrics appear instead to be more robust (Choi and Shin, 2017; Béres et al., 2019). Notwithstanding this, other studies used a sample frequency of 200 Hz obtaining reliable results (Sun et al., 2012; Elgendi et al., 2016). Thus it can be argued that the above-mentioned recommendations for ECG could also be valid for PPG.

Most studies consider PRV and HRV to be exchangeable (Blackford et al., 2016; Pernice et al., 2018; Singh et al., 2018), although sometimes different measurements were detected when people (mostly adults) were under postural (45° head-up tilt test) or mental (arithmetic test) stress, especially for RMSSD, LF, HF, and LF/HF (Pernice et al., 2019b).

In the context of NICU, PPG provides accurate data in addition to the ease of use. A recent study showed that, through specific algorithms, PPG could detect the movement artifacts, remove them from the recordings, and use them to evaluate the onset and duration of the newborn's movements: this measurement could help assess the motor development and even the cognitive growth related to motor control (Zuzarte et al., 2019). These elements could favor the use of PPG over other devices. However, some studies showed lack of sufficient data sampling to perform adequate statistical analysis, calling, therefore, for more robust data collection (Kevat et al., 2017; Henry et al., 2020).

In the last years, non-contact video-photoplethysmography (vPPG) has been introduced: a non-invasive optical technology able to remotely detect the blood volume changes, using natural light. A digital camera measures the little cutaneous light intensity changes, which derive from the cardiovascular rhythm of the skin blood perfusion (Blanik et al., 2016; Valenza et al., 2018). vPPG could accurately monitor the newborn's movements (Cobos-Torres et al., 2018) and even help prevent sudden infant death syndrome (SIDS) (Zhao et al., 2016).

Since HR tends to significantly decrease over minutes or hours before SIDS occurrence, a technology that could continuously monitor HR, in particular, during night-time or with low ambient light—SIDS happens especially in these conditions—could efficiently prevent SIDS. Using specific algorithms, vPPG seems to make accurate HR measurements during night-time; besides, since vPPG does not rely on skin sensors, it could avoid fatal errors linked to their detachment due to the baby's movements (Zhao et al., 2016).

From the available evidence on newborns and adults, vPPG and ECG would seem to agree in the detection of HR and several HRV metrics (e.g., LF, HF, SDNN, SampEn, and Lyapunov exponent): the statistical analyses (e.g., Bland–Altman tests and Pearson's r) showed high correlations, and the measurement errors between the two technologies were similar to those between ECG and other technologies, such as pulse oximeter (Blanik et al., 2014; Villarroel et al., 2014; Blackford et al., 2016; Valenza et al., 2018). Other studies found vPPG and PPG to give similar measurements in the assessment of HR, LF, and HF: since PPG is usually considered as reliable as ECG, the authors inferred that vPPG could also agree with ECG (Sun et al., 2012; Aarts et al., 2013; McDuff et al., 2014; Paul et al., 2020). However, more studies on newborns are needed to improve vPPG and to better evaluate its usefulness (Cobos-Torres et al., 2018; Valenza et al., 2018).

Before proceeding further, we briefly mention ballistography (BSG), another contactless and unobtrusive technology that could help HRV assessment. BSG can detect the mechanical forces exerted by the body, including body movements, breathing motion, and heartbeat, through sensors placed in the bedding or mattress under the infant's body or even in the incubator rack. From the mechanical signals recorded by the sensors (e.g., including electromechanical film sensors, load-cells, and accelerometers), BSG successfully extrapolated reliable respiratory waveforms and HR recordings in preterm newborns (Nukaya et al., 2014; Lee et al., 2016; Joshi et al., 2018, 2019a). While in neonatology, BSG represents a new technology that should be improved to optimally and precisely detect the weak forces of the baby's body; in adults, BSG gave reliable HRV measurements as compared with ECG (Shin et al., 2011; Wang et al., 2015).

## Factors That Can Alter HRV Assessment

To optimally use HRV, NICU professionals must be aware there are factors that can influence HRV recording, including the sleep stage, the infant's position, and the NICU/incubator environment (De Jonckheere and Storme, 2019; Weber and Harrison, 2019).

HRV in newborns is often measured during sleep, which shows two main stages: the quiet sleep (QS), characterized by the absence of movements and slow rhythmic breathing, and the active sleep (AS), characterized by myoclonic twitching, facial, eye, and head movements, and irregular heart and breath rates (Walusinski, 2006; Dereymaeker et al., 2017).

AS and QS show, respectively, a sympathetic and a parasympathetic predominance (Frasch et al., 2007; Yiallourou et al., 2012), with HRV metrics, such as SDNN, LF/HF, LF, and TP higher in AS than in QS. Sometimes, parasympathetic-related metrics were found to be higher during AS than QS (although by a lower degree than the sympathetic-related metrics), maybe to balance the sympathetic activity (Yiallourou et al., 2012; Stéphan-Blanchard et al., 2013; Fyfe et al., 2015; Thiriez et al., 2015; Schneider et al., 2018).

If this were the case, altered parasympathetic-related metrics during AS could reveal that the vagal system and the ANS still have to fully develop (Yiallourou et al., 2012). An immature PNS may, thus, fail to balance the SNS and the hypothalamic–pituitary–adrenal axis activity under stressful conditions, thus increasing the risk of sepsis, necrotizing enterocolitis (NEC), or even SIDS (Yiallourou et al., 2012; Stone et al., 2013; Sullivan et al., 2014; Mulkey et al., 2018).

Indeed, healthy newborns show an increase in several metrics during a head-up tilt test, thus recruiting both ANS branches to counter the hypotensive stressor (Yiallourou et al., 2012). Besides, newborns who later displayed abnormalities, such as altered neurological development (i.e., cerebral palsy, language or mental retardation, vision or hearing disability, or attention disorders) showed altered HRV (lower total and non-harmonic power) just during AS (Thiriez et al., 2015).

Although the studies do not perfectly agree (Fyfe et al., 2015), the prone position correlates with lower SDRR and RMSSD (Galland et al., 2006; Lucchini et al., 2016) and also lower sympathetic tone measured through LF and TP (Jean-Louis et al., 2004): these results could indicate an altered ANS response, possibly clarifying the higher risk of SIDS during prone sleep (Elhaik, 2016). The side position seems to stabilize HR and prevent oxygen desaturation during feeding in newborns with GA < 34 weeks (Thoyre et al., 2012), although a recent study failed to find the same results (Raczyńska and Gulczyńska, 2019).

Concerning the NICU environment, temperature, light, and noise could be a significant source of stress that can affect infants' HRV (De Jonckheere and Storme, 2019; Weber and Harrison, 2019).

Warm incubators correlated with higher HR, lower parasympathetic activity, HF, and shorter RR intervals (Franco et al., 2000). When the incubator temperature was 2°C lower than the newborn's skin temperature, the parasympathetic-related metric RMSSD increased in all sleep stages, together with SDNN and HF, whereas the sympathetic-related metric CSI decreased; the opposite findings were obtained when the incubator was 2°C warmer (Stéphan-Blanchard et al., 2013). Therapeutic hypothermia in case of hypoxic–ischemic encephalopathy (HIE) could induce an increase in LF, root mean square from detrended fluctuation analysis, which describes short-term fluctuations ($RMS_S$), root mean square from detrended fluctuation analysis, which describes long-term fluctuations ($RMS_L$), and DFA $\alpha_S$ (Massaro et al., 2017). Extreme variations of environmental or core temperature in both directions can, lastly, destabilize the ANS (as revealed by HRV alteration) and induce possible complications (Fox and Matthews, 1989; Mowery et al., 2011).

Light and sound can alter the newborn's cardiorespiratory functions, increase stress level (Williams et al., 2009; Ozawa et al., 2010; Weber and Harrison, 2019), and modify HRV. Reducing light exposure through eye-mask and covering incubators/cribs can induce a QS with a stable respiratory rate (Shiroiwa et al., 1986; Venkataraman et al., 2018). For sound regulation, positive stimuli, such as human voice, rather than forcefully excluding stressful events, may represent a better option (Weber and Harrison, 2019). The use of tools, such as earmuffs, showed ambiguous results: some studies revealed higher oxygen saturation and quieter sleep, whereas other studies proved higher stress and lower HF (Zahr and de Traversay, 1995; Aita et al., 2013; Almadhoob and Ohlsson, 2020).

The incubator can influence newborns' HRV also through the emission of electromagnetic fields, which indeed correlate with adverse health consequences in infancy (Li et al., 2012). When the incubator power is turned on, LF/HF increases, whereas HF decreases (Bellieni et al., 2008). Positioning the newborn as far away as possible from the incubator power can limit and reverse these effects (Bellieni et al., 2008; Passi et al., 2017).

From the HeRO experience, we also know that some drugs (i.e., dexamethasone, paralytics, anesthetics, and anticholinergics), surgery, and initiation of mechanical ventilation can significantly alter various HRV metrics (Fairchild and O'Shea, 2010; Fairchild, 2013).

If all these factors can impact HRV, thereby biasing its measurement, a real-time monitor could overcome these biases by recording how HRV changes before, during, and after the occurrence of these factors. Again, ML algorithms could help cope with these confounding factors: by weighting and integrating their effects, such technology could help reduce false alarms, exactly as those algorithms that can detect artifacts due to baby's movements (Ostojic et al., 2020).

## The Clinical Usefulness of Real-Time HRV to Monitor Newborns

As with HR and $SpO_2$, HRV could be monitored in real-time to provide information about the infant's current conditions.

Real-time HRV helped identify stress-related behaviors, which are difficult to visually recognize: for example, LF increased during stress behaviors, whereas HF increased with self-consoling behaviors (Gardner et al., 2018). Moreover, real-time HRV monitoring—as revealed by changes in short- and long-term metrics, such as RMSSD, LF/HF, SampEn, and DFA α1 (but also SDNN, LF, HF, SD1, SD2, ApEn, and DFA α2) or in the newborn infant parasympathetic evaluation (NIPE)—correlated with nociceptive events, such as heel stick (Weissman et al., 2012; Butruille et al., 2015).

Derived from short-term HRV metrics related to PNS and reflecting HFn variations, the NIPE aims to assess in real-time the level of the newborn's comfort and nociception (Butruille et al., 2015). Although it failed to correlate with pain scales measuring acute neonatal pain (Cremillieux et al., 2018) and with hemodynamic change following endotracheal intubation in children aged 1–24 months (Zhang et al., 2019), the NIPE succeeded in evaluating prolonged neonatal pain (Buyuktiryaki et al., 2018) and the balance between nociception and antinociception (Weber et al., 2019; Zhang et al., 2019).

Studies that continuously recorded HRV for more than 24 h, or that reviewed those recordings if available, led to discovering that HRV alteration can precede adverse outcomes even by 24 h (Griffin and Moorman, 2001; Stone et al., 2013; Kumar et al., 2020). Many of the studies about the predictive power of real-time HRV, in particular, in case of infections, relied on the HeRO monitor (Andersen et al., 2019; Kumar et al., 2020). The NIPE technology represents another attempt of introducing real-time HRV monitoring in NICUs, but it needs further evidence (Weber et al., 2019; Zhang et al., 2019).

It was revealed that HRV measured with the HRC index decreased 6 h before medical NEC and even 16 h before surgical NEC, thus also indicating the severity of the newborn's condition (Stone et al., 2013). Abnormal heart rate characteristics (HRC) correlated with a several-fold increase in the risk of sepsis, urinary tract infections, and death in the day and week next to alteration (Griffin et al., 2005), and it could also precede the occurrence of SIDS (Zhao et al., 2016). In infants aged 28–35 weeks, low HF (less than 4.68 $ms^2$) was found to correlate with the incidence of NEC stage 2+ (Doheny et al., 2014).

In a recent study, HRV combined with respiratory variability analysis changed significantly during the 24 h before the diagnosis of late-onset sepsis, especially in the last 6 h where real-time

monitoring showed signals of cardiorespiratory instability. The reduction in RMSSD, accompanied by a decrease in average acceleration response (AAR, a robust index evaluating the heart capacity to increase its rhythm) and an increase in respiratory variability-related ApEn, predicted late-onset sepsis the most (Joshi et al., 2020).

The HeRO monitor, allowing NICU professionals to continuously observe the HR characteristics variation, reduced relative mortality due to sepsis even more than 20% (Fairchild, 2013; Kumar et al., 2020), especially when birth weight was lower than 1,000 g (Moorman et al., 2011).

A system that assesses real-time HRV can thus have significant clinical implications. Such a system could display both short- and long-term HRV metrics, thus augmenting HRV predictive usefulness (Voss et al., 2013) and overcoming the limits of short-time metrics, which are highly sensitive to artifacts (Stapelberg et al., 2017). Due to being non-invasive, real-time HRV would also be preferable to other assessment procedures that can cause discomfort to newborns and take time to be performed (De Jonckheere and Storme, 2019).

However, what could be the best way to make HRV an innovative tool that could augment the newborn's management?

# THE FUTURE PERSPECTIVES FOR THE CLINICAL USE OF HRV IN THE NICU

## Technological Innovation to Monitor HRV in Real-Time

HRV monitoring is more accessible and less invasive than other diagnostic and predicting methods, such as blood sampling for measuring inflammatory cytokines and predicting sepsis (Fairchild, 2013). Real-time HRV monitoring could, thus, represent a significant step forward to improve baby care, especially when clinical decision-making is based on the sole experience of NICU professionals (Mowery et al., 2011; Oliveira et al., 2019a; Weber and Harrison, 2019).

Relying upon the instruments already present in NICUs is paramount (Rajalakshmi et al., 2019); in particular, due to the advantages over ECG, PPG could be the instrument to choose, although PPG-based computation can suffer from precision issues compared with ECG-based computation (Kevat et al., 2017; Pernice et al., 2019b; Henry et al., 2020).

For the sake of simplicity and for attracting clinicians to the use of HRV (King, 2020), the first step could be introducing in NICUs a PRV/HRV monitor that shows a single metric able to correlate with the infants' clinical conditions or development. Although the literature shows that the combination of more metrics can perform better than the use of single metrics (as detailed in previous sections), the literature also shows that single metrics can display good performance (more details in the next sections).

In infants, RMSSD correlated with several outcomes in both the short and the long term: ANS stage of development in the case of prematurity (Hoyer et al., 2009; Lucchini et al., 2016; Aye et al., 2018; Schneider et al., 2018), late-onset sepsis (Joshi et al., 2020), extubation success (Latremouille et al., 2018), recovering after admission in intensive care units (Marsillio et al., 2019), pain behavior (Weissman et al., 2012), short-term neurological development when measured during low blood pressure episodes (Semenova et al., 2018), and later neurological development at 2 years (Dimitrijević et al., 2016). RMSSD showed also to change according to the incubator temperature, thus revealing how infant's HRV changes due to environmental temperature (Stéphan-Blanchard et al., 2013).

RMSSD seems a reliable index of HRV modulation by vagal activity (Shaffer and Ginsberg, 2017) and has good statistical properties (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996), and in adult studies, it was associated with adult health more than immune, inflammatory, and metabolic blood markers (Jarczok et al., 2015).

The good relationship with vagal activity makes RMSSD particularly interesting for neonatologists, for three main reasons: (a) the PNS develops during the last weeks of gestation—when preterm birth can occur—and after birth (Schneider et al., 2018; Patural et al., 2019), (b) the PNS activity correlates with the infant's conditions and development (Mulkey and du Plessis, 2019), and (c) since vagal activity correlates positively with inflammation regulation (Fairchild and O'Shea, 2010), RMSSD could help detect newborn's inflammation (as shown in animal fetuses, Frasch et al., 2016). The relation of RMSSD with vagal-related HRV modulation could help clinician in easily interpreting RMSSD changes: the lower the RMSSD, the lower the modulation by the vagal activity, and thus, the worse the infant's conditions.

As a time-domain metric, RMSSD has the advantage over frequency-domain metrics of being a more "direct" measure: RMSSD is obtained by merely calculating the root mean square value of successive time differences between heartbeats (Shaffer and Ginsberg, 2017). Hence, it does not suffer from the conceptual biases that could arise with frequency-domain metrics due to choosing a frequency threshold not validated in humans (Hayano and Yuda, 2019).

Therefore, the NICU real-time monitor could report RMSSD tracking in the previous 24 h, 4–5 min, and also 10–30 s—ultra-short RMSSD recordings are as reliable as short ones (Munoz et al., 2015). The monitor could be equipped with an alarm that activates when the actual RMSSD value changes over a threshold from the newborn's past mean value. The alarm could activate when RMSSD decreases—lower modulation by vagal activity and, thus, risk of worse outcomes—but also when it rises significantly. For instance, an increase in RMSSD could represent pathological conditions, such as severe unconjugated hyperbilirubinemia (in association with low minimum HR) (Özdemir et al., 2018), or the occurrence of an antenatal/intrapartum hypoxia–ischemia that could lead to brain injury (Frasch, 2018; Yamaguchi et al., 2018).

To make HRV a gold standard, however, there is the need to deepen the knowledge about RMSSD or any other metric: more robust studies on larger samples are needed (Stéphan-Blanchard

et al., 2013; De Jonckheere and Storme, 2019) as studies still show contradictory findings that merit careful analysis (Wang et al., 2016; Joshi et al., 2020).

A further crucial step is to integrate HRV signals with other vital signs, such as $SpO_2$ and blood pressure, to improve its predictive power and reduce mortality (Sullivan et al., 2018; Kumar et al., 2020). Even further is the integration of information about the infant's gestation, labor, and perinatal period is essential: since NICU professionals have to deal with complex pathologies, including SIDS (Siren, 2017), technological tools that can elaborate all these types of information could become paramount to improve clinical care (Hemphill et al., 2011).

Therefore, in parallel with introducing a real-time HRV monitoring system, there might be the possibility to start using the tools already present in NICUs to collect a large amount of data about all the babies' vital signs (Kumar et al., 2020). This data could then be analyzed by ML algorithms, whose results the bedside monitors would display as one or more metrics, graphs, or models (Bravi et al., 2011; Hemphill et al., 2011; Seely et al., 2011).

Indeed, several fields in medicine share this need of collecting and using a large amount of data to improve patient care. An example is the neurocritical care where, as any other intensive care unit, "extreme complexity reigns": continuous multimodal monitoring is vital to guide an efficient decision-making process with the purpose of preventing complications and warranting survival, especially in case of emergency (Hemphill et al., 2011; Lara and Püttgen, 2018).

However, this means revolutionizing the NICU itself. First, the data must be stored and automatically controlled for artifacts. Then, all the medical devices have to communicate and to be synchronized with each other to assure the collected data are coherent; they could also connect to a central computer/hub that autonomously synchronizes the data process. Synchronization is essential: otherwise, data interpretation would become flawed. Lastly, clinicians could add laboratory values, imaging results, and medical documentation to the patient's record (Hemphill et al., 2011).

Advanced bioinformatics could then follow with the use of the many available techniques of data mining coming from statistics, ML, and AI. For example, ML or Deep Learning approaches can be used, including the ones based on decision trees and neural networks, especially dynamic Bayesian neural networks, which capture well non-linear phenomena with complex multimodal relationships. Based on the big-data of other patients, such approaches could elaborate the past and present information of the actual patient to predict the evolution of the clinical conditions. Moreover, they could visualize this prediction in more useful ways (e.g., from numerical probability to specific types of graphs) than raw data or simple statistical metrics (Hemphill et al., 2011).

Multimodal monitoring and the efficient application of data mining algorithms in medicine have yet to grow. In fact, many obstacles (e.g., the availability of resources) may hinder such development, but the complexity of intensive care units makes a strong call to action, and the stakeholders in the healthcare systems must be aware of the opportunities informatics could give to clinical care (Hemphill et al., 2011; Lara and Püttgen, 2018).

The stakeholders must also know that it is paramount to establish tight collaborations between the obstetric, neonatal, and pediatric departments: sharing data about patients' health and medical devices among these departments would elicit high-quality research that would help clinicians to correlate neonatal outcomes to their fetal development and past history, thus truly improving neonatal care—otherwise, how would it be possible to treat neonatal pathologies ignoring their fetal origin?

In the next sections, it is argued as HRV analysis could indeed represent a useful tool to help both in decision-making and in monitoring the infant's condition.

## HRV as an Innovative Tool in Neonatal Decision-Making Intervention

Because various metrics, such as RMSSD, relate to HRV modulation by vagal tone, which plays a significant role in infant's condition, HRV monitoring could give reliable information about the infant's level of stress (Mulkey and du Plessis, 2019).

Indeed, since LF and HF inversely correlated with salivary cortisol levels in both healthy and NICU admitted newborns (Hashiguchi et al., 2020), real-time HRV monitoring could assess the actual stress level of newborns. Moreover, since stress- and pain-related behaviors in newborns are difficult to discriminate, real-time HRV could represent a valuable tool to assess both neonatal stress and pain (Cremillieux et al., 2018; Zhang et al., 2019).

As neonatal stress and pain can adversely influence neurodevelopment and brain maturation, as well as future health and longevity (Holsti et al., 2006; Reynolds, 2013; Pillai Riddell et al., 2015; Simeoni et al., 2018), every procedure that can improve the newborn's condition is paramount, especially in case of prematurity or pathological conditions. Knowing when and how to apply such procedures is even more essential: a real-time HRV monitor that gives information about the actual newborn's level of stress could elicit NICU professionals to promptly adjust the NICU environment to improve the newborn's wellbeing and comfort.

Real-time HRV would help nurses to adopt correct behaviors for reducing visual and auditory overstimulation: in a complex environment, such as the NICU, it is indeed difficult to know exactly when to perform these procedures (Aita and Goulet, 2003).

Based on the value of the index shown by the HRV monitor, NICU staff could increase or decrease the temperature of the incubator, change the illumination intensity or type, and introduce positive stimuli: for instance, human voice or songs, even recorded, cloths with parent-scent, eye contact, and gentle touch (Ozawa et al., 2010; Stéphan-Blanchard et al., 2013; Weber and Harrison, 2019).

Real-time HRV could also help choose between "positive" procedures, such as facilitated tucking—manually supporting the baby by holding the upper and lower extremities in flexion—plus human voice or skin-to-skin contact between the mother and the newborn: these procedures, if well

performed, should increase the level of newborn's comfort, and a HRV monitor could be able to show their actual benefit (Alexandre et al., 2013; Butruille et al., 2017; Marvin et al., 2019).

As shown in sections "The ANS Development and Its Relationship With HRV" and " Factors That Can Alter HRV Assessment", HRV metrics could change according to environmental factors. However, this knowledge must be deepened, and the faster way to achieve this result could be introducing HRV monitoring in as many NICUs as possible and by collecting a large amount of data, from which ML applications could extrapolate models or metrics suitable and clinically-sound for the above-mentioned conditions.

A real-time HRV monitor could help understand when newborns need analgesic medications to withstand better invasive and noxious procedures (Weber et al., 2019) or adjuvant neuroprotective therapies in case of HIE—infants who subsequently died due to HIE showed lower LF and higher HF—(Massaro et al., 2014), and it could also help choose better how to administer surfactant in case of respiratory distress syndrome—the NIPE index showed different values according to the technique used (Okur et al., 2019).

In the same way, HRV could guide the choice among various respiratory supports post-extubation, besides predicting the outcome of extubation. Indeed, RMSSD, SDNN, pNN50, TP, and VLF were higher during non-synchronized non-invasive ventilation than during nasal continuous positive airway pressure (CPAP) in extreme preterms who were reintubated a few hours later (Latremouille et al., 2018). Such difference was not found in infants who showed successful extubation, even though every HRV metric analyzed was higher (but not significantly) during ventilation than nasal CPAP. Therefore, the authors speculated that those variations could correlate with the failure in extubation, and that non-invasive ventilation could better modulate the ANS of less stable infants than nasal CPAP (Latremouille et al., 2018). HRV measured as SDRR was also found to increase during high flow nasal cannula, but not during nasal CPAP, in extremely preterm infants who succeeded in being extubated (Latremouille et al., 2019).

## HRV as an Index to Monitor Clinical Improvement

To be an all-round innovative tool, real-time HRV should not only predict future outcomes with good accuracy but should also provide useful information to monitor the current condition of the newborn.

The question is: could HRV help understand whether the baby's condition is deteriorating or improving?

A real-time HRV monitoring system could give a clear answer. Besides, this is another field in which more data and the use of ML software could dramatically increase the clinical usefulness of HRV, and thus another reason to introduce HRV monitors in NICU and to integrate their recordings with the infant's development over time.

HRV—measured through several metrics including HRC index, DFA $\alpha_S$, $RMS_S$, $RMS_L$, LF, and HF—could change greatly in case of pathological conditions, such as congenital heart disease, cardiac failure in persistent ductus arteriosus, respiratory deterioration with apnea, respiratory distress syndrome, inflammation, hyperbilirubinemia, respiratory syncytial virus, and sepsis. Indeed, inflammatory cytokines (e.g., IL-6, IL-8, and IL-13), bacterial toxins, and free bilirubin could pass through the immature brain–blood barrier and exert neurotoxic activity, thus negatively influencing the nervous system. The same negative effect can be induced by the cardiorespiratory pathologies (van Ravenswaaij-Arts et al., 1991; Prietsch et al., 1992; Stock et al., 2010; Raynor et al., 2012; Sullivan et al., 2014; Uhrikova et al., 2015; Al-Shargabi et al., 2017; Mulkey et al., 2020).

Since HRV modifications correlated with inflammatory cytokine levels (Al-Shargabi et al., 2017), real-time HRV could be a useful marker of clinical acuity: indeed, HRV alterations—a decrease in $RMS_S$, $RMS_L$, DFA $\alpha_S$, LF, HF, and TP or an increase in HRC index—correlated with the pathology severity (Sullivan et al., 2014; Al-Shargabi et al., 2018). Variations in LF, HF, and LF/HF correlated with HIE severity, although more studies are needed to resolve conflicting results between studies (Andersen et al., 2019). In a recent paper on a pediatric population, compared with the time of admission in the intensive care units, RMSSD, SDNN, and pNN50 continuously rose as the time of discharge approached, that is, as the infants recovered (Marsillio et al., 2019).

The correlation between HRV alterations, inflammation, and inflammation-related pathologies can be better understood through the cholinergic anti-inflammatory pathway (CAP). Briefly, the CAP begins with the afferent vagus nerve that detects inflammatory cytokines or bacterial toxins and transmits these signals to several brain nuclei, which then induce an anti-inflammatory response through the release of acetylcholine. This response involves complex interactions between both ANS branches and plays a paramount role in finely tuning the immune response and avoiding tissue damages in case of inflammation (Garzoni et al., 2013; Bonaz et al., 2017).

The CAP can fail to properly function in case of ANS dysfunction, but also due to pathogens and inflammation: pathogens can rapidly activate but desensitize the CAP (i.e., alter cholinergic receptors) (Fairchild et al., 2011), whereas prolonged inflammation can induce apoptosis in the brainstem vagal nuclei (Fritze et al., 2014). As a result, HRV decreases, and adverse outcomes can occur: indeed, low vagal activity and reduced CAP efficiency correlate with increased inflammation, morbidity, and mortality, in both infants and adults (Garzoni et al., 2013; Bonaz et al., 2017).

In fetuses, the CAP, although at the early stage of maturation, might also modulate local and systemic inflammation (Garzoni et al., 2013). Indeed, animal studies suggested that in ovine fetuses, CIMVA applied to fHRV found several metrics that uniquely predicted, 1.5 days in advance, M1 and M2 macrophages activation and occludin expression (i.e., increased leakiness) in the terminal ileum (Liu et al., 2016). Therefore,

if cytokines and toxins can alter HRV through the CAP, it might be intuitive why HRV analysis can inform about the global health of the infant and predict future inflammation-related development.

HRV modifications could also reflect the effect of treatments, such as ventilation, antibiotics, and phototherapy. To date, a clear correlation is still lacking, and better research is needed, although initial evidence is available (Sullivan et al., 2014; Uhrikova et al., 2015; Al-Shargabi et al., 2017; Latremouille et al., 2018). For instance, as $RMS_S$, $RMS_L$, DFA $\alpha_S$, LF, HF, and TP decreased around 48 h before NEC occurred, the same metrics recovered to their baseline values during the first 60 h after NEC diagnosis (Al-Shargabi et al., 2018). Monitoring HRV in the 60 min prior to extubation could help predict which babies will have an adverse outcome, since they could show much lower TP, LF, HF, LF/HF, and VLF than babies who will succeed in being extubated (Kaczmarek et al., 2013).

Other indications about the clinical usefulness of HRV, and real-time monitoring, come from the studies on HIE severity and ANS in infants. During therapeutic hypothermia for HIE, lower $RMS_S$, $RMS_L$, DFA $\alpha_S$, and LF and higher HF indicated a higher risk of secondary energy failure, brain injury, and death, thus helping in identifying the infants who failed to respond to hypothermia and needed adjuvant neuroprotective therapies (Govindan et al., 2014; Massaro et al., 2014; Metzler et al., 2017). The analysis of DFA $\alpha_S$, $RMS_S$, and $RMS_L$ could also prove useful to discriminate among different types of brain injury, such as white matter injury, watershed stroke, basal ganglia injury, or global injury (Metzler et al., 2017). As shown with fHRV analysis, HRV monitoring, especially during labor, could also predict which newborns are at risk of developing neuroinflammation and, thus, brain injury—neuroinflammation is correlated to severe acidemia, and fHRV monitoring could detect it with 1 h of advance. Since therapeutic hypothermia is poorly applied, either because often the brain injury has already developed or because neonatologists lack tools to properly recognize which newborns require hypothermia, HRV monitoring could overcome this predicament (Frasch et al., 2014; Xu et al., 2014; Gold et al., 2019).

Real-time HRV is shown to be useful for monitoring how infants react to routine-care procedures, such as diaper changes or pupil examination, and for revealing neurological abnormalities. Indeed, infants with HIE and impaired ANS—defined as at least one alteration in LF, HF, DFA $\alpha_S$, $RMS_S$, or $RMS_L$—showed HR, blood pressure, and cerebral blood flow alteration during those procedures. Moreover, the more the number of altered HRV metrics, the worse the outcome, i.e., moderate severe brain injury or death (Campbell et al., 2018).

Real-time HRV could also help mothers and fathers to monitor the interaction with the baby: using a feedback and feedforward process, parents could see how their actions affect their baby (Van Puyvelde et al., 2019a,b; these studies, however, examined how touch influenced RSA and RR intervals, not specific HRV metrics).

Furthermore, real-time HRV could help control how the infant's condition progresses in different positions (supine, prone, or side-lying). This monitoring would be useful, for example, to balance the need for proper oxygenation and avoiding adverse outcomes, such as SIDS. Infants who succumbed to SIDS showed lower RSA during all sleep stages (Kluge et al., 1988) and altered Poincaré plots compared with controls (Schechtman et al., 1992). Those infants showed also higher basal HR during QS (Kelly et al., 1986), lower HF, and higher LF before pre-apneic sighs than those infants who did not die from SIDS (Franco et al., 2003).

Newborns who develop SIDS seem to show an autonomic instability that impedes them from facing a life-threatening event (Elhaik, 2016; Zhao et al., 2016). Real-time HRV could help prevent this adverse scenario. Besides, due to the uncertainty still surrounding SIDS—for instance, infants who succumb to SIDS could even show the same RSA and HR, but higher LF than controls (Gordon et al., 1984)—, continuous monitoring data analyzed by AI algorithms could help shed more light on SIDS occurrence by giving useful information about the newborn's ANS condition.

Speaking of which, an ML algorithm based on boosted decision trees increased the predictive power of HRV during episodes of low mean blood pressure in preterm infants with a GA between 23 and 31 weeks. Although good results were obtained even with single metrics—among time, frequency, and non-linear ones, RMSSD gave the best area under the curve (0.87)—combining all the metrics through the mentioned algorithm gave an area under the curve of 0.97 for predicting short-term neurological outcomes (i.e., grade III/IV intraventricular hemorrhage or cystic periventricular leukomalacia, NEC, bronchopulmonary dysplasia, infection, and retinopathy) (Semenova et al., 2018).

Lastly, real-time HRV monitoring could give information about the neurological development. Indeed, newborn's HRV predicted the occurrence of neurological abnormalities—cerebral palsy, language or mental retardation, vision or hearing disability, or attention disorders—at 2 years (Thiriez et al., 2015; Dimitrijević et al., 2016). Twenty-four hour RMSSD, SDANN, and SDNN were particularly good predictors, respectively, 88.9, 83.3, and 83.3% specificity and 100% sensibility using the threshold values of 17, 38, and 47 ms—and also improved the prognostic value of general movements assessment: among newborns with poor repertoire general movements, those with higher RMSSD, SDANN, and SDNN showed better neurological development (Dimitrijević et al., 2016).

## HRV Potential Usefulness in Middle- and Low-Income Realities

HRV could be a useful non-invasive tool to assess infant's conditions and to reduce the risk of adverse outcomes and, as the HeRO monitor showed, mortality from sepsis. This last result is worth noting since neonatal sepsis remains one of the major causes of neonatal mortality, together with prematurity and birth-related complications, and this holds especially in middle- and low-income countries (WHO, 2016).

Indeed, only 15 in 48 Asian and African countries reported having an indicator about sepsis management in their Health Management Information Systems (WHO, 2017). In countries,

such as South Africa and India, about 1 infant in 35 dies before the first birthday, even more than ten times compared with developed countries, such as Japan, Sweden, or Italy (about 1 in 350–500) (OECD, 2020). Closing this large gap by reducing worldwide neonatal mortality to less than 1 infant in 100 was deemed by the WHO as one of the top priorities in 2015 (WHO, 2016). Of notice, the first 28 days after birth shows the highest mortality risk (United Nations Inter-agency Group for Child Mortality Estimation (UN IGME), 2019).

The studies that investigated the reasons behind the difference in mortality highlighted that the barriers to efficient care were twofold. On the one hand, people tend to delay seeking care due to lack of knowledge about neonatal health, sociocultural behaviors (e.g., relying on traditional practices), and several concerns about the cost of healthcare, the attitude of NICU staff, and the lack of appropriate medical equipment (Watson et al., 2020). On the other hand, obstetrics and neonatal evidence-based practices fail to be introduced due to lack of financial resources, workforce capacity, and specific training in case of emergency. The effect is a low-quality screening for pathological conditions, such as IUGR or sepsis, which can lead to severe neonatal health problems and even death (Hoyer et al., 2017; Otieno et al., 2018).

To date, real-time HRV monitoring cannot solve all these complex problems, but it could surely address financial- and efficiency-related obstacles. Indeed, HRV monitors based on PPG, contactless vPPG, or even BSG could be low-cost and easy-to-use devices (Sun et al., 2012; Sun and Thakor, 2016; Joshi et al., 2018) that allow midwifery and NICU staff to efficiently monitor the newborn's development, screen for pathological conditions, and choose the best treatment (Hoyer et al., 2017). A recent paper showed that the HeRO monitor could also help reduce, even if a modest effect was shown, the use of antibiotics and blood culture tests for suspicion of sepsis in the first 120 days of life (King, 2020).

Moreover, if relying on big-data and the Internet, the HRV monitors could even be simple systems that monitor the babies, send information to a central hub (e.g., through the Cloud), and receive the corresponding elaboration, thus having the same efficiency of HRV monitors in high-income countries (Werth, 2019).

## CONCLUSION

The present paper reviewed the use of HRV in the neonatal field and the utility of real-time HRV monitoring to assess the newborn's clinical conditions, showing that several metrics and computed metrics change in conjunction with stress-/pain-related behaviors, inflammation, pathological conditions, such as cardiac failure, respiratory distress syndrome, hyperbilirubinemia, NEC, and sepsis, and neurological development.

The paper also reviewed the NICU technology to evaluate how to measure real-time HRV efficiently. Indeed, a system based on PPG could be the optimal solution due to being low-cost, easy-to-use, and non-invasive, although PPG-based computation seems less precise than ECG-based computation. Therefore, future studies will have to carefully assess if the outcomes reviewed in this paper might be influenced by this difference in precision between PPG and ECG.

In the next decade, introducing real-time HRV in NICUs would be a great step forward in the improvement of neonatal care, especially if supported by the advancements in bioinformatics, which could easily extrapolate accurate predicting models from all the data collected in the NICUs, although several concerns and limitations have to be overcome before fully implementing the system into a daily NICU routine care.

## AUTHOR CONTRIBUTIONS

MC, FCe, AC, NB, and AM contributed equally to the conceptualization and to the writing of the manuscript. DB, FCa, and CC revised and edited the final draft of the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

## REFERENCES


Aarts, L. A. M., Jeanne, V., Cleary, J. P., Lieber, C., Nelson, J. S., Bambang Oetomo, S., et al. (2013). Non-contact heart rate monitoring utilizing camera photoplethysmography in the neonatal intensive care unit — A pilot study. *Early Hum. Dev.* 89, 943–948. doi: 10.1016/j.earlhumdev.2013.09.016

Abry, P., Roux, S. G., Chudacek, V., Borgnat, P., Goncalves, P., and Doret, M. (2013). "Hurst exponent and intrapartum fetal heart rate: impact of decelerations," in *Proceedings of the 26th IEEE International Symposium on Computer-Based Medical Systems*, Port: IEEE, 131–136. doi: 10.1109/CBMS.2013.6627777

Aita, M., and Goulet, C. (2003). Assessment of neonatal nurses' behaviors that prevent overstimulation in preterm infants. *Intensive Crit. Care Nurs.* 19, 109–118. doi: 10.1016/s0964-3397(03)00023-5

Aita, M., Johnston, C., Goulet, C., Oberlander, T. F., and Snider, L. (2013). Intervention minimizing preterm infants' exposure to NICU light and noise. *Clin. Nurs. Res.* 22, 337–358. doi: 10.1177/1054773812469223

Alexandre, C., De Jonckheere, J., Rakza, T., Mur, S., Carette, D., Logier, R., et al. (2013). [Impact of cocooning and maternal voice on the autonomic nervous system activity in the premature newborn infant]. *Arch. Pediatr.* 20, 963–968. doi: 10.1016/j.arcped.2013.06.006

Almadhoob, A., and Ohlsson, A. (2020). Sound reduction management in the neonatal intensive care unit for preterm or very low birth weight infants. *Cochrane Database Syst. Rev.* 1:CD010333. doi: 10.1002/14651858.CD010333.pub3

Alonzo, C. J., Nagraj, V. P., Zschaebitz, J. V., Lake, D. E., Moorman, J. R., and Spaeder, M. C. (2018). Heart rate ranges in premature neonates using high resolution physiologic data. *J. Perinatol.* 38, 1242–1245. doi: 10.1038/s41372-018-0156-1

Als, H., Butler, S., Kosta, S., and McAnulty, G. (2005). The assessment of preterm infants' behavior (APIB): furthering the understanding and measurement of neurodevelopmental competence in preterm and full-term infants. *Ment. Retard. Dev. Disabil. Res. Rev.* 11, 94–102. doi: 10.1002/mrdd.20053

Al-Shargabi, T., Govindan, R. B., Dave, R., Metzler, M., Wang, Y., du Plessis, A., et al. (2017). Inflammatory cytokine response and reduced heart rate variability

in newborns with hypoxic-ischemic encephalopathy. *J. Perinatol.* 37, 668–672. doi: 10.1038/jp.2017.15

Al-Shargabi, T., Reich, D., Govindan, R. B., Shankar, S., Metzler, M., Cristante, C., et al. (2018). Changes in autonomic tone in premature infants developing necrotizing enterocolitis. *Am. J. Perinatol.* 35, 1079–1086. doi: 10.1055/s-0038-1639339

Andersen, M., Andelius, T. C. K., Pedersen, M. V., Kyng, K. J., and Henriksen, T. B. (2019). Severity of hypoxic ischemic encephalopathy and heart rate variability in neonates: a systematic review. *BMC Pediatr.* 19:242. doi: 10.1186/s12887-019-1603-7

Aye, C. Y. L., Lewandowski, A. J., Oster, J., Upton, R., Davis, E., Kenworthy, Y., et al. (2018). Neonatal autonomic function after pregnancy complications and early cardiovascular development. *Pediatr. Res.* 84, 85–91. doi: 10.1038/s41390-018-0021-0

Ayres-de-Campos, D., and Bernardes, J. (2010). Twenty-five years after the FIGO guidelines for the use of fetal monitoring: time for a simplified approach? *Int. J. Gynecol. Obstet.* 110, 1–6. doi: 10.1016/j.ijgo.2010.03.011

Babenko, O., Kovalchuk, I., and Metz, G. A. (2015). Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. *Neurosci. Biobehav. Rev.* 48, 70–91. doi: 10.1016/j.neubiorev.2014.11.013

Bellieni, C. V., Acampa, M., Maffei, M., Maffei, S., Perrone, S., Pinto, I., et al. (2008). Electromagnetic fields produced by incubators influence heart rate variability in newborns. *Arch. Dis. Child Fetal Neonatal Ed.* 93, F298–F301. doi: 10.1136/adc.2007.132738

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin. Proc.* 68, 988–1001. doi: 10.1016/s0025-6196(12)62272-1

Béres, S., Holczer, L., and Hejjel, L. (2019). On the minimal adequate sampling frequency of the photoplethysmogram for pulse rate monitoring and heart rate variability analysis in mobile and wearable technology. *Meas. Sci. Rev.* 19, 232–240. doi: 10.2478/msr-2019-0030

Biban, P. (2010). From neonatal to paediatric intensive care: an educational pathway. *Minerva Pediatr.* 62(3 Suppl. 1), 129–131.

Billman, G. E. (2011). Heart rate variability - a historical perspective. *Front. Physiol.* 2:86. doi: 10.3389/fphys.2011.00086

Blackford, E. B., Piasecki, A. M., and Estepp, J. R. (2016). Measuring pulse rate variability using long-range, non-contact imaging photoplethysmography. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2016, 3930–3936. doi: 10.1109/EMBC.2016.7591587

Blanik, N., Abbas, A. K., Venema, B., Blazek, V., and Leonhardt, S. (2014). Hybrid optical imaging technology for long-term remote monitoring of skin perfusion and temperature behavior. *J. Biomed. Opt.* 19:016012. doi: 10.1117/1.JBO.19.1.016012

Blanik, N., Heimann, K., Pereira, C., Paul, M., Blazek, V., Venema, B., et al. (2016). Remote vital parameter monitoring in neonatology - robust, unobtrusive heart rate detection in a realistic clinical scenario. *Biomed. Tech.* 61, 631–643. doi: 10.1515/bmt-2016-0025

Bonaz, B., Sinniger, V., and Pellissier, S. (2017). The vagus nerve in the neuro-immune axis: implications in the pathology of the gastrointestinal tract. *Front. Immunol.* 8:1452. doi: 10.3389/fimmu.2017.01452

Bravi, A., Longtin, A., and Seely, A. J. (2011). Review and classification of variability analysis techniques with clinical applications. *Biomed. Eng. Online* 10:90. doi: 10.1186/1475-925X-10-90

Butruille, L., Blouin, A., De Jonckheere, J., Mur, S., Margez, T., Rakza, T., et al. (2017). Impact of skin-to-skin contact on the autonomic nervous system in the preterm infant and his mother. *Infant Behav. Dev.* 49, 83–86. doi: 10.1016/j.infbeh.2017.07.003

Butruille, L., De Jonckheere, J., Marcilly, R., Boog, C., Bras da Costa, S., Rakza, T., et al. (2015). Development of a pain monitoring device focused on newborn infant applications: the NeoDoloris project. *IRBM* 36, 80–85. doi: 10.1016/j.irbm.2015.01.005

Buyuktiryaki, M., Uras, N., Okur, N., Oncel, M. Y., Simsek, G. K., Isik, S. O., et al. (2018). Evaluation of prolonged pain in preterm infants with pneumothorax using heart rate variability analysis and EDIN (Échelle Douleur Inconfort Nouveau-Né, neonatal pain and discomfort scale) scores. *Korean J. Pediatr.* 61, 322–326. doi: 10.3345/kjp.2017.05939

Campbell, H., Govindan, R. B., Kota, S., Al-Shargabi, T., Metzler, M., Andescavage, N., et al. (2018). Autonomic dysfunction in neonates with hypoxic ischemic encephalopathy undergoing therapeutic hypothermia impairs physiological responses to routine care events. *J. Pediatr.* 196, 38–44. doi: 10.1016/j.jpeds.2017.12.071

Cardoso, S., Silva, M. J., and Guimarães, H. (2017). Autonomic nervous system in newborns: a review based on heart rate variability. *Childs. Nerv. Syst.* 33, 1053–1063. doi: 10.1007/s00381-017-3436-8

Cesarelli, M., Romano, M., Ruffo, M., Bifulco, P., and Pasquariello, G. (2010). Foetal heart rate variability frequency characteristics with respect to uterine contractions. *J. Biomed. Sci. Eng.* 03, 1014–1021. doi: 10.4236/jbise.2010.310132

Cheng, G., Zhou, X., Qu, J., Ashwell, K. W., and Paxinos, G. (2004). Central vagal sensory and motor connections: human embryonic and fetal development. *Auton. Neurosci.* 114, 83–96. doi: 10.1016/j.autneu.2004.06.008

Chiew, C. J., Liu, N., Tagami, T., Wong, T. H., Koh, Z. X., and Ong, M. E. H. (2019). Heart rate variability based machine learning models for risk prediction of suspected sepsis patients in the emergency department. *Medicine* 98:e14197. doi: 10.1097/MD.0000000000014197

Chock, V. Y., Davis, A. S., and Hintz, S. R. (2015). the roles and responsibilities of the neonatologist in complex fetal medicine: providing a continuum of care. *Neoreviews* 16, e9–e15. doi: 10.1542/neo.16-1-e9

Choi, A., and Shin, H. (2017). Photoplethysmography sampling frequency: pilot assessment of how low can we go to analyze pulse rate variability with reliability? *Physiol. Meas.* 38, 586–600. doi: 10.1088/1361-6579/aa5efa

Chudacek, V., Anden, J., Mallat, S., Abry, P., and Doret, M. (2014). Scattering transform for intrapartum fetal heart rate variability fractal analysis: a case-control study. *IEEE Trans. Biomed. Eng.* 61, 1100–1108. doi: 10.1109/TBME.2013.2294324

Clairambault, J., Curzi-Dascalova, L., Kauffmann, F., Médigue, C., and Leffler, C. (1992). Heart rate variability in normal sleeping full-term and preterm neonates. *Early Hum. Dev.* 28, 169–183. doi: 10.1016/0378-3782(92)90111-s

Cobos-Torres, J. C., Abderrahim, M., and Martínez-Orgado, J. (2018). Non-contact, simple neonatal monitoring by photoplethysmography. *Sensors* 18:4362. doi: 10.3390/s18124362

Cremillieux, C., Makhlouf, A., Pichot, V., Trombert, B., and Patural, H. (2018). Objective assessment of induced acute pain in neonatology with the Newborn Infant Parasympathetic Evaluation index. *Eur. J. Pain.* 22, 1071–1079. doi: 10.1002/ejp.1191

Cunningham, S., Symon, A. G., Elton, R. A., Zhu, C., and McIntosh, N. (1999). Intra-arterial blood pressure reference ranges, death and morbidity in very low birthweight infants during the first seven days of life. *Early Hum. Dev.* 56, 151–165. doi: 10.1016/s0378-3782(99)00038-9

Daunhawer, I., Kasser, S., Koch, G., Sieber, L., Cakal, H., Tütsch, J., et al. (2019). Enhanced early prediction of clinically relevant neonatal hyperbilirubinemia with machine learning. *Pediatr. Res.* 86, 122–127. doi: 10.1038/s41390-019-0384-x

De Jonckheere, J., and Storme, L. (2019). NIPE is related to parasympathetic activity. Is it also related to comfort? *J. Clin. Monit. Comput.* 33, 747–748. doi: 10.1007/s10877-019-00276-1

de Souza Filho, L. F. M., de Oliveira, J. C. M., Ribeiro, M. K. A., Moura, M. C., Fernandes, N. D., de Sousa, R. D., et al. (2019). Evaluation of the autonomic nervous system by analysis of heart rate variability in the preterm infants. *BMC Cardiovasc. Disord.* 19:198. doi: 10.1186/s12872-019-1166-4

DiPietro, J. A., and Voegtline, K. M. (2017). The gestational foundation of sex differences in development and vulnerability. *Neuroscience* 342, 4–20. doi: 10.1016/j.neuroscience.2015.07.068

Dereymaeker, A., Pillay, K., Vervisch, J., De Vos, M., Van Huffel, S., Jansen, K., et al. (2017). Review of sleep-EEG in preterm and term neonates. *Early Hum. Dev.* 113, 87–103. doi: 10.1016/j.earlhumdev.2017.07.003

Dimitrijević, L., Bjelaković, B., Čolović, H., Mikov, A., Živković, V., Kocić, M., et al. (2016). Assessment of general movements and heart rate variability in prediction of neurodevelopmental outcome in preterm infants. *Early Hum. Dev.* 99, 7–12. doi: 10.1016/j.earlhumdev.2016.05.014

DiPietro, J. A., Hodgson, D. M., Costigan, K. A., Hilton, S. C., and Johnson, T. R. (1996). Fetal neurobehavioral development. *Child. Dev.* 67, 2553–2567. doi: 10.1111/j.1467-8624.1996.tb01874.x

Doheny, K. K., Palmer, C., Browning, K. N., Jairath, P., Liao, D., He, F., et al. (2014). Diminished vagal tone is a predictive biomarker of necrotizing enterocolitis-risk in preterm infants. *Neurogastroenterol. Motil.* 26, 832–840. doi: 10.1111/nmo.12337

Durosier, L. D., Herry, C. L., Cortes, M., Cao, M., Burns, P., Desrochers, A., et al. (2015). Does heart rate variability reflect the systemic inflammatory response in a fetal sheep model of lipopolysaccharide-induced sepsis? *Physiol. Meas.* 36, 2089–2102. doi: 10.1088/0967-3334/36/10/2089

Eiselt, M., Curzi-Dascalova, L., Clairambault, J., Kauffmann, F., Médigue, C., and Peirano, P. (1993). Heart-rate variability in low-risk prematurely born infants reaching normal term: a comparison with full-term newborns. *Early Hum. Dev.* 32, 183–195. doi: 10.1016/0378-3782(93)90011-I

Elgendi, M., Norton, I., Brearley, M., Dokos, S., Abbott, D., and Schuurmans, D. (2016). A pilot study: can heart rate variability (HRV) be determined using short-term photoplethysmograms? *F1000Res.* 5:2354. doi: 10.12688/f1000research.9556.1

Elhaik, E. (2016). A "Wear and Tear" hypothesis to explain sudden infant death syndrome. *Front. Neurol.* 7:180. doi: 10.3389/fneur.2016.00180

Fairchild, K., and Aschner, J. (2012). HeRO monitoring to reduce mortality in NICU patients. *Res. Rep. Neonatol.* 2, 65–76. doi: 10.2147/RRN.S32570

Fairchild, K. D. (2013). Predictive monitoring for early detection of sepsis in neonatal ICU patients. *Curr. Opin. Pediatr.* 25, 172–179. doi: 10.1097/MOP.0b013e32835e8fe6

Fairchild, K. D., and O'Shea, T. M. (2010). Heart rate characteristics: physiomarkers for detection of late-onset neonatal sepsis. *Clin. Perinatol.* 37, 581–598. doi: 10.1016/j.clp.2010.06.002

Fairchild, K. D., Srinivasan, V., Randall Moorman, J., Gaykema, R. P. A., and Goehler, L. E. (2011). Pathogen-induced heart rate changes associated with cholinergic nervous system activation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R330–R339. doi: 10.1152/ajpregu.00487.2010

Ferrario, M., Signorini, M. G., Magenes, G., and Cerutti, S. (2006). Comparison of entropy-based regularity estimators: application to the fetal heart rate signal for the identification of fetal distress. *IEEE Trans. Biomed. Eng.* 53, 119–125. doi: 10.1109/TBME.2005.859809

Forte, G., and Casagrande, M. (2019). Heart rate variability and cognitive function: a systematic review. *Front. Neurosci.* 13:710. doi: 10.3389/fnins.2019.00710

Fox, G. P., and Matthews, T. G. (1989). Autonomic dysfunction at different ambient temperatures in infants at risk of sudden infant death syndrome. *Lancet* 2, 1065–1067. doi: 10.1016/s0140-6736(89)91080-5

Franco, P., Szliwowski, H., Dramaix, M., and Kahn, A. (2000). Influence of ambient temperature on sleep characteristics and autonomic nervous control in healthy infants. *Sleep* 23, 401–407.

Franco, P., Verheulpen, D., Valente, F., Kelmanson, I., de Broca, A., Scaillet, S., et al. (2003). Autonomic responses to sighs in healthy infants and in victims of sudden infant death. *Sleep Med.* 4, 569–577. doi: 10.1016/s1389-9457(03)00107-2

Frasch, M. G. (2018). Saving the brain one heartbeat at a time: perspectives. *J. Physiol.* 596, 5503–5504. doi: 10.1113/JP275776

Frasch, M. G. (2020). Heart rate variability code: does it exist and can we hack it? *ArXiv* [Preprint], Available online at: http://arxiv.org/abs/2001.08264 (accessed July 25, 2020).

Frasch, M. G., Herry, C. L., Niu, Y., and Giussani, D. A. (2020). First evidence that intrinsic fetal heart rate variability exists and is affected by hypoxic pregnancy. *J. Physiol.* 598, 249–263. doi: 10.1113/JP278773

Frasch, M. G., Szynkaruk, M., Prout, A. P., Nygard, K., Cao, M., Veldhuizen, R., et al. (2016). Decreased neuroinflammation correlates to higher vagus nerve activity fluctuations in near-term ovine fetuses: a case for the afferent cholinergic anti-inflammatory pathway? *J. Neuroinflammation* 13:103. doi: 10.1186/s12974-016-0567-x

Frasch, M. G., Xu, Y., Stampalija, T., Durosier, L. D., Herry, C., Wang, X., et al. (2014). Correlating multidimensional fetal heart rate variability analysis with acid-base balance at birth. *Physiol. Meas.* 35, L1–L12. doi: 10.1088/0967-3334/35/12/L1

Frasch, M. G., Zwiener, U., Hoyer, D., and Eiselt, M. (2007). Autonomic organization of respirocardial function in healthy human neonates in quiet and active sleep. *Early Hum. Dev.* 83, 269–277. doi: 10.1016/j.earlhumdev.2006.05.023

Fraser, A. G. (2017). A manifesto for cardiovascular imaging: addressing the human factor. *Eur. Heart J. Cardiovasc. Imaging* 18, 1311–1321. doi: 10.1093/ehjci/jex216

Fritze, D., Zhang, W., Li, J.-Y., Chai, B., and Mulholland, M. (2014). Thrombin mediates vagal apoptosis and dysfunction in inflammatory bowel disease. *J. Gastrointest. Surg.* 18, 1495–1506. doi: 10.1007/s11605-014-2565-6

Fyfe, K. L., Yiallourou, S. R., Wong, F. Y., Odoi, A., Walker, A. M., and Horne, R. S. C. (2015). The effect of gestational age at birth on post-term maturation of heart rate variability. *Sleep* 38, 1635–1644. doi: 10.5665/sleep.5064

Galland, B. C., Taylor, B. J., Bolton, D. P., and Sayers, R. M. (2006). Heart rate variability and cardiac reflexes in small for gestational age infants. *J. Appl. Physiol.* 100, 933–939. doi: 10.1152/japplphysiol.01275.2005

Gardner, F. C., Adkins, C. S., Hart, S. E., Travagli, R. A., and Doheny, K. K. (2018). Preterm stress behaviors, autonomic indices, and maternal perceptions of infant colic. *Adv. Neonatal. Care* 18, 49–57. doi: 10.1097/ANC.0000000000000451

Garzoni, L., Faure, C., and Frasch, M. G. (2013). Fetal cholinergic anti-inflammatory pathway and necrotizing enterocolitis: the brain-gut connection begins in utero. *Front. Integr. Neurosci.* 7:57. doi: 10.3389/fnint.2013.00057

Georgieva, A., Payne, S. J., Moulden, M., and Redman, C. W. G. (2013). Artificial neural networks applied to fetal monitoring in labour. *Neural Comput. Appl.* 22, 85–93. doi: 10.1007/s00521-011-0743-y

Gold, N., Herry, C. L., Wang, X., and Frasch, M. G. (2019). Fetal cardiovascular decompensation during labor predicted from the individual heart rate: a prospective study in fetal sheep near term and the impact of low sampling rate. *ArXiv* [Preprint], Available online at: http://arxiv.org/abs/1911.01304 (accessed August 21, 2020).

Gonçalves, H., Amorim-Costa, C., Ayres-de-Campos, D., and Bernardes, J. (2017). Gender-specific evolution of fetal heart rate variability throughout gestation: a study of 8823 cases. *Early Hum. Dev.* 115, 38–45. doi: 10.1016/j.earlhumdev.2017.09.002

Gonçalves, H., Rocha, A. P., Ayres-de-Campos, D., and Bernardes, J. (2006). Linear and nonlinear fetal heart rate analysis of normal and acidemic fetuses in the minutes preceding delivery. *Med. Biol. Eng. Comput.* 44, 847–855. doi: 10.1007/s11517-006-0105-6

Gordon, D., Cohen, R. J., Kelly, D., Akselrod, S., and Shannon, D. C. (1984). Sudden infant death syndrome: abnormalities in short term fluctuations in heart rate and respiratory activity. *Pediatr. Res.* 18, 921–926. doi: 10.1203/00006450-198410000-00001

Govindan, R. B., Massaro, A. N., Al-Shargabi, T., Andescavage, N. N., Chang, T., Glass, P., et al. (2014). Detrended fluctuation analysis of non-stationary cardiac beat-to-beat interval of sick infants. *EPL Europhys. Lett.* 108:40005. doi: 10.1209/0295-5075/108/40005

Griffin, M. P., Lake, D. E., Bissonette, E. A., Harrell, F. E. Jr., O'Shea, T. M., and Moorman, J. R. (2005). Heart rate characteristics: novel physiomarkers to predict neonatal infection and death. *Pediatrics* 116, 1070–1074. doi: 10.1542/peds.2004-2461

Griffin, M. P., and Moorman, J. R. (2001). Toward the early diagnosis of neonatal sepsis and sepsis-like illness using novel heart rate analysis. *Pediatrics* 107, 97–104. doi: 10.1542/peds.107.1.97

Hashiguchi, K., Kuriyama, N., Koyama, T., Matsui, D., Ozaki, E., Hasegawa, T., et al. (2020). Validity of stress assessment using heart rate variability in newborns. *Pediatr. Int.* 62, 694–700. doi: 10.1111/ped.14149

Hay, W. W. (1987). The uses, benefits, and limitations of pulse oximetry in neonatal medicine: consensus on key issues. *J. Perinatol.* 7, 347–349.

Hayano, J., and Yuda, E. (2019). Pitfalls of assessment of autonomic function by heart rate variability. *J. Physiol. Anthropol.* 38:3. doi: 10.1186/s40101-019-0193-2

Heathers, J. A. (2012). Sympathovagal balance from heart rate variability: an obituary. *Exp. Physiol.* 97:556. doi: 10.1113/expphysiol.2011.063867

Hejjel, L. (2017). Comment on 'Photoplethysmography sampling frequency: pilot assessment of how low can we go to analyze pulse rate variability with reliability?'. *Physiol. Meas.* 38, 2249–2251. doi: 10.1088/1361-6579/aa9303

Hemphill, J. C., Andrews, P., and De Georgia, M. (2011). Multimodal monitoring and neurocritical care bioinformatics. *Nat. Rev. Neurol.* 7, 451–460. doi: 10.1038/nrneurol.2011.101

Henry, C., Shipley, L., Ward, C., Mirahmadi, S., Liu, C., Morgan, S., et al. (2020). Accurate neonatal heart rate monitoring using a new wireless, cap mounted device. *Acta Paediatr.* doi: 10.1111/apa.15303 [Epub ahead of print].

Herry, C. L., Burns, P., Desrochers, A., Fecteau, G., Durosier, L. D., Cao, M., et al. (2019). Vagal contributions to fetal heart rate variability: an omics approach. *Physiol. Meas.* 40:065004. doi: 10.1088/1361-6579/ab21ae

Herry, C. L., Cortes, M., Wu, H.-T., Durosier, L. D., Cao, M., Burns, P., et al. (2016). Temporal patterns in sheep fetal heart rate variability correlate to systemic cytokine inflammatory response: a methodological exploration of monitoring potential using complex signals bioinformatics. *PLoS One* 11:e0153515. doi: 10.1371/journal.pone.0153515

Holsti, L., Grunau, R. E., Whifield, M. F., Oberlander, T. F., and Lindh, V. (2006). Behavioral responses to pain are heightened after clustered care in preterm infants born between 30 and 32 weeks gestational age. *Clin. J. Pain* 22, 757–764. doi: 10.1097/01.ajp.0000210921.10912.47

Holzman, J. B., and Bridgett, D. J. (2017). Heart rate variability indices as biomarkers of top-down self-regulatory mechanisms: a meta-analytic review. *Neurosci. Biobehav. Rev.* 74(Pt A), 233–255. doi: 10.1016/j.neubiorev.2016

Hoyer, D., Heinicke, E., Jaekel, S., Tetschke, F., Di Pietro Paolo, D., Haueisen, J., et al. (2009). Indices of fetal development derived from heart rate patterns. *Early Hum. Dev.* 85, 379–386. doi: 10.1016/j.earlhumdev.2009.01.002

Hoyer, D., Schmidt, A., Gustafson, K. M., Lobmaier, S. M., Lakhno, I., van Leeuwen, P., et al. (2019). Heart rate variability categories of fluctuation amplitude and complexity: diagnostic markers of fetal development and its disturbances. *Physiol. Meas.* 40:064002. doi: 10.1088/1361-6579/ab205f

Hoyer, D., Schneider, U., Kowalski, E.-M., Schmidt, A., Witte, O. W., Schleußner, E., et al. (2015). Validation of functional fetal autonomic brain age score fABAS in 5 min short recordings. *Physiol. Meas.* 36, 2369–2378. doi: 10.1088/0967-3334/36/11/2369

Hoyer, D., Tetschke, F., Jaekel, S., Nowack, S., Witte, O. W., Schleußner, E., et al. (2013). Fetal functional brain age assessed from universal developmental indices obtained from neuro-vegetative activity patterns. *PLoS One* 8:e74431. doi: 10.1371/journal.pone.0074431

Hoyer, D., Żebrowski, J., Cysarz, D., Gonçalves, H., Pytlik, A., Amorim-Costa, C., et al. (2017). Monitoring fetal maturation—objectives, techniques and indices of autonomic function. *Physiol. Meas.* 38, R61–R88. doi: 10.1088/1361-6579/aa5fca

Jarczok, M. N., Kleber, M. E., Koenig, J., Loerbroks, A., Herr, R. M., Hoffmann, K., et al. (2015). Investigating the associations of self-rated health: heart rate variability is more strongly associated than inflammatory and other frequently used biomarkers in a cross sectional occupational sample. *PLoS One* 10:e0117196. doi: 10.1371/journal.pone.0117196

Javorka, K., Lehotska, Z., Kozar, M., Uhrikova, Z., Kolarovszki, B., Javorka, M., et al. (2017). Heart rate variability in newborns. *Physiol. Res.* 66(Suppl. 2), S203–S214. doi: 10.33549/physiolres.933676

Jean-Louis, M., Anwar, M., Rosen, H., Craelius, W., Hiatt, M., and Hegyi, T. (2004). Power spectral analysis of heart rate in relation to sleep position. *Biol. Neonate* 86, 81–84. doi: 10.1159/000077782

Jeng, S. F., Yau, K. I., Chen, L. C., and Hsiao, S. F. (2000). Alberta infant motor scale: reliability and validity when used on preterm infants in Taiwan. *Phys. Ther.* 80, 168–178. doi: 10.1093/ptj/80.2.168

Jennings, J. R., Allen, B., Gianaros, P. J., Thayer, J. F., and Manuck, S. B. (2015). Focusing neurovisceral integration: cognition, heart rate variability, and cerebral blood flow. *Psychophysiology* 52, 214–224. doi: 10.1111/psyp.12319

Joshi, R., Bierling, B., Feijs, L., van Pul, C., and Andriessen, P. (2019a). Monitoring the respiratory rate of preterm infants using an ultrathin film sensor embedded in the bedding: a comparative feasibility study. *Physiol. Meas.* 40:045003. doi: 10.1088/1361-6579/ab1595

Joshi, R., Kommers, D., Guo, C., Bikker, J.-W., Feijs, L., van Pul, C., et al. (2019b). Statistical modeling of heart rate variability to unravel the factors affecting autonomic regulation in preterm infants. *Sci. Rep.* 9:7691. doi: 10.1038/s41598-019-44209-z

Joshi, R., Bierling, B. L., Long, X., Weijers, J., Feijs, L., Van Pul, C., et al. (2018). A ballistographic approach for continuous and non-obtrusive monitoring of movement in neonates. *IEEE J. Transl. Eng. Health Med.* 6, 1–10. doi: 10.1109/JTEHM.2018.2875703

Joshi, R., Kommers, D., Oosterwijk, L., Feijs, L., van Pul, C., and Andriessen, P. (2020). Predicting neonatal sepsis using features of heart rate variability, respiratory characteristics, and ECG-Derived estimates of infant motion. *IEEE J. Biomed. Health Inform.* 24, 681–692. doi: 10.1109/JBHI.2019.2927463

Kaczmarek, J., Chawla, S., Marchica, C., Dwaihy, M., Grundy, L., and Sant'Anna, G. M. (2013). Heart rate variability and extubation readiness in extremely preterm infants. *Neonatology* 104, 42–48. doi: 10.1159/000347101

Kasai, M., Lear, C. A., Davidson, J. O., Beacom, M. J., Drury, P. P., Maeda, Y., et al. (2019). Early sinusoidal heart rate patterns and heart rate variability to assess hypoxia-ischaemia in near-term fetal sheep. *J. Physiol.* 597, 5535–5548. doi: 10.1113/JP278523

Kelly, D. H., Golub, H., Carley, D., and Shannon, D. C. (1986). Pneumograms in infants who subsequently died of sudden infant death syndrome. *J. Pediatr.* 109, 249–254. doi: 10.1016/s0022-3476(86)80380-8

Kevat, A. C., Bullen, D. V., Davis, P. G., and Kamlin, C. O. (2017). A systematic review of novel technology for monitoring infant and newborn heart rate. *Acta Paediatr.* 106, 710–720. doi: 10.1111/apa.13786

Kim, H. G., Cheon, E. J., Bai, D. S., Lee, Y. H., and Koo, B. H. (2018). Stress and heart rate variability: a meta-analysis and review of the literature. *Psychiatry Investig.* 15, 235–245. doi: 10.30773/pi.2017.08.17

King, W. E. (2020). HeRO monitoring: does it lead to unnecessary testing and treatment? *Neonatology Today* 15, 33–38.

Kluge, K. A., Harper, R. M., Schechtman, V. L., Wilson, A. J., Hoffman, H. J., and Southall, D. P. (1988). Spectral analysis assessment of respiratory sinus arrhythmia in normal infants and infants who subsequently died of sudden infant death syndrome. *Pediatr. Res.* 24, 677–682. doi: 10.1203/00006450-198812000-00005

Koutcherov, Y., Mai, J. K., and Paxinos, G. (2003). Hypothalamus of the human fetus. *J. Chem. Neuroanat.* 26, 253–270. doi: 10.1016/j.jchemneu.2003.07.002

Kumar, N., Akangire, G., Sullivan, B., Fairchild, K., and Sampath, V. (2020). Continuous vital sign analysis for predicting and preventing neonatal diseases in the twenty-first century: big data to the forefront. *Pediatr. Res.* 87, 210–220. doi: 10.1038/s41390-019-0527-0

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lakhno, I. (2017). Autonomic imbalance captures maternal and fetal circulatory response to pre-eclampsia. *Clin. Hypertens* 23:5. doi: 10.1186/s40885-016-0061-x

Lara, L. R., and Püttgen, H. A. (2018). Multimodality monitoring in the neurocritical care unit: contin. *Lifelong Learn. Neurol.* 24, 1776–1788. doi: 10.1212/CON.0000000000000671

Latremouille, S., Al-Jabri, A., Lamer, P., Kanbar, L., Shalish, W., Kearney, R. E., et al. (2018). Heart rate variability in extremely preterm infants receiving nasal CPAP and non-synchronized noninvasive ventilation immediately after extubation. *Respir. Care* 63, 62–69. doi: 10.4187/respcare.05672

Latremouille, S., Shalish, W., Kanbar, L., Lamer, P., Rao, S., Kearney, R. E., et al. (2019). The effects of nasal continuous positive airway pressure and high flow nasal cannula on heart rate variability in extremely preterm infants after extubation: a randomized crossover trial. *Pediatr. Pulmonol.* 54, 788–796. doi: 10.1002/ppul.24284

Lee, W., Yoon, H., Han, C., Joo, K., and Park, K. (2016). Physiological signal monitoring bed for infants based on load-cell sensors. *Sensors* 16:409. doi: 10.3390/s16030409

Li, D. K., Ferber, J. R., Odouli, R., and Quesenberry, C. P. Jr. (2012). A prospective study of in-utero exposure to magnetic fields and the risk of childhood obesity. *Sci. Rep.* 2:540. doi: 10.1038/srep00540

Li, X., Xu, Y., Herry, C., Durosier, L. D., Casati, D., Stampalija, T., et al. (2015). Sampling frequency of fetal heart rate impacts the ability to predict pH and BE at birth: a retrospective multi-cohort study. *Physiol. Meas.* 36, L1–L12. doi: 10.1088/0967-3334/36/5/L1

Li, X., Zheng, D., Zhou, S., Tang, D., Wang, C., and Wu, G. (2005). Approximate entropy of fetal heart rate variability as a predictor of fetal distress in women at term pregnancy. *Acta Obstet. Gynecol. Scand.* 84, 837–843. doi: 10.1111/j.0001-6349.2005.00773.x

Lin, T., Khalpey, Z., and Aras, S. (2020). Heart rate variability: a possible machine learning biomarker for mechanical circulatory device complications and heart recovery. *VAD J.* 6. [Epub ahead of print].

Liu, H. L., Garzoni, L., Herry, C., Durosier, L. D., Cao, M., Burns, P., et al. (2016). Can monitoring fetal intestinal inflammation using heart rate variability analysis signal incipient necrotizing enterocolitis of the neonate?: pediatr. *Crit. Care Med.* 17, e165–e176. doi: 10.1097/PCC.0000000000000643

Lobmaier, S. M., Müller, A., Zelgert, C., Shen, C., Su, P. C., Schmidt, G., et al. (2020). Fetal heart rate variability responsiveness to maternal stress, non-invasively detected from maternal transabdominal ECG. *Arch. Gynecol. Obstet.* 301, 405–414. doi: 10.1007/s00404-019-05390-8

Lucchini, M., Fifer, W. P., Sahni, R., and Signorini, M. G. (2016). Novel heart rate parameters for the assessment of autonomic nervous system function in premature infants. *Physiol. Meas.* 37, 1436–1446. doi: 10.1088/0967-3334/37/9/1436

Malacova, E., Tippaya, S., Bailey, H. D., Chai, K., Farrant, B. M., Gebremedhin, A. T., et al. (2020). Stillbirth risk prediction using machine learning for a large cohort of births from Western Australia, 1980-2015. *Sci. Rep.* 10:5354. doi: 10.1038/s41598-020-62210-9

Marsillio, L. E., Manghi, T., Carroll, M. S., Balmert, L. C., and Wainwright, M. S. (2019). Heart rate variability as a marker of recovery from critical illness in children. *PLoS One* 14:e0215930. doi: 10.1371/journal.pone.0215930

Marvin, M. M., Gardner, F. C., Sarsfield, K. M., Travagli, R. A., and Doheny, K. K. (2019). Increased frequency of skin-to-skin contact is associated with enhanced vagal tone and improved health outcomes in preterm neonates. *Am. J. Perinatol.* 36, 505–510. doi: 10.1055/s-0038-1669946

Massaro, A. N., Campbell, H. E., Metzler, M., Al-Shargabi, T., Wang, Y., du Plessis, A., et al. (2017). Effect of temperature on heart rate variability in neonatal ICU Patients with hypoxic-ischemic encephalopathy. *Pediatr. Crit. Care Med.* 18, 349–354. doi: 10.1097/PCC.0000000000001094

Massaro, A. N., Govindan, R. B., Al-Shargabi, T., Andescavage, N. N., Metzler, M., Chang, T., et al. (2014). Heart rate variability in encephalopathic newborns during and after therapeutic hypothermia. *J. Perinatol.* 34, 836–841. doi: 10.1038/jp.2014.108

McDuff, D., Gontarek, S., and Picard, R. W. (2014). Improvements in remote cardiopulmonary measurement using a five band digital camera. *IEEE Trans. Biomed. Eng.* 61, 2593–2601. doi: 10.1109/TBME.2014.2323695

McDuff, D. J., Blackford, E. B., and Estepp, J. R. (2018). Fusing partial camera signals for noncontact pulse rate variability measurement. *IEEE Trans. Biomed. Eng.* 65, 1725–1739. doi: 10.1109/TBME.2017.2771518

Metzler, M., Govindan, R., Al-Shargabi, T., Vezina, G., Andescavage, N., Wang, Y., et al. (2017). Pattern of brain injury and depressed heart rate variability in newborns with hypoxic ischemic encephalopathy. *Pediatr. Res.* 82, 438–443. doi: 10.1038/pr.2017.94

Mizumoto, H., Tomotaki, S., Shibata, H., Ueda, K., Akashi, R., Uchio, H., et al. (2012). Electrocardiogram shows reliable heart rates much earlier than pulse oximetry during neonatal resuscitation. *Pediatr. Int.* 54, 205–207. doi: 10.1111/j.1442-200X.2011.03506.x

Moorman, J. R., Carlo, W. A., Kattwinkel, J., Schelonka, R. L., Porcelli, P. J., Navarrete, C. T., et al. (2011). Mortality reduction by heart rate characteristic monitoring in very low birth weight neonates: a randomized trial. *J. Pediatr.* 159, 900.e1–906.e1. doi: 10.1016/j.jpeds.2011.06.044

Mowery, N. T., Morris, J. A. Jr., Jenkins, J. M., Ozdas, A., and Norris, P. R. (2011). Core temperature variation is associated with heart rate variability independent of cardiac index: a study of 278 trauma patients. *J. Crit. Care* 26, 534.e9–534.e17. doi: 10.1016/j.jcrc.2010.11.008

Mulkey, S. B., and du Plessis, A. (2018). The critical role of the central autonomic nervous system in fetal-neonatal transition. *Semin. Pediatr. Neurol.* 28, 29–37. doi: 10.1016/j.spen.2018.05.004

Mulkey, S. B., and du Plessis, A. J. (2019). Autonomic nervous system development and its impact on neuropsychiatric outcome. *Pediatr. Res.* 85, 120–126. doi: 10.1038/s41390-018-0155-0

Mulkey, S. B., Govindan, R., Metzler, M., Swisher, C. B., Hitchings, L., Wang, Y., et al. (2020). Heart rate variability is depressed in the early transitional period for newborns with complex congenital heart disease. *Clin. Auton. Res.* 30, 165–172. doi: 10.1007/s10286-019-00616-w

Mulkey, S. B., Kota, S., Swisher, C. B., Hitchings, L., Metzler, M., Wang, Y., et al. (2018). Autonomic nervous system depression at term in neurologically normal premature infants. *Early Hum. Dev.* 123, 11–16. doi: 10.1016/j.earlhumdev.2018.07.003

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., et al. (2015). Validity of (Ultra-)short recordings for heart rate variability measurements. *PLoS One* 10:e0138921. doi: 10.1371/journal.pone.0138921

Nukaya, S., Sugie, M., Kurihara, Y., Hiroyasu, T., Watanabe, K., and Tanaka, H. (2014). A noninvasive heartbeat, respiration, and body movement monitoring system for neonates. *Artif. Life Robot.* 19, 414–419. doi: 10.1007/s10015-014-0179-4

OECD (2020). *Infant Mortality Rates (indicator)*. OECD: Paris, doi: 10.1787/83dea506-en

Okur, N., Uras, N., Buyuktiryaki, M., Oncel, M. Y., Saria, F. N., and Yarci, E. (2019). Neonatal pain and heart rate variability in preterm infants treated with surfactant: a pilot study. *Arch. Argent. Pediatr.* 117, 397–401. doi: 10.5546/aap.2019.eng.397

Oliveira, V., Martins, R., Liow, N., Teiserskas, J., von Rosenberg, W., Adjei, T., et al. (2019a). Prognostic accuracy of heart rate variability analysis in neonatal encephalopathy: a systematic review. *Neonatology* 115, 59–67. doi: 10.1159/000493002

Oliveira, V., von Rosenberg, W., Montaldo, P., Adjei, T., Mendoza, J., Shivamurthappa, V., et al. (2019b). Early postnatal heart rate variability in healthy newborn infants. *Front. Physiol.* 10:922. doi: 10.3389/fphys.2019.00922

Ostojic, D., Guglielmini, S., Moser, V., Fauchère, J. C., Bucher, H. U., Bassler, D., et al. (2020). "Reducing false alarm rates in neonatal intensive care: a new machine learning approach," in *Oxygen Transport to Tissue XLI Advances in Experimental Medicine and Biology*, eds P.-D. Ryu, J. C. LaManna, D. K. Harrison, and S.-S. Lee (Cham: Sringer International ublishing), 285–290. doi: 10.1007/978-3-030-34461-0_36

Otieno, P., Waiswa, P., Butrick, E., Namazzi, G., Achola, K., Santos, N., et al. (2018). Strengthening intrapartum and immediate newborn care to reduce morbidity and mortality of preterm infants born in health facilities in Migori County, Kenya and Busoga Region, Uganda: a study protocol for a randomized controlled trial. *Trials* 19:313. doi: 10.1186/s13063-018-2696-2

Ozawa, M., Sasaki, M., and Kanda, K. (2010). Effect of procedure light on the physiological responses of preterm infants. *Jpn. J. Nurs. Sci.* 7, 76–83. doi: 10.1111/j.1742-7924.2010.00142.x

Özdemir, R., Olukman, Ö, Karadeniz, C., Çelik, K., Katipoğlu, N., Muhtar Yılmazer, M., et al. (2018). Effect of unconjugated hyperbilirubinemia on neonatal autonomic functions: evaluation by heart rate variability. *J. Matern. Fetal Neonatal Med.* 31, 2763–2769. doi: 10.1080/14767058.2017.1355901

Pagani, M., Lucini, D., and Porta, A. (2012). Sympathovagal balance from heart rate variability: time for a second round? *Exp. Physiol.* 97, 1141–1142. doi: 10.1113/expphysiol.2012.066977

Passi, R., Doheny, K. K., Gordin, Y., Hinssen, H., and Palmer, C. (2017). Electrical grounding improves vagal tone in preterm infants. *Neonatology* 112, 187–192. doi: 10.1159/000475744

Patural, H., Pichot, V., Flori, S., Giraud, A., Franco, P., Pladys, P., et al. (2019). Autonomic maturation from birth to 2 years: normative values. *Heliyon* 5:e01300. doi: 10.1016/j.heliyon.2019.e01300

Paul, M., Karthik, S., Joseph, J., Sivaprakasam, M., Kumutha, J., Leonhardt, S., et al. (2020). Non-contact sensing of neonatal pulse rate using camera-based imaging: a clinical feasibility study. *Physiol. Meas.* 41:024001. doi: 10.1088/1361-6579/ab755c

Pernice, R., Javorka, M., Krohova, J., Czippelova, B., Turianikova, Z., Busacca, A., et al. (2018). Reliability of short-term heart rate variability indexes assessed through photoplethysmography. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2018, 5513–5610. doi: 10.1109/EMBC.2018.8513634

Pernice, R., Javorka, M., Krohova, J., Czippelova, B., Turianikova, Z., Busacca, A., et al. (2019a). A validity and reliability study of conditional entropy measures of pulse rate variability. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2019, 5568–5571. doi: 10.1109/EMBC.2019.8856594

Pernice, R., Javorka, M., Krohova, J., Czippelova, B., Turianikova, Z., Busacca, A., et al. (2019b). Comparison of short-term heart rate variability indexes evaluated through electrocardiographic and continuous blood pressure monitoring. *Med. Biol. Eng. Comput.* 57, 1247–1263. doi: 10.1007/s11517-019-01957-4

Phillipos, E., Solevåg, A. L., Pichler, G., Aziz, K., van Os, S., O'Reilly, M., et al. (2016). Heart rate assessment immediately after birth. *Neonatology* 109, 130–138. doi: 10.1159/000441940

Pichot, V., Roche, F., Celle, S., Barthélémy, J. C., and Chouchou, F. (2016). HRV analysis: a free software for analyzing cardiac autonomic activity. *Front. Physiol.* 7:557. doi: 10.3389/fphys.2016.00557

Pillai Riddell, R. R., Racine, N. M., Gennis, H. G., Turcotte, K., Uman, L. S., Horton, R. E., et al. (2015). Non-pharmacological management of infant and young child procedural pain. *Cochrane Database Syst. Rev.* 2015:CD006275. doi: 10.1002/14651858.CD006275.pub3

Pinaya, W. H. L., Mechelli, A., and Sato, J. R. (2019). Using deep autoencoders to identify abnormal brain structural patterns in neuropsychiatric disorders: a large-scale multi-sample study. *Hum. Brain Mapp.* 40, 944–954. doi: 10.1002/hbm.24423

Prietsch, V., Maier, R., Schmitz, L., and Obladen, M. (1992). Long-term variability of heart rate increases with successful closure of patent ductus arteriosus in preterm infants. *Biol. Neonate* 61, 142–149. doi: 10.1159/000243736

Raczyńska, A., and Gulczyńska, E. (2019). The impact of positioning on bottle-feeding in preterm infants (= 34 GA). A comparative study of the semi-elevated and the side-lying position - a pilot study. *Dev. Period Med.* 23, 117–124.

Rajalakshmi, A., Sunitha, K. A., and Venkataraman, R. (2019). A survey on neonatal incubator monitoring system. *J. Phys. Conf. Ser.* 1362:012128. doi: 10.1088/1742-6596/1362/1/012128

Rashwan, N. I., Hassan, M. H., Mohey El-Deen, Z. M., and Ahmed, A. E. (2019). Validity of biomarkers in screening for neonatal sepsis - a single center -hospital based study. *Pediatr. Neonatol.* 60, 149–155. doi: 10.1016/j.pedneo.2018.05.001

Raynor, L. L., Saucerman, J. J., Akinola, M. O., Lake, D. E., Moorman, J. R., and Fairchild, K. D. (2012). Cytokine screening identifies NICU patients with Gram-negative bacteremia. *Pediatr. Res.* 71, 261–266. doi: 10.1038/pr.2011.45

Rees, C. A. (2014). Lost among the trees? The autonomic nervous system and paediatrics. *Arch. Dis. Child.* 99, 552–562. doi: 10.1136/archdischild-2012-301863

Reynolds, R. M. (2013). Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis–2012 Curt Richter Award Winner. *Psychoneuroendocrinology* 38, 1–11. doi: 10.1016/j.psyneuen.2012.08.012

Rivolta, M. W., Stampalija, T., Casati, D., Richardson, B. S., Ross, M. G., Frasch, M. G., et al. (2014). Acceleration and deceleration capacity of fetal heart rate in an in-vivo sheep model. *PLoS One* 9:e104193. doi: 10.1371/journal.pone.0104193

Romano, M., Bifulco, P., Cesarelli, M., Sansone, M., and Bracale, M. (2006). Foetal heart rate power spectrum response to uterine contraction. *Med. Biol. Eng. Comput.* 44, 188–201. doi: 10.1007/s11517-006-0022-8

Sahni, R., Gupta, A., Ohira-Kist, K., and Rosen, T. S. (2003). Motion resistant pulse oximetry in neonates. *Arch. Dis. Child Fetal Neonatal Ed.* 88, F505–F508. doi: 10.1136/fn.88.6.f505

Schechtman, V. L., Raetz, S. L., Harper, R. K., Garfinkel, A., Wilson, A. J., Southall, D. P., et al. (1992). Dynamic analysis of cardiac R-R intervals in normal infants and in infants who subsequently succumbed to the sudden infant death syndrome. *Pediatr. Res.* 31, 606–612. doi: 10.1203/00006450-199206000-00014

Schmidt, A., Schukat-Talamazzini, E. G., Zöllkau, J., Pytlik, A., Leibl, S., Kumm, K., et al. (2018). Universal characteristics of evolution and development are inherent in fetal autonomic brain maturation. *Auton. Neurosci.* 212, 32–41. doi: 10.1016/j.autneu.2018.02.004

Schneider, U., Bode, F., Schmidt, A., Nowack, S., Rudolph, A., Dölker, E. M., et al. (2018). Developmental milestones of the autonomic nervous system revealed via longitudinal monitoring of fetal heart rate variability. *PLoS One* 13:e0200799. doi: 10.1371/journal.pone.0200799

Seely, A. J. E., Green, G. C., and Bravi, A. (2011). "Continuous multiorgan variability monitoring in critically ill patients — complexity science at the bedside," in *2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, Boston, MA: IEEE, 5503–5506. doi: 10.1109/IEMBS.2011.6091404

Semenova, O., Carra, G., Lightbody, G., Boylan, G., Dempsey, E., and Temko, A. (2018). "Heart rate variability during periods of low blood pressure as a predictor of short-term outcome in preterms," in *2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Honolulu, HI: IEEE, 5614–5517. doi: 10.1109/EMBC.2018.8513600

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shaffer, F., McCraty, R., and Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front. Psychol.* 5:1040. doi: 10.3389/fpsyg.2014.01040

Shin, J. H., Hwang, S. H., Chang, M. H., and Park, K. S. (2011). Heart rate variability analysis using a ballistocardiogram during Valsalva manoeuvre and post exercise. *Physiol. Meas.* 32, 1239–1264. doi: 10.1088/0967-3334/32/8/015

Shiroiwa, Y., Kamiya, Y., Uchibori, S., Inukai, K., Kito, H., Shibata, T., et al. (1986). Activity, cardiac and respiratory responses of blindfold preterm infants in a neonatal intensive care unit. *Early Hum. Dev.* 14, 259–265. doi: 10.1016/0378-3782(86)90187-8

Shuffrey, L. C., Myers, M. M., Odendaal, H. J., Elliott, A. J., du Plessis, C., Groenewald, C., et al. (2019). Fetal heart rate, heart rate variability, and heart rate/movement coupling in the safe passage study. *J. Perinatol.* 39, 608–618. doi: 10.1038/s41372-019-0342-9

Signorini, M. G., Fanelli, A., and Magenes, G. (2014). Monitoring fetal heart rate during pregnancy: contributions from advanced signal processing and wearable technology. *Comput. Math. Methods Med.* 2014, 1–10. doi: 10.1155/2014/707581

Simeoni, U., Armengaud, J. B., Siddeek, B., and Tolsa, J. F. (2018). Perinatal origins of adult disease. *Neonatology* 113, 393–399. doi: 10.1159/000487618

Singh, N., Moneghetti, K. J., Christle, J. W., Hadley, D., Plews, D., and Froelicher, V. (2018). Heart rate variability: an old metric with new meaning in the era of using mhealth technologies for health and exercise training guidance. part one: physiology and methods. *Arrhythm. Electrophysiol. Rev.* 7, 193–198. doi: 10.15420/aer.2018.27.2

Siren, P. M. A. (2017). SIDS-CDF hypothesis revisited: cause vs. Contributing factors. *Front. Neurol.* 7:244. doi: 10.3389/fneur.2016.00244

Spassov, L., Curzi-Dascalova, L., Clairambault, J., Kauffmann, F., Eiselt, M., Médigue, C., et al. (1994). Heart rate and heart rate variability during sleep in small-for-gestational age newborns. *Pediatr. Res.* 35, 500–505. doi: 10.1203/00006450-199404000-00022

Spilka, J., Chudáèek, V., Koucký, M., Lhotská, L., Huptych, M., Janků, P., et al. (2012). Using nonlinear features for fetal heart rate classification. *Biomed. Signal Process. Control* 7, 350–357. doi: 10.1016/j.bspc.2011.06.008

Spyridou, K., Chouvarda, I., Hadjileontiadis, L., and Maglaveras, N. (2018). Linear and nonlinear features of fetal heart rate on the assessment of fetal development in the course of pregnancy and the impact of fetal gender. *Physiol. Meas.* 39:015007. doi: 10.1088/1361-6579/aa9e3c

Stapelberg, N. J. C., Neumann, D. L., Shum, D. H. K., McConnell, H., and Hamilton-Craig, I. (2017). The sensitivity of 38 heart rate variability measures to the addition of artifact in human and artificial 24-hr cardiac recordings. *Ann. Noninvasive Electrocardiol.* 23:e12483. doi: 10.1111/anec.12483

Stenson, B. J. (2016). Oxygen saturation targets for extremely preterm infants after the NeOProM trials. *Neonatology* 109, 352–358. doi: 10.1159/000444913

Stéphan-Blanchard, E., Chardon, K., Léké, A., Delanaud, S., Bach, V., and Telliez, F. (2013). Heart rate variability in sleeping preterm neonates exposed to cool and warm thermal conditions. *PLoS One* 8:e68211. doi: 10.1371/journal.pone.0068211

Stock, C., Teyssier, G., Pichot, V., Goffaux, P., Barthelemy, J. C., and Patural, H. (2010). Autonomic dysfunction with early respiratory syncytial virus-related infection. *Auton. Neurosci.* 156, 90–95. doi: 10.1016/j.autneu.2010.03.012

Stone, M. L., Tatum, P. M., Weitkamp, J. H., Mukherjee, A. B., Attridge, J., McGahren, E. D., et al. (2013). Abnormal heart rate characteristics before clinical diagnosis of necrotizing enterocolitis. *J. Perinatol.* 33, 847–850. doi: 10.1038/jp.2013.63

Sullivan, B. A., Grice, S. M., Lake, D. E., Moorman, J. R., and Fairchild, K. D. (2014). Infection and other clinical correlates of abnormal heart rate characteristics in preterm infants. *J. Pediatr.* 164, 775–780. doi: 10.1016/j.jpeds.2013.11.038

Sullivan, B. A., Wallman-Stokes, A., Isler, J., Sahni, R., Moorman, J. R., Fairchild, K. D., et al. (2018). Early pulse oximetry data improves prediction of death and adverse outcomes in a two-center cohort of very low birth weight infants. *Am. J. Perinatol.* 35, 1331–1338. doi: 10.1055/s-0038-1654712

Sun, Y., Hu, S., Azorin-Peris, V., Kalawsky, R., and Greenwald, S. (2012). Noncontact imaging photoplethysmography to effectively access pulse rate variability. *J. Biomed. Opt.* 18:061205. doi: 10.1117/1.JBO.18.6.061205

Sun, Y., and Thakor, N. (2016). Photoplethysmography revisited: from contact to noncontact, from point to imaging. *IEEE Trans. Biomed. Eng.* 63, 463–477. doi: 10.1109/TBME.2015.2476337

Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology (1996). Heart rate variability. standards of measurement, physiological interpretation, and clinical. (Use). *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/s0165-0327(00)00338-4

Thiriez, G., Mougey, C., Vermeylen, D., Wermenbol, V., Lanquart, J. P., Lin, J. S., et al. (2015). Altered autonomic control in preterm newborns with impaired neurological outcomes. *Clin. Auton. Res.* 25, 233–242. doi: 10.1007/s10286-015-0298-6

Thoyre, S. M., Holditch-Davis, D., Schwartz, T. A., Melendez Roman, C. R., and Nix, W. (2012). Coregulated approach to feeding preterm infants with lung disease: effects during feeding. *Nurs. Res.* 61, 242–251. doi: 10.1097/NNR.0b013e31824b02ad

Topalidou, A., Ali, N., Sekulic, S., and Downe, S. (2019). Thermal imaging applications in neonatal care: a scoping review. *BMC Pregnancy Childbirth.* 19:381. doi: 10.1186/s12884-019-2533-y

Tribe, R. M., Taylor, P. D., Kelly, N. M., Rees, D., Sandall, J., and Kennedy, H. P. (2018). Parturition and the perinatal period: can mode of delivery impact on the future health of the neonate? *J. Physiol.* 596, 5709–5722. doi: 10.1113/JP275429

Tu, W., Chen, P. A., Koenig, N., Gomez, D., Fujiwara, E., Gill, M. J., et al. (2020). Machine learning models reveal neurocognitive impairment type and prevalence are associated with distinct variables in HIV/AIDS. *J. Neurovirol.* 26, 41–51. doi: 10.1007/s13365-019-00791-6

Turova, V., Sidorenko, I., Eckardt, L., Rieger-Fackeldey, E., Felderhoff-Müser, U., Alves-Pinto, A., et al. (2020). Machine learning models for identifying preterm infants at risk of cerebral hemorrhage. *PLoS One* 15:e0227419. doi: 10.1371/journal.pone.0227419

Uhrikova, Z., Zibolen, M., Javorka, K., Chladekova, L., and Javorka, M. (2015). Hyperbilirubinemia and phototherapy in newborns: effects on cardiac autonomic control. *Early Hum. Dev.* 91, 351–356. doi: 10.1016/j.earlhumdev.2015.03.009

United Nations Inter-agency Group for Child Mortality Estimation (UN IGME) (2019). *). Levels & Trends in Child Mortality: Report 2019, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation*. New York, NY: United Nations Children's Fund.

Urfer-Maurer, N., Ludyga, S., Stalder, T., Brand, S., Holsboer-Trachsler, E., Gerber, M., et al. (2018). Heart rate variability and salivary cortisol in very preterm children during school age. *Psychoneuroendocrinology* 87, 27–34. doi: 10.1016/j.psyneuen.2017.10.004

Valenza, G., Iozzia, L., Cerina, L., Mainardi, L., and Barbieri, R. (2018). Analysis of instantaneous linear, nonlinear and complex cardiovascular dynamics from videophotoplethysmography. *Methods Inf. Med.* 57, 135–140. doi: 10.3414/ME17-02-0013

van Laar, J., Peters, C., Vullings, R., Houterman, S., Bergmans, J., and Oei, S. (2010). Fetal autonomic response to severe acidaemia during labour: fetal autonomic stress response during labour. *BJOG Int. J. Obstet. Gynaecol.* 117, 429–437. doi: 10.1111/j.1471-0528.2009.02456.x

van Laar, J. O. E. H., Peters, C. H. L., Houterman, S., Wijn, P. F. F., Kwee, A., and Oei, S. G. (2011). Normalized spectral power of fetal heart rate variability is associated with fetal scalp blood pH. *Early Hum. Dev.* 87, 259–263. doi: 10.1016/j.earlhumdev.2011.01.028

Van Leeuwen, P., Werner, L., Hilal, Z., Schiermeier, S., Hatzmann, W., and Grönemeyer, D. (2014). Fetal electrocardiographic measurements in the assessment of fetal heart rate variability in the antepartum period. *Physiol. Meas.* 35, 441–454. doi: 10.1088/0967-3334/35/3/441

Van Puyvelde, M., Collette, L., Gorissen, A. S., Pattyn, N., and McGlone, F. (2019a). Infants autonomic cardio-respiratory responses to nurturing stroking touch delivered by the mother or the father. *Front. Physiol.* 10:1117. doi: 10.3389/fphys.2019.01117

Van Puyvelde, M., Gorissen, A. S., Pattyn, N., and McGlone, F. (2019b). Does touch matter? The impact of stroking versus non-stroking maternal touch on cardio-respiratory processes in mothers and infants. *Physiol. Behav.* 207, 55–63. doi: 10.1016/j.physbeh.2019.04.024

van Ravenswaaij-Arts, C. M., Hopman, J. C., Kollée, L. A., van Amen, J. P., and Stoelinga, G. B. (1991). The influence of respiratory distress syndrome on heart rate variability in very preterm infants. *Early Hum. Dev.* 27, 207–221. doi: 10.1016/0378-3782(91)90195-9

Vassar, R., Schadl, K., Cahill-Rowley, K., Yeom, K., Stevenson, D., and Rose, J. (2020). Neonatal brain microstructure and machine-learning-based prediction of early language development in children born very preterm. *Pediatr. Neurol.* 108, 86–92. doi: 10.1016/j.pediatrneurol.2020.02.007

Venkataraman, R., Kamaluddeen, M., Amin, H., and Lodha, A. (2018). Is Less noise, light and parental/caregiver stress in the neonatal intensive care unit better for neonates? *Indian Pediatr.* 55, 17–21. doi: 10.1007/s13312-018-1220-9

Verklan, M. T., and Walden, M. (2014). *Core Curriculum for Neonatal Intensive Care Nursing*, 5th Edn. St. Louis, MO: Elsevier.

Villarroel, M., Guazzi, A., Jorge, J., Davis, S., Watkinson, P., Green, G., et al. (2014). Continuous non-contact vital sign monitoring in neonatal intensive care unit. *Healthc. Technol. Lett.* 1, 87–91. doi: 10.1049/htl.2014.0077

Voss, A., Schroeder, R., Vallverdú, M., Schulz, S., Cygankiewicz, I., Vázquez, R., et al. (2013). Short-term vs. long-term heart rate variability in ischemic cardiomyopathy risk stratification. *Front. Physiol.* 4:364. doi: 10.3389/fphys.2013.00364

Walusinski, O. (2006). Yawning: unsuspected avenue for a better understanding of arousal and interoception. *Med. Hypotheses* 67, 6–14. doi: 10.1016/j.mehy.2006.01.020

Wang, K., Zhu, T., Zhang, X., Yu, C., Cao, X., Tang, J., et al. (2015). [Comparison of heart rate variability measurements between ballistocardiogram and electrocardiography]. *Zhonghua Xin Xue Guan Bing Za Zhi* 43, 448–451.

Wang, Y., Carrault, G., Beuchee, A., Costet, N., Shu, H., and Senhadji, L. (2016). Heart rate variability and respiration signal as diagnostic tools for late onset sepsis in neonatal intensive care units. *ArXiv* [Preprint] Available online at: https://arxiv.org/abs/1605.05247 (accessed July 25, 2020).

Warmerdam, G. J. J., Vullings, R., Van Laar, J. O. E. H., Van der Hout-Van der Jagt, M. B., Bergmans, J. W. M., Schmitt, L., et al. (2016). Using uterine activity to improve fetal heart rate variability analysis for detection of asphyxia during labor. *Physiol. Meas.* 37, 387–400. doi: 10.1088/0967-3334/37/3/387

Warmerdam, G. J. J., Vullings, R., Van Laar, J. O. E. H., Van der Hout-Van der Jagt, M. B., Bergmans, J. W. M., Schmitt, L., et al. (2018). Detection rate of fetal distress using contraction-dependent fetal heart rate variability analysis. *Physiol. Meas.* 39:025008. doi: 10.1088/1361-6579/aaa925

Watson, G., Patel, K., Leng, D., Vanna, D., Khut, S., Prak, M., et al. (2020). Barriers and facilitators to neonatal health and care-seeking behaviours in rural Cambodia: a qualitative study. *BMJ Open* 10:e035449. doi: 10.1136/bmjopen-2019-035449

Weber, A., and Harrison, T. M. (2019). Reducing toxic stress in the neonatal intensive care unit to improve infant outcomes. *Nurs. Outlook* 67, 169–189. doi: 10.1016/j.outlook.2018.11.002

Weber, F., Roeleveld, H. G., Geerts, N. J. E., Warmenhoven, A. T., Schröder, R., and de Leeuw, T. G. (2019). The heart rate variability-derived Newborn Infant Parasympathetic Evaluation (NIPE$^{TM}$) Index in pediatric surgical patients from 0 to 2 years under sevoflurane anesthesia-A prospective observational pilot study. *Paediatr. Anaesth.* 29, 377–384. doi: 10.1111/pan.13613

Weese-Mayer, D. E., Corwin, M. J., Peucker, M. R., Di Fiore, J. M., Hufford, D. R., Tinsley, L. R., et al. (2000). Comparison of apnea identified by respiratory inductance plethysmography with that detected by end-tidal CO(2) or thermistor. The CHIME Study Group. *Am. J. Respir. Crit. Care Med.* 162(2 Pt 1), 471–480. doi: 10.1164/ajrccm.162.2.9904029

Weissman, A., Zimmer, E. Z., Aranovitch, M., and Blazer, S. (2012). Heart rate dynamics during acute pain in newborns. *Pflugers. Arch.* 464, 593–599. doi: 10.1007/s00424-012-1168-x

Werth, J. V. S. W. (2019). *On the Automated Analysis of Preterm Infant Sleep States From Electrocardiography*. Eindhoven: Technische Universiteit Eindhoven.

Werther, T., Aichhorn, L., Baumgartner, S., Berger, A., Klebermass-Schrehof, K., and Salzer-Muhar, U. (2018). Discrepancy between invasive and non-invasive

blood pressure readings in extremely preterm infants in the first four weeks of life. *PLoS One* 13:e0209831. doi: 10.1371/journal.pone.0209831

WHO (2016). *World Health Statistics 2016. Monitoring Health for The SDGs*. Avaliable online at: http://apps.who.int/iris/bitstream/10665/206498/1/9789241565264_eng.pdf (accessed July 28, 2020).

WHO (2017). *Reaching the Every Newborn National 2020 Milestones: Country Progress, Plans and Moving Forward.* Avaliable online at: https://apps.who.int/iris/bitstream/handle/10665/255719/9789241512619-eng.pdf (accessed July 28, 2020).

Williams, A. L., Sanderson, M., Lai, D., Selwyn, B. J., and Lasky, R. E. (2009). Intensive care noise and mean arterial blood pressure in extremely low-birth-weight neonates. *Am. J. Perinatol.* 26, 323–329. doi: 10.1055/s-0028-1104741

Williams, D. P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M. N., Sternberg, E. M., et al. (2019). Heart rate variability and inflammation: a meta-analysis of human studies. *Brain Behav. Immun.* 80, 219–226. doi: 10.1016/j.bbi.2019.03.009

Xu, A., Durosier, L. D., Ross, M. G., Hammond, R., Richardson, B. S., and Frasch, M. G. (2014). Adaptive brain shut-down counteracts neuroinflammation in the near-term ovine fetus. *Front. Neurol.* 5:110. doi: 10.3389/fneur.2014.00110

Yamaguchi, K., Lear, C. A., Beacom, M. J., Ikeda, T., Gunn, A. J., and Bennet, L. (2018). Evolving changes in fetal heart rate variability and brain injury after hypoxia-ischaemia in preterm fetal sheep. *J. Physiol.* 596, 6093–6104. doi: 10.1113/JP275434

Yiallourou, S. R., Sands, S. A., Walker, A. M., and Horne, R. S. (2012). Maturation of heart rate and blood pressure variability during sleep in term-born infants. *Sleep* 35, 177–186. doi: 10.5665/sleep.1616

Zahr, L. K., and de Traversay, J. (1995). Premature infant responses to noise reduction by earmuffs: effects on behavioral and physiologic measures. *J. Perinatol.* 15, 448–455.

Zhang, K., Wang, S., Wu, L., Song, Y., Cai, M., Zhang, M., et al. (2019). Newborn infant parasympathetic evaluation (NIPE) as a predictor of hemodynamic response in children younger than 2 years under general anesthesia: an observational pilot study. *BMC Anesthesiol.* 19:98. doi: 10.1186/s12871-019-0774-y

Zhao, F., Li, M., Jiang, Z., Tsien, J. Z., and Lu, Z. (2016). Camera-based, non-contact, vital-signs monitoring technology may provide a way for the early prevention of SIDS in infants. *Front. Neurol.* 7:236. doi: 10.3389/fneur.2016.00236

Zöllkau, J., Dölker, E.-M., Schmidt, A., Schneider, U., and Hoyer, D. (2019). Dependencies between maternal and fetal autonomic tone. *J. Perinat. Med.* 47, 323–330. doi: 10.1515/jpm-2018-0221

Zuzarte, I., Indic, P., Sternad, D., and Paydarfar, D. (2019). Quantifying movement in preterm infants using photoplethysmography. *Ann. Biomed. Eng.* 47, 646–658. doi: 10.1007/s10439-018-02135

**Conflict of Interest:** DB was employed by the company BioTekna.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

PERSPECTIVE
published: 07 October 2020
doi: 10.3389/fnins.2020.579365

# Exploring the Effects of Osteopathic Manipulative Treatment on Autonomic Function Through the Lens of Heart Rate Variability

*Luca Carnevali*[1,2]*, *Luca Lombardi*[2], *Mauro Fornari*[2] and *Andrea Sgoifo*[1,2]

[1] Stress Physiology Lab, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy, [2] Stress Control Lab, Collegio Italiano di Osteopatia, Parma, Italy

The osteopathic community has long hypothesized that the autonomic nervous system (ANS) represents one of the putative substrates through which osteopathic manipulative treatment (OMT) can improve body functions that have been altered by musculoskeletal alterations. Heart rate variability (HRV) is an important physiological measure of cardiac ANS activity. Emerging evidence suggests that OMT is associated with HRV changes that (i) are indicative of a larger cardiac vagal modulation, (ii) are independent from the part of the body needing treatment, (iii) occur even in the absence of musculoskeletal alterations. Yet, many questions remain unanswered, the duration of these effects and the specificity of HRV responses to different OMT techniques being perhaps the most critical. Therefore, this paper discusses prospects for future applications of HRV for the study of the influence of OMT on ANS function. Moreover, based on existing studies and preliminary data on the effects of OMT on HRV in specific pathological (hypertension) and physiological (stress exposure and recovery from sport competition) conditions that are commonly associated with increased sympathetic and/or decreased vagal activity, we propose that HRV analysis could be exploited to evaluate the effectiveness of OMT as a preventive or complementary strategy in clinical and non-clinical conditions characterized by ANS imbalance.

**Keywords: osteopathy, heart rate variability, autonomic, hypertension, stress, fatigue status**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Eleonora Tobaldini, University of Milan, Italy*
*Moacir Fernandes Godoy, Faculty of Medicine of São José do Rio Preto, Brazil*
*Markus Raab, German Sport University Cologne, Germany*
*Valerie Eckardt, Deutsche Sporthochschule Köln, in collaboration with reviewer MR*

***Correspondence:**
*Luca Carnevali*
*luca.carnevali@unipr.it*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *02 July 2020*
***Accepted:*** *17 September 2020*
***Published:*** *07 October 2020*

***Citation:***
*Carnevali L, Lombardi L, Fornari M and Sgoifo A (2020) Exploring the Effects of Osteopathic Manipulative Treatment on Autonomic Function Through the Lens of Heart Rate Variability. Front. Neurosci. 14:579365. doi: 10.3389/fnins.2020.579365*

## INTRODUCTION

Osteopathic manipulative treatment (OMT), a non-invasive form of manual therapy, has evolved as a therapeutic approach aimed at correcting alterations in musculoskeletal structures that are having direct or indirect negative effects on the perfusion of body tissues and inherent physiological function. This perspective article is centered around the belief, within the osteopathic community, that the autonomic nervous system (ANS) represents one of the putative substrates of the action of OMT and its favorable effects on body functions (Henley et al., 2008; Rechberger et al., 2019). Somatic dysfunctions—which in the osteopathic terminology imply "impaired or altered function of related components of the body framework system: skeletal, arthrodial and myofascial structures, and related vascular, lymphatic and neural elements" (Glossary Review Committee, for the Educational Council on Osteopathic Principles and the American Association of Colleges of Osteopathic Medicine, 2017)—are treated using a wide variety

of manual techniques, alone or in combination. The description of these techniques, which include articular and myofascial techniques, balanced ligamentous tension and craniosacral techniques, just to name a few, falls beyond the scope of this paper and can be found elsewhere (e.g., Campbell et al., 2012). Here, the focus is directed to the theoretical association between OMT and ANS, which is being supported by emerging empirical evidence obtained through physiological measures of ANS activity (Rechberger et al., 2019). Specifically, in the next sections we will discuss studies that have adopted heart rate variability (HRV) measures to evaluate the relationship between OMT and ANS activity in healthy subjects and in specific pathological and physiological conditions characterized by ANS imbalance, and we will offer new prospects for future applications of HRV for the study of the influence of OMT on ANS function.

## AUTONOMIC FUNCTION AND HRV

Autonomic regulation of most visceral organs reflects the balance between sympathetic and parasympathetic (vagal) modulation. This is particularly apparent in the neural control of the heart, where the balance between sympathetic excitation and vagal inhibition of sinoatrial node activity contributes to beat-to-beat heart rate fluctuations, also known as HRV. Traditionally, several HRV indices and methods have been used to quantify cardiac sympathetic and vagal influences (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Shaffer and Ginsberg, 2017). However, it is now widely recognized that the association between ANS balance and HRV is non-trivial, particularly in light of the non-linear and non-reciprocal relationship between sympathetic and vagal activity, and caution has been advised with loose interpretations of HRV metrics (Goldstein et al., 2011; Billman, 2013; Reyes Del Paso et al., 2013; Laborde et al., 2017). While we do not want to dig into the intricacies of HRV research, it is worth mentioning for the purposes of this paper that measures of HRV that reflect fast changes are reliably interpreted as surrogate indexes of cardiac vagal function (Laborde et al., 2017). Commonly reported measures of vagally mediated HRV include the root mean square of successive beat-to-beat interval differences (RMSSD)—a time-domain measure—and the high frequency HRV (HF-HRV). Moreover, the low frequency (LF) to HF ratio (LF/HF) was long considered as representing the sympathovagal balance, although this stance has been highly criticized (e.g., Billman, 2013). In fact, given that the LF band does not reflect sympathetic activity (Goldstein et al., 2011; Rahman et al., 2011; Heathers, 2012), there is now a consensus to say that the precise physiological underpinnings of the LF/HF ratio are still unclear, making the interpretation of LF/HF data problematic at best (Laborde et al., 2017).

The use of HRV as a proxy for ANS function has become a popular approach in several clinical and investigational domains (e.g., Thayer et al., 2010, 2012; Kemp and Quintana, 2013). In fact, an optimal level of vagally mediated HRV is generally associated with health, self-regulatory capacity, and adaptability or resilience, whereas low vagally mediated HRV is considered a risk factor for adverse physical and psychological health outcomes (Thayer et al., 2012; Beauchaine and Thayer, 2015; Sgoifo et al., 2015; Williams et al., 2015; Carnevali et al., 2018). More recently, HRV analysis has also captured the attention of manual therapy research in an attempt to understand how different manual approaches can influence ANS function, including, as we will detail below, OMT.

## OMT AND HRV IN HEALTHY SUBJECTS

To the best of our knowledge, the first investigation of the link between OMT and HRV dates back to 2008 (Henley et al., 2008). This crossover study employed one particular type of OMT technique, cervical myofascial release, and explored its effect on ECG-based HRV responses to a passive 50° head-up tilt test in a small group of healthy male and female subjects (19–50 years old). The OMT technique was administered for 2 min after the beginning of the tilt test and was compared with sham manipulation and no-touch control. Results indicated that OMT was associated with higher HF-HRV values and a lower LF/HF ratio during the last 5 min of the tilt test, thus providing the first quantitative demonstration of the potential influence of OMT on reactivity measures of HRV during passive standing. This was followed by the investigation of the effects of a single session of OMT on ECG-based resting measures of HRV in small samples of healthy male and female subjects (22–32 years old) (Shi et al., 2011; Giles et al., 2013). An increase in vagally mediated HRV (i.e., HF-HRV) compared to baseline was observed during 4 min of cranial OMT—with no differences compared with sham therapy (Shi et al., 2011)—and specifically during the last 6 min of a 15 min cervical OMT protocol compared with sham therapy and time control in a crossover design (Giles et al., 2013). Notably, the latter effect was reported in healthy subjects free of injury or somatic dysfunction and therefore cannot be attributed to the correction of underlying musculoskeletal conditions. Moreover, the increase in vagally mediated HRV during OMT was not associated with concomitant changes in respiratory rate. This is relevant in light of the known influence of respiratory rate on cardiac vagal tone and particularly on the HF-HRV component (Berntson et al., 1997; Hill and Siebenbrock, 2009; Laborde et al., 2017). To improve the clinical generalizability of these findings, a subsequent sham-controlled crossover study adopted different OMT techniques to correct specific somatic dysfunctions found on structural evaluation—instead of a pre-determined OMT technique—in a healthy sample ($n$ = 57) of asymptomatic adults (18–35 years old, 51% males). Interestingly, an increase in measures of vagally mediated HRV obtained via plethysmography was observed during both the 15-min OMT treatment and the following 5 min when no hand contact was provided (Ruffini et al., 2015). These results were recently replicated by the same group (Cerritelli et al., 2020a), and appear consistent with HRV responses to other forms of manual therapy, including spinal manipulative therapy and manual cranial therapy, both

in asymptomatic adults (Budgell and Hirano, 2001; Budgell and Polus, 2006; Welch and Boone, 2008) and children (Bayo-Tallon et al., 2019). Together, these studies provide preliminary evidence that manual therapy techniques, and OMT in particular for our purposes, are associated with HRV changes which seem to: (i) be indicative of a larger cardiac vagal modulation under several conditions (i.e., resting and passive standing), (ii) be independent from the part of the body needing treatment, (iii) occur even in the absence of somatic dysfunctions, and (iv) be evident in different age and sex groups. In our view, one major limitation of these studies is that HRV measures were obtained during or immediately after a single-session treatment, and therefore it is not possible to estimate the extent to which OMT-associated increases in vagally mediated HRV can endure over time. Moreover, considering that there are many types of OMT techniques, it would be interesting to test whether they are associated with different HRV responses in the same individual.

## OMT AND HRV IN PATHOLOGICAL AND PHYSIOLOGICAL CONDITIONS CHARACTERIZED BY ANS IMBALANCE

In light of the above reported HRV responses in healthy individuals, the use of OMT in conditions characterized by ANS imbalance seems intuitive, yet little quantitative data currently supports its effectiveness. Here, we present preliminary evidence of the effects of OMT on HRV in specific pathological (hypertension) and physiological (stress exposure and recovery from sport competition) conditions that are generally associated with increased sympathetic and/or decreased vagal activity.

### Hypertension

Hypertension is commonly associated with increased sympathetic and decreased vagal activity (Julius, 1991), and reduced HRV has been described in hypertensive patients compared to normotensive individuals (Huikuri et al., 1996; Singh et al., 1998). In a non-randomized exploratory trial in which hypertensive subjects underwent OMT every fortnight for a period of 1 year, alongside routine hypertensive treatment, the authors found preliminary evidence of an association between OMT and reduced systolic BP (SBP), but not diastolic BP (DBP), compared to a control condition (Cerritelli et al., 2011). However, ANS function was not investigated in this study. More recently, BP and HRV were assessed in hypertensive and normotensive men aged 40–60 years before and after a single-session cranial OMT (Curi et al., 2018). Analysis of BP and HRV (derived from a cardiac monitor) revealed a decrease in SBP and DBP in hypertensive, but not normotensive, subjects, and a modest increase in vagally mediated HRV in both groups during the 15 min that followed OMT (Curi et al., 2018). However, as the authors acknowledged, the absence of a sham intervention and the short HRV and BP monitoring period after OMT limited the interpretation of these results. In a pilot and non-controlled study conducted in our lab, we evaluated BP and HRV in a small sample of hypertensive men undergoing four sessions of OMT, once a week for 4 weeks. The OMT intervention consisted of cervical myofascial release to improve vagus nerve function (Magoun, 1976; Henley et al., 2008), thoracic myofascial release and rib raising/articulation techniques to inhibit the sympathetic ganglionic chain, and lymphatic drainage techniques to contribute to body fluid homeostasis. BP and HRV assessment was conducted before the beginning of the OMT protocol and 48 h after the last OMT session, both at rest and in response to a passive 70° head-up tilt test. Our preliminary results indicate the presence of reduced resting values of SBP and DBP 48 h after the completion of the OMT protocol (**Figures 1A,B**). Moreover, we observed a larger reduction in HF-HRV values (**Figure 1C**) and a larger increase in the LF/HF ratio (**Figure 1D**) in response to passive standing compared with the pre-treatment assessment. Notably, previous studies have indicated that the reflex increase in sympathetic activity during passive standing is impaired in hypertension, probably because the sympathetic system is already hyperactive (Piccirillo et al., 1996; Heiskanen et al., 2011). Thus, keeping the limitations of the LF/HF in mind, our HRV data might hint at an improvement in cardiac ANS reactivity following OMT in hypertensive subjects. We believe that these results, although limited by their exploratory nature and clear methodological shortcomings, warrant new investigations involving randomized controlled trials on the effectiveness of OMT as a preventive and/or complementary treatment for hypertension and on the underlying ANS mechanisms.

### Stress Exposure

It is widely known that stressors tip the ANS balance toward a larger sympathetic prevalence and activate the hypothalamic-pituitary-adrenal axis (i.e., increase cortisol levels). In a between-subject study conducted by our group, a single-session of OMT using craniosacral techniques was performed immediately after an acute mental stressor on a small sample of healthy young men (20–30 years old) (Fornari et al., 2017). We found a smaller reduction in HF-HRV values (obtained from ECG analysis) and a much lower cortisol increment in response to the mental stressor in the OMT group compared with sham treatment (Fornari et al., 2017). While these results must be interpreted with caution given the exploratory nature of this study, in our view the association between OMT and reduced cardiac vagal withdrawal and cortisol rise in response to an acute stress represents an intriguing health-related outcome. In fact, building on the neurovisceral integration model (Thayer and Lane, 2000, 2009; Smith et al., 2017), sustained ANS imbalance is conceived as one feature of the biology of chronic stress that contributes to the development and progression of stress-related disorders. Relatedly, there is extensive evidence of reduced vagally mediated HRV in a number of stress-related emotional dysregulations, psychological disorders, and physical dysfunctions (Thayer et al., 2010, 2012; Beauchaine and Thayer, 2015; Sgoifo et al., 2015; Williams et al., 2015). Therefore, further investigation into the effects of OMT on HRV responses to acute and chronic stress conditions will provide novel insights into the potential utility of OMT as a

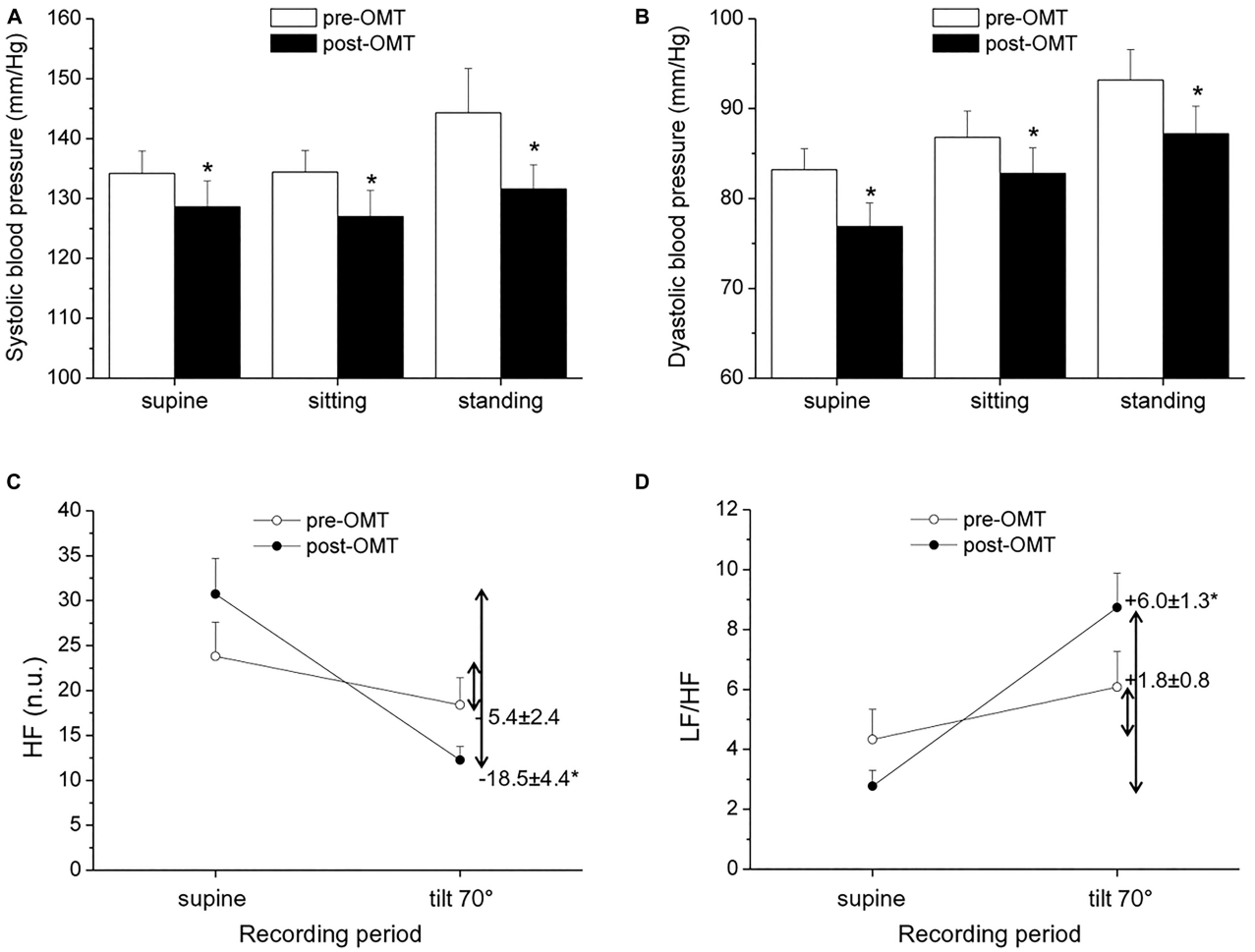



**FIGURE 1 |** Pilot study conducted on 10 men with a confirmed diagnosis of hypertension [age: 54 ± 2 years; body mass index = 25.8 ± 1.0 kg/m$^2$; non-smokers; no other clinical conditions; under regular antihypertensive treatment with calcium channel blockers ($n$ = 6) and ACE inhibitors ($n$ = 4)]. Blood pressure (BP) and heart rate variability (HRV) measures were obtained prior to and 48 h after an osteopathic manipulative treatment (OMT) protocol, consisting of four sessions of OMT, once a week for 4 weeks. Systolic **(A)** and diastolic **(B)** BP were measured with an electronic sphygmomanometer (A&D Medical: Model UA-631 V). High-frequency (HF, **C**) and low frequency (LF) /HF **(D)** data of HRV were calculated from ECG signals recorded with a BT16Plus device (Francesco Marazza Hardware & Software). ECG recordings lasted for 10 min in a supine position on a motorized table and during passive head-up tilting at 70°. Inner numbers in **(C,D)** represent delta changes (i.e., tilt value—supine value). Values are expressed as mean ± SEM. *indicates a significant difference (paired $t$-test $p < 0.05$).

preventive or complementary strategy in clinical and non-clinical settings associated with life stress (e.g., Dixon et al., 2020).

## Recovery From Sport Competition

Conditions of ANS imbalance characterized by decreased vagally mediated HRV have also been described in athletes in the aftermath of a sport competition or in the presence of overtraining syndrome (Iellamo et al., 2002; Mourot et al., 2004; Gratze et al., 2005; Bricout et al., 2010; Hellard et al., 2011; Boullosa et al., 2012; Edmonds et al., 2013). Consistent with this literature, in a recent study conducted by our group we found signs of reduced vagally mediated HRV (RMSSD and HF-HRV obtained from ECG analysis) and elevated mean arterial pressure 18–20 h after a rugby match in male players (Carnevali et al., 2020). Remarkably, in this sham-controlled crossover trial we showed that signs of cardiac vagal withdrawal and elevated mean arterial pressure were corrected by a players' need-based OMT treatment addressing specific somatic dysfunctions found on structural evaluation (Carnevali et al., 2020). Previous studies have shown that OMT can help relieve pain and have an impact on various kinematic parameters that could be beneficial to athletes' health and performance (Licciardone et al., 2005; Brolinson et al., 2012). Performing at high level also requires an optimal interplay of sympathetic and vagal activity (Hedelin et al., 2001; Pagani and Lucini, 2009; Hug et al., 2014). Therefore, our preliminary results might suggest wider opportunities for the use of OMT in athletes, thus opening the way for future randomized controlled trials aimed at testing the effectiveness of OMT as a recovery strategy to restore athletes' optimal ANS function in the aftermath of a competition and/or during conditions of overtraining syndrome.

## POTENTIAL PATHOPHYSIOLOGICAL LINKS BETWEEN OMT AND HRV

The specific pathophysiological mechanisms underlying the influence of OMT on ANS function, and specifically HRV, are currently unknown. However, we may put forward several hypotheses. For example, musculoskeletal alterations may increase sympathetic activity via proinflammatory mediators (Pongratz and Straub, 2014), and/or cause a compressive effect on the vagus nerve, given the anatomical relationship of vagal efferents to the musculoskeletal structures at the occiput (Giles et al., 2013). Consequently, the correction of musculoskeletal alterations with OMT may help restore the ANS balance. Moreover, it is becoming increasingly clear that the ANS, particularly the vagus nerve, is involved in the regulation of the inflammatory reflex that controls innate immune responses when tissue is injured or there is a pathogen invasion (Pavlov and Tracey, 2012). The inflammatory reflex has an afferent vagal component that is activated by cytokines and relay information to the hypothalamus. A subsequent efferent signal via the vagus initiates an anti-inflammatory response that prevents the release of inflammatory products into the blood stream (Tracey, 2002). Relatedly, we recently published a meta-analysis demonstrating the presence of a negative relationship between HRV and markers of inflammation (Williams et al., 2019). It is therefore also plausible that the correction of alterations in the musculoskeletal structures the surround the vagus may improve its ability to contribute to anti-inflammatory responses- another way of describing the "structure-function concept" of osteopathy- resulting in increased HRV. However, findings of increased HRV following OMT also in asymptomatic individuals free of injury or somatic dysfunction suggest that other mechanisms may be involved. For example, OMT might directly activate c-tactile fiber afferent projections to brain stem nuclei involved in the regulation of cardiac ANS control. In fact, growing evidence suggests that touch-based interventions such as OMT may play an interoceptive role via c-tactile afferents (McGlone et al., 2017; Edwards et al., 2018; Cerritelli et al., 2020b). It must be noted, however, that in the Edwards' study (Edwards et al., 2018), changes in interoceptive accuracy following OMT mobilization of the temporomandibular joint were not accompanied by concomitant changes in HRV in healthy subjects. Therefore, future mechanistic research is needed to unveil the precise pathophysiological links between OMT and HRV.

## CONCLUSION

The study of the relationship between OMT and the ANS is very much in its early days. However, the analysis of HRV in experimental investigations on this link has started to provide preliminary insights into the ability of OMT to tip the ANS balance toward a relatively larger cardiac vagal modulation in healthy subjects. Many questions remain unanswered, the duration of this effect and the specificity of HRV responses to different OMT techniques being perhaps the most critical. We therefore encourage future randomized controlled trials adopting different treatment protocols and longer HRV assessment to address these questions in healthy populations and individuals with diagnosed somatic dysfunctions. Specifically, we would advise researchers to adopt the three Rs design (resting, reactivity, recovery) mentioned in Laborde et al. (2017) for a better understanding of the effects of a single session of OMT on vagally mediated HRV, and also of a multiple-session OMT intervention (Hottenrott et al., 2019). We believe that the results of these trials will provide a more precise quantitative evaluation of the effects of OMT on cardiac ANS function, and will also help identify or refine specific protocols for the use of OMT in conditions characterized by ANS imbalance. In fact, we have presented here preliminary and promising examples of the favorable effects of OMT on HRV in pathological and physiological conditions that are commonly associated with larger sympathetic dominance, namely hypertension, stress exposure, and recovery from sport competition. We are aware that there is still a long road ahead, but, in our view, further substantiation of the influence of OMT on ANS function using HRV will represent the first step toward novel applications of OMT as a preventive and/or complementary strategy in these and potentially other clinical and non-clinical conditions characterized by ANS imbalance. Relatedly, more effort should be directed to the exploration of the mechanisms linking OMT to HRV. Of these, we propose that mechanisms that rely on c-tactile fiber signaling may represent the most promising candidate.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional review board of the Collegio Italiano di Osteopatia in Parma. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

LC and AS were involved in the conception and design of this article. LC, LL, and MF conducted the pilot study. LC wrote the first draft. LL, MF, and AS revised the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.

## REFERENCES


Bayo-Tallon, V., Esquirol-Caussa, J., Pamias-Massana, M., Planells-Keller, K., and Palao-Vidal, D. J. (2019). Effects of manual cranial therapy on heart rate variability in children without associated disorders: translation to clinical practice. *Complement. Ther. Clin Pract.* 36, 125–141. doi: 10.1016/j.ctcp.2019.06.008

Beauchaine, T. P., and Thayer, J. F. (2015). Heart rate variability as a transdiagnostic biomarker of psychopathology. *Int. J. Psychophysiol.* 98, 338–350. doi: 10.1016/j.ijpsycho.2015.08.004

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26. doi: 10.3389/fphys.2013.00026

Boullosa, D. A., Abreu, L., Tuimil, J. L., and Leicht, A. S. (2012). Impact of a soccer match on the cardiac autonomic control of referees. *Eur. J. Appl. Physiol.* 112, 2233–2242. doi: 10.1007/s00421-011-2202-y

Bricout, V. A., Dechenaud, S., and Favre-Juvin, A. (2010). Analyses of heart rate variability in young soccer players: the effects of sport activity. *Auton. Neurosci.* 154, 112–116. doi: 10.1016/j.autneu.2009.12.001

Brolinson, P. G., Smolka, M., Rogers, M., Sukpraprut, S., Goforth, M. W., Tilley, G., et al. (2012). Precompetition manipulative treatment and performance among Virginia Tech athletes during 2 consecutive football seasons: a preliminary, retrospective report. *J. Am. Osteopath. Assoc.* 112, 607–615.

Budgell, B., and Hirano, F. (2001). Innocuous mechanical stimulation of the neck and alterations in heart-rate variability in healthy young adults. *Auton. Neurosci.* 91, 96–99. doi: 10.1016/S1566-0702(01)00306-X

Budgell, B., and Polus, B. (2006). The effects of thoracic manipulation on heart rate variability: a controlled crossover trial. *J. Manipulative Physiol. Ther.* 29, 603–610. doi: 10.1016/j.jmpt.2006.08.011

Campbell, S. M., Winkelmann, R. R., and Walkowski, S. (2012). Osteopathic manipulative treatment: novel application to dermatological disease. *J. Clin. Aesther. Dermatol.* 5, 24–32.

Carnevali, L., Cerritelli, F., Guolo, F., and Sgoifo, A. (2020). Osteopathic manipulative treatment and cardiovascualr autonomic parameters in rugby players: a randomized, sham-controlled trial. *J. Manipulative Physiol. Ther.* (in press).

Carnevali, L., Koenig, J., Sgoifo, A., and Ottaviani, C. (2018). Autonomic and brain morphological predictors of stress resilience. *Front. Neurosci.* 12:228. doi: 10.3389/fnins.2018.00228

Cerritelli, F., Cardone, F., Pirino, A., Merla, A., and Scoppa, F. (2020a). Does osteopathic manipulation treatment induce autonomic chnages in healthy participants? A thermal imaging study. *Front. Neurosci.* 14:887. doi: 10.3389/fnins.2020.00887

Cerritelli, F., Carinci, F., Pizzolorusso, G., Turi, P., Renzetti, C., Pizzolorusso, F., et al. (2011). Osteopathic manipulation as a complementary treatment for the prevention of cardiac complications: 12-Months follow-up of intima media and blood pressure on a cohort affected by hypertension. *J. Bodyw. Mov. Ther.* 15, 68–74. doi: 10.1016/j.jbmt.2010.03.005

Cerritelli, F., Chiacchiaretta, P., Gambi, F., Perrucci, M. G., Barassi, G., Visciano, C., et al. (2020b). Effect of manual approaches with osteopathic modality on brain correlates of interoception: an fMRI study. *Sci. Rep.* 10:3214. doi: 10.1038/s41598-020-60253-6

Curi, A. C. C., Maior Alves, A. S., and Silva, J. G. (2018). Cardiac autonomic response after cranial technique of the fourth ventricle (cv4) compression in systemic hypertensive subjects. *J. Bodyw. Mov. Ther.* 22, 666–672. doi: 10.1016/j.jbmt.2017.11.013

Dixon, L., Fotinos, K., Sherifi, E., Lokuge, S., Fine, A., Furtado, M., et al. (2020). Effect of osteopathic manipulative therapy on generalized anxiety disorder. *J. Am. Osteopath. Assoc.* 120, 133–143. doi: 10.7556/jaoa.2020.026

Edmonds, R. C., Sinclair, W. H., and Leicht, A. S. (2013). Effect of a training week on heart rate variability in elite youth rugby league players. *Int. J. Sports Med.* 34, 1087–1092. doi: 10.1055/s-0033-1333720

Edwards, D. J., Young, H., Curtis, A. S., and Johnston, R. (2018). The immediate effect of therapeutic touch and deep touch pressure on range of motion, interoceptive accuracy and heart rate variability: a randomized controlled trial with moderation analysis. *Front. Integr. Neurosci.* 12:41. doi: 10.3389/fnint.2018.00041

Fornari, M., Carnevali, L., and Sgoifo, A. (2017). Single osteopathic manipulative therapy session dampens acute autonomic and neuroendocrine responses to mental stress in healthy male participants. *J. Am. Osteopath. Assoc.* 117, 559–567. doi: 10.7556/jaoa.2017.110

Giles, P. D., Hensel, K. L., Pacchia, C. F., and Smith, M. L. (2013). Suboccipital decompression enhances heart rate variability indices of cardiac control in healthy subjects. *J. Altern. Complement. Med.* 19, 92–96. doi: 10.1089/acm.2011.0031

Glossary Review Committee, for the Educational Council on Osteopathic Principles and the American Association of Colleges of Osteopathic Medicine (2017). *Glossary of OsteopathicTerminology*. Available online at: https://www.aacom.org/docs/defaultsource/insideome/got2011ed.pdf (accessed September 3, 2020)

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Gratze, G., Rudnicki, R., Urban, W., Mayer, H., Schlogl, A., and Skrabal, F. (2005). Hemodynamic and autonomic changes induced by Ironman: prediction of competition time by blood pressure variability. *J. Appl. Physiol.* 99, 1728–1735. doi: 10.1152/japplphysiol.00487.2005

Heathers, J. A. (2012). Sympathovagal balance from heart rate variability: an obituary. *Exp. Physiol.* 97:556. doi: 10.1113/expphysiol.2011.063867

Hedelin, R., Bjerle, P., and Henriksson-Larsen, K. (2001). Heart rate variability in athletes: relationship with central and peripheral performance. *Med. Sci. Sports Exerc.* 33, 1394–1398. doi: 10.1097/00005768-200108000-00023

Heiskanen, N., Saarelainen, H., Karkkainen, H., Valtonen, P., Lyyra-Laitinen, T., Laitinen, T., et al. (2011). Cardiovascular autonomic responses to head-up tilt in gestational hypertension and normal pregnancy. *Blood Press.* 20, 84–91. doi: 10.3109/08037051.2010.532313

Hellard, P., Guimaraes, F., Avalos, M., Houel, N., Hausswirth, C., and Toussaint, J. F. (2011). Modeling the association between HR variability and illness in elite swimmers. *Med. Sci. Sports Exerc.* 43, 1063–1070. doi: 10.1249/MSS.0b013e318204de1c

Henley, C. E., Ivins, D., Mills, M., Wen, F. K., and Benjamin, B. A. (2008). Osteopathic manipulative treatment and its relationship to autonomic nervous system activity as demonstrated by heart rate variability: a repeated measures study. *Osteopath. Med. Prim. Care* 2:7. doi: 10.1186/1750-4732-2-7

Hill, L. K., and Siebenbrock, A. (2009). Are all measures created equal? Heart rate variability and respiration – biomed 2009. *Biomed. Sci. Instrum.* 45, 71–76.

Hottenrott, L., Ketelhut, S., and Hottenrott, K. (2019). Commentary: vagal tank theory: the three Rs of cardiac vagal control functioning – resting, reactivity, and recovery. *Front. Neurosci.* 13:1300. doi: 10.3389/fnins.2019.01300

Hug, B., Heyer, L., Naef, N., Buchheit, M., Wehrlin, J. P., and Millet, G. P. (2014). Tapering for marathon and cardiac autonomic function. *Int. J. Sports Med.* 35, 676–683. doi: 10.1055/s-0033-1361184

Huikuri, H. V., Ylitalo, A., Pikkujamsa, S. M., Ikaheimo, M. J., Airaksinen, K. E., Rantala, A. O., et al. (1996). Heart rate variability in systemic hypertension. *Am. J. Cardiol.* 77, 1073–1077. doi: 10.1016/s0002-9149(96)00135-x

Iellamo, F., Legramante, J. M., Pigozzi, F., Spataro, A., Norbiato, G., Lucini, D., et al. (2002). Conversion from vagal to sympathetic predominance with strenuous training in high-performance world class athletes. *Circulation* 105, 2719–2724. doi: 10.1161/01.cir.0000018124.01299.ae

Julius, S. (1991). Autonomic nervous system dysregulation in human hypertension. *Am. J. Cardiol.* 67, 3B–7B. doi: 10.1016/0002-9149(91)90813-z

Kemp, A. H., and Quintana, D. S. (2013). The relationship between mental and physical health: insights from the study of heart rate variability. *Int. J. Psychophysiol.* 89, 288–296. doi: 10.1016/j.ijpsycho.2013.06.018

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Licciardone, J. C., Brimhall, A. K., and King, L. N. (2005). Osteopathic manipulative treatment for low back pain: a systematic review and meta-analysis of

randomized controlled trials. *BMC Musculoskelet. Dis.* 6:43. doi: 10.1186/1471-2474-6-43

Magoun, H. I. (1976). *Osteopathy in the Cranial Field*. Kirksville, MO: The Journal Printing Company.

McGlone, F., Cerritelli, F., Walker, S., and Esteves, J. (2017). The role of gentle touch in perinatal osteopathic manual theraphy. *Neurosci. Biobehav. Rev.* 72, 1–9. doi: 10.1016/j.neubiorev.2016.11.009

Mourot, L., Bouhaddi, M., Perrey, S., Cappelle, S., Henriet, M. T., Wolf, J. P., et al. (2004). Decrease in heart rate variability with overtraining: assessment by the Poincare plot analysis. *Clin. Physiol. Funct. Imaging* 24, 10–18. doi: 10.1046/j.1475-0961.2003.00523.x

Pagani, M., and Lucini, D. (2009). Can autonomic monitoring predict results in distance runners? *Am. J. Physiol. Heart Circ. Physiol.* 296, H1721–H1722. doi: 10.1152/ajpheart.00337.2009

Pavlov, V. A., and Tracey, K. J. (2012). The vagus nerve and the inflammatory reflex–linking immunity and metabolism. *Nat. Rev. Endocrinol.* 8, 743–754. doi: 10.1038/nrendo.2012.189

Piccirillo, G., Munizzi, M. R., Fimognari, F. L., and Marigliano, V. (1996). Heart rate variability in hypertensive subjects. *Int. J. Cardiol.* 53, 291–298. doi: 10.1016/0167-5273(95)02538-3

Pongratz, G., and Straub, R. H. (2014). The sympathetic nervous response in inflammation. *Arthritis Res. Ther.* 16:504. doi: 10.1186/s13075-014-0504-2

Rahman, F., Pechnik, S., Gross, D., Sewell, L., and Goldstein, D. S. (2011). Low frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Clin. Auton. Res.* 21, 133–141. doi: 10.1007/s10286-010-0098-y

Rechberger, V., Biberschick, M., and Porthun, J. (2019). Effectiveness of an osteopathic treatment on the autonomic nervous system: a systematic review of the literature. *Eur. J. Med. Res.* 24:36. doi: 10.1186/s40001-019-0394-5

Reyes Del Paso, G. A., Langewitz, W., Mulder, L. J., Van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Ruffini, N., D'alessandro, G., Mariani, N., Pollastrelli, A., Cardinali, L., and Cerritelli, F. (2015). Variations of high frequency parameter of heart rate variability following osteopathic manipulative treatment in healthy subjects compared to control group and sham therapy: randomized controlled trial. *Front. Neurosci.* 9:272. doi: 10.3389/fnins.2015.00272

Sgoifo, A., Carnevali, L., Pico-Alfonso, M. A., and Amore, M. (2015). Autonomic dysfunction and heart rate variability in depression. *Stress* 18, 343–352. doi: 10.3109/10253890.2015.1045868

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shi, X., Rehrer, S., Prajapati, P., Stoll, S. T., Gamber, R. G., and Downey, H. F. (2011). Effect of cranial osteopathic manipulative medicine on cerebral tissue oxygenation. *J. Am. Osteopath. Assoc.* 111, 660–666.

Singh, J. P., Larson, M. G., Tsuji, H., Evans, J. C., O'donnell, C. J., and Levy, D. (1998). Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham heart study. *Hypertension* 32, 293–297. doi: 10.1161/01.hyp.32.2.293

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/s0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Tracey, K. J. (2002). The inflammatory reflex. *Nature* 420, 853–859. doi: 10.1038/nature01321

Welch, A., and Boone, R. (2008). Sympathetic and parasympathetic responses to specific diversified adjustments to chiropractic vertebral subluxations of the cervical and thoracic spine. *J. Chiropr. Med.* 7, 86–93. doi: 10.1016/j.jcm.2008.04.001

Williams, D. P., Cash, C., Rankin, C., Bernardi, A., Koenig, J., and Thayer, J. F. (2015). Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. *Front. Psychol.* 6:261. doi: 10.3389/fpsyg.2015.00261

Williams, D. P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M. N., Sternberg, E. M., et al. (2019). Heart rate variability and inflammation: a meta-analysis of human studies. *Brain Behav. Immun.* 7, 219–226. doi: 10.1016/j.bbi.2019.03.009

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer ET declared a past co-authorship with several of the authors LC, AS to the handling Editor

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 07 October 2020
doi: 10.3389/fnins.2020.569359

# Heart Rate Variability Moderates the Association Between Beliefs About Worry and Generalized Anxiety Disorder Symptoms

*Grace M. Fishback[1], Lyvia Chriki[2], Julian F. Thayer[3] and Michael W. Vasey[4]**

[1] Department of Neurology, University of Colorado School of Medicine, Denver, CO, United States, [2] Private Practice, Newton, MA, United States, [3] Department of Psychological Science, School of Social Ecology, University of California, Irvine, Irvine, CA, United States, [4] Department of Psychology, The Ohio State University, Columbus, OH, United States

OPEN ACCESS

**Edited by:**
Sylvain Laborde,
German Sport University Cologne, Germany

**Reviewed by:**
Luca Carnevali,
University of Parma, Italy
Dorota Zyśko,
Wrocław Medical University, Poland

***Correspondence:**
Michael W. Vasey
vasey.1@osu.edu

**Specialty section:**
This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience

**Received:** 03 June 2020
**Accepted:** 07 September 2020
**Published:** 07 October 2020

**Citation:**
Fishback GM, Chriki L, Thayer JF and Vasey MW (2020) Heart Rate Variability Moderates the Association Between Beliefs About Worry and Generalized Anxiety Disorder Symptoms. Front. Neurosci. 14:569359. doi: 10.3389/fnins.2020.569359

Paradoxically, some individuals who experience pathological worry also have good capacity for top-down control over their thoughts. Why such individuals would nevertheless worry excessively remains unclear. One explanation is suggested by research showing that those experiencing pathological worry are set apart from healthy controls by their beliefs that worry has utility and that effective worrying requires them to consider all possibilities before terminating a worry bout. This suggests that worriers with good capacity for cognitive control may engage in prolonged worry because they believe it is adaptive to do so. In a sample of 109 college students, among whom individuals reporting pathological worry were overrepresented, we tested this hypothesis using an objective index of top-down control capacity (i.e., resting vagally mediated heart rate variability [vmHRV]) and self-report measures of beliefs about worry and generalized anxiety disorder (GAD) symptom severity/status. As predicted, GAD symptom severity and vmHRV interacted to predict beliefs about worry. Specifically, high GAD symptoms were most strongly associated with beliefs that worry has utility at *higher* levels of vmHRV. Furthermore, this pattern was mostly a function of the belief that worry serves to distract the worrier from more emotional things. Similarly, high GAD symptoms were most strongly associated with endorsement of an 'as many as can' (AMAC) problem-solving rule when vmHRV was *high*. From the opposite perspective, both worry utility beliefs and AMAC rule endorsement were associated with the highest GAD symptom severity at higher levels of vmHRV. This was also true for the belief that worry distracts from more emotional things predicting analog GAD status. These results suggest that worriers who have higher levels of top-down control capacity may initiate and persist in worry, at least initially, because they value it. However, why they nevertheless rate their worry as excessive and uncontrollable is an important question for future research.

**Keywords: heart rate variability, worry, generalized anxiety disorder, worry beliefs, cognitive control capacity**

## INTRODUCTION

Generalized anxiety disorder (GAD), is characterized by uncontrollable and excessive worry (i.e., pathological worry; American Psychiatric Association [APA], 2013). It is common, debilitating, and persistent over many years (Kessler et al., 2012). Furthermore, many individuals with GAD fail to respond to current treatments and those who do respond often fail to maintain improvement over several years (e.g., Cuijpers et al., 2014), suggesting there may be sources of heterogeneity that moderate treatment response. One candidate domain of heterogeneity is the capacity for top-down control over cognition (Toh and Vasey, 2017; Vasey et al., 2017). Understandably, scholars have linked pathological worry to deficits in such cognitive control (e.g., Borkovec et al., 1983; Hirsch and Mathews, 2012). However, studies of top-down control capacity in worriers and individuals with GAD reveal substantial heterogeneity in their results. For example, evidence suggests that such individuals vary widely in their self-reported levels of attentional control (AC) or, more broadly, the dimension of effortful control (EC; see Vasey et al., 2017). Whereas some studies have found significant negative correlations between GAD status/symptoms and self-reported AC/EC and similar constructs (e.g., Armstrong et al., 2011; Olatunji et al., 2011), others have found no association (e.g., Bienvenu et al., 2004) or even a significant positive association (e.g., Rosellini and Brown, 2011).

Studies using behavioral measures of top-down control also present a mixed picture. Although individuals with GAD sometimes perform worse than controls on tests of AC and cognitive flexibility (e.g., Olatunji et al., 2011; Stefanopoulou et al., 2014), other studies have found no difference (e.g., Hoehn-Saric et al., 1989). Indeed, in two separate studies, Yiend et al. (2014) found individuals with GAD to be significantly *faster* than controls in disengaging attention from threat cues. Consistent with such mixed findings, Derryberry and Reed (2002) found that high trait-anxious college students reporting high AC failed to show the difficulty disengaging attention from threat cues seen among their low AC counterparts. Lonigan and Vasey (2009) found similar results in a youth sample.

Neuroimaging studies also reveal heterogeneity in both structural and functional measures of brain regions involved in cognitive control among pathological worriers. For example, Makovac et al. (2016a) and Carnevali et al. (2019) found individuals with GAD to have lower average gray matter volume than healthy controls in regions of the PFC involved in top-down control. However, in contrast, Mohlman et al. (2009) found medial orbital PFC volume to be *positively* associated with scores on the Penn State Worry Questionnaire (PSWQ). Makovac et al. (2016b) found individuals with GAD had lower functional connectivity than healthy controls at baseline between the amygdala and regulatory regions of the PFC. In contrast, Etkin et al. (2009) found individuals with GAD to show atypical *heightened* functional connectivity at rest between the amygdala and the dorsolateral PFC, a region that is associated with cognitive control. Similar heterogeneity is seen in response to tasks involving processing of negative stimuli. For example, Price et al. (2011) found individuals with GAD to show hypoactivity in the PFC compared to controls during an emotional Stroop task. In contrast, Makovac et al. (2016b) found that functional connectivity between the amygdala and regulatory areas in the PFC *increased* among individuals with GAD following a perseverative cognition induction.

Psychophysiological studies measuring vagally mediated heart rate variability (vmHRV) also reveal heterogeneity among pathological worriers. As articulated in the Neurovisceral Integration Model (NIM; Thayer and Lane, 2000) and Polyvagal Theory (Porges, 2008), measures of vmHRV provide an index of activity in the parasympathetic nervous system, which is associated in turn with activity in brain regions and circuits involved in inhibitory control (Lane et al., 2009; Nugent et al., 2011; Thayer et al., 2012). For example, higher vmHRV at rest predicts better performance on tasks requiring top-down control such as the think/no-think task, which requires control over memory retrieval (Gillie et al., 2014), and the thought-suppression paradigm, which requires control over ongoing thought (Gillie et al., 2015). Furthermore, studies show that higher vmHRV is associated specifically with better capacity to regulate attention with respect to threat-stimuli. For example, higher vmHRV predicts greater ability to disengage attention from fearful face distractors (Park et al., 2013) and better inhibition of return to fearful versus neutral faces (Park et al., 2012).

Unsurprisingly given such findings, studies have linked low resting vmHRV to pathological worry (e.g., Thayer et al., 1996; Carnevali et al., 2019). A meta-analysis by Chalmers et al. (2014) shows that individuals with GAD do indeed have lower resting vmHRV on average than controls (Hedge's $g = -0.55$). However, even an effect of such magnitude leaves more than 75% overlap between groups. Thus, it is not surprising that some studies have failed to find a difference (e.g., Kollai and Kollai, 1992; Hammel et al., 2011; Aldao and Mennin, 2012; Fisher and Newman, 2013; Levine et al., 2016). Studies comparing high and low worriers have produced similar variability, with some studies finding the expected difference (e.g., Brosschot et al., 2007), others finding no difference (e.g., Knepp and Friedman, 2008; Mankus et al., 2013) and at least one finding high worriers to have significantly *higher* vmHRV at rest than low worriers (Davis et al., 2002 [study 2]). The high end of the range of vmHRV scores in Mankus et al.'s (2013) analog GAD group (absolute value of mean successive differences [$|MSD|$] range = 4.09–170.38) versus their low GAD symptoms group ($|MSD|$ range = 4.58–82.41) illustrates the presence of individuals with high vmHRV among those high in GAD symptoms.

Given that some individuals reporting high levels of GAD symptoms also have high capacity for cognitive control, we must ask why such individuals nevertheless experience excessive worry. One explanation is that they intentionally initiate and persist in worry because they believe it serves primary adaptive goals (Freeston et al., 1994). Specifically, such individuals may believe that worry has positive effects [e.g., enhanced problem-solving (Davey, 1994)], or that it fosters avoidance of or preparation for anticipated catastrophic outcomes (Davey et al., 1996; Cartwright-Hatton and Wells, 1997). Consistent with this

view, Borkovec and Roemer (1995) interviewed individuals with GAD and identified six beliefs about functions served by worry. Specifically, the GAD group tended to believe that worry can (1) enhance motivation to complete tasks, (2) aid in problem-solving, (3) help one prepare for the worst, (4) aid in planning to avoid negative events, or (5) serve to distract from more anxiety-provoking thoughts. Sixth, they tended to hold the superstitious belief that worrying about something makes it is less likely to happen or at least feel that way.

It is easy to see how worriers might come to regard worry as serving such functions. Since feared outcomes rarely happen, their non-occurrence following a period of worry may negatively reinforce worry as a coping strategy (Davey and Meeten, 2016). Similarly, worry can be reinforced by virtue of its ability to blunt autonomic arousal (Borkovec et al., 2004) or foster avoidance of aversive emotional contrasts (Newman and Llera, 2011). Furthermore, if worriers believe that worry helps them prepare for the worst and they are able to handle feared outcomes better than they expected when they do occur, then worry can seem effective even if they would have weathered the event just as well without worrying. As noted by Freeston et al. (1994), such beliefs may help explain why worriers continue to worry even though it is an aversive experience. One implication of this is that worriers who hold such beliefs and who have good cognitive control ability may channel that capacity toward persisting in worry despite its unpleasantness. Similarly, worriers who have good cognitive control but who believe that worry has utility may feel it would be bad to try to limit their worrying (Cartwright-Hatton and Wells, 1997).

Such beliefs set those experiencing pathological worry apart from controls. A review by Hebert et al. (2014) showed that worry utility beliefs characterize individuals diagnosed with GAD (e.g., Borkovec and Roemer, 1995; Ladouceur et al., 1998; Newman and Llera, 2011), GAD-analogs (e.g., Freeston et al., 1994), and high worriers (e.g., Davey et al., 1996; Laugesen et al., 2003). Furthermore, such beliefs are associated with higher levels of worry in response to stressful events (Iijima and Tanno, 2013). They may also interfere with readiness for change in therapy. In a highly anxious community sample, Covin et al. (2008) found that positive beliefs about worry were significantly negatively associated with readiness for change. Similarly, Laberge et al. (2000) found that worry decreased when positive beliefs about worry were targeted in cognitive-behavior therapy (CBT) for GAD. Importantly, they found that the more positive beliefs changed, the more change in worry severity was seen over time.

Evidence suggests that worriers especially regard worry as a way of regulating anxiety through distraction. In two studies comparing GAD analogs (i.e., individuals who met diagnostic criteria for GAD based on questionnaire responses) to controls, Borkovec and Roemer (1995) found that those high in GAD symptoms were especially characterized by the belief that their worries effectively distract them from even more emotional things. Indeed, only that belief significantly differentiated the analog GAD samples from comparison groups in both studies. These "more emotional things" may be images that activate heightened autonomic arousal symptoms (Borkovec et al., 2004) or they may be unpredictable contrasting spikes in negative emotion (Newman and Llera, 2011). Given its distinctiveness, the current study included a special focus on this belief.

Beyond holding beliefs in worry's utility, pathological worriers are set apart from controls by their problem-solving orientation (Davey et al., 1992; Freeston et al., 1994). Not only are they unusually likely to rate worry as useful for problem-solving (Ladouceur et al., 1998), they also tend to believe that such a purpose is best served when they consider as many possibilities as they can when worrying (Davey and Meeten, 2016). That is, they follow an 'as many as can' (AMAC) rule when worrying rather than stopping when they no longer 'feel like continuing' (FLC). Evidence suggests that following an AMAC rule fosters perseveration during worry whereas following an FLC rule is associated with termination of a worry bout (Davey and Meeten, 2016). We suggest further that adherence to an AMAC rule should especially foster perseverative worry among worriers having good capacity for cognitive control, which permits them to persist in worrying despite its unpleasantness.

In this study, we tested these predictions using a measure of resting vmHRV as an objective index of top-down control capacity. Specifically, we predicted that (1) GAD symptom severity should be most strongly, positively correlated with worry utility beliefs among individuals with high levels of resting vmHRV because they are able to use their capacity for cognitive control in the service of worrying. In contrast, GAD symptoms should tend to be unrelated to such beliefs among those with low levels of resting vmHRV because they should worry excessively mainly because they can't help it. Furthermore, based on the findings of Borkovec and Roemer (1995), we predicted that (2) this pattern should hold especially for the belief that worry distracts from more emotional things. Similarly, we predicted that (3) GAD symptoms should be most strongly positively correlated with endorsement of an AMAC approach to worry among individuals with high vmHRV. From the opposite point of view, we predicted (4) that beliefs in the utility of worry should be most strongly, positively correlated with GAD symptom severity and GAD status among those with high vmHRV and (5) that should be true especially for the belief that worry distracts from more emotional things. So too did we predict (6) that endorsement of an AMAC rule would be most strongly, positively correlated with GAD severity/status among those with higher levels of vmHRV.

# MATERIALS AND METHODS

## Participants

Participants were recruited from among students taking introductory psychology at The Ohio State University. Potential participants were screened using the Generalized Anxiety Disorder Questionnaire – IV (GAD-Q-IV; Newman et al., 2002) to maximize the number reporting high levels of GAD symptoms. Specifically, all individuals who endorsed at least four of the five dichotomous items on the GAD-Q-IV were sent an email message inviting them to participate. We additionally invited a random subsample of the remainder to ensure that the sample included the full range of GAD symptoms. This resulted

in 58 (47.2%) individuals who met the screening criteria at the time they participated in the study and 65 (52.8%) who did not.

The final sample was drawn from 123 participants who completed at least the first laboratory session in a larger, multi-session study. This maximized our sample size despite attrition in later sessions. Additionally, we limited the current sample to those participants having useable heart rate data. Such data were missing for 14 participants due to equipment failure or experimenter error. This resulted in a final sample of 109 participants, in which 65.1% self-identified as female and age ranged from 18-28 years ($M$ = 19.3, $SD$ = 2.1). They self-identified primarily as Caucasian (71.6%, African American: 7.3%, Asian American: 6.4%, Latino: 3.7%, Multiple Categories: 5.5%, Other: 5.5%). All participants received course credit for their participation.

## Procedure

Upon arrival in the laboratory, after giving informed consent, participants were fitted with the Polar watch and chest belt through which the ECG signal was recorded. Following completion of a brief neutral computer task, participants sat in a quiet room for 5-minutes before their resting ECG was recorded for 5-minutes. They then completed self-report questionnaires in random order, among which were all measures used in the current study.

## Measures

### Self-Report Questionnaires

*Generalized Anxiety Disorder Questionnaire – IV (*GAD-Q-IV; Newman et al., 2002): The GAD-Q-IV is a self-report questionnaire assessing the diagnostic criteria for GAD based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association [APA], 1994). It is comprised of five yes/no questions assessing frequency and duration of excessive and uncontrollable worry, a checklist of associated symptoms, an open-ended list of worry topics, and two 9-point Likert scale items (ranging from 0 = "none" to 8 = "severe") regarding level of interference and distress. The GAD-Q-IV can be scored in several ways. Under the approach used by Newman et al. (2002), several items were skipped if a participant's worry had not persisted for at least 6-months. However, we chose to have all participants answer all questions and to include them in the total score as suggested by Rodebaugh et al. (2008). We otherwise scored the GADQ-IV according to the Newman et al. (2002) formula and used the resulting continuous score as a measure of GAD symptom severity. This continuous score has good psychometric properties (see Rodebaugh et al., 2008) and had good internal consistency in the current sample (Cronbach's alpha = 0.79). We also used the approach described by Moore et al. (2014) to identify participants who met *DSM-IV* diagnostic criteria for GAD based on their GAD-Q-IV responses. This resulted in an analog GAD subgroup of 26 participants.

*GAD-7 Scale* (Spitzer et al., 2006). The GAD-7 is a 7-item self-report questionnaire assessing GAD symptom severity. The items are based on the *DSM-IV* diagnostic criteria. Answers are ranked on a 4-point Likert scale from 0 (not at all) to 3 (nearly every day). The GAD-7 demonstrates excellent internal consistency (Cronbach's alpha = 0.92) and good convergent validity. In the current sample, Cronbach's alpha was 0.90.

*The Overall Anxiety Severity and Impairment Scale (*OASIS; Norman et al., 2006). The OASIS is a 5-item self-report questionnaire assessing the extent to which individuals experience their anxiety as intrusive and impairing. Answers are rated on a 5-point Likert scale ranging from "None" to "Extreme". Subjects are asked about the frequency of feeling anxious, intensity of the anxiety, and interference of anxiety in their functioning. Norman et al. (2006) reported that the scale has good convergent validity and good test-retest reliability over a one-month period. In the current sample, Cronbach's alpha was 0.83.

*Reasons to Worry Questionnaire (*RWQ; Borkovec and Roemer, 1995). The RWQ is a 6-item self-report questionnaire assessing reasons why people may worry. Questions ask about six worry functions: motivation to complete tasks, aids in problem solving, preparation for negative events, avoidance of negative events, distraction from emotional topics, and superstitious effects on feared outcomes. Respondents indicate how much each item applies to them using a 5-point Likert scale ranging from "not at all" to "very much". GAD status correlates with higher scores on each of the six items (Borkovec and Roemer, 1995). In the current sample Cronbach's alpha was 0.80.

*Why Worry? Questionnaire (*WWQ; Freeston et al., 1994). The WWQ is a 20-item self-report questionnaire regarding a person's motivations for worrying. Items pertain to ways in which worry prevents negative outcomes or has positive effects. Respondents rate each item on a 5-point Likert scale ranging from "not at all characteristic of me" to "entirely characteristic of me". We used the total score to represent overall beliefs about the utility of worry. Freeston et al. (1994) demonstrated the WWQ has good agreement with similar measures as well as good internal consistency. Results from Freeston et al. (1994) also show the WWQ to have good ability to distinguish pathological worriers from healthy controls. In the current sample, Cronbach's alpha for the total score was 0.91.

*Problem Solving* Inventory (PSI; Heppner and Petersen, 1982). The PSI is a 35 item self-report questionnaire measuring participants' confidence in their ability to solve problems, their tendency to approach or avoid problem solving, and their perception of their degree of control over emotions and behaviors they achieve during problem solving. Items are answered on a 6-point Likert scale (ranging from "Strongly Disagree" to "Strongly Agree"). For the current study we focused only on item #7, which was used as a measure of endorsement of an AMAC rule. Item #7 reads, "When I have a problem, I think of as many possible ways to handle it as I can until I can't come up with any more ideas."

### Vagally Mediated Heart Rate Variability (vmHRV)

Resting vmHRV was estimated using a 5-minute ECG segment recorded using a Polar RS8000 Running Computer Wristwatch and standard Wearlink chest belt. The Polar watch collects data at a 1000 Hz sampling rate and provides a reliable and valid ECG signal (Quintana et al., 2012). We examined the ECG signal and removed artifacts using the KUBIOS HRV analysis package 2.2 (Tarvainen et al., 2009). KUBIOS was also used to

compute the root mean square of successive differences (RMSSD) in intervals between heartbeats, which is a time-domain measure of vagally-mediated (parasympathetic) changes in heart rate (Shaffer and Ginsberg, 2017). Higher RMSSD values indicate higher HRV. Values of RMSSD were natural log transformed to better approximate a normal distribution.[1]

## Data Analytic Strategy

Because the RWQ and WWQ both measure worry utility beliefs and were highly correlated ($r$ = 0.65, $p < 0.001$; see **Table 1**), we chose to consolidate them into a composite Worry Utility Beliefs score, which was created by averaging the standardized total scores from each measure. Similarly, because scores on the GAD-Q-IV, GAD7, and OASIS were highly intercorrelated (see **Table 1**), we created a composite GAD Symptom Severity score by averaging the standardized total scores from the three measures (Cronbach's alpha = 0.92).

As noted previously, Borkovec and Roemer (1995) found that their GAD samples were set apart from their comparison groups by their endorsement of one belief, represented by item #5 on the RWQ ("Worrying about most of the things I worry about is a way to distract myself from worrying about even more emotional things, things I don't want to think about"). Therefore, we focused specifically on that belief. Because item #2 on the WWQ is very similar ("Worrying about less important things distracts me from more emotional subjects that I don't want to think about") and because the two items were strongly correlated ($r$ = 0.58, $p < 0.001$; see **Table 1**), we created a composite "Worry Distracts" score by averaging their scores.

All hypotheses were tested via multiple linear regression (MLR) analyses using SPSS Version 25 for Macintosh. For example, to predict the Worry Utility Beliefs score, the GAD Symptom Severity score, vmHRV (i.e., Ln[RMSSD]), and the GAD Symptom Severity x vmHRV interaction were included in the model. Because statistical power to detect interactions is limited in small sample (McClelland and Judd, 1993), we sought to maximize power by limiting the number of predictors in the model to preserve degrees of freedom.[2] Regression diagnostics were examined for each analysis to identify cases that might be exerting excessive influence on overall model fit or on individual beta weights. Specifically, we used ±1.0 as a cutoff for standardized Dffits and Dfbeta values for each case (Cohen et al., 2002). No high influence cases were identified in any analysis.

All interaction effects with $p < 0.10$ were probed using the PROCESS utility for SPSS (Hayes, 2013; freely available at http://www.afhayes.com). PROCESS estimates simple slopes at specific values of the moderator. We chose to illustrate all interactions by depicting simple slopes for each predictor at high (+ 1 *SD*) and low (−1 *SD*) levels of the moderator. However, PROCESS also implements the Johnson-Neyman technique for deriving regions of significance for the simple slope of the predictor at

[1]We also used the coefficient of variation for RMSSD to take into account differences in heart rate (see de Geus et al., 2019) but results were unchanged. Therefore, we report only the results based on RMSSD.

[2]Models. including sex and age as covariates did not substantively alter the pattern of results.

**TABLE 1** | Zero-order correlations and descriptive statistics.

| Variable | GAD-Q-IV | GAD7 | OASIS | GAD symptom severity | vmHRV | RWQ | WWQ | Worry utility score | RWQ item #5 | WWQ item #2 | Worry distracts score | Mean | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GAD-Q-IV | – | | | | | | | | | | | 6.34 | 3.69 |
| GAD7 | **0.85** | – | | | | | | | | | | 6.78 | 5.29 |
| OASIS | **0.79** | **0.82** | – | | | | | | | | | 6.06 | 3.55 |
| GAD Composite | **0.96** | **0.96** | **0.83** | – | | | | | | | | 0.00 | 1.00 |
| vmHRV [Ln(RMSSD)] | −0.02 | 0.01 | 0.02 | −0.01 | – | | | | | | | 3.58 | 0.98 |
| RWQ | **0.36** | **0.38** | **0.26** | **0.36** | 0.04 | – | | | | | | 14.38 | 5.08 |
| WWQ | **0.64** | **0.62** | **0.54** | **0.66** | −0.05 | **0.65** | – | | | | | 49.54 | 16.12 |
| Worry Utility Score | **0.55** | **0.53** | **0.44** | **0.56** | −0.01 | **0.91** | **0.91** | – | | | | 0.00 | 1.00 |
| RWQ Item #5 | **0.36** | **0.36** | **0.32** | **0.37** | 0.10 | **0.54** | **0.45** | **0.54** | – | | | 1.96 | 1.21 |
| WWQ Item #2 | **0.33** | **0.36** | **0.34** | **0.36** | 0.08 | **0.43** | **0.51** | **0.52** | **0.58** | – | | 2.34 | 1.22 |
| Worry Distracts Score | **0.38** | **0.41** | **0.37** | **0.41** | 0.10 | **0.55** | **0.54** | **0.60** | **0.89** | **0.90** | – | 0.00 | 1.00 |
| PSI Item #7 | 0.08 | 0.04 | 0.15 | 0.06 | 0.08 | −0.06 | −0.08 | −0.08 | **0.22** | −0.02 | 0.11 | 2.81 | 1.36 |

*All bold correlations significant at $p < 0.05$.*

**TABLE 2 |** Summary of regression analyses predicting Worry Utility, Worry Distracts, and 'As Many As Can.'

| | Dependent variable | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Worry utility score ($R^2$ = 0.327***)** | | **Worry distracts score ($R^2$ = 0.235***)** | | **Worry utility with worry distracts as a covariate ($R^2$ = 0.492***)** | | **'As Many As Can' ($R^2$ = 0.052)** | |
| **Variable** | **B (*SE*)** | ***sr*** | **B (*SE*)** | ***sr*** | **B (*SE*)** | ***sr*** | **B (*SE*)** | ***sr*** |
| Constant | 0.000 (0.072) | – | 0.000 (0.085) | – | 0.001 (0.070) | – | 0.008 (0.097) | – |
| GAD Symptom Composite | 0.501*** (0.073) | 0.550*** | 0.424*** (0.085) | 0.424*** | 0.396*** (0.078) | 0.357*** | 0.091 (0.101) | 0.088 |
| vmHRV | -0.035 (0.074) | −0.038 | 0.061 (0.086) | 0.061 | −0.060 (0.071) | −0.059 | 0.067 (0.096) | 0.068 |
| GAD Composite x vmHRV | 0.145* (0.063) | 0.184* | 0.226** (0.074) | 0.234** | 0.095 (0.062) | 0.107 | 0.175* (0.084) | 0.205* |
| Worry Distracts Score | – | – | – | – | 0.416*** (0.080) | 0.364*** | – | – |

*B = unstandardized regression coefficient; SE = standard error; sr = semi-partial correlation coefficient. ***p < 0.001;*p < 0.01;*p < 0.05; †p < 0.10.*

all observed values of the moderator (see Hayes, 2013, pp. 307–315). For each interaction we report the region of significance in terms of standard deviations from the mean of the moderator, along with the percentile of the distribution corresponding to the region of significance.

# RESULTS

## Preliminary Analyses

All analyses were conducted using 109 participants having complete vmHRV data (88.6% of the original data set). Those participants also had complete data on the other measures with the exception of the PSQ, which was available for 104 participants because it was added after the study began. In the full sample of 123 participants, vmHRV and PSQ data were missing completely at random (Little's Missing Completely at Random test $p = 0.263$). According to their GAD-Q-IV responses, 23.9% (n = 26) met *DSM-IV* GAD criteria. Based on the GAD-7, 29.3% (n = 32) scored above the clinical cut-off whereas the OASIS identified 36.7% (n = 40) who fell in the clinical range. **Table 1** shows descriptive statistics and correlations for all variables. Notably, GAD symptom severity was uncorrelated with resting vmHRV ($r = -0.01$, $p = 0.948$). Unexpectedly, item #7 of the PSI did not correlate significantly with GAD symptom severity. However, it was significantly positively correlated with the "worry distracts from more emotional things" item (#5) on the RWQ ($r = 0.22$, $p < 0.05$).

## Primary Analyses

### Predictions 1 and 2: GAD Symptom Severity Interacts With vmHRV to Predict Worry Utility Beliefs

As shown in **Table 2**, the model predicting Worry Utility Beliefs from GAD Symptom Severity, vmHRV, and their interaction was significant ($R^2 = 0.327$, p < 0.001). Although GAD Symptom Severity was significantly positively correlated with Worry Utility Beliefs on average (semi-partial r [$sr = 0.550$, $p < 0.0001$), a significant interaction showed that association to be conditional upon level of vmHRV ($sr = 0.184$, $p = 0.024$). As shown in **Figure 1**, when vmHRV was high (i.e., + 1 *SD*), the simple slope for GAD Symptom Severity was significant (B = 0.71, $p < 0.0001$). When vmHRV was low (i.e., −1 *SD*), the simple slope remained significant but was weaker in magnitude (B = 0.39, $p = 0.0003$). The Johnson-Neyman technique revealed that GAD Symptom Severity was significantly positively correlated with Worry Utility Beliefs except for vmHRV < -1.71 *SD*s (percentile = 8.3). This correlation was strongest when vmHRV was highest.

**Table 2** also shows that the same model predicting the Worry Distracts score was significant ($R^2 = 0.235$, p < 0.001). Although GAD Symptom Severity was significantly positively correlated with Worry Distracts on average ($sr = 0.424$, $p < 0.0001$), a significant interaction showed that association varied depending on level of vmHRV ($sr = 0.234$, $p = 0.007$). As shown in **Figure 2**, when vmHRV was high, the simple slope for GAD Symptom Severity was significant (B = 0.63, $p < 0.0001$). When vmHRV was low, the simple slope remained significant but was weaker in magnitude (B = 0.22, $p = 0.048$). The Johnson-Neyman technique revealed that GAD Symptom Severity was significantly positively correlated with Worry Distracts except for vmHRV < -1.01 *SD*s (percentile = 12.8). This correlation was strongest when vmHRV was highest.

Finally, **Table 2** shows that when the Worry Distracts score was entered as a covariate into the model predicting the Worry

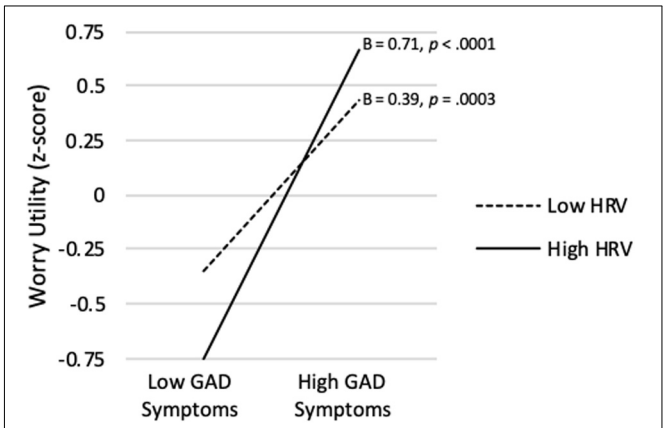



**FIGURE 1 |** GAD symptom severity predicting worry utility beliefs score at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

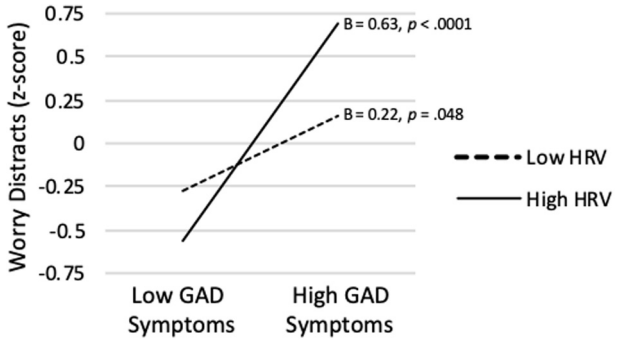



**FIGURE 2 |** GAD symptom severity predicting worry distracts score at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

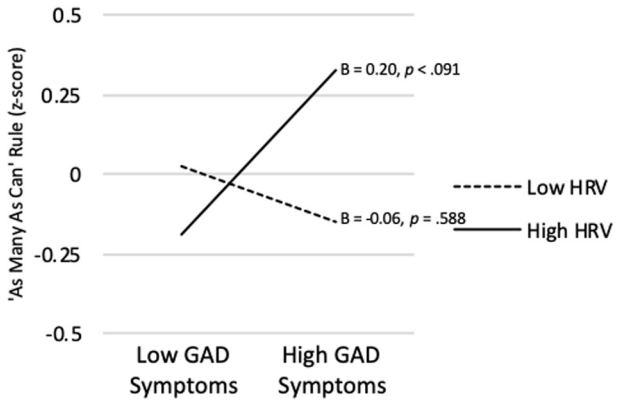



**FIGURE 3 |** GAD symptom severity predicting 'As Many As Can' rule endorsement at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

Utility score, the GAD Symptom Severity x vmHRV interaction became non-significant ($sr = 0.107$, $p = 0.130$). Thus, the variance in the Worry Utility score predicted by the GAD Symptom Severity x vmHRV interaction was accounted for largely by the belief that worry distracts from more emotional things.

## Prediction 3: GAD Symptom Severity Interacts With vmHRV to Predict AMAC Rule Endorsement

**Table 2** also shows that the model predicting the AMAC rule from GAD Symptom Severity, vmHRV, and their interaction was not significant ($R^2 = 0.052$, p = 0.144). Nevertheless, as predicted, the AMAC rule x vmHRV interaction was significant ($sr = 0.205$, $p = 0.0395$). As shown in **Figure 3**, when vmHRV was high, the simple slope for the AMAC rule was positive and approached significance (B = 0.204, $p = 0.091$). When vmHRV was low, it was non-significant (B = −0.06 $p = 0.588$). The Johnson-Neyman technique revealed that GAD Symptom Severity was significantly positively correlated with strength of AMAC rule endorsement only for vmHRV > 1.15 *SD*s (percentile = 95.2). This correlation was strongest when vmHRV was highest.

### Predictions 4 and 5: Worry Utility Beliefs Interact With vmHRV to Predict GAD Symptom Severity and Analog GAD Status

As shown in **Table 3**, the model predicting GAD Symptom Severity from Worry Utility Beliefs, vmHRV, and their interaction was significant ($R^2 = 0.313$, p < 0.0001). Worry Utility Beliefs were significantly positively correlated with GAD Symptom Severity on average ($sr = 0.516$, $p < 0.0001$). Although the Worry Utility Beliefs x vmHRV interaction did not achieve significance ($sr = 0.142$, $p = 0.083$), its pattern was consistent with expectation. As shown in **Figure 4**, when vmHRV was at + 1 *SD*, the simple slope for Worry Utility Beliefs was significant (B = 0.70, $p < 0.0001$). When vmHRV was at −1 *SD*, the simple slope remained significant but was weaker in magnitude (B = 0.34, $p = 0.014$). The Johnson-Neyman technique revealed that Worry Utility Beliefs were significantly positively correlated with GAD Symptom Severity except for vmHRV < -1.21 *SD*s (percentile = 11.9). This correlation was strongest when vmHRV was highest.

**Table 3** also shows that the model predicting GAD Symptom Severity from Worry Distracts, vmHRV, and their interaction was significant ($R^2 = 0.223$, p < 0.0001). Although Worry Distracts was significantly positively correlated with GAD Symptom Severity on average ($sr = 0.360$, $p < 0.0001$), a significant interaction term showed that association varied depending on level of vmHRV ($sr = 0.226$, $p = 0.001$). As shown in **Figure 5**,

**TABLE 3 |** Summary of regression analyses predicting GAD symptom severity.

| | Predictor Variable | | | | | |
|---|---|---|---|---|---|---|
| | Worry Utility ($R^2$ = 0.313***) | | Worry Distracts ($R^2$ = 0.223***) | | 'As Many As Can' ($R^2$ = 0.058) | |
| **Variable** | **B (*SE*)** | ***sr*** | **B (*SE*)** | ***sr*** | **B (*SE*)** | ***sr*** |
| Constant | 0.000 (0.081) | – | −0.027 (0.086) | – | −0.031 (0.094) | – |
| Predictor | 0.521*** (0.082) | 0.516*** | 0.370*** (0.088) | 0.360*** | 0.054 (0.095) | 0.056 |
| vmHRV | −0.054 (0.082) | −0.050 | −0.015 (0.086) | −0.015 | 0.003 (0.095) | 0.003 |
| Predictor x vmHRV | 0.176†(0.101) | 0.142† | 0.268** (0.102) | 0.226** | 0.218* (0.093) | 0.229* |

*N = 109 except for 'as many as can' analysis for which N = 104. B = unstandardized regression coefficient; SE = standard error; sr = semi-partial correlation coefficient. ***p < 0.001;*p < 0.01;* p < 0.05; †p < 0.10.*

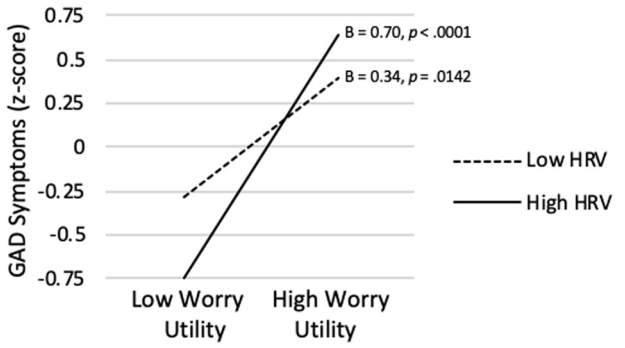



**FIGURE 4 |** Worry utility beliefs score predicting GAD symptom severity at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

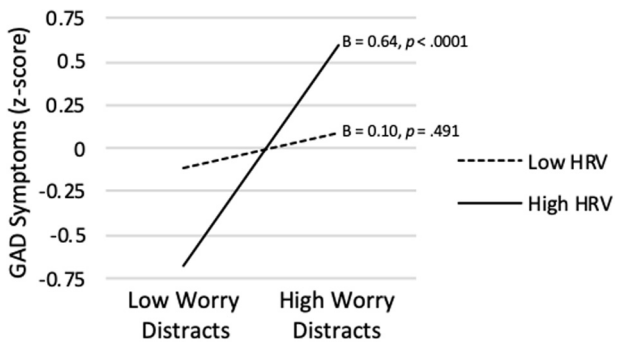



**FIGURE 5 |** Worry distracts score predicting GAD symptom severity at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

when vmHRV was high, the simple slope for Worry Distracts was significantly positive (B = 0.64, $p < 0.0001$). The simple slope was non-significant when vmHRV was low (B = 0.10, $p = 0.491$). The Johnson-Neyman analysis revealed that Worry Distracts was significantly correlated with GAD Symptom Severity except for vmHRV < −0.54 *SD*'s (percentile = 18.4). This correlation was strongest when vmHRV was highest.

As shown in **Table 4**, the binary logistic regression model predicting analog GAD Status from Worry Utility Beliefs, vmHRV, and their interaction was significant (Nagelkerke $R^2 = 0.447$, p < 0.0001). Worry Utility Beliefs were significantly positively correlated with analog GAD Status on average ($p = 0.0011$). However, the Worry Utility Beliefs x vmHRV interaction did not approach significance ($p = 0.189$) and was not interpreted further.

**Table 4** also shows that the model predicting analog GAD Status from Worry Distracts, vmHRV, and their interaction was significant (Nagelkerke $R^2 = 0.223$, p < 0.0001). Although Worry Distracts was significantly positively correlated with analog GAD Status on average ($p = 0.001$), a significant interaction term showed that association varied depending on level of vmHRV ($p = 0.034$). As shown in **Figure 7**, when vmHRV was high, the simple slope for Worry Distracts was significantly positive (B = 1.57, $p = 0.0001$). The simple slope was non-significant when vmHRV was low (B = 0.21, $p = 0.620$). The Johnson-Neyman analysis revealed that Worry Distracts was significantly positively correlated with analog GAD Status except for vmHRV < -0.42 *SD*s (percentile = 18.3). This correlation was strongest when vmHRV was highest.

## Prediction 6: AMAC Rule Interacts With vmHRV to Predict GAD Symptom Severity and Analog GAD Status

As shown in **Table 3**, the model predicting GAD Symptom Severity from the AMAC rule, vmHRV, and their interaction was not significant ($R^2 = 0.058$, p = 0.112). However, as predicted, the AMAC rule x vmHRV interaction was significant ($sr = 0.229$, $p = 0.020$). The Johnson-Neyman technique revealed that strength of endorsement of the AMAC rule was significantly positively correlated with GAD Symptom Severity only for vmHRV > 0.865 *SD*s (percentile = 88.5). Additionally, the simple slope was significantly negative for vmHRV < -2.76 *SD*s (percentile = 3.85). As shown in **Figure 6**, when vmHRV at + 1 *SD*, the simple slope for the AMAC rule was significant (B = 0.27, $p = 0.038$). When vmHRV was at −1 *SD*, it was non-significant (B = −0.17, $p = 0.217$).

As shown in **Table 4**, the model predicting analog GAD Status from the AMAC rule, vmHRV, and their interaction was not significant (Nagelkerke $R^2 = 0.077$, p = 0.141). However, as predicted, the AMAC rule x vmHRV interaction was significant

**TABLE 4 |** Summary of binary logistic regression analyses predicting GAD status.

| | Predictor Variable | | | | | |
|---|---|---|---|---|---|---|
| | Worry Utility (Nagelkerke $R^2 = 0.447$, $p < 0.0001$) | | Worry Distracts (Nagelkerke $R^2 = 0.273$, $p = 0.0001$) | | 'As Many As Can' (Nagelkerke $R^2 = 0.077$, $p = 0.141$) | |
| **Variable** | **B (*SE*)** | ***p*-value** | **B (*SE*)** | ***p*-value** | **B (*SE*)** | ***p*-value** |
| Constant | -1.827 (0.346) | 0.0000 | -1.490 (0.283) | 0.0000 | -1.316 (0.251) | 0.0000 |
| Predictor | 1.760 (0.385) | 0.0011 | 0.889 (0.272) | 0.0011 | -0.092 (0.253) | 0.715 |
| vmHRV | -0.428 (0.329) | 0.193 | -0.214 (0.240) | 0.373 | 0.078 (0.285) | 0.785 |
| Predictor x vmHRV | 0.507 (0.386) | 0.189 | 0.676 (0.319) | 0.034 | 0.552 (0.267) | 0.039 |

*N = 109 except for 'as many as can' analysis for which N = 104. B = unstandardized regression coefficient; SE = standard error; coefficients are expressed in a log-odds metric.*

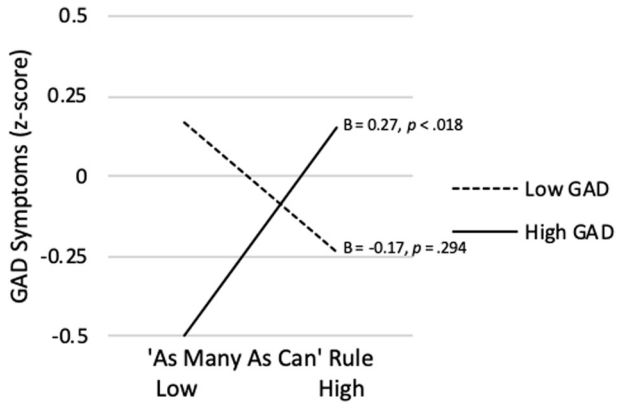



**FIGURE 6 |** 'As Many As Can' rule endorsement predicting GAD symptom severity at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

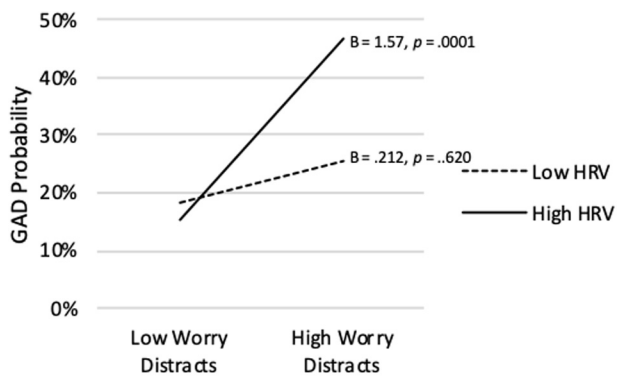



**FIGURE 7 |** Worry distracts score predicting probability of analog GAD diagnosis at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

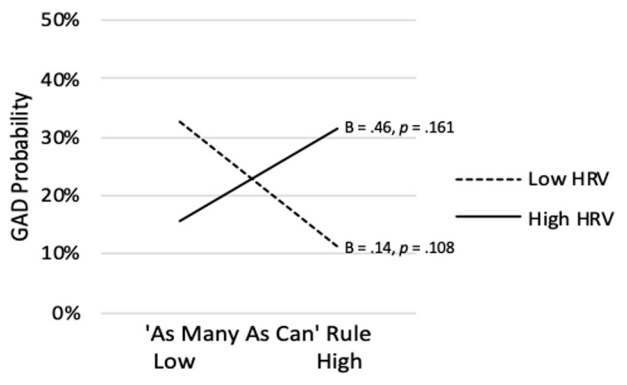



**FIGURE 8 |** 'As Many As Can' rule endorsement predicting analog GAD at high (+ 1 *SD*) and low (−1 *SD*) vmHRV.

(*sr* = 0.552, $p$ = 0.039). As shown in **Figure 8**, when vmHRV was at + 1 *SD*, the simple slope for the AMAC rule was positive but not significant (B = 0.455, $p$ = 0.161). When vmHRV was at −1 *SD*, it was negative but also non-significant (B = −0.665, $p$ = 0.108). The Johnson-Neyman technique revealed that strength of endorsement of the AMAC rule was significantly negatively associated with analog GAD status when vmHRV was low (i.e., <-2.930 SDs; percentile = 2.88). Although this simple slope shifted to a positive association at higher vmHRV, it did not achieve significance.

## DISCUSSION

This study's aim was to help explain why some individuals experience pathological worry despite having good capacity for top-down control over cognition. We hypothesized that such individuals use that capacity to initiate and persist in worrying despite its aversiveness because they believe it has important benefits and/or they believe in the importance of exhaustively considering all possibilities when worrying. In contrast, such beliefs should be less relevant among worriers having poor cognitive control capacity, who instead should experience excessive worry mainly because they can't help it.

Our results are consistent with this perspective. GAD symptom severity was indeed most strongly associated with beliefs in worry's utility when vmHRV was highest. This correlation was weaker at lower vmHRV, becoming non-significant when vmHRV was in the bottom 8.3% of the sample. This pattern was especially apparent for the belief that worry distracts from more emotional things, which research suggests is especially characteristic of pathological worriers (Borkovec and Roemer, 1995). This pattern also emerged when predicting strength of endorsement of an AMAC problem-solving rule. Although the overall regression model in that case was not significant, the interaction term was. Again, the association was strongest when vmHRV was highest. Thus, as expected, worriers are most likely to believe in worry's utility and endorse an exhaustive approach to problem solving when their resting vmHRV suggests they have good capacity for top-down control of cognition.

In our view these findings suggest that worry utility beliefs and endorsement of an AMAC problem-solving rule foster persistence in worrying among worriers having high capacity for top-down control. That is, we believe that such individuals use their cognitive control capacity to initiate and persist in worrying despite its aversiveness because they believe doing so has important payoffs. Our results support this perspective, especially for the belief that worry is useful because it distracts from more emotional things. Specifically, strong endorsement of that belief predicted the highest GAD symptom severity when vmHRV was *highest*, becoming non-significant when vmHRV was below the 18.4th percentile. Similarly, among those endorsing the strongest belief that worry distracts from more emotional things, the probability of meeting GAD diagnostic criteria based on GAD-Q-IV responses was maximized when vmHRV was *highest*, becoming non-significant when vmHRV was below the 18.3rd percentile. This supports the view of Borkovec and Roemer (1995), that the belief that worry distracts from more emotional things plays a pivotal role in pathological worry. However, our findings suggest further that this is true only

for worriers who possess sufficient capacity for top-down control to persist in worrying in an effort to achieve that goal.

This same pattern was also observed for broader worry utility beliefs. Although the hypothesized worry utility belief x vmHRV interaction only approached significance, as expected, such beliefs predicted the highest GAD symptom severity when vmHRV was highest and became non-significant when vmHRV was below the 11.9th percentile. Results were similar but weaker when predicting analog GAD status.

This pattern was also found for the AMAC approach to problem solving. Endorsement of that approach did interact significantly with vmHRV to predict GAD symptom severity. High endorsement of an AMAC approach was significantly positively associated with GAD symptoms only when vmHRV was above the 75th percentile. This association was significantly negative when vmHRV was lower than -2.8 SDs. However, that region of significance applied to only 3.8% of the sample and should be interpreted cautiously. A similar but weaker pattern was found predicting GAD status. Thus, our findings are consistent with the view that an AMAC approach to problem-solving fosters perseverative worry among individuals with high capacity for cognitive control. This is consistent with Meeten et al.'s (2016) finding that AMAC rule endorsement is associated with increased connectivity between the amygdala and PFC during resting state fMRI. Meeten and colleagues interpret that increased connectivity as reflecting attempts by high worriers to engage in goal-directed worry. In this regard it is important to note that higher connectivity between the amygdala and the PFC is associated with higher vmHRV (Sakaki et al., 2016). Higher vmHRV is also linked to higher inhibition of return to threat, which may foster the type of exhaustive search for novel solutions implied by the AMAC rule (Park et al., 2012).

Given our results, we must ask why someone having good cognitive control capacity might nevertheless learn to worry and come to hold such beliefs about its functions and form. One avenue is through parental influences. Specifically, parents who are worriers may encourage their children to worry, reinforce its occurrence, and model its use as a coping strategy (Aktar et al., 2017). In such ways they may inculcate their children with their beliefs about worry's utility and there tendency to follow an AMAC rule when worrying.

No matter how worriers having good capacity for cognitive control come to worry initially, their beliefs in worry's utility and in the need to worry exhaustively may be especially likely to strengthen as a result of worrying. Because they are able to persist in worrying despite its aversiveness, they may be more likely to experience reinforcement for worrying, which should, in turn, reinforce their beliefs about its functions and form. For example, the catastrophic outcomes anticipated by worriers rarely occur and when they do, worriers typically weather them better than they feared they might. That may be especially true for worriers having good capacity for top-down control. Therefore, such a worrier may be more likely to be negatively reinforced by virtue of concluding that worrying helped prevent, or prepare them for, a feared event even if the outcome would have been the same had they not worried (Davey and Meeten, 2016). Similarly, worry can be reinforced by virtue of its ability to blunt autonomic arousal (Borkovec et al., 2004) or foster avoidance of aversive emotional contrasts (Newman and Llera, 2011). These outcomes may be more likely if a worrier is able to draw upon their cognitive control capacity to persist in worrying despite its aversiveness (e.g., see Vasey et al., 2017). Such circumstances also seem likely to create the conditions for beliefs about worry's functions and form to be strengthened through the process of effort justification (Kitayama and Tompson, 2015). By virtue of their belief in worry's benefits and the importance of worrying exhaustively, such individuals are motivated to initiate and persist in worrying despite its aversiveness. However, that aversiveness should produce strong cognitive dissonance, which can be reduced by increasing one's commitment to the beliefs in question. Thus, the more such individuals worry, the more they should come to value it and the more firmly they should be committed to the reasons they have learned to worry and the exhaustive manner in which they think worry should proceed.

Our results suggest several questions for future research. First, the defensive stance toward the world that characterize worriers has been linked to lower levels of vmHRV within the NIM (Thayer and Lane, 2000) and Polyvagal Theory (Porges, 2008). Thus, it remains unclear how a worrier can adopt such a stance toward the world and nevertheless exhibit higher levels of vmHRV. A second important question for future research is why worriers with good capacity for top-down control nevertheless report that their worry is excessive and uncontrollable. One possibility is that such worriers may believe it would be bad to try to limit their worrying as suggested by Cartwright-Hatton and Wells (1997), even though they have the capacity to do so. However, whereas that might lead them to worry excessively it would not explain why they perceive worry to be uncontrollable. Instead, high cognitive control worriers may find their worry spinning out of control and proceeding involuntarily because worry depletes the very cognitive control resources they had initially used to persist in worrying. Evidence suggests that worry does indeed deplete such resources (e.g., Hayes et al., 2008; Stefanopoulou et al., 2014). This is also consistent with findings by Levine et al. (2016) showing that whereas individuals with GAD did *not* differ in vmHRV from healthy controls at baseline, they showed greater reductions in vmHRV during worry. Meeten et al. (2016) provide further evidence supporting such a process. Specifically, they found that higher AMAC rule endorsement predicted stronger declines in vmHRV in individuals with GAD following a perseverative cognition induction. Furthermore, research shows that worriers tend to shift from an AMAC rule at the outset of a catastrophizing worry task to an FLC rule at the end of such a task (Davey et al., 2007). However, it is likely that cognitive control resources are required to implement a goal of stopping worrying following such a rule shift. Consequently, since worrying consumes such resources, worriers having sufficient capacity to initially persist in worrying in accordance with the AMAC rule or their beliefs about worry's utility should find it difficult to stop the process once they no longer feel like continuing. However, it should be noted that Makovac et al. (2016b) found that higher vmHRV at baseline among individuals with GAD predicted weaker declines

in vmHRV following a perseverative cognition induction. This suggests that worriers having high cognitive control may be initially protected from worry-induced declines in cognitive control. If so, they may be able to engage in longer bouts of worry before losing control.

## Limitations

Our study had several limitations. First, given the study's design, we cannot draw firm conclusions regarding the direction of the associations observed or their causal status. Future research should attempt to resolve questions of directionality. Second, generalizability of our results may be limited by the fact that participants were college students characterized by a narrow age range and limited ethnic diversity. Furthermore, although individuals reporting high GAD symptom severity were well-represented in the current sample, future studies should include clinically diagnosed, treatment seeking cases. Third, we lacked information concerning medications that participants were taking that could alter their resting levels of vmHRV. Since some participants reported high GAD symptoms, it is possible that some may have been taking medications for anxiety and/or depression. Thus, it is possible that medication effects may have contributed to our findings. However, insofar as some such medications can reduce vmHRV whereas others can cause it to increase (see Kemp et al., 2014 and Kemp et al., 2016) such effects seem unlikely to account for our findings.

Finally, given our small sample size, statistical power was limited, especially for detecting interactions (McClelland and Judd, 1993). Consequently, some regression models and interaction effects did not achieve significance despite accounting for substantial percentages of variance. This is especially relevant in the case of the AMAC rule, which was assessed using a single questionnaire item. That undoubtedly increased measurement error and thus further reduced power. Since the item asked about consideration of all possible problem solutions rather than specifically about an AMAC approach to worrying, it may also have failed to measure that construct adequately. Past research has shown that AMAC rule endorsement is associated with worry severity (Davey and Meeten, 2016). In this study item #7 on the PSI did not correlate significantly with GAD symptom severity at the zero-order level. However, that correlation was significant at high levels of vmHRV. AMAC rule endorsement is also correlated with worry utility beliefs (Davey and Meeten, 2016). Thus, it is notable that Item #7 on the PSI was significantly correlated with the worry distracts item on the RWQ. However, it is also notable that the item in question did not correlate significantly with our broader measures of worry utility beliefs. Thus, it remains unclear how well item #7 of the PSI represented AMAC rule endorsement. Future research should utilize a more psychometrically sound measure of this construct, such as the Worry Stop Rule Checklist (Davey et al., 2005).

## Conclusion

Our results suggest that worriers who have good top-down control capacity initiate and persist in worry because they value it. However, why they nevertheless rate their worry as excessive and uncontrollable is an important question for future research.

# DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

# ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Behavioral and Social Sciences Institutional Review Board of The Ohio State University. The patients/participants provided their written informed consent to participate in this study.

# AUTHOR CONTRIBUTIONS

GF and MV conducted the data analyses and wrote the first draft of the manuscript. LC collected the data and designed the larger study from which the data were drawn. JT contributed to the critical design of the study and assisted with data analysis. All authors provided important intellectual content in revising the manuscript and approved the final version before submission.

# REFERENCES


Aktar, E., Nikolic, M., and Bögels, S. (2017). Environmental transmission of generalized anxiety disorder from parents to children: worries, experiential avoidance, and intolerance of uncertainty. *Dialogues Clin. Neurosci.* 19, 137–146.

Aldao, A., and Mennin, D. S. (2012). Paradoxical cardiovascular effects of implementing adaptive emotion regulation strategies in generalized anxiety disorder. *Behav. Res. Ther.* 50, 122–130. doi: 10.1016/j.brat.2011.12.004

American Psychiatric Association [APA] (1994). *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edn. Washington, DC: American Psychiatric Association.

American Psychiatric Association [APA] (2013). *Diagnostic and Statistical Manual of Mental Disorders*, 5th Edn. Washington, DC: American Psychiatric Association.

Armstrong, T., Zald, D. H., and Olatunji, B. O. (2011). Attentional control in OCD and GAD: specificity and associations with core cognitive symptoms. *Behav. Res. Ther.* 49, 756–762. doi: 10.1016/j.brat.2011.08.003

Bienvenu, O. J., Samuels, J. F., Costa, P. T., Reti, I. M., Eaton, W. W., and Nestadt, G. (2004). Anxiety and depressive disorders and the five-factor model of personality: a higher- and lower-order personality trait investigation in a community sample. *Depress. Anxiety* 20, 92–97. doi: 10.1002/da.20026

Borkovec, T. D., Alcaine, O. M., and Behar, E. (2004). "Avoidance theory of worry and generalized anxiety disorder," in *Generalized Anxiety Disorder: Advances in Research and Practice*, eds R. G. Heimberg, C. L. Turk, and D. S. Mennin, (New York, NY: The Guilford Press), 77–109.

Borkovec, T. D., Robinson, E., Pruzinsky, T., and DePree, J. A. (1983). Preliminary exploration of worry: some characteristics and processes. *Behav. Res. Ther.* 21, 9–16. doi: 10.1016/0005-7967(83)90121-3

Borkovec, T. D., and Roemer, L. (1995). Perceived functions of worry among generalized anxiety disorder subjects: distraction from more emotionally distressing topics? *J. Behav. Ther. Exp. Psychol.* 26, 25–30. doi: 10.1016/0005-7916(94)00064-S

Brosschot, J. F., Van Dijk, E., Thayer, J. F., and Van Dijk, E. (2007). Daily worry is related to low heart rate variability during waking and the subsequent nocturnal sleep period. *Int. J. Psychophysiol.* 63, 39–47. doi: 10.1016/j.ijpsycho.2006.07.016

Carnevali, L., Mancini, M., Koenig, J., Makovac, E., Watson, D. R., Meeten, F., et al. (2019). Cortical morphometric predictors of autonomic dysfunction in generalized anxiety disorder. *Auton. Neurosci.* 217, 41–48. doi: 10.1016/j.autneu.2019.01.001

Cartwright-Hatton, S., and Wells, A. (1997). Beliefs about worry and intrusions: the meta-cognitions questionnaire and its correlates. *J. Anxiety Disord.* 11, 279–296. doi: 10.1016/S0887-6185(97)00011-X

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., and Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Front. Psychiatry* 5:80. doi: 10.3389/fpsyt.2014.00080

Cohen, J., Cohen, E., West, S. G., and Aiken, L. S. (2002). *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*, 3rd Edn. Mahwah, NJ: Erlbaum.

Covin, R., Ouimet, A. J., Seeds, P. M., and Dozois, D. J. A. (2008). A meta-analysis of CBT for pathological worry among clients with GAD. *J. Anxiety Disord.* 22, 108–116. doi: 10.1016/j.janxdis.2007.01.002

Cuijpers, P., Sijbrandij, M., Koole, S., Huibers, M., Berking, M., and Andersson, G. (2014). Psychological treatment of generalized anxiety disorder: a meta-analysis. *Clin. Psychol. Rev.* 34, 130–140. doi: 10.1016/j.cpr.2014.01.002

Davey, G. C. L. (1994). "Pathological worrying as exacerbated problem solving," in *Worrying Perspectives on Theory, Assessment and Treatment*, eds G. C. L. Davey, and F. Tallis, (Chichester: John Wiley).

Davey, G. C. L., Eldridge, F., Drost, J., and MacDonald, B. A. (2007). What ends a worry bout? An analysis of changes in mood and stop rule use across the catastrophizing interview task. *Behav. Res. Ther.* 45, 1231–1243. doi: 10.1016/j.brat.2006.08.024

Davey, G. C. L., Hampton, J., Farrell, J., and Davidson, S. (1992). Some characteristics of worrying: evidence for worrying and anxiety as separate constructs. *J. Pers. Individ. Dif.* 13, 133–147. doi: 10.1016/0191-8869(92)90036-O

Davey, G. C. L., and Meeten, F. (2016). The perseverative worry bout: a review of cognitive, affective, and motivational factors that contribute to worry perseveration. *Biol. Psychiatry* 121, 233–243. doi: 10.1016/j.biopsycho.2016.04.003

Davey, G. C. L., Startup, H. M., MacDonald, C. B., Jenkins, D., and Patterson, K. (2005). The use of "as many as can" versus "feel like continuing" stop rules during worrying. *Cogn. Ther. Res.* 29, 155–169. doi: 10.1007/s10608-005-3162-5

Davey, G. C. L., Tallis, F., and Capuzzo, N. (1996). Beliefs about the consequences of worrying. *Cogn. Ther. Res.* 20, 499–420. doi: 10.1007/BF02227910

Davis, M., Montgomery, I., and Wilson, G. (2002). Worry and heart rate variables: autonomic rigidity under challenge. *J. Anxiety Disord.* 16, 639–659. doi: 10.1016/S0887-6185(02)00132-9

de Geus, E. J. C., Gianaros, P. J., Brindle, R. C., Jennings, J. R., and Berntson, G. G. (2019). Should heart rate variability be "corrected" for heart rate? Biological, quantitative, and interpretive considerations. *Psychophysiology* 56:e13287. doi: 10.1111/psyp.13287

Derryberry, D., and Reed, M. A. (2002). Anxiety-related attentional biases and their regulation by attentional control. *J. Abnorm. Psychol.* 111, 225–236. doi: 10.1037//0021-843x.111.2.225

Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., and Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Arch. Gen. Psychiatry* 66, 1361–1372. doi: 10.1001/archgenpsychiatry.2009.104

Fisher, A. J., and Newman, M. G. (2013). Heart rate and autonomic response to stress after experimental induction of worry versus relaxation in healthy, high-worry, and generalized anxiety disorder individuals. *Biol. Psychol.* 93, 65–74. doi: 10.1016/j.biopsycho.2013.01.012

Freeston, M. H., Rhéaume, J., Letarte, H., Dugas, M. J., and Ladouceur, R. (1994). Why do people worry? *Pers. Individ. Dif.* 17, 791–802. doi: 10.1016/0191-8869(94)90048-5

Gillie, B. L., Vasey, M. W., and Thayer, J. F. (2014). Heart rate variability predicts control over memory retrieval. *Psychol. Sci.* 25, 458–465. doi: 10.1111/psyp.12443

Gillie, B. L., Vasey, M. W., and Thayer, J. F. (2015). Individual differences in heart rate variability moderate thought suppression success. *Psychophysiology* 52, 1149–1160. doi: 10.1177/0956797613508789

Hammel, J. C., Smitherman, T. A., McGlynn, F. D., Mulfinger, A. M. M., Lazarte, A. A., and Gothard, K. D. (2011). Vagal influence during worry and cognitive challenge. *Anxiety Stress Coping* 24, 121–136. doi: 10.1080/10615806.2010.490912

Hayes, A. F. (2013). *Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach*. New York, NY: Guilford.

Hayes, S., Hirsch, C., and Mathews, A. (2008). Restriction of working memory capacity during worry. *J. Abnorm. Psychol.* 117, 712–717. doi: 10.1037/a0012908

Hebert, E. A., Dugas, M. J., and Tulloch, T. G. (2014). Positive beliefs about worry: a psychometric evaluation of the why worry-II. *Pers. Individ. Dif.* 56, 3–8. doi: 10.1016/j.paid.2013.08.009

Heppner, P. P., and Petersen, C. H. (1982). The development and implications of a personal problem-solving inventory. *J. Couns. Psychol.* 29, 66–75. doi: 10.1037/0022-0167.29.1.66

Hirsch, C. R., and Mathews, A. (2012). A cognitive model of pathological worry. *Behav. Res. Ther.* 50, 636–646. doi: 10.1016/j.brat.2012.06.007

Hoehn-Saric, R., Mcleod, D. R., and Zimmerli, W. D. (1989). Somatic manifestations in women with generalized anxiety disorder. Psychophysiological responses to psychological stress. *Arch. Gen. Psychiatry* 46, 1113–1119. doi: 10.1001/archpsyc.1989.01810120055009

Iijima, Y., and Tanno, Y. (2013). The moderating role of positive beliefs about worry in the relationship between stressful events and worry. *J. Pers. Individ. Dif.* 55, 1003–1006. doi: 10.1016/j.paid.2013.08.004

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., Carvalho de Figueiredo, R., et al. (2014). Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. *Am. J. Psychiatry* 171, 1328–1334. doi: 10.1176/appi.ajp.2014.13121605

Kemp, A. H., Fráguas, R., Brunoni, A. R., Bittencourt, M. S., Nunes, M. A., and Dantas, E. M. (2016). Differential association of specific selective serotonin reuptake inhibitors with resting-state heart rate and heart rate variability: implications for health and well-being. *Psychosom. Med.* 78, 810–818. doi: 10.1097/PSY.0000000000000336

Kessler, R. C., Petuhkova, M., Sampson, N. A., Zaslavsky, A. M., and Wittchen, H.-U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int. J. Methods Psychiatr. Res.* 21, 169–184. doi: 10.1002/mpr.1359

Kitayama, S., and Tompson, S. (2015). A biosocial model of affective decision making: implications for dissonance, motivation, and culture. *Adv. Exp. Soc. Psychol.* 52, 71–137.

Knepp, M. M., and Friedman, B. H. (2008). Cardiovascular activity during laboratory tasks in women with high and low worry. *Biol. Psychol.* 79, 287–293. doi: 10.1016/j.biopsycho.2008.07.002

Kollai, M., and Kollai, B. (1992). Cardiac vagal tone in generalised anxiety disorder. *Br. J. Psychiatry* 161, 831–835. doi: 10.1192/bjp.161.6.831

Laberge, M., Dugas, M. J., and Ladouceur, R. (2000). Modification of beliefs relative to worriers following treatment for generalized anxiety disorder. *Can. J. Behav. Sci.* 32, 91–96. doi: 10.1037/h0087104

Ladouceur, R., Blais, F., Freeston, M. H., and Dugas, M. J. (1998). Problem solving and problem orientation in generalized anxiety disorder. *J. Anxiety Disord.* 12, 139–152. doi: 10.1016/S0887-6185(98)00002-4

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., and Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage* 44, 213–222. doi: 10.1016/j.neuroimage.2008.07.056

Laugesen, N., Dugas, M. J., and Bukowski, W. M. (2003). Understanding adolescent worry: the application of a cognitive model. *J. Abnorm. Child Psychol.* 31, 55–64. doi: 10.1023/A:1021721332181

Levine, J. C., Fleming, R., Piedmont, J. I., Cain, S. M., and Chen, W.-J. (2016). Heart rate variability and generalized anxiety disorder during laboratory-induced worry and aversive imagery. *J. Affect. Disord.* 205, 207–215. doi: 10.1016/j.jad.2016.07.019

Lonigan, C. J., and Vasey, M. W. (2009). Negative affectivity, effortful control, and attention to threat-relevant stimuli. *J. Abnorm. Child Psychol.* 37, 387–399. doi: 10.1007/s10802-008-9284-y

Makovac, E., Meeten, F., Watson, D. R., Garfinkel, S. N., Critchley, H. D., and Ottaviani, C. (2016a). Neurostructural abnormalities associated with axes of emotion dysregulation in generalized anxiety. *Neuroimage Clin.* 10, 172–181. doi: 10.1016/j.nicl.2015.11.022

Makovac, E., Meeten, F., Watson, D. R., Herman, A., Garfinkel, S. N., Critchley, H. D., et al. (2016b). Alternations in amygdala-prefrontal functional connectivity account for excessive worry and autonomic dysregulation in generalized anxiety disorder. *Biol. Psychiatry* 80, 786–795. doi: 10.1016/j.biopsych.2015.10.013

Mankus, A. M., Aldao, A., Kerns, C., Mayville, E. W., and Mennin, D. S. (2013). Mindfulness and heart rate variability in individuals with high and low generalized anxiety symptoms. *Behav. Res. Ther.* 51, 386–391. doi: 10.1016/j.brat.2013.03.005

McClelland, G. H., and Judd, C. M. (1993). Statistical difficulties of detecting interactions and moderator effects. *Psychol. Bull.* 114, 376–390. doi: 10.1037/0033-2909.114.2.376

Meeten, F., Davey, G. C. L., Makovac, E., Watson, D. R., Garfinkel, S. N., Critchley, H. D., et al. (2016). Goal directed worry rules are associated with distinct patterns of amygdala functional connectivity and vagal modulation during perseverative cognition. *Front. Hum. Neurosci.* 10:553. doi: 10.3389/fnhum.2016.00553

Mohlman, J., Price, R. B., Eldreth, D. A., Chazin, D., Glover, D. M., and Kates, W. R. (2009). The relation of worry to prefrontal cortex volume in older adults with and without generalized anxiety disorder. *Psychiatry Res.* 173, 121–127. doi: 10.1016/j.pscychresns.2008.09.010

Moore, M. T., Anderson, N. L., Barnes, J. M., Haigh, E. A. P., and Fresco, D. M. (2014). Using the GAD-Q-IV to identify generalized anxiety disorder in psychiatric treatment seeking and primary care medical samples. *J. Anxiety Disord.* 28, 25–30. doi: 10.1016/j.janxdis.2013.10.009

Newman, M. G., and Llera, S. J. (2011). A novel theory of experiential avoidance in generalized anxiety disorder: a review and synthesis of research supporting a contrast avoidance model of worry. *Clin. Psychol. Rev.* 31, 371–382. doi: 10.1016/j.cpr.2011.01.008

Newman, M. G., Zuellig, A. R., Kachin, K. E., Constantino, M. J., Przeworski, A., Erickson, T., et al. (2002). Preliminary reliability and validity of the generalized anxiety disorder questionnaire-IV: a revised self-report diagnostic measure of generalized anxiety disorder. *Behav. Ther.* 33, 215–233. doi: 10.1016/S0005-7894(02)80026-0

Norman, S. B., Cissell, S. H., Means-Christensen, A. J., and Stein, M. B. (2006). Development and validation of an overall anxiety sensitivity and impairment scale (OASIS). *Depress. Anxiety* 23, 245–249. doi: 10.1002/da.20182

Nugent, A. C., Bain, E. E., Thayer, J. F., Sollers, J. J., and Drevets, W. C. (2011). Heart rate variability during motor and cognitive tasks in females with major depressive disorder. *Psychiatry Res. Neuroimaging* 191, 1–8. doi: 10.1016/j.pscychresns.2010.08.013

Olatunji, B. O., Ciesielski, ÄB. G., Armstrong, T., Zhao, M., and Zald, D. H. (2011). Making something out of nothing: neutral content modulates attention in generalized anxiety disorder. *Depress. Anxiety* 434, 427–434. doi: 10.1002/da.20806

Park, G., Van Bavel, J. J., Vasey, M. W., and Thayer, J. F. (2012). Cardiac vagal tone predicts inhibited attention to fearful faces. *Emotion* 12, 1292–1302. doi: 10.1037/a0028528

Park, G., Van Bavel, J. J., Vasey, M. W., and Thayer, J. F. (2013). Cardiac vagal tone predicts attentional engagement to and disengagement from fearful faces. *Emotion* 13, 645–656. doi: 10.1037/a0032971

Porges, S. W. (2008). The polyvagal perspective. *Biol. Psychol.* 74, 116–143.

Price, R. B., Eldreth, D. A., and Mohlman, J. (2011). Deficient prefrontal attentional control in late-life generalized anxiety disorder: an fMRI investigation. *Transl. Psychiatry* 1:e46. doi: 10.1038/tp.2011.46

Quintana, D. S., Heathers, J. A. J., and Kemp, A. H. (2012). On the validity of using the Polar RS800 heart rate monitor for heart rate variability research. *Eur. J. Appl. Physiol.* 112, 4179–4180. doi: 10.1007/s00421-012-2453-2

Rodebaugh, T. L., Holaway, R. M., and Heimberg, R. G. (2008). The factor structure and dimensional scoring of the generalized anxiety disorder questionnaire for DSM-IV. *Assessment* 15, 343–350. doi: 10.1177/1073191107312547

Rosellini, A. J., and Brown, T. A. (2011). The NEO five-factor inventory: latent structure and relationships with dimensions of anxiety and depressive disorders in a large clinical sample. *Assessment* 18, 27–38. doi: 10.1177/1073191110382848

Sakaki, M., Yoo, H. J., Nga, L., Lee, T.-H., Thayer, J. F., and Mather, M. (2016). Heart rate variability is associated with amygdala functional connectivity with MPFC across younger and older adults. *Neuroimage* 139, 44–52. doi: 10.1016/j.neuroimage.2016.05.076

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Spitzer, R. L., Kroenke, K., Williams, J. B. W., and Lowe, B. (2006). A brief measure for assessing generalized anxiety disorder. *Arch. Inter. Med.* 166, 1092–1097. doi: 10.1001/archinte.166.10.1092

Stefanopoulou, E., Hirsch, C. R., Hayes, S., Adlam, A., and Coker, S. (2014). Are attentional control resources reduced by worry in generalized anxiety disorder? *J. Abnorm. Psychol.* 123, 330–335. doi: 10.1037/a0036343

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-aho, P. O., and Karjalainen, P. A. (2009). "Kubios HRV – A software for advanced heart rate variability analysis," in *Proceedings of the 4th European Conference of the International Federation for Medical and Biological Engineering*, Vol. 22, eds J. Vander Sloten, P. Verdonck, M. Nyssen, and J. Haueisen, (Berlin: Springer), 1022–1025.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., Friedman, B. H., and Borkovec, T. D. (1996). Autonomic characteristics of generalized anxiety disorder and worry. *Biol. Psychiatry* 39, 255–266. doi: 10.1016/0006-3223(95)00136-0

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Toh, G. Y., and Vasey, M. W. (2017). Heterogeneity in autonomic arousal level in perseverative worry: the role of cognitive control and verbal thought. *Front. Hum. Neurosci.* 11:108. doi: 10.3389/fnhum.2017.00108

Vasey, M. W., Chriki, L., and Toh, G. Y. (2017). Cognitive control and anxious arousal in worry and generalized anxiety: an initial test of an integrative model. *Cogn. Ther. Res.* 41, 155–169. doi: 10.1007/s10608-016-9809-6

Yiend, J., Mathews, A., Burns, T., Dutton, K., Fernandez-Martin, A., Georgiou, G. A., et al. (2014). Mechanisms of selective attention in generalized anxiety disorder. *Clin. Psychol. Sci.* 3, 758–771. doi: 10.1177/2167702614545216

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 08 October 2020
doi: 10.3389/fnins.2020.570400

# A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback

*Fred Shaffer[1]* and Zachary M. Meehan[2]*

[1] *Center for Applied Psychophysiology, Truman State University, Kirksville, MO, United States,* [2] *Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, United States*

Heart rate variability (HRV) represents fluctuations in the time intervals between successive heartbeats, which are termed interbeat intervals. HRV is an emergent property of complex cardiac-brain interactions and non-linear autonomic nervous system (ANS) processes. A healthy heart is not a metronome because it exhibits complex non-linear oscillations characterized by mathematical chaos. HRV biofeedback displays both heart rate and frequently, respiration, to individuals who can then adjust their physiology to improve affective, cognitive, and cardiovascular functioning. The central premise of the HRV biofeedback resonance frequency model is that the adult cardiorespiratory system has a fixed resonance frequency. Stimulation at rates near the resonance frequency produces large-amplitude blood pressure oscillations that can increase baroreflex sensitivity over time. The authors explain the rationale for the resonance frequency model and provide detailed instructions on how to monitor and assess the resonance frequency. They caution that patterns of physiological change must be compared across several breathing rates to evaluate candidate resonance frequencies. They describe how to fine-tune the resonance frequency following an initial assessment. Furthermore, the authors critically assess the minimum epochs required to measure key HRV indices, resonance frequency test-retest reliability, and whether rhythmic skeletal muscle tension can replace slow paced breathing in resonance frequency assessment.

**Keywords: biofeedback, complexity, emotional self-regulation, heart rate variability, neurocardiology, resonance, performance**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Uirassu Borges, German Sport University Cologne, Germany*
*Moacir Fernandes Godoy, Faculty of Medicine of São José do Rio Preto, Brazil*
*Donald James Noble, Emory University, United States*

****Correspondence:***
*Fred Shaffer*
*fredricshaffer@gmail.com*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *07 June 2020*
***Accepted:*** *10 September 2020*
***Published:*** *08 October 2020*

***Citation:***
*Shaffer F and Meehan ZM (2020) A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback. Front. Neurosci. 14:570400. doi: 10.3389/fnins.2020.570400*

## INTRODUCTION

Slow paced breathing is a central component of HRV biofeedback because respiratory sinus arrhythmia (RSA) amplitude (peak-to-trough heart rate difference across the breathing cycle) increases with slow breathing (Cooke et al., 1998). The resonance frequency training model identifies the respiration rate that produces the greatest heart rate oscillations by stimulating the *baroreflex*, which is the homeostatic system that regulates blood pressure using *baroreceptors* (blood pressure receptors; Swenne, 2013). This protocol measures HRV changes as adult clients breathe from 6.5 to 4.5 breaths per min (bpm) in 0.5-bpm steps (Lehrer et al., 2003).

The purpose of this article is to describe Lehrer and colleagues' resonance frequency assessment protocol in detail, illustrate the challenges in choosing between several potential resonance frequencies, and address issues like test-retest validity that require further research. We organized

this article so that sections build on each other. These include: HRV, Baroreflex, Vaschillo Two Closed-Loop Model, Resonance Frequency Model, Importance of Resonance Frequency Assessment, Individualized Frequencies, Resonance Frequency Assessment Protocol, Resonance Frequency Selection, and Unanswered Questions.

The HRV section begins with an explanation of HRV, its relationship to HR, how breathing can produce large-scale HR oscillations called RSA, and the clinical and performance applications of resonance frequency training. The authors emphasize that HRV indexes neurocardiac function and autonomic functioning, as well as the mobilization and use of scarce self-regulatory resources. Finally, this section reviews how healthy variability contributes to regulatory capacity and adaptability.

The Baroreflex section explains the baroreflex mechanism and how breathing at slow rates (e.g., 4.5 to 6.5 bpm) produces the large-scale heart rate changes observed in RSA. The Vaschillo Two Closed-Loop Model section introduces an evidence-based model of how activities like slow paced breathing and rhythmic skeletal muscle tension can stimulate the baroreflex and increase RSA. The Resonance Frequency Model section introduces the concept of resonance, explains that the volume of blood in the vascular tree determines each individual's resonance frequency, and emphasizes that stimulation at the resonance frequency maximizes RSA and HRV.

The Importance of Resonance Frequency Assessment section summarizes preliminary evidence of the benefits of breathing at an individual's unique resonance frequency. The Individualized Frequencies section explains the relationship between respiration rate and *peak frequency* (largest amplitude frequency) and stresses that the respiration rate that produces a peak frequency depends on the location of the resonance frequency between 4.5 and 6.5 bpm. Resonance frequency training does not always reward 6 bpm (0.1 Hz) breathing because the resonance frequency may be lower or higher.

The Resonance Frequency Assessment Protocol section describes sensor channels and parameters monitored, terms and definitions, normative values, client orientation, and how to conduct practice breathing and resonance frequency assessment trials. The Resonance Frequency Selection section summarizes resonance frequency assessment criteria and explains the importance of *phase synchrony* (alignment of heart rate and respiration rate signal peaks) and *HR Max – HR Min* (the mean difference between the highest and lowest heart rate across all breathing cycles). This section describes procedures for breaking ties and confirming the resonance frequency during the second session with a client.

The Unanswered Questions section explores why resonance frequency assessment is necessary given that slow paced breathing increases HRV and achieves clinical gains without HRV biofeedback. We revisit evidence that training at a client's resonance frequency may improve systolic and mood. Resonance Frequency Test-Reliability presents evidence of acceptable 2-week test-retest reliability for the Lehrer et al. (2003) resonance frequency assessment protocol and stresses the need for replication studies with more robust samples. Finally, Rhythmic Skeletal Muscle Tension summarizes evidence that this technique can also increase RSA and HRV and calls for research concerning its comparability to resonance frequency breathing and when resonance frequency assessment using this method can achieve acceptable test-retest validity.

## HRV

Heart rate, HRV, and RSA calculations depend on the time intervals between heartbeats (Task Force Report, 1996). Heart rate is the number of heartbeats each min. Along with associated metrics, it provides detailed information that clinicians can apply in a variety of medical and psychological interventions (Lehrer et al., 2020a). Frequently used as a target in clinical and performance interventions, HRV represents fluctuations in the time intervals between successive heartbeats, which are termed interbeat intervals (Task Force Report, 1996). Clinicians measure these interbeat intervals in milliseconds (ms). For example, a 60-bpm heart rate corresponds to an interbeat interval of 1000 ms since there are sixty 1000-ms intervals in a min. HRV biofeedback presents heart rate and sometimes also directly some HRV parameters to individuals to improve their affective, cognitive, and cardiovascular functioning (McCraty and Shaffer, 2015). These changes may be mediated by increased cardiac vagal tone, RSA, and activation of integrated homeostatic systems. The goal of HRV biofeedback is to increase RSA, which is heart rate acceleration and deceleration across the breathing cycle (Eckberg, 1983), in order to enhance autonomic homeostatic capacity (Vaschillo et al., 2002, p. 4). RSA involves respiration-driven changes in heart rate that are mediated by the *vagus nerve*, which conveys baroreceptor inputs to the brain and then returns them to the heart after integration in the brain (Kollai and Mizsei, 1990). When we inhale, the cardiovascular center inhibits vagal firing and heart rate speeds (Yasuma and Hayano, 2004). Conversely, when we exhale, the cardiovascular center restores vagal inhibition and heart rate slows (Eckberg and Eckberg, 1982; Berntson et al., 1997). HRV biofeedback teaches clients to increase RSA by creating sinusoidal phase-synchronous patterns of heart rate and respiration (Lehrer and Gevirtz, 2014). HRV biofeedback is extensively used to treat an array of disorders (e.g., asthma and depression) and enhance performance in a variety of contexts (e.g., sports; Gevirtz, 2013; Tan et al., 2016; Lehrer et al., 2020a). While the final targets of these applications may differ, HRV biofeedback increases cardiac vagal activity (Vaschillo et al., 2006; Lehrer et al., 2020a) and stimulates the negative feedback loops that are responsible for homeostasis (Lehrer and Eddie, 2013).

The neurovisceral integration model describes HRV as an emergent property of complex cardiac-brain interactions and non-linear autonomic nervous system (ANS) processes. HRV provides a window into neurocardiac function. HRV may reflect medial prefrontal cortex (mPFC) integration with brainstem regulation of the heart by the nucleus tractus solitarius (Thayer et al., 2012). The neurovisceral integration model describes the interrelationship between the prefrontal cortex, HRV, and executive function (Thayer et al., 2009). In turn, increased

HRV may enhance top-down mPFC control of emotional health (Mather and Thayer, 2018).

Heart rate variability is generated by interdependent regulatory systems with widely varying rhythms that enable us to adapt to physical and psychological challenges (Thayer and Lane, 2000). Short-term (∼5 min) HRV measurements are produced by interactions among the autonomic, cardiovascular, central nervous, endocrine, and respiratory systems. These integrated systems utilize feedback from *baroreceptors* (receptors that detect blood pressure changes) and *chemoreceptors* (receptors that monitor chemicals like blood gases; Kougias et al., 2010).

Heart rate variability is a marker for the regulation of integrated functions and efficient allocation of limited self-regulatory resources. HRV appears to index autonomic functioning, blood pressure, cardiac functioning, digestion, oxygen and carbon dioxide exchange, vascular tone (diameter of resistance vessels), and possibly facial muscle regulation (Gevirtz et al., 2016). HRV reflects the vagal contribution to executive functions, affective control, and social self-regulation (Byrd et al., 2015; Laborde et al., 2017; Mather and Thayer, 2018). Indeed, Laborde et al. (2018) *vagal tank theory* proposes that vagal traffic to the heart indicates how efficiently we mobilize and use scarce self-regulatory resources.

Both regulatory capacity and adaptability depend on healthy variability due to increased vagal traffic. "A healthy heart is not a metronome" (Shaffer et al., 2014, p. 5). Variability enables adaptability. Multiple overlapping system oscillations, characterized by mathematical chaos, produce the complex non-linear oscillations of a healthy heart. "Oscillatory patterns with greater complexity, such as those that occur when a number of oscillatory patterns overlap, are described as 'chaotic.' Chaos reflects the simultaneous operation of numerous control processes" (Lehrer and Eddie, 2013, p. 145). The integrated action of multiple control systems contributes stability in response to challenges like exercise and stressors. Practically, the interdependence of these control systems means that interventions like slow paced breathing can initiate system-wide changes to increase HRV (Goldberger, 1991; Lehrer and Eddie, 2013). Slow paced breathing at the rate of 6 breaths per min produces large-scale increases in cardiorespiratory synchrony (Noble and Hochman, 2019). Healthy variability allows rapid responses to changing workloads and unpredictable environmental challenges (Beckers et al., 2006) and contributes to regulatory capacity (Grossman and Taylor, 2007). Whereas healthy biological systems show spatial and temporal complexity, diseases like cardiac conduction disorders can decrease or increase complexity (Vaillancourt and Newell, 2002). Increased HRV is only desirable when it is produced by increased cardiac vagal tone instead of cardiac conduction abnormalities (Stein et al., 2005).

## THE BAROREFLEX

The *baroreflex* is central to understanding HRV biofeedback because maneuvers like slow paced breathing and rhythmic skeletal muscle tension stimulate it to increase RSA. Rhythmic skeletal muscle tension involves the simultaneous contraction of the hands and feet at rates from 6.5 to 4.5 contractions per min (cpm) while sitting. Both slow paced breathing and rhythmic skeletal muscle tension are hypothesized to increase HRV by stimulating a ∼0.1-Hz resonance in the cardiovascular system (Vaschillo et al., 2011). The baroreflex, which provides homeostatic control of acute changes, continuously operates through the interaction of multiple regulatory systems. Cardiorespiratory control of blood pressure and HRV depends on baroreceptors found in the aortic arch and carotid sinuses. While these blood pressure sensors continuously generate action potentials, blood pressure modulates their firing rate. Rising blood pressure increases and falling blood pressure decreases afferent transmission via glossopharyngeal (IX) and vagus (X) nerves that targets the nucleus tractus solitarius in the dorsomedial medulla. The nucleus tractus solitarius, in turn, directs the medulla's vasomotor and cardiac control centers to adjust vascular tone and heart rate, respectively (**Figure 1**; Cutsforth-Gregory and Benarroch, 2017; Fox and Rompolski, 2019). The baroreflex integrates blood pressure, heart rate, and vascular tone control systems (Vaschillo et al., 2002) and contributes to RSA (Karemaker, 2009) along with a brainstem respiratory central pattern generator (Berntson et al., 1997; Eckberg, 2003) and pulmonary afferents (Taha et al., 1995; Koh et al., 1998). Respiration produces blood pressure and heart rate oscillations at an individual's resonance frequency. "... as you inhale, HR rises and BP falls, but the baroreflex causes an immediate augmentation of the respiration-induced HR increase, with the opposite happening as you exhale, causing high-amplitude HR oscillations" (Lehrer and Vaschillo, 2008, p. 12; **Figure 2**).

## VASCHILLO'S TWO CLOSED-LOOP MODEL

Vaschillo's two closed-loop model explains how HRV biofeedback procedures like slow paced breathing and rhythmic skeletal muscle tension can stimulate the baroreflex and amplify RSA. Vaschillo et al. (2002) describe the vascular tone and heart rate baroreflexes as closed loops and propose that stimulating one closed loop activates its counterpart. HRV biofeedback typically utilizes *slow paced breathing*, which is breathing at a target rate such as 6 bpm, to stimulate the baroreflex and increase RSA (Gevirtz et al., 2016). Respiration can produce blood pressure oscillations via changes in thoracic pressure (Pinsky, 2018) that can stimulate the closed loops. Rhythmic skeletal muscle tension may produce comparable changes by stimulating blood pressure, heart rate, and vascular tone control systems without the requirement of slower-than-normal respiration (Vaschillo et al., 2011).

## RESONANCE FREQUENCY MODEL

*Resonance* is an amplification process in which stimulating a negative feedback, self-corrective system at its intrinsic frequency

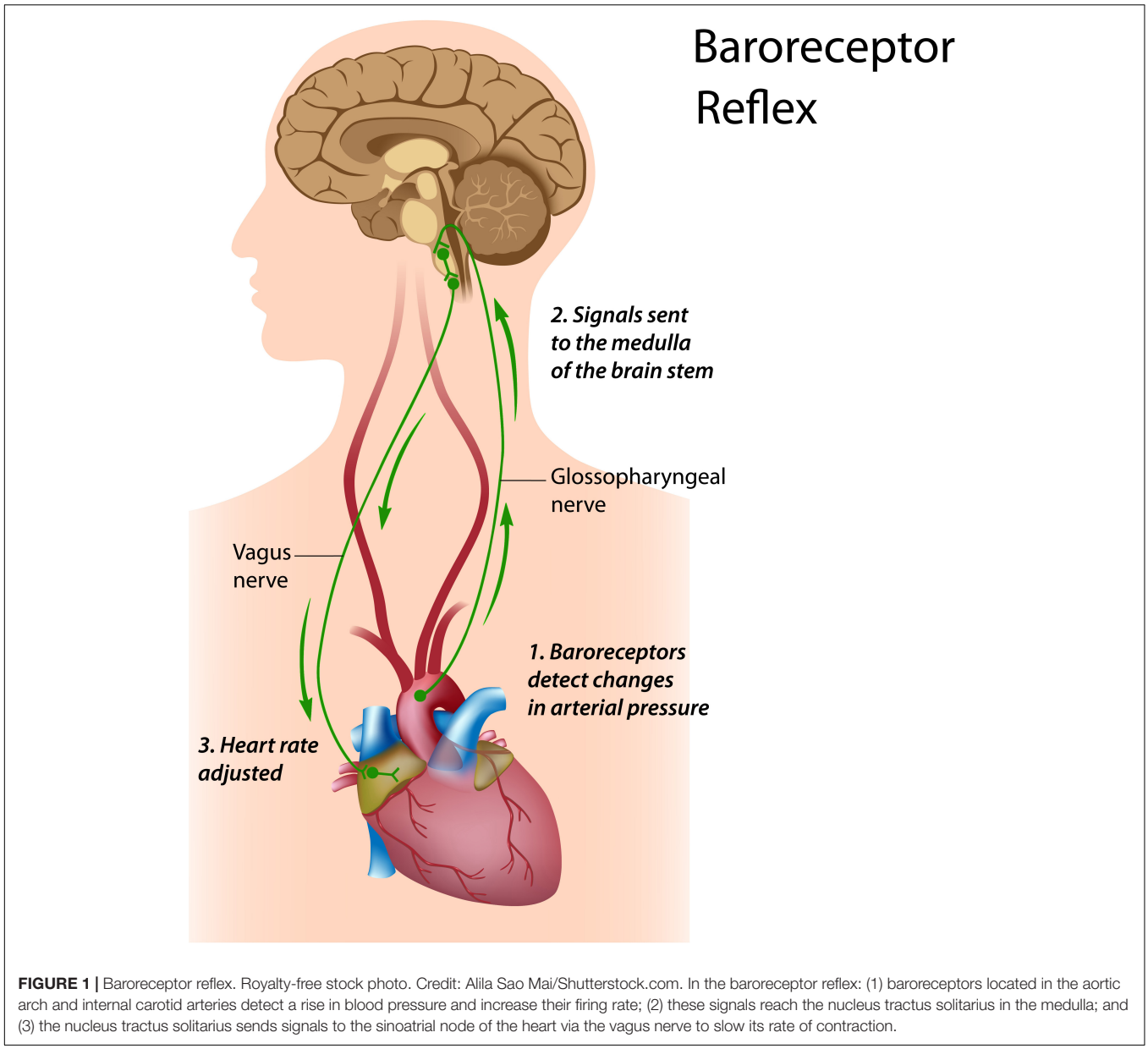



**FIGURE 1 |** Baroreceptor reflex. Royalty-free stock photo. Credit: Alila Sao Mai/Shutterstock.com. In the baroreceptor reflex: (1) baroreceptors located in the aortic arch and internal carotid arteries detect a rise in blood pressure and increase their firing rate; (2) these signals reach the nucleus tractus solitarius in the medulla; and (3) the nucleus tractus solitarius sends signals to the sinoatrial node of the heart via the vagus nerve to slow its rate of contraction.

generates high-amplitude oscillations at that frequency (Başar, 1998; Lehrer and Gevirtz, 2014). Resonance is a property of the baroreflex system (Vaschillo et al., 2002, 2006). The mechanisms responsible for this amplification are complex:

> The mechanism for this effect lies in a confluence of processes: (1) phase relationships between heart rate oscillations and breathing at specific frequencies, (2) phase relationships between heart rate oscillations and breathing at specific frequencies, (3) activity of the baroreflex, and (4) resonance characteristics of the cardiovascular system (Lehrer and Gevirtz, 2014, p. 1).

The resonance frequency model predicts that we can best stimulate the baroreflex and increase RSA and HRV at an individual's unique resonance frequency. In the cardiovascular system, the volume of blood in the vascular tree is responsible for the delay in the entire baroreflex loop across inhalation and exhalation (Vaschillo et al., 2011; Sakakibara et al., 2020). The *resonance frequency model* proposes that breathing, rhythmic skeletal muscle tension, and emotional stimulation (e.g., viewing positive and negative emotionally charged slides) at the resonance frequency (~ 0.1 Hz) can increase RSA and HRV (Vaschillo et al., 2006). This phenomenon resembles striking a bell that continues to resonate. At the resonance frequency in adults, when heart rate rises during inhalation blood pressure starts to fall ~ 5 s later. The strength of heart rate oscillations increases 4–10 times from resting baselines (difference between minimum and maximum heart rate) (Vaschillo et al., 2002; Lehrer et al., 2020b). External stimulation such as slow paced breathing or rhythmic skeletal muscle tension near an individual's precise resonance frequency produces the greatest RSA and HRV and increases *baroreflex gain*

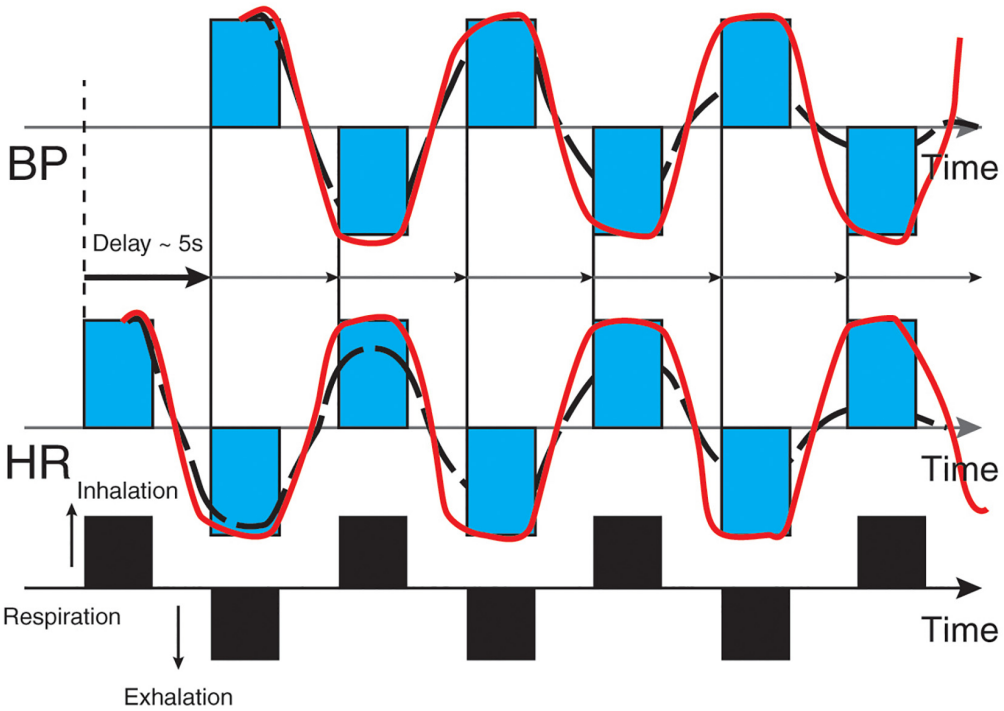



**FIGURE 2 |** Heart rate and blood pressure oscillations elicited by respiration. Credit and permissions: adapted from Evgeny Vaschillo. Original publication: Lehrer and Vaschillo (2008). The future of HRV biofeedback. *Biofeedback* 36(1), 11–14. This graphic depicts blood pressure oscillations on the top and heart rate oscillations on the bottom. Inhalation causes an immediate rise in heart rate, followed (~5 s) by increased blood pressure and baroreceptor firing. Exhalation results in an immediate decrease in heart rate followed (~5 s) by decreased blood pressure and baroreceptor firing.

(heart rate change per 1 mmHg change in blood pressure; Lehrer and Gevirtz, 2014).

## THE IMPORTANCE OF RESONANCE FREQUENCY ASSESSMENT

The purpose of assessment is to discover the baroreflex resonance frequency. While all HRV biofeedback training protocols are designed to stimulate the baroreflex, resonance frequency and 6-bpm slow paced breathing approaches may target different frequencies when adults are taller. Although several researchers train individuals to breathe at their resonance frequency (Lehrer et al., 2004; Lin et al., 2012; Steffen et al., 2017), others simply instruct them to breathe at slower-than-normal rates (Zautra et al., 2010; Cullins et al., 2013). There is compelling evidence that breathing near an individual's resonance frequency – but not at the exact rate – increases RSA amplitude and baroreflex gain compared with other frequencies (Vaschillo et al., 2002, 2004; Lehrer et al., 2020a). However, we lack conclusive evidence that resonance frequency breathing produces superior clinical outcomes in treatment of most disorders (Lehrer et al., 2020a). Researchers have started to address this question and initial findings support the importance of training at the resonance frequency. For instance, 5 weeks of resonance frequency training produced greater systolic blood pressure reductions in prehypertensive participants than slow paced breathing (Lin et al., 2012). In a second study, 15 min of slow paced breathing at the resonance frequency produced more positive mood than resonance frequency + 1 bpm or control groups and lower systolic blood pressure than the control group during the Paced Auditory Serial Addition Task (Steffen et al., 2017).

## HRV BIOFEEDBACK TRAINS CLIENTS AT INDIVIDUALIZED FREQUENCIES

Heart rate variability biofeedback stimulates the baroreflex at a given rate to produce a peak frequency in the LF range through exercises such as slow paced breathing (Gevirtz et al., 2016). Respiration rate determines the *electrocardiogram's* (ECG's) peak frequency (Shaffer and Ginsberg, 2017). For example, breathing at 6 bpm produces a peak frequency of 0.1 Hz because (6 breaths/min)/(60 s/min) = 0.1 breaths/s [0.1 Hz]. We show adult peak frequencies and their corresponding respiration rates (bpm) in **Table 1**. Depending on where an adult's resonance frequency lies between 4.5 and 6.5 bpm, slow paced breathing could produce a peak frequency between 0.075 and 0.11 Hz (Vaschillo et al., 2002, 2006). In practice, we provide auditory and visual feedback to reward increases in the amplitude of this frequency, which may differ from 0.1 Hz, to maximize RSA amplitude and baroreflex gain (Lehrer and Gevirtz, 2014).

**TABLE 1 |** Respiration rates and corresponding ECG peak frequencies.

| Respiration rate | Peak frequency (Hz) |
|---|---|
| 4.5 | 0.075 |
| 5.0 | 0.08 |
| 5.5 | 0.09 |
| 6.0 | 0.10 |
| 6.5 | 0.11 |
| 7.0 | 0.12 |
| 7.5 | 0.13 |

*Credit: Center for Applied Psychophysiology. Respiration rate, breaths per min; peak frequency, highest amplitude electrocardiogram frequency.*

# A RESONANCE FREQUENCY ASSESSMENT PROTOCOL

Resonance frequency assessment identifies the unique breathing rate that best stimulates the baroreflex and maximizes RSA amplitude before initiating HRV biofeedback. The resonance frequency ranges from 4.5 to 6.5 bpm for adults and 6.5 to 9.5 bpm for children. The difference by age group arises because children are typically smaller than adults, and therefore, have smaller vascular trees and less inertia due to blood volume (Lehrer and Gevirtz, 2014). Resonance frequency measurement (Lehrer et al., 2013) has greatly influenced HRV biofeedback practice and can be readily adapted for different age groups and morbidities. The main adjustments for children include simplified instructions, providing entertaining displays to engage them, and running their slow paced breathing trials from 9.5 to 6.5 bpm instead of the adult range from 6.5 to 4.5 bpm. No special procedural adjustments are required for healthy older adults. They tend to have lower resting RSA and smaller RSA increases following HRV biofeedback (Lehrer et al., 2020a). Adult complaints like asthma, Generalized Anxiety Disorder, and low back pain, as well as dysfunctional breathing behaviors like overbreathing may be associated with rapid breathing. Postpone resonance frequency assessment until you successfully train them to breathe effortlessly (Peper et al., 2008) between 4.5 and 6.5 bpm. Because slower breathing may be difficult for some clients, they may overbreathe and expel excessive $CO_2$. If they report that they feel faint or that their heart is beating too hard, instruct them to take shallower and smoother breaths (Lehrer et al., 2013). Resonance frequency assessment is contraindicated for clients whose sinus rhythm is driven by a pacemaker because this device externally regulates HRV. Assessment may also be contraindicated for clients whose overbreathing compensates for increased acidity in the blood due to conditions like kidney disease. Slow paced breathing would increase $CO_2$ levels in the blood and dangerously increase acidosis (Khazan, 2013).

## Sensor Channels and Parameters Monitored

In practice, the resonance frequency measurement protocol requires the ability to display instantaneous HR and respiration in real time. A clinician monitors HR using an ECG (or a *photoplethysmograph* (PPG), which optically measures the maximum value of the pulse wave to calculate instantaneous heart rate and interbeat intervals. Both ECG and PPG sensors obtain equivalent interbeat interval values under resting conditions with normal tissue perfusion (Giardino et al., 2002; Schafer and Vagedes, 2013). During slow paced breathing, PPG monitoring from the toes (2.1 beats), thumb (2.9 beats), and earlobes (3.4 beats) produces increasing phase delay with respect to the ECG. There is bilateral symmetry in phase delay between homologous recording sites (Allen, 2019). During resting conditions following 1 min of deep breathing, PPG recordings from the ear lobe achieve good agreement with ECG measurements (Weinschenk et al., 2016); however, the PPG method may measure HRV less accurately during slow paced breathing (Jan et al., 2019). In addition, ECG values are more accurate than PPG values when there is marked sympathetic activation – typically occurring in disorders such as anxiety – as peripheral vasoconstriction affects detection of the peak of the blood pressure wave from the digits but not R-spike detection from the chest, torso, or wrists (Giardino et al., 2002; Schafer and Vagedes, 2013; Shaffer and Combatalade, 2013). To determine when the ECG method is more appropriate, a clinician should evaluate the raw PPG waveform before data collection to decide whether it is flat or low amplitude (Shaffer and Combatalade, 2013).

Respiratory feedback serves several functions during resonance frequency assessment as adults breathe from 6.5 to 4.5 bpm in 0.5-bpm steps: pacing, respiration rate confirmation, and identification of dysfunctional breathing. A respiration display guides clients to breathe at prescribed rates and confirms their success. Both actions are critical because we cannot evaluate the effects of breathing at 5.5 bpm if a client actually breathed at 6 bpm. Respiratory monitoring is also essential to identify dysfunctional breathing behaviors like *apnea* (breath-holding) and *overbreathing* (excessive exhaling of $CO_2$), which can interfere with HRV biofeedback. Although HRV analysis software (e.g., Kubios) can extract respiration data *after* a slow paced breathing trial, resonance frequency assessment using slow paced breathing requires the real-time display of respiration to ensure that clients breathe at precise rates. A better solution than extracting respiration data after each slow paced breathing trial is to monitor breathing using a *respirometer* (flexible sensor band) that measures abdominal or thoracic expansion and contraction to acquire the respiratory waveform (Shaffer and Moss, 2019). A clinician should continuously monitor all raw waveforms (ECG or PPG, and respirometer) for artifact (false values) during each slow paced breathing trial so that they can immediately repeat contaminated trials.

## Terms and Definitions

Data from heart rate, respiration, and their synchrony provide detailed information for resonance frequency assessment. Clinicians who assess resonance frequency are concerned with the smoothness and regularity of heart rate signals. *Heart rate-respiration phase synchrony* is the phase angle of the peaks and troughs of the heart rate and respiration rate signals. *HRV frequency-domain metrics* calculate the spectral distribution of

signal energy. HRV biofeedback is concerned with the *very-low-frequency* (VLF; 0.0033–0.04 Hz), *low-frequency* (LF; 0.04–0.15 Hz), and *high-frequency* (HF; 0.15–0.40 Hz) bands. Although there is uncertainty regarding the sources of VLF power in short-term measurements (Kleiger et al., 2005), sympathetic activation due to effortful breathing is a possible source (Bernardi et al., 1996). There is also disagreement about the sources of LF power in short-term measurements (Akselrod et al., 1981; Goldstein et al., 2011; Reyes del Paso et al., 2013). LF power is an important indicator of HRV biofeedback training success for several reasons. First, slow paced breathing, which is one method of stimulating the baroreflex, increases LF power by increasing cardiac vagal tone (Kromenacker et al., 2018). Second, increased LF power is associated with greater RSA and HRV (Vaschillo et al., 2002). Increased RSA and HRV occur in the LF range because the baroreceptor reflex's resonance frequency resides within this range (Lehrer et al., 2020a). HF power is due to parasympathetic activity, and the natural logarithm of HF power indexes cardiac vagal tone (Task Force Report, 1996). *Absolute power* is the signal energy within a frequency band expressed in $ms^2$/Hz. *Normalized power* is the percentage of total power. For example, normalized LF power is LF/(LF + HF) or LF/(VLF + LF + HF). *Peaks* are the highest-amplitude frequencies within a band like LF. Resonance frequency assessment examines both the magnitude and number of LF peaks (Shaffer and Ginsberg, 2017). LF peak amplitude and the number of LF peaks are among six resonance frequency selection criteria (Lehrer et al., 2013). Larger peaks indicate greater resonance effects due to increased breathing and heart rate synchrony. Several LF peaks may occur when individuals breathe at a single rate outside of their resonance frequency. This can produce separate peaks at the baroreflex frequency and their actual respiratory rate. While breathing at adjacent rates may still stimulate the baroreflex, it will produce smaller resonance effects (Lehrer et al., 2020a). When clients do not precisely follow instructions, changing respiration rates can also produce multiple peaks and weaker resonance effects. Breathing in a narrow frequency range around the resonance frequency better stimulates the baroreflex and increases RSA than breathing in a wider frequency range (Vaschillo et al., 2002).

*HRV time-domain indices* quantify the amount of variability in a series of interbeat intervals. For example, *HR Max-HR Min* is the average change between the highest and lowest heart rate across all breathing cycles (Cipresso et al., 2019).

During resonance frequency assessment, clinicians can measure respiration rate, heart rate, heart rate-respiration phase synchrony, heart rate peak-trough amplitude, mean LF power, the magnitude and number of peaks within the LF band, and the smoothness of the heart rate curve envelope during each breathing trial. These data will allow clinicians to compare the differential effects of breathing rates on HRV parameters to identify each client's resonance frequency (Lehrer et al., 2013). Where clinical or peak performance interventions require more comprehensive information, clinicians can integrate a sphygmomanometer, capnometer, electrodermograph, and electromyograph (EMG) into psychophysiological assessment. A *sphygmomanometer* measures systolic and diastolic blood pressure. A *capnometer*, which monitors *end-tidal* $CO_2$ (alveolar $CO_2$ concentration at the conclusion of a breath), can detect overbreathing. This dysfunctional breathing behavior involves excessive $CO_2$ exhalation due to mouth breathing, rapid deep breathing, and sighs, and yawns (Khazan, 2013, 2019a). An *electrodermograph*, which measures eccrine sweat gland activity, can disclose increased sympathetic nervous system arousal that may accompany dysfunctional breathing. *Skin conductance level* (SCL) is a tonic measure of eccrine sweat gland activity. Furthermore, an *EMG*, which monitors skeletal muscle action potentials, can likewise indicate dysfunctional breathing if frontales or breathing accessory muscles exceed normal resting values of ≤3 microvolts (μV; Shaffer, 2020). For example, elevated frontales, scalene, or trapezius EMG activity may signal excessive breathing effort (Khazan, 2013).

The decision to add these modalities involves a cost/benefit analysis. Is the extra information worth the cost in equipment and time? Clinicians might answer this question on a case-by-case basis, guided by the client's training goals and whether the intervention is 6-bpm slow paced breathing or resonance frequency biofeedback. For example, if the training goal is to lower systolic blood pressure, a sphygmomanometer can show whether one breathing rate produces greater reductions than an adjacent rate. When treating panic disorder, clinicians can use a capnometer and electrodermograph to determine which rate produces optimal end-tidal CO2 and reductions in sympathetic activation, respectively. Finally, clinicians can monitor breathing accessory muscles (trapezius and scalene) to detect overuse, as this issue may need correction regardless of the breathing rate chosen. In all these examples, clinicians should interpret patterns of psychophysiological change using adult normative values (Khazan, 2019b). Normative values obtained during *resting conditions* – no breathing instructions, feedback, or task – enable clinicians to interpret patterns of psychophysiological change during resonance frequency assessment. Because HRV time domain and frequency domain norms are influenced by age, sex, and fitness (Koenig and Thayer, 2016; Shaffer and Ginsberg, 2017), we encourage readers to consult several studies that provide representative values (Umetani et al., 1998; Berkoff et al., 2007; Nunan et al., 2010). Blood pressure should be less than 120/80 mmHg (Fox and Rompolski, 2019). End-tidal $CO_2$ should range between 35 and 45 mmHg or torr. SCL should be ≤5 microsiemens (μS). Finally, EMG activity should be ≤3 μV with a wide bandpass (e.g., 20–1000 Hz; Khazan, 2019b).

## Orientation for Resonance Frequency Assessment

The resonance frequency assessment protocol (Lehrer et al., 2013) described in this article is simple to administer and provides intuitive directions:

> Today, I am going to introduce you to a method that will help you control your symptoms. We will be using a number of measuring devices, and wearing them may feel a little strange in the beginning. This introduction will allow you to become familiar with what it feels like to wear the sensors, and to watch the body signals they are measuring on the screen, before we start your biofeedback training. I will attach all of the sensors to your body and then you will see what they are measuring on your monitor. These sensors will simply be measuring your physiological activity

and will not cause any harm to you. I will briefly explain what each measurement is (p. 98).

Attach and test each sensor, start displaying physiological activity, and explain the meaning of the graphs and numerical values. For example:

In this top graph, the red line is your heart rate in terms of beats per minute, and the blue line shows your breathing. You'll notice that the blue line moves up as you breathe in and down as you breathe out (p. 99).

Before you start resonance frequency assessment trials, invite questions and then provide a brief overview of the assessment process. As before, we encourage you to modify Lehrer et al. (2013) explanation:

Today we are going to find out the speed of breathing that should best help you to cope with your symptoms. This breathing frequency is different for each person.

When you breathe at this rate, your breathing will produce strong effects on your nervous and cardiovascular systems that should be very good for you and should help you to control your symptoms (p. 99).

Your heart rate varies with each breath, and with various other processes in your body, including the baroreflex. This variability is good and is a sign of health. We will now find your "resonance frequency" – the speed of breathing at which your HRV is the highest. In this task, we will ask you to breathe at five rates for periods of about 2 min each. You should not find this task difficult. However, if you feel uncomfortable at any time, you can simply stop the task and tell us. When we begin, we will ask you to breathe in and out at a 10-s breathing rate. Then we will ask you to breathe at various other rates, so we can find the exact frequency at which your cardiovascular system resonates. This will be your own resonance breathing frequency. You will be able to use this breathing rate to best help your symptoms. Breathe easily and comfortably, but not too deeply. Do not try too hard. Do you have any questions? (p. 99).

## Practice Breathing Before Resonance Frequency Trials

Clinicians should provide their clients with breathing practice before conducting resonance frequency trials because the protocol requires breathing rates that are slower than normal, especially for clinical populations such as clients diagnosed with chronic pain. Although a healthy resting adult breathes from 12 to 20 bpm (Khazan, 2019a), a resonance frequency assessment protocol instructs adults to breathe at less than half that rate. Allow clients to practice relaxed breathing from 5.5 to 6 bpm before starting resonance frequency trials. When a respiration rate is difficult, instruct them to increase or decrease it by 1/2 bpm. For example, if a client typically breathes at 18 bpm, instruct them to decrease their breathing rate every few seconds from 18 bpm to 17.5 bpm to 17 bpm, and so forth. Clinicians should standardize the inhalation-to-exhalation ratio across breathing trials. Longer exhalation than inhalation is recommended in resonance frequency assessment (Lehrer et al., 2013) and may increase RSA from baseline values due to a greater increase in cardiac vagal tone (Strauss-Blasche et al., 2000; Van Diest et al., 2014). However, several studies (Zerr et al., 2015; Meehan et al., 2017) found no difference between resting HRV metrics (e.g., HR Max-HR Min, pNN50, RMSSD, SDNN, and LF power) when participants breathed at 1:1 and 1:2 inhalation-to-exhalation ratios.

## Resonance Frequency Trials

Instruct your client to breathe for 2-min intervals from 6.5 to 4.5 bpm, decreasing in 0.5 bpm-steps with 2-min rest periods. Record physiological activity during slow paced breathing as separate 2-min epochs. Create a display for each resonance frequency trial and capture 2 min of raw breathing and heart rate waveforms for each respiration rate (**Figure 3**). Record each trial's measurement parameters as shown in **Table 2** (Lehrer et al., 2013). Valid resonance frequency assessment requires careful artifact removal because one invalid interbeat interval can significantly distort metrics like HR Max-HR Min and SDNN (Berntson et al., 1997). Although automatic artifacting can identify suspect interbeat intervals, manual artifacting may produce superior results. Please review excellent discussions of interbeat interval editing for manual artifacting (Peltola, 2012; Laborde et al., 2017), as an explanation of these strategies is outside of the scope of this article.

Consider the following directions when introducing each respiration rate: "Now try breathing at this frequency (following the pacer)" (Lehrer et al., 2013 p. 101). After your client completes 2 min of paced breathing, check on their comfort and verify that they followed the pacer by confirming the average respiration rate for that trial. Repeat trials if the clients were 0.25 bpm too fast or slow. *Resonance frequency assessment without a respirometer lacks this quality control; in such cases, we cannot verify that clients have breathed at the target rates.*

Check for artifactual interbeat intervals and repeat invalid epochs after the client has rested for 2 min. Examine the segment spectral display for the location of LF peaks. When a peak occurs at 4.5 or 6.5 bpm, extend assessment with trials 0.5 bpm above and below this inflection point until LF amplitude decreases.

# RESONANCE FREQUENCY SELECTION

The goal of resonance frequency selection is to identify the frequency that best stimulates the baroreflex system and thereby increases RSA. Clinicians use six weighted criteria to evaluate adult breathing rates between 4.5 and 6.5 bpm. They prioritize these criteria by their association with resonance effects. This selection process requires careful analysis because a single breathing rate may not maximize all six criteria. When this happens, clinicians select the frequency that satisfies the majority of these criteria. The resonance frequency estimate represents the "best convergence" of the selection criteria (Lehrer et al., 2013, p. 102). Researchers have not validated these weights and they require experimental confirmation:

(1) Phase synchrony. In adults, when respiration and heart rate signals rise and fall at the same time (0°), this maximally stimulates the baroreflex and increases RSA (Vaschillo et al., 2004; Lehrer and Gevirtz, 2014; Lehrer et al., 2020a). Whether RSA optimizes pulmonary gas exchange efficiency

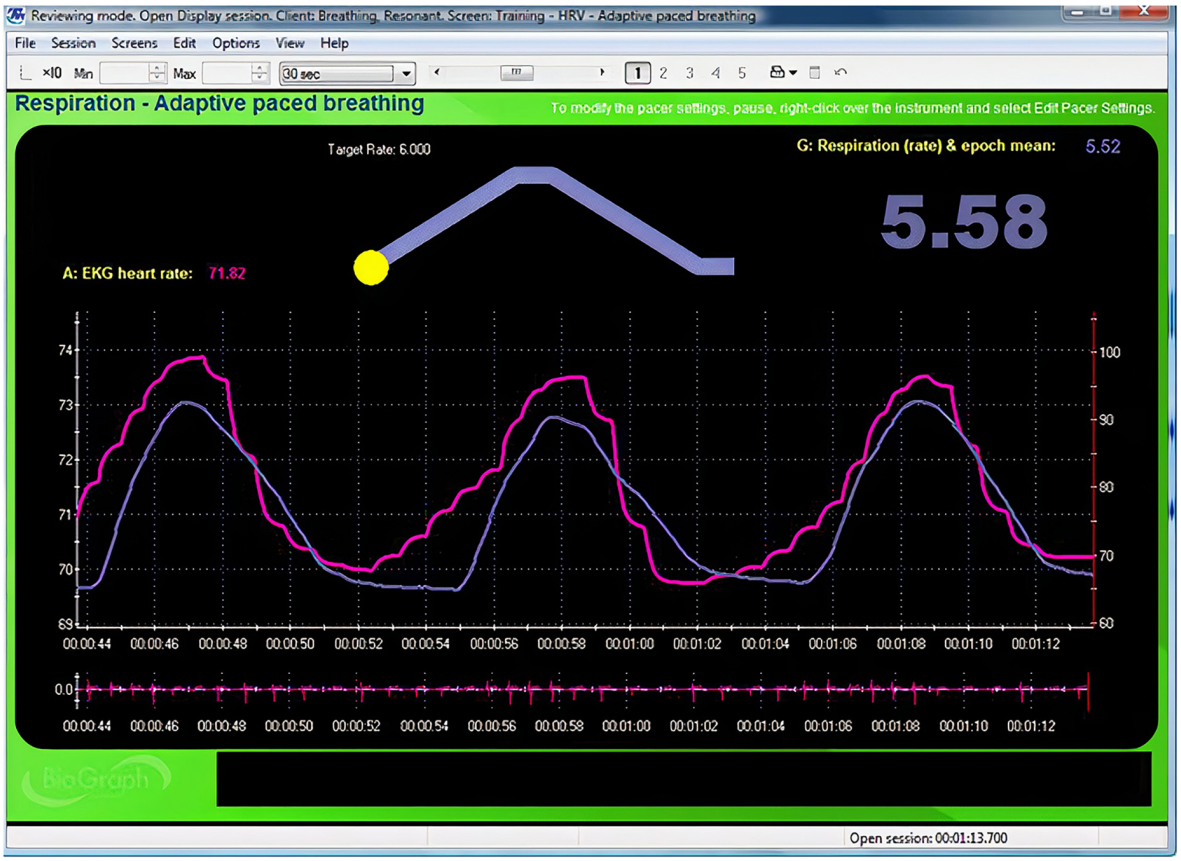



**FIGURE 3 |** Animated pacing display. Credit and permissions: Center for Applied Psychophysiology. The top display with the moving yellow ball is designed to help clients breathe at 6 bpm. The exhalation is followed by a post-expiratory pause. The current respiration rate (5.58 bpm) appears on the right. The graph immediately below shows instantaneous heart rate (pink) and respiration (purple). Note the degree to which the waveform peaks and troughs coincide since this graphically represents phase synchrony. A raw ECG waveform is displayed toward the bottom of the screen.

**TABLE 2 |** Resonance frequency assessment check-list for each trial.

| Resonance frequency trial parameters |
|---|
| pacing target bpm |
| actual bpm |
| respiration-HR phase |
| HR Max-HR Min |
| absolute LF power |
| normalized LF power |
| highest amplitude LF peak |
| number of distinct LF peaks |
| sinusoidal waveform |
| client difficulty |

*Credit: Center for Applied Psychophysiology. bpm, breaths per min; LF, low frequency; peak, highest amplitude frequency.*

is currently unclear (Buchheit, 2010). Software measures the phase synchrony between the respirometer and heart rate waveforms: 0° means that heart rate begins to rise at the start of an inhalation; 90° means that heart rate begins to increase during the middle of an inhalation and to decrease during the middle of an exhalation; 180° means that heart rate decreases during inhalation and increases during exhalation. Phase synchrony (∼0°) carries the greatest weight because it enables clients to achieve the greatest resonance effects. Strong resonance effects, in turn, increase RSA and many HRV metrics, and allow HRV biofeedback training to more effectively stimulate and strengthen the baroreflex (Lehrer et al., 2003, 2013).

(2) Peak-trough amplitude. Higher heart rate peak-trough amplitudes are better because greater RSA can increase baroreflex sensitivity over weeks of HRV resonance frequency training (Lehrer et al., 2003; Lehrer and Gevirtz, 2014). *HR Max – HR Min* is one method of quantifying peak-trough amplitude (Cipresso et al., 2019). Clinicians measure HR Max – HR Min using a respirometer to determine when each breathing cycle starts and ends. Peak-trough amplitude is second because larger peak-trough differences signal greater resonance effects and contribute to more effective baroreflex activation (Vaschillo et al., 2002).

(3) LF power. The baroreflex system exhibits resonance because it is a feedback system with a constant delay (Lehrer, 2013). Its resonance frequency lies within the

LF range. Higher absolute and percent total LF power are desirable because they increase as the respiration rate approaches the resonance frequency and more effectively stimulates the baroreflex (Vaschillo et al., 2002). Further, cardiac vagal activity increases when individuals engage in slow paced breathing within the LF range (Kromenacker et al., 2018). Clinicians measure absolute LF power of the 0.04–0.15 Hz range in $ms^2$/Hz. They calculate percent total LF power as LF/(LF + HF) or LF/(VLF + LF + HF; Lehrer et al., 2013). LF power is third because it confirms that clients are breathing at rates between 4.5 and 6.5 bpm, which are necessary to produce the greatest resonance effects and possibly RSA as well.

(4) Maximum LF amplitude peak. Larger LF peaks reflect stronger resonance effects due to greater synchrony between breathing and heart rate. Clinicians use spectral analysis to identify the LF peak with the largest absolute power ($ms^2$/Hz). Maximum LF amplitude peak is fourth because the LF spectral peak is higher at the resonance frequency than at any other respiratory frequency (Lehrer et al., 2013). When clients breathe at a consistent rate within the LF range, this increases resonance effects and RSA.

(5) Smoothness of the heart rate curve envelope. Smooth heart rate waveforms are best because they permit closer phase synchrony with respiration waveforms and therefore allows clients to achieve the greatest resonance effects and RSA (Lehrer and Gevirtz, 2014). Clinicians visually inspect heart rate curve envelope for their smoothness. Signals that resemble sine waves are smooth, whereas jagged waveforms are irregular (Lehrer et al., 2013). Smoothness of the heart rate curve envelope is fifth because it reflects the breathing mechanics required to achieve the greatest resonance effects and RSA.

(6) Fewest LF peaks. Fewer peaks are better than more peaks because they are generated by breathing within a narrower frequency band about the resonance frequency within the LF range. Clients can generate multiple peaks when they breathe slightly faster or slower than their resonance frequency. This can result in a peak at the respiratory rate and another at the baroreflex frequency. In contrast to breathing at various frequencies in the LF range, breathing at a single frequency better enables phase synchrony between breathing and heart rate, stimulates the baroreflex, and increases RSA (Vaschillo et al., 2002). Clinicians can count the number of LF peaks by visually inspecting a spectral display of the LF range. The fewest LF peaks is sixth because this demonstrates that the client is *consistently* breathing within a narrow band within the LF range, which increases resonance effects and RSA.

Although it would be ideal if one respiration rate produced the greatest increases in phase synchrony, peak-trough amplitude, LF power, maximum LF amplitude peak, and heart rate curve smoothness, and the fewest LF peaks, it is unlikely. Adjacent respiration rates may optimize different selection criteria. The six criteria provide a strategy for identifying potential resonance frequencies. There were two candidate resonance frequencies in

**Table 3**: 5.0 bpm for 53-bpm HR Max-HR Min and 5.5 bpm for 7°phase synchrony (Shaffer, 2020).

Cardiac vagal tone should be one of the resonance frequency assessment criteria since increasing this parameter is one of the goals of HRV biofeedback training (Vaschillo et al., 2006). There is evidence that both LF power and RMSSD index cardiac vagal tone when breathing at slow rates. Slow paced breathing increases LF power by increasing cardiac vagal tone (Kromenacker et al., 2018) and RMSSD reflects cardiac vagal firing with minimal confounding by respiration rate (Penttilä et al., 2001).

## How to Break Ties and Confirm the Resonance Frequency

Consider your clients' perspective when breaking ties between nearby breathing rates and then reconfirm that rate during the first training session. Which rate feels most comfortable? If your clients struggle with breathing at 4.5 bpm, this pace may result in overbreathing and *vagal withdrawal*, in which increased sympathetic firing inhibits parasympathetic regulation (Porges, 1995; Thayer et al., 2012). Which rate brings your clients closest to their training goal? If your clients entered training to lower blood pressure, consider the rate that produces the greatest decreases. In response to the previous example, breathing at 5.0 bpm reduced blood pressure by 9/12 mmHg compared with 5.5 bpm (Shaffer, 2020). When you collaborate with your clients to break ties, you can strengthen your relationship and increase the likelihood that they will practice resonance frequency breathing outside of the clinic. After preliminary resonance frequency measurement, clinicians should monitor 3–5 min of breathing at the resonance frequency while watching for signs of overbreathing like faintness (Lehrer et al., 2013). If such symptoms are present, clinicians should encourage shallower breathing to reduce the $CO_2$ loss that is responsible for them. Next, they should ask clients to breathe at rates that are 1/2-bpm faster and slower for 3–5 min each. This step allows clients to compare their subjective comfort one more time during each breathing rate. Finally, after artifacting, clinicians should evaluate the three trials – resonance frequency, resonance frequency + 1/2-bpm, and resonance frequency − 1/2-bpm – using the previous resonance frequency criteria.

# UNANSWERED QUESTIONS

Four major questions regarding resonance frequency assessment require further research. These questions include whether resonance frequency training is more effective than 6-bpm slow paced breathing, the minimum epoch required for valid resonance frequency measurements, the Lehrer protocol's test-retest reliability, and whether rhythmic skeletal muscle tension can replace slow paced breathing in resonance frequency assessment.

*Does resonance frequency training produce superior outcomes in adults compared with 6-bpm slow paced breathing?* This question is an "elephant in the room" that researchers need to more completely address. While initial studies (Lin et al., 2012; Steffen et al., 2017) found evidence that resonance frequency

**TABLE 3 |** Resonance frequency assessment of a healthy undergraduate.

| Respiration rate | Phase synchrony (°) | HR Max-HR Min (bpm) | Normalized LF power (%) | Number of LF peaks | SCL (μS) | Systolic blood pressure | Diastolic blood pressure |
|---|---|---|---|---|---|---|---|
| 7.5 | 25 | 40 | 83 | + | 14 | 103 | 61 |
| 7.0 | 22 | 38 | 94 | – | 14 | 118 | 65 |
| 6.5 | 27 | 43 | 93 | – | 15 | 133 | 56 |
| 6.0 | 13 | 46 | 95 | + | 15 | 106 | 73 |
| 5.5 | 7 | 49 | 90 | + | 16 | 116 | 70 |
| 5.0 | −30 | 53 | 94 | – | 19 | 107 | 58 |
| 4.5 | −32 | 51 | 94 | – | 20 | 101 | 72 |

*Credit: Center for Applied Psychophysiology. Respiration rate, breaths per min; phase synchrony, phase relationship between the peaks and troughs of heart rate and respirometer signals; HR Max-HR Min, the mean difference between the fastest and slowest heart rates across each respiratory cycle; normalized LF power, division of LF power by the sum of LF and HF power; the number of LF peaks, the number of highest amplitude frequencies within the LF range where + means fewest peaks; SCL, skin conductance level; systolic blood pressure, maximum arterial pressure during left ventricle contraction; diastolic blood pressure, minimum arterial pressure during ventricular relaxation.*

training produces greater systolic blood pressure reductions and positive mood, a recent meta-analysis (Lehrer et al., 2020a) found non-significant effects on diastolic or systolic blood pressure.

*What minimum epoch is required to obtain valid resonance frequency measurements?* Although clinicians may assume that 2-min recordings achieve acceptable concurrent validity with respect to 5-min recordings, no peer-reviewed study has demonstrated this result for the most important resonance frequency criteria: heart rate-respiration phase synchrony and HR Max-HR Min. Different epoch lengths may be required for acceptable concurrent validity of related HRV metrics like LF and normalized LF power. Shaffer et al. (2019) evaluated the concurrent validity of these indices in 38 healthy undergraduates. Their concurrent validity criteria included a Pearson's correlation value $\geq$0.90 and a Bland–Altman limits of agreement allowable difference of $\pm$ 5% of the 5-min value range. Whereas 90-s epochs were sufficient to measure LF power, 180-s records were needed to estimate 5-min normalized LF power. Researchers should investigate the concurrent validity of all four measures with a larger, more representative sample.

*How reliable is resonance frequency assessment?* Evidence of resonance frequency test-retest reliability is severely limited but encouraging. Fuller et al. (2011) reported that the resonance frequency was stable in 21 undergraduates. The authors demonstrated acceptable test-retest reliability ($r$ = 0.73. $d$ = 2.14) for participants assessed 2 weeks apart. The question of test-retest reliability is pivotal for resonance frequency assessment. *Why invest an entire session to measure the resonance frequency if it significantly changes across training sessions?* Researchers should replicate this finding with a larger and more representative sample. Resonance frequency assessment may achieve greater test-retest reliability in taller, rather than shorter individuals. Taller adults tend to have lower resonance frequencies due to the greater time required for blood pressure adjustment following baroreflex-mediated heart rate changes (Vaschillo et al., 2006).

*Could rhythmic skeletal muscle tension replace slow paced breathing in resonance frequency assessment?* Rhythmic skeletal muscle tension can stimulate the baroreflex like resonance frequency breathing and increase LF HRV power (Vaschillo et al., 2011). In this study, participants placed in a semi-recumbent position rhythmically contracted their hands and feet 3, 6, and 12 times per min. The rhythmic skeletal muscle tension only produced high-amplitude oscillations in blood pressure, heart rate, and vascular tone at 6 contractions per min (cpm) – which is a frequency of 0.1 Hz. These findings raise the possibility that clinicians could use rhythmic skeletal muscle tension in place of slow paced breathing to measure resonance frequency and deliver HRV biofeedback training. The rhythmic skeletal muscle tension protocol would avoid the challenging requirement that individuals breathe at unusually slow rates (e.g., 4.5–6.5 bpm). Before this protocol can be adopted, research will have to prove that it achieves acceptable criterion validity – confirmation that test scores accurately estimate scores of validated measures (Gulliksen, 1987) – with respect to slow paced breathing and test-retest validity.

## CONCLUSION

Variability in the timing of interbeat intervals may promote adaptive capacity. Cardiovascular health and optimal affective, cognitive, and social functioning depend on complex non-linear oscillations produced by complex neurocardiac interactions and non-linear ANS processes (Segerstrom and Ness, 2007). ANS and cardiorespiratory system plasticity make HRV biofeedback possible. The premises of the resonance frequency model of HRV biofeedback are that younger adults have a unique resonance frequency determined by the volume of blood in the vascular tree (and its inertia), and heart rate and blood pressure are 180° out of phase in younger adults at that frequency, which lies between 4.5 and 6.5 bpm or cpm. Stimulation of the baroreflex by breathing and rhythmic skeletal muscle tension near the resonance frequency can produce immediate large-scale increases in RSA compared with that at resting baselines. Weeks of HRV biofeedback training at the resonance frequency can increase baroreflex gain and cardiac vagal tone to treat clinical disorders and promote optimal performance (Lehrer et al., 2020a).

Determination of the resonance frequency is a prerequisite for HRV biofeedback resonance frequency training because adult peak frequencies range between 0.075 and 0.11 Hz. Because there is evidence that 6-bpm slow paced breathing maximizes

RSA and baroreflex sensitivity (Russo et al., 2017; Zaccaro et al., 2018), is individualized training near the resonance frequency worth the expense of resonance frequency assessment and psychophysiological monitoring equipment? To date, there is preliminary evidence (Lin et al., 2012; Steffen et al., 2017) that HRV biofeedback resonance frequency training produces greater systolic blood pressure reductions than 6-bpm slow paced breathing, resonance frequency + 1 HRV biofeedback, or control conditions. Further research is needed to demonstrate the value of resonance frequency assessment and training.

The resonance frequency protocol described in this article can be readily adapted for different ages and morbidities. Resonance frequency assessment is contraindicated in medical conditions that produce acidosis and where a pacemaker externally controls the sinus rhythm. This protocol requires monitoring of the heart rate and respirometer waveforms to measure heart rate-respiration phase synchrony and HR Max-HR Min, which are its most important criteria. Although both ECG and PPG methods produce comparable results when clients breathe at normal rates with healthy tissue perfusion, the ECG is more accurate during slow paced breathing and when sympathetic activation results in vasoconstriction and smaller pulse wave peaks. The resonance frequency should meet most of Lehrer et al. (2013) weighted criteria after data have been carefully artifacted. Clinicians should incorporate both LF power and RMSSD as selection criteria since they index cardiac vagal tone. Because several breathing rates may maximize different resonance frequency criteria, clinicians may break ties by considering client comfort, preference, and training goals.

We have raised several important questions. Does resonance frequency training produce superior outcomes in adults compared with 6-bpm slow paced breathing? Are 2 min sufficient to measure the HRV indices used to determine the resonance frequency? What is the 2-week test-retest reliability for the resonance frequency? Can rhythmic skeletal muscle tension replace paced breathing in resonance frequency assessment? Answers to these questions could refine Lehrer and colleagues' assessment protocol and increase confidence in its results.

## ETHICS STATEMENT

Written informed consent was obtained from the individual(s), and/or minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

FS reviewed the literature, wrote the initial manuscript, and made subsequent revisions following feedback from ZM. ZM reviewed the literature, created and maintained a UST publication database, and made editorial suggestions for all drafts. FS and ZM discussed the resonance frequency assessment literature and developed the central themes of this review manuscript. Both authors contributed to the article and approved the submitted version.

## FUNDING

This work was funded by the Shawn and Jacqui Bergman Psychology Research Fund.

## ACKNOWLEDGMENTS

The authors want to express their profound thanks to Richard Gevirtz, Paul Lehrer, Evgeny and Bronya Vaschillo, and Christopher Zerr for their generous contributions to this article.

## REFERENCES

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C., and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: aquantitative probe of beat-to-beat cardiovascular control. *Science* 213, 220–222. doi: 10.1126/science.6166045

Allen, J. (2019). Quantifying the delays between multi-site photoplethysmography pulse and electrocardiogram R-R interval changes under slow-paced breathing. *Front. Physiol.* 10:1190. doi: 10.3389/fphys.2019.01190

Başar, E. (1998). "Resonance phenomena in the brain, physical systems, and nature," in *Brain Functions and Oscillations*, ed. E. Başar (Berlin: Springer Verlag). doi: 10.1007/978-3-642-72192-2

Beckers, F., Verheyden, B., and Aubert, A. E. (2006). Aging and nonlinear heart rate control in a healthy population. *Am. J. Physiol. Heart Circ. Physiol.* 290, H2560–H2570. doi: 10.1152/ajpheart.00903.2005

Berkoff, D. J., Cairns, C. B., Sanchez, L. D., and Moorman, C. T. (2007). Heart rate variability in elite American track-and-field athletes. *J. Strength Cond. Res.* 21, 227–231. doi: 10.1519/00124278-200702000-00041

Bernardi, L., Valle, F., Coco, M., Calciati, A., and Sleight, P. (1996). Physical activity influences heart rate variability and very-low-frequency components in Holter electrocardiograms. *Cardiovasc. Res.* 32, 234–237. doi: 10.1016/0008-6363(96)00081-8

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Buchheit, M. (2010). Respiratory sinus arrhythmia and pulmonary gas exchange efficiency: time for a reappraisal. *Exp. Physiol.* 95:767. doi: 10.1113/expphysiol.2010.053470

Byrd, D. L., Reuther, E. T., McNamara, J. P. H., DeLucca, T. L., and Berg, W. K. (2015). Age differences in high frequency phasic heart rate variability and performance response to increased executive function load in three executive function tasks. *Front. Psychol.* 5:1470. doi: 10.3389/fpsyg.2014.01470

Cipresso, P., Colombo, D., and Riva, G. (2019). Computational psychometrics using psychophysiological measures for the assessment of acute mental stress. *Sensors* 19:781. doi: 10.3390/s19040781

Cooke, W. H., Cox, J. F., Diedrich, A. M., Taylor, J. A., Beightol, L. A., Ames, J. E., et al. (1998). Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am. J. Physiol.* 274, H709–H718. doi: 10.1152/ajpheart.1998.274.2.H709

Cullins, S. W., Gevirtz, R. N., Poeltler, D. M., Cousins, L. M., Harpin, R., and Muench, F. (2013). An exploratory analysis of the utility of adding cardiorespiratory biofeedback in the standard care of pregnancy-induced hypertension. *Appl. Psychophysiol. Biofeedback* 38, 161–170. doi: 10.1007/s10484-013-9219-4

Cutsforth-Gregory, J. K., and Benarroch, E. E. (2017). Nucleus of the solitary tract, medullary reflexes, and clinical implications. *Neurology* 88, 1187–1196. doi: 10.1212/WNL.0000000000003751

Eckberg, D. L. (1983). Human sinus arrhythmia as an index of vagal cardiac outflow. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 54, 961–966. doi: 10.1152/jappl.1983.54.4.961

Eckberg, D. L. (2003). The human respiratory gate. *J. Physiol.* 548, 339–352. doi: 10.1113/jphysiol.2003.037192

Eckberg, D. L., and Eckberg, M. J. (1982). Human sinus node responses to repetitive, ramped carotid baroreceptor stimuli. *Am. J. Physiol.* 242, H638–H644. doi: 10.1152/ajpheart.1982.242.4.H638

Fox, S. I., and Rompolski, K. (2019). *Human Physiology*. New York, NY: McGraw-Hill Education.

Fuller, J., Wally, C., Westermann-Long, A., Korenfeld, D., and Carrell, D. (2011). Resonance frequency measurements are reliable. *Appl. Psychophysiol. Biofeedback* 36:219.

Gevirtz, R. (2013). The promise of heart rate variability biofeedback: evidence-based applications. *Biofeedback* 41, 110–120. doi: 10.5298/1081-5937-41.3.01

Gevirtz, R. N., Lehrer, P. M., and Schwartz, M. S. (2016). "Cardiorespiratory biofeedback," in *Biofeedback: A Practitioner's Guide*, eds M. S. Schwartz and F. Andrasik (New York, NY: The Guilford Press), 196–213.

Giardino, N. D., Lehrer, P. M., and Edelberg, R. (2002). Comparison of finger plethysmograph to ECG in the measurement of heart rate variability. *Psychophysiology* 39, 246–253. doi: 10.1111/1469-8986.3920246

Goldberger, A. L. (1991). Is the normal heartbeat chaotic or homeostatic? *News Physiol. Sci.* 6, 87–91. doi: 10.1152/physiologyonline.1991.6.2.87

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). Low frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Grossman, P., and Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsy

Gulliksen, H. (1987). *Theory of Mental Tests*. Hillsdale, NJ: Erlbaum.

Jan, H. Y., Chen, M. F., Fu, T. C., Lin, W. C., Tsai, C. L., and Lin, K. P. (2019). Evaluation of coherence between ECG and PPG derived parameters on heart rate variability and respiration in healthy volunteers with/without controlled breathing. *J. Med. Biol. Eng.* 39, 783–795. doi: 10.1007/s40846-019-00468-9

Karemaker, J. M. (2009). Counterpoint: respiratory sinus arrhythmia is due to the baroreflex mechanism. *J. Appl. Psychol.* 106, 1742–1743. doi: 10.1152/japplphysiol.91107.2008a

Khazan, I. (2019a). *Biofeedback and Mindfulness in Everyday Life: Practical Solutions for Improving Your Health and Performance*. New York, NY: W. W. Norton & Company.

Khazan, I. (2019b). "A guide to normal values in biofeedback," in *Physiological Recording Technology and Applications in Biofeedback and Neurofeedback*, eds D. Moss and F. Shaffer (Oakbrook Terrace, IL: Association for Applied Psychophysiology and Biofeedback), 2–6.

Khazan, I. Z. (2013). *The Clinical Handbook of Biofeedback: A Step-By-Step Guide for Training and Practice with Mindfulness*. Malden, MA: Wiley-Blackwell. doi: 10.1002/9781118485309

Kleiger, R. E., Stein, P. K., and Bigger, J. T. Jr. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474X.2005.10101.x

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Koh, J., Brown, T. E., Beightol, L. A., and Eckberg, D. L. (1998). Contributions of tidal lung inflation to human R-R interval and arterial pressure fluctuations. *J. Auton. Nerv. Syst.* 68, 89–95. doi: 10.1016/s0165-1838(97)00114-8

Kollai, M., and Mizsei, G. (1990). Respiratory sinus arrhythmia is a limited measure of cardiac parasympathetic control in man. *J. Physiol.* 434, 329–342. doi: 10.1113/jphysiol.1990.sp018070

Kougias, P., Weakley, S. M., Yao, Q., Lin, P. H., and Chen, C. (2010). Arterial baroreceptors in the management of systemic hypertension. *Med. Sci. Monit.* 16, RA1–RA8.

Kromenacker, B. W., Sanova, A. A., Marcus, F. I., Allen, J. J. B., and Lane, R. D. (2018). Vagal mediation of low-frequency heart rate variability during slow yogic breathing. *Psychosom. Med.* 80, 581–587. doi: 10.1097/psy.0000000000000603

Laborde, S., Mosley, E., and Mertgen, A. (2018). Vagal tank theory: the three Rs of cardiac vagal control functioning – resting, reactivity, and recovery. *Front. Neursci.* 12:458. doi: 10.3389/fnins.2018.00458

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lehrer, P. (2013). How does heart rate variability biofeedback work? resonance, the baroreflex, and other mechanisms. *Biofeedback* 41, 26–31. doi: 10.5298/1081-5937-41.1.02

Lehrer, P., and Eddie, D. (2013). Dynamic processes in regulation and some implications for biofeedback and biobehavioral interventions. *Appl. Psychophysiol. Biofeedback* 38, 143–155. doi: 10.1007/s10484-013-9217-6

Lehrer, P., Kaur, K., Sharma, A., Shah, K., Huseby, R., Bhavsar, J., et al. (2020a). Heart rate variability biofeedback improves emotional and physical health and performance: a systematic review and meta-analysis. *Appl. Psychophysiol. Biofeedback* 45, 109–129. doi: 10.1007/s10484-020-09466-z

Lehrer, P., Vaschillo, B., Zucker, T., Graves, J., Katsamanis, M., Aviles, M., et al. (2013). Protocol for heart rate variability biofeedback training. *Biofeedback* 41, 98–109. doi: 10.5298/1081-5937-41.3.08

Lehrer, P. M., and Gevirtz, R. (2014). Heart rate variability: How and why does it work? *Front. Psychol.* 5:756. doi: 10.3389/fpsyg.2014.00756

Lehrer, P. M., and Vaschillo, E. (2008). The future of heart rate variability biofeedback. *Biofeedback* 36, 11–14.

Lehrer, P. M., Vaschillo, E., Vaschillo, B., Lu, S.-E., Eckberg, D. L., Edelberg, R., et al. (2003). Heart rate variability biofeedback increases baroreflex gain and peak expiratory flow. *Psychosom. Med.* 65, 796–805. doi: 10.1097/01.PSY.0000089200.81962.19

Lehrer, P. M., Vaschillo, E., Vaschillo, B., Lu, S.-E., Scardella, A., Siddique, M., et al. (2004). Biofeedback treatment for asthma. *Chest* 126, 352–361. doi: 10.1378/chest.126.2.352

Lehrer, P. M., Vaschillo, E., and Vidali, V. (2020b). Heart rate and breathing are not always in phase during resonance frequency breathing. *Appl. Psychophysiol. Biofeedback* 45, 145–152. doi: 10.1007/s10484-020-09459-y

Lin, G., Xiang, Q., Fu, X., Wang, S., Wang, S., Chen, S., et al. (2012). Heart rate variability biofeedback decreases blood pressure in prehypertensive subjects by improving autonomic function and baroreflex. *J. Altern. Complement. Med.* 18, 143–152. doi: 10.1089/acm.2010.0607

Mather, M., and Thayer, J. (2018). How heart rate variability affects emotion regulation brain networks. *Curr. Opin. Behav. Sci.* 19, 98–104. doi: 10.1016/j.cobeha.2017.12.017

McCraty, R., and Shaffer, F. (2015). Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Glob. Adv. Health Med.* 4, 46–61. doi: 10.7453/gahmj.2014.073

Meehan, Z., Muesenfechter, N., Gravett, N., Watson, T., Smith, A., Shearman, S., et al. (2017). A 1:2 inhalation-to-exhalation ratio does not increase heart rate variability during 6-bpm breathing [Abstract]. *Appl. Psychophysiol. Biofeedback* 45, 110–111. doi: 10.1007/s10484-018-9390-8

Noble, D. J., and Hochman, S. (2019). Hypothesis: pulmonary afferent activity patterns during slow, deep breathing contribute to the neural induction of physiological relaxation. *Front. Physiol.* 10:1176. doi: 10.3389/fphys.2019.01176

Nunan, D., Sandercock, G. R. H., and Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing Clin. Electrophysiol.* 33, 1407–1417. doi: 10.1111/j.1540-8159.2010.02841.x

Peltola, M. A. (2012). Role of editing of R–R intervals in the analysis of heart rate variability. *Front. Physiol.* 3:148. doi: 10.3389/fphys.2012.00148

Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol.* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Peper, E., Gibney, K. H., Tylova, H., Harvey, R., and Combatalade, D. (2008). *Biofeedback Mastery: An Experiential Teaching and Self-Training Manual*. Wheat Ridge, CO: AAPB.

Pinsky, M. R. (2018). Cardiopulmonary interactions: physiological basis and clinical applications. *Ann. Am. Thorac. Soc.* 15, S45–S48. doi: 10.1513/AnnalsATS.201704-339FR

Porges, S. W. (1995). Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A polyvagal theory. *Psychophysiology* 32, 301–318. doi: 10.1111/j.1469-8986.1995.tb01213.x

Reyes del Paso, G. A., Langewitz, W., Mulder, L. J. M., Van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Russo, M. A., Santarelli, D. M., and O'Rourke, D. (2017). The physiological effects of slow breathing in the healthy human. *Breathe* 13, 298–309. doi: 10.1183/20734735.009817

Sakakibara, M., Kaneda, M., and Oikawa, L. O. (2020). Efficacy of paced breathing at the low-frequency peak on heart rate variability and baroreflex sensitivity. *Appl. Psychophysiol. Biofeedback* 45, 31–37. doi: 10.1007/s10484-019-09453-z

Schafer, A., and Vagedes, J. (2013). How accurate is pulse rate variability as an estimate of heart rate variability? a review on studies comparing photoplethysmographic technology with an electrocardiogram. *Int. J. Cardiol.* 166, 15–29. doi: 10.1016/j.ijcard.2012.03.119

Segerstrom, S. C., and Ness, L. S. (2007). Heart rate variability reflects self-regulatory strength, effort, and fatigue. *Psychol. Sci.* 18, 275–281. doi: 10.1111/j.1467-9280.2007.01888.x

Shaffer, F. (2020). Resonance frequency assessment: the challenge of standardizing heart rate variability biofeedback research. *Biofeedback* 48, 7–15. doi: 10.5298/1081-5937-48.01.06

Shaffer, F., and Combatalade, D. (2013). Don't add or miss a beat: a guide to cleaner heart rate variability recordings. *Biofeedback* 41, 121–130. doi: 10.5298/1081-5937-41.3.04

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability (HRV) metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shaffer, F., McCraty, R., and Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front. Psychol.* 5:1040. doi: 10.3389/fpsyg.2014.01040

Shaffer, F., and Moss, D. (2019). "Biofeedback," in *Brain and Heart Dynamics*, eds D. Bugada, V. Bellini, E. G. Bignami, and L. F. Lorini (Cham: Springer), 1–15.

Shaffer, F., Shearman, S., Meehan, Z., Gravett, N., and Urban, H. (2019). "The promise of ultra-short-term (UST) heart rate variability measurements: a comparison of Pearson product-moment correlation coefficient and limits of agreement (LoA) concurrent validity criteria," in *Physiological Recording Technology and Applications in Biofeedback and Neurofeedback*, eds D. Moss and F. Shaffer (Oakbrook Terrace, IL: Association for Applied Psychophysiology and Biofeedback), 214–220.

Steffen, P. R., Austin, T., DeBarros, A., and Brown, T. (2017). The impact of resonance frequency breathing on measures of heart rate variability, blood pressure, and mood. *Front. Public Health* 5:222. doi: 10.3389/fpubh.2017.00222

Stein, P. K., Domitrovich, P. P., Hui, N., Rautaharju, P., and Gottfdiener, J. (2005). Sometimes higher heart rate variability is not better heart rate variability: results of graphical and nonlinear analyses. *J. Cardiovasc. Electrophysiol.* 16, 954–959. doi: 10.1111/j.1540-8167.2005.40788.x

Strauss-Blasche, G., Moser, M., Voica, M., McLeod, D., Klammer, N., and Marktl, W. (2000). Relative timing of inspiration and expiration affects respiratory sinus arrhythmia. *Clin. Exp. Pharmacol. Physiol.* 27, 601–606. doi: 10.1046/j.1440-1681.2000.03306.x

Swenne, C. A. (2013). Baroreflex sensitivity: mechanisms and measurement. *Neth. Heart J.* 21, 58–60. doi: 10.1007/s12471-012-0346-y

Taha, B. H., Simon, P. M., Dempsey, J. A., Skatrud, J. B., and Iber, C. (1995). Respiratory sinus arrhythmia in humans: an obligatory role for vagal feedback from the lungs. *J. Appl. Physiol.* 78, 638–645. doi: 10.1152/jappl.1995.78.2.638

Tan, G., Shaffer, F., Lyle, R., and Teo, I. (eds) (2016). *Evidence-Based Practice in Biofeedback and Neurofeedback*, 3rd Edn. Wheat Ridge, CO: Association for Applied Psychophysiology.

Task Force Report. (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnson, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Umetani, K., Singer, D. H., McCraty, R., and Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. Coll. Cardiol.* 31, 593–601. doi: 10.1016/S0735-1097(97)00554-8

Vaillancourt, D. E., and Newell, K. M. (2002). Changing complexity in human behavior and physiology through aging and disease. *Neurobiol. Aging* 23, 1–11. doi: 10.1016/S0197-4580(01)00247-0

Van Diest, I., Verstappen, K., Aubert, A. E., Widjaja, D., Vansteenwegen, D., and Vlemincx, E. (2014). Inhalation/exhalation ratio modulates the effect of slow breathing on heart rate variability and relaxation. *Appl. Psychophysiol. Biofeedback* 39, 171–180. doi: 10.1007/s10484-014-9253-x

Vaschillo, E., Lehrer, P., Rishe, N., and Konstantinov, M. (2002). Heart rate variability biofeedback as a method for assessing baroreflex function: a preliminary study of resonance in the cardiovascular system. *Appl. Psychophysiol. Biofeedback* 27, 1–27. doi: 10.1023/A:1014587304314

Vaschillo, E. G., Vaschillo, B., and Lehrer, P. M. (2004). Heartbeat synchronizes with respiratory rhythm only under specific circumstances. *Chest* 126, 1385–1386. doi: 10.1016/S0012-3692(15)31329-5

Vaschillo, E. G., Vaschillo, B., and Lehrer, P. M. (2006). Characteristics of resonance in heart rate variability stimulated by biofeedback. *Appl. Psychophysiol. Biofeedback* 31, 129–142. doi: 10.1007/s10484-006-9009-3

Vaschillo, E. G., Vaschillo, B., Pandina, R. J., and Bates, M. E. (2011). Resonances in the cardiovascular system caused by rhythmical muscle tension. *Psychophysiology* 48, 927–936. doi: 10.1111/j.1469-8986.2010.01156.x

Weinschenk, S. W., Beise, R. D., and Lorenz, J. (2016). Heart rate variability (HRV) in deep breathing tests and 5-min short-term recordings: agreement of ear photoplethysmography with ECG measurements, in 343 subjects. *Eur. J. Appl. Physiol.* 116, 1527–1535. doi: 10.1007/s00421-016-3401-3

Yasuma, F., and Hayano, J. (2004). Respiratory sinus arrhythmia: Why does the heartbeat synchronize with respiratory rhythm? *Chest* 125, 638–690. doi: 10.1378/chest.125.2.683

Zaccaro, A., Piarulli, A., Laurino, M., Garbella, E., Menicucci, D., Neri, B., et al. (2018). How breath-control can change your life: a systematic review on psycho-physiological correlates of slow breathing. *Front. Hum. Neurosci.* 12:353. doi: 10.3389/fnhum.2018.00353

Zautra, A. J., Fasman, R., Davis, M. C., and Craig, A. D. B. (2010). The effects of slow breathing on affective responses to pain stimuli: an experimental study. *Pain* 149, 12–18. doi: 10.1016/j.pain.2009.10.001

Zerr, C., Kane, A., Vodopest, T., Allen, J., Hannan, J., Fabbri, M., et al. (2015). Does inhalation-to-exhalation ratio matter in heart rate variability biofeedback? [Abstract]. *Appl. Psychophysiol. Biofeedback* 40:135.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

CORRECTION
published: 01 December 2020
doi: 10.3389/fnins.2020.627512

# Corrigendum: A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback

*Fred Shaffer*[1]* *and Zachary M. Meehan*[2]

[1] Center for Applied Psychophysiology, Truman State University, Kirksville, MO, United States, [2] Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, United States

**Keywords: biofeedback, complexity, emotional self-regulation, heart rate variability, neurocardiology, resonance, performance**

**A Corrigendum on**

**A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback**
*by Shaffer, F., and Meehan, Z. M. (2020). Front. Neurosci. 14:570400. doi: 10.3389/fnins.2020.570400*

In the original article, there was an error. The high-frequency band's upper limit was reversed.

A correction has been made to "A Resonance Frequency Assessment Protocol section," "Terms and Definitions subsection."

Data from heart rate, respiration, and their synchrony provide detailed information for resonance frequency assessment. Clinicians who assess resonance frequency are concerned with the smoothness and regularity of heart rate signals. Heart rate-respiration phase synchrony is the phase angle of the peaks and troughs of the heart rate and respiration rate signals. HRV frequency-domain metrics calculate the spectral distribution of signal energy. HRV biofeedback is concerned with the very-low-frequency (VLF; 0.0033–0.04 Hz), low-frequency (LF; 0.04–0.15 Hz), and high-frequency (HF; 0.15–0.40 Hz) bands. Although there is uncertainty regarding the sources of VLF power in short-term measurements (Kleiger et al., 2005), sympathetic activation due to effortful breathing is a possible source (Bernardi et al., 1996). There is also disagreement about the sources of LF power in short-term measurements (Akselrod et al., 1981; Goldstein et al., 2011; Reyes del Paso et al., 2013). LF power is an important indicator of HRV biofeedback training success for several reasons. First, slow paced breathing, which is one method of stimulating the baroreflex, increases LF power by increasing cardiac vagal tone (Kromenacker et al., 2018). Second, increased LF power is associated with greater RSA and HRV (Vaschillo et al., 2002). Increased RSA and HRV occur in the LF range because the baroreceptor reflex's resonance frequency resides within this range (Lehrer et al., 2020). HF power is due to parasympathetic activity, and the natural logarithm of HF power indexes cardiac vagal tone (Task Force Report, 1996). Absolute power is the signal energy within a frequency band expressed in $ms^2$/Hz. Normalized power is the percentage of total power. For example, normalized LF power is LF/(LF + HF) or LF/(VLF + LF + HF). Peaks are the highest-amplitude frequencies within a band like LF. Resonance frequency assessment examines both the magnitude and number of LF peaks (Shaffer and Ginsberg, 2017). LF peak amplitude and the number of LF peaks are among six resonance frequency selection criteria (Lehrer et al., 2013). Larger peaks indicate greater resonance effects due to increased breathing and heart rate synchrony. Several LF peaks may occur when individuals breathe at a single rate outside of their resonance frequency. This can produce separate peaks at the baroreflex frequency and their actual respiratory rate. While breathing at adjacent rates may still stimulate the baroreflex, it will produce smaller resonance effects (Lehrer et al., 2020). When clients do not precisely follow instructions, changing respiration rates can also produce multiple peaks and weaker resonance effects. Breathing

OPEN ACCESS

***Approved by:***
*Frontiers Editorial Office, Frontiers Media SA, Switzerland*

***Correspondence:**
*Fred Shaffer*
*fredricshaffer@gmail.com*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *09 November 2020*
***Accepted:*** *13 November 2020*
***Published:*** *01 December 2020*

***Citation:***
*Shaffer F and Meehan ZM (2020) Corrigendum: A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback. Front. Neurosci. 14:627512. doi: 10.3389/fnins.2020.627512*

in a narrow frequency range around the resonance frequency better stimulates the baroreflex and increases RSA than breathing in a wider frequency range (Vaschillo et al., 2002).

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

## REFERENCES


Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C., and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: aquantitative probe of beat-to-beat cardiovascular control. *Science* 213, 220–222. doi: 10.1126/science.6166045

Bernardi, L., Valle, F., Coco, M., Calciati, A., and Sleight, P. (1996). Physical activity influences heart rate variability and very-low-frequency components in Holter electrocardiograms. *Cardiovasc. Res.* 32, 234–237. doi: 10.1016/0008-6363(96)00081-8

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). Low frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Kleiger, R. E., Stein, P. K., and Bigger, J. T. Jr. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474X.2005.10101.x

Kromenacker, B. W., Sanova, A. A., Marcus, F. I., Allen, J. J. B., and Lane, R. D. (2018). Vagal mediation of low-frequency heart rate variability during slow yogic breathing. *Psychosom. Med.* 80, 581–587. doi: 10.1097/psy.0000000000000603

Lehrer, P., Vaschillo, B., Zucker, T., Graves, J., Katsamanis, M., Aviles, M., et al. (2013). Protocol for heart rate variability biofeedback training. *Biofeedback* 41, 98–109. doi: 10.5298/1081-5937-41.3.08

Lehrer, P. M., Vaschillo, E., and Vidali, V. (2020). Heart rate and breathing are not always in phase during resonance frequency breathing. *Appl. Psychophysiol. Biofeedback* 45, 145–152. doi: 10.1007/s10484-020-09459-y

Reyes del Paso, G. A., Langewitz, W., Mulder, L. J. M., Van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability (HRV) metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Task Force Report (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Vaschillo, E., Lehrer, P., Rishe, N., and Konstantinov, M. (2002). Heart rate variability biofeedback as a method for assessing baroreflex function: a preliminary study of resonance in the cardiovascular system. *Appl. Psychophysiol. Biofeedback* 27, 1–27. doi: 10.1023/A:1014587304314

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 22 October 2020
doi: 10.3389/fnins.2020.564123

# Assessing New Methods to Optimally Detect Episodes of Non-metabolic Heart Rate Variability Reduction as an Indicator of Psychological Stress in Everyday Life: A Thorough Evaluation of Six Methods

*Stephen B. R. E. Brown[1,2,3*†], Jos F. Brosschot[1,4†], Anke Versluis[1,5†], Julian F. Thayer[6†] and Bart Verkuil[2,4†]*

[1] Department of Health, Medical, and Neuropsychology, Leiden University, Leiden, Netherlands, [2] Leiden Institute for Brain and Cognition, Leiden, Netherlands, [3] Humanities and Social Sciences, Department of Psychology, Red Deer College, Red Deer, AB, Canada, [4] Department of Clinical Psychology, Leiden University, Leiden, Netherlands, [5] Department of Public Health and Primary Care, Leiden University Medical Centre, Leiden, Netherlands, [6] Department of Psychological Science, University of California, Irvine, Irvine, CA, United States

OPEN ACCESS

**Edited by:**
*Vitor Engracia Valenti, São Paulo State University, Brazil*

**Reviewed by:**
*Andreas Richard Schwerdtfeger, University of Graz, Austria*
*Phyllis Kravet Stein, Washington University in St. Louis, United States*

***Correspondence:**
*Stephen B. R. E. Brown*
*stephen.brown@rdc.ab.ca*

*† These authors have contributed equally to this work*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

**Received:** *20 May 2020*
**Accepted:** *30 September 2020*
**Published:** *22 October 2020*

**Citation:**
*Brown SBRE, Brosschot JF, Versluis A, Thayer JF and Verkuil B (2020) Assessing New Methods to Optimally Detect Episodes of Non-metabolic Heart Rate Variability Reduction as an Indicator of Psychological Stress in Everyday Life: A Thorough Evaluation of Six Methods. Front. Neurosci. 14:564123. doi: 10.3389/fnins.2020.564123*

Frequent or chronic reduction in heart rate variability (HRV) is a powerful predictor of cardiovascular disease, and psychological stress has been suggested to be a co-determinant of this reduction. Recently, we evaluated various methods to measure additional HRV reduction in everyday life and to relate these reductions to psychological stress. In the current paper, we thoroughly evaluate these methods and add two new methods in both newly acquired and reanalyzed datasets. All of these methods use a subset of 24 h worth of HRV and movement data to do so: either the first 10 min of every hour, the full 24 h, a combination of 10 min from three consecutive hours, a classification of level of movement, the data from day *n* to detect episodes in day *n* + 1, or a range of activities during lab calibration. The method that used the full 24 h worth of data detected the largest percentage of episodes of reduced additional HRV that matched with self-reported stress levels, making this method the most promising, while using the first 10 min from three consecutive hours was a good runner-up.

**Keywords: additional HRV, psychological stress, worry, cardiovascular disease, heart rate variability**

## INTRODUCTION

With cardiovascular disease being the dominant cause of death in the world (Alwan, 2011), studying predictors of this serious ailment is imperative. The development of cardiovascular disease can be powerfully predicted by frequent or chronic reductions in the variation of time between successive heart beats (i.e., by reductions in so-called heart rate variability or HRV; Bosma et al., 1998; Orth-Gomér et al., 2000; Matthews and Gump, 2002; Rosengren et al., 2004; Kivimäki et al., 2006) as well

as to precede the development of several risk factors, like hypertension, high cholesterol, diabetes, and immunological markers of pathogenic states (Thayer and Lane, 2007; Thayer et al., 2010; Jarczok et al., 2019). The risk of negative cardiovascular events may be increased by as much as 32–45% by low HRV (Hillebrand et al., 2013) and a potential cause of such HRV reductions may be prolonged exposure to psychological stress (McEwen, 2001). Stress can be considered to be a complicated, multidimensional phenomenon that may be strongly related to the consistency of a person's emotional response to life events (e.g., with happiness, anger, etc., Lazarus, 1993).

The relationship between stress and changes in physiological parameters that are not due to changes in physical activity was demonstrated first by Blix et al. (1974) in helicopter pilots during take-off. Our lab has recently developed a technique to detect episodes of reduced HRV in ambulatory participants (Verkuil et al., 2016), and to relate these physiological episodes to the participants' self-reported episodes of psychological stress and worrying (Verkuil et al., 2016; Brown et al., 2018). We had participants wear an ECG sensor for 24 h, as they went about their daily doings. Each experimental session started with a short calibration period in the lab, during which participants engaged in four classes of energy-expending physical activity that might also be performed during a regular day: standing, cycling, climbing stairs, and lying down. The participants' HRV was measured during each of these classes of activity. We used these data to compute an HRV baseline during various levels of physical activity[1]. Some studies have excluded all periods of high physical activity (Brosschot et al., 2007; Pieper et al., 2007, 2010), and/or have identified epochs of non-movement by using accelerometer readings (Sowder et al., 2010), but the interrelatedness of activity levels and HRV (Rennie et al., 2003) makes it imperative to take levels of physical activity into account when studying HRV. Research has consistently shown that during episodes of physical activity, heart rate increases are associated with HRV decreases (for an extensive review, see Michael et al., 2017). A number of other factors influence HRV (reviewed in Fattison et al., 2016), and we therefore only include participants who neither smoked, nor were on antihypertensive or cardiological medication like beta-blockers (also see the Discussion). Our approach enabled us to estimate the amount of HRV that is not due purely to physical activity (i.e., *additional physiology*, in the current study, HRV: the term *additional* was coined by Blix et al., 1974) and this, in turn, allowed us to examine the relationship between psychological factors such as stress and emotions and physiological activity.

In the Verkuil et al. (2016) study, the HRV and physical activity data that were collected during a calibration period in the lab, were used to compute personalized algorithms for each participant, which could then be utilized to detect episodes associated with reductions in additional HRV. Given that these episodes of reduced additional HRV were not associated with concurrent levels of movement, they can therefore considered to be related to psychological stress (Myrtek et al., 2005). Furthermore, participants were prompted hourly to fill out some questionnaires on mobile phones while wearing their ECG sensors. The questionnaires assessed whether participants had experienced stress or worry during the previous hour, enabling us to associate physiological stress markers and psychological stress markers.

Techniques to estimate additional physiology are involved methods that have only occasionally been used in ambulatory emotion-related studies; furthermore, heart rate was typically studied instead of HRV and, crucially, no *individualized algorithms* were utilized (Myrtek and Brügner, 1996; Myrtek, 2004; Myrtek et al., 2005; Ebner-Priemer et al., 2007; Prill and Fahrenberg, 2007). Verkuil et al. (2016) developed a two-step process to detect episodes of additional HRV reductions: first, the relationship between HRV and movement (i.e., walking, cycling, etc., all expressed as acceleration) was formalized by fitting an inverse regression model to the data that were acquired during the calibration period. This was done separately for each participant. The obtained model parameters were then utilized to detect episodes of reduced additional HRV (see below, as well as Verkuil et al., 2016, for more detail). In a follow-up study, we then related these episodes of reduced additional HRV to episodes of worry or stress that were self-reported by participants (Brown et al., 2018). In the same study, we explored alternative methods to that used by Verkuil et al. (2016). All of these methods, which are detailed in the "Materials and Methods" section below, are based on a similar principle: the construction of an inverse regression model that quantifies the relationship between HRV and movement. However, these alternative methods use different subsets of the data to compute those inverse regression models. For example, instead of using data obtained during a calibration phase, as Verkuil et al. (2016) did, in one of our alternative methods, we used the first 10 min worth of data from every available hour to create our inverse regression model. One alternative method seemed particularly promising: simply using all available data (in the Verkuil et al., 2016, study, that yielded 24 h worth of data) for a given participant led to a considerably better match between physiological episodes of reduced additional HRV and self-reported psychological episodes of worry and stress than using the data from the lab calibration.

We have evaluated these alternative methods by reanalyzing data and found promising results, so further tests on a dataset that was acquired for that specific purpose was in order. We have therefore acquired data for six participants, who were subjected to three 24 h test sessions. Not only did this allow us to test our methods on a dedicated dataset, but it also allowed us to evaluate two more methods to further explore the optimal method to estimate reductions in additional HRV. First of all, we have added a class of activity to Verkuil et al. (2016) calibration phase in the lab: participants were required to read a complicated text out loud and they had to clench their fists and tense their shoulders for 3 min. We expected that reading out loud while the experimenter listened would evoke feelings of stress in the participants, while clenching their fists and tensing their shoulders might reduce their HRV, which is common for such isometric activity (i.e., clenching muscles without actually moving; Stewart et al., 2007).

[1]By physical activity, we mean bodily movement of various intensities (e.g., casual walking vs. climbing stairs).

Incorporating this "stress-induction" class of activity into the lab calibration phase may therefore improve the sensitivity to detect episodes of reduced additional HRV in the method that used lab calibration data. Having access to 72 h worth of data for every participant allowed us to introduce and explore one further method: it might be possible to use the data of day $n$ to detect episodes of reduced additional HRV in day $n + 1$. This method has the advantage of avoiding "double dipping" into the data by using an inverse regression model that was established on one dataset (i.e., one 24 h period) to detect episodes in another dataset (i.e., another 24 h period) of the same participant.

Given the modest sample size of the current study—although the results were clear and in line with earlier work—we have also reanalyzed a dataset from our lab, which also included 72 h worth of data for participants (Versluis et al., 2018). However, we were unable to test all of our hypotheses in this dataset alone, as the participants in this dataset were not subjected to the laboratory calibration phase that was used by Verkuil et al. (2016).

The goal of the current paper is to further explore the optimal way to study and quantify the relationship between additional HRV reductions and movement, to learn more about this powerful and important predictor of cardiovascular disease. We will compare the performance of the various methods in a number of ways and, crucially, we will determine which of the methods is best able to detect episodes of reduced additional HRV, as demonstrated by a high correspondence between such method-identified physiological episodes and participant-reported episodes of psychological stress and worrying. Given our previous findings, we expect the method that used all available data for a given session to perform best. The method that used the first 10 min of three consecutive hours may also perform well, based on previous findings. Our two new methods (extended lab calibration that incorporated stress induction and using the data of day $n$ to detect episodes of reduced additional HRV in day $n + 1$) may, in turn, outperform these two methods.

# MATERIALS AND METHODS

## Study Design

We reanalyzed one dataset (Versluis et al., 2018, institutional review board approval number 4689348773) and we acquired data from six new participants to evaluate a number of methods to optimally detect episodes of reductions in additional HRV.

## Setting

Participants for the new dataset were tested at Leiden University in 2015 and 2016. Participants were recruited via posters in the building and through Leiden University's digital participant recruitment system. For the Versluis et al. (2018) dataset, please see the section "Materials and Methods" section in the relevant paper.

The six new participants we tested for this study were invited into the lab, were fitted with an ECG sensor, mounted on a chest strap, and were provided with an Android-based Motorola Razr cell phone which prompted them once an hour, at random moments within that hour, to fill out a set of psychological questionnaires about whether they had experienced stress and worry during the past hour, and how long these episodes had lasted (for more information, see Verkuil et al., 2016, whose procedure was followed). Participants then undertook the following types of (physical) activity, without breaks in between: (1) sitting down for 3 min while watching a relaxing video; (2) standing up for 3 min while counting in steps of two (to keep participants' minds off of ruminating); (3) lying down for 3 min while counting in steps of two; (4) cycling on a stationary bike for 3 min; (5) sitting down, clenching one's fists and tensing the shoulders for 3 min; (6) reading a text on the history of Leiden University out loud for 3 min, while the experimenter listened; (7) climbing four flights of stairs (63 steps). Two types of activity were added to the Verkuil et al. (2016) lab calibration: clenching one's fists and tensing the shoulders, which was expected to reduce HRV, and reading a complicated text out loud, which was expected to evoke feelings of stress. After engaging in all of these types of activity, participants were sent home, with instructions to wear the ECG monitor for 24 h and fill in the hourly questionnaires on the smartphones.

## Participants

We tested six participants (four females, mean age 26.5 years), who were each subjected to three 24-h test sessions. Unfortunately, one 24-h dataset was not recorded by the ECG sensor due to technical reasons; this left us with five complete 72-h datasets and one dataset of 48 h. We have included the participant for whom we had 48 h worth of data in our analyses and considered one 24 h dataset to be statistically "missing data" that were not replaced. We have also reanalyzed data from Versluis et al. (2018). Due to technical reasons which have resulted in noisy data, we were only able to analyze 5 participants from the control condition of the study from Versluis et al. (2018) (all females, mean age 26 years), which obviously limits the power of the concomitant analyses; we consider this to be a set of exploratory analyses. All participants signed informed consent before being included and they were financially compensated according to Leiden University's policy for the remuneration of participants. For both studies, participants who smoked, or who were on antihypertensive or cardiological medication (like beta-blockers) were not allowed to participate in the studies.

## Variables

In the current study, we have evaluated six alternative methods to the additional HRV detection method that was described by Verkuil et al. (2016). All of these methods used a similar approach: an inverse regression model was fitted for every individual to quantify the relationship between HRV, expressed as the root mean squares of successive differences, RMSSD[2],

[2]There are multiple methods to estimate HRV either in the time or frequency domains. We selected this specific method because it is commonly used (Task Force of The European Society of Cardiology and The North American Society of Pacing, and Electrophysiology, 1996; Berntsen et al., 2017, p. 196) and recently, single RMSSD values were shown to be powerful predictors of health risk factors in a very large sample (Jarczok et al., 2019).

and movement, which was expressed as acceleration in $g$ (the averaged acceleration in three axes), according to Eq. 1.

$$Expected\ RMSSD_{i,j} = B0_i + \frac{B1_i}{acceleration_{i,j}} \quad (1)$$

In this inverse regression model, an expected RMSSD value for participant $i$ at 30-s sampling interval $j$ was computed as the sum of the value of RMSSD while no acceleration was present (i.e., the intercept, $B0_i$) and the change in RMSSD that was due to acceleration (i.e., the slope, $B1_i$). The standard error of the mean of RMSSD was also computed, to be used in later computations (see section "Data Sources/Measurement" below).

RMSSD was computed for 30-s intervals throughout the entire 24-h test session and was then averaged over samples that spanned different amounts of time, depending on the particular method used (see below). $g$, as used in this study, has been demonstrated to be a valid method to measure movement, especially walking, jogging, sitting, and lying down (Lugade et al., 2014). We then fitted the inverse regression model to a subset of all the data points that were available for a given participant; for example, Verkuil et al. (2016) fitted such a model to the HRV and movement data that were acquired during a laboratory calibration period while participants performed various activities. The parameters from these regression models were then utilized, for each individual participant, to predict HRV levels as a function of movement levels. Whenever actual HRV levels fell two standard errors below predicted HRV levels, and such a difference lasted at least 7.5 consecutive minutes[3], we considered this to represent an episode of decreased additional HRV (formulae are presented in Verkuil et al., 2016). In line with Verkuil et al. (2016), if multiple such episodes were identified within a given hour, we only used the first episode that was detected within that hour in further analyses.

## Data Sources/Measurement

All 24-h ECG data and movement data were collected with an ecgMove sensor (ECGMove 3, Movisens, GmbH, Karlsruhe, Germany). Data were processed offline in Movisens DataAnalyzer version 1.12 (preprocessing and artifact rejection were described in Verkuil et al., 2016); the Data-Analyzer software uses automated algorithms to detect and remove artifacts in the data. Data were then analyzed in MATLAB™ (MathWorks, Natick, Massachusetts). Analysis scripts are available from the corresponding author. The methods that were used to detect episodes of reduced additional HRV are described next.

### Method 1: First 10 Min of Every Hour

This method, described previously in Brown et al. (2018), computed inverse regression models based on the first 10 min of every hour for which data were collected. For example, this yielded 24 separate inverse regression models if 24 h worth of data were available. Episodes of reduced additional HRV (see above) were detected for every hour, using the model parameters for that specific hour. An advantage of this method is that episodes of reduced additional HRV within a given hour were detected with a model that was based on data from that specific hour. This "double dipping" is also this method's disadvantage: (part of) the data from hour $n$ were used to detect additional HRV episodes in hour $n$. Such episodes were also looked for in the first 10 min of data, which was the time period used to construct the inverse regression model that was utilized for this detection process in the first place. Nevertheless, this appears to be a fairly minor concern: should an episode of reduced additional HRV be identified in the first 10 min of a given hour, then there seems to be no empirical reason to question whether a person actually experienced worry or stress during those 10 min (also see section "Discussion").

### Method 2: Full Dataset

This method used the entire period for which data were collected during a given test session to compute an inverse regression model. For example, if 24 h worth of data were available, a single inverse regression model was computed, based on that entire 24-h period. The parameters from this model were then used to identify episodes of reduced additional HRV separately for every hour in that dataset. The inverse regression models in this method are based on a large number of data points, rendering these models more robust than models based on just 10 min worth of data, which is an advantage this method offers. This advantage is slightly offset by the levels of movement and HRV being averaged over the total available time period and them not being modeled separately for each hour. This method has been described previously in Brown et al. (2018).

### Method 3: First 10 Min of Three Consecutive Hours

As discussed previously in Brown et al. (2018), this method computed an inverse regression model based on the first 10 min worth of data for three consecutive hours, which allowed us to compensate for fluctuations over time in movement or HRV. In this method, every regression model was therefore based on 30 min worth of data. The inverse regression models for the first and last hours of a dataset were based on the first 10 min of the first and second hours of the dataset and on the first 10 min of the penultimate and final hours, respectively. So, if 24 h worth of data were available, we computed 24 separate inverse regression models. An advantage of this method is that changes in HRV or movement levels over three consecutive hours were taken into account. Furthermore, the inverse regression models were based on an average of the first 10 min of three consecutive hours, which leads to more reliable model parameter estimations than 10 using only 10 min worth of data would (cf. Method 1, above). However, using HRV and movement data from three consecutive hours is also a disadvantage: more data may be used to compute inverse regression models, but movement and HRV levels are autocorrelated over time, which would reduce variation in these levels over three consecutive hours.

[3]The Verkuil et al. method was developed in a first attempt to identify episodes of reduced additional HRV in an ambulatory setting, and it has guided the current work. Requiring actual HRV to be two standard errors below the predicted HRV for 7.5 min is a carefully considered but potentially seemingly arbitrary decision. Verkuil et al. reasoned that two standard errors would provide a pronounced enough difference between the two variables, and that 7.5 min would be enough time to qualify a period as representing worrying or stress. For a discussion of these decisions, see Brown et al. (2018).

### Method 4: Movement Level Bins

This method, discussed previously in Brown et al. (2018), used the natural variation in a participant's movement levels throughout the day. We therefore binned movement data based on quartiles that were defined per participant, thus creating four bins that classified levels of movement that ranged from relatively very low to relatively very high. Each bin contained 5 consecutive minutes' worth of data: a participant's level of movement within these 5 min had to be in between two quartiles to be assigned to a specific bin. Given our desire to identify all four bins for as large a number of participants as possible, we chose to base bins on periods of five consecutive minutes; making bins wider (i.e., encompassing more time), would attenuate the number of participants for whom all four bins could be identified (see below). Our analysis was restricted to the 5 min bin of every movement class that occurred first. For example, if four clusters of quartile-1 movement were identified for a given participant, only the first cluster was used in subsequent analyses, so that each bin contained the same amount of data for each participant (e.g., not 5 min for one participant and 80 min for another participant). Inverse regression models were based on all available quartile data, so a maximum of 20 min worth of data. By representing four levels of movement in the inverse regression models, this method takes variations in an individual's movement into account, which is an advantage that is not offered by methods that only use the data from the first 10 min of every hour, which are, of course, unlikely to contain each possible level of movement by mere chance. Another advantage of this method is that it only requires the computation of a single inverse regression model, as opposed to methods that require models for every hour of data. Unfortunately, not all four bins could be identified in one of the six newly tested participants. Of course, this participant did have movement data that fell between two quartiles, but s/he simply did not have 5 consecutive minutes' worth of such data. However, the other three bins could be identified in this participant, yielding 15 min worth of data that were usable to compute an inverse regression model. The same applied to the five reanalyzed participants from the Versluis et al. dataset. In a way, this activity bin method is comparable to the laboratory-based method of Verkuil et al. (2016), which was based on four predetermined physical activity categories: lying down, standing, cycling and climbing stairs. However, the activity bin method it is data-driven and does not depend on a laboratory-based calibration data, which might not be available to interested researchers, and this renders the activity bin method more versatile.

### Method 5: Extended Calibration

This method is highly comparable to the original method by Verkuil et al. (2016): we computed an inverse regression model on the data acquired during the lab calibration, but we added two types of activity: reading a complicated text out loud and clenching one's fists while tensing one's shoulders. We expected these two activities to be stressful and to lower HRV, respectively. Therefore, adding these two types of activity may lead to the formulation of a model that has a better fit than Verkuil et al. (2016) original models, as (simulated) stress and an HRV-lowering procedure are now also included in the calibration phase. A disadvantage of this method is that it is difficult to find a text that is difficult to read for all participants; although most of our participants stumbled over such words as "string galvanometer" and the Latin names of ancient professors (e.g., Jacobus Arminius, Daniel Heinsius, etc.), other participants read through these texts with few issues.

### Method 6: Next-Day Prediction

An important question following the work that was reported by Brown et al. (2018) was whether it might be possible to use the inverse regression model of day $n$ to identify episodes of reduced additional HRV in day $n + 1$. This method was performed on a subset of all available data: we used all the data for a participant's first testing day to detect episodes of reduced additional HRV in his or her second day, and all the data from his or her second day to detect episodes in the third day. The advantage of this method is that there is no double dipping into the data, as the model used to detect episodes of reduced additional HRV in a given day is based on data from a completely different day. The obvious disadvantage was that no episodes of reduced additional HRV could be detected in the first test session ($n$), because that would require an inverse regression model to be computed for day $n - 1$; theoretically, one could use the data from, for example, the last testing day for that purpose. Given our specific interest in the efficacy of "next-day" predictions, we have not explored that option further (cf. section "Discussion").

### Episodes Detected During Sleep

Occasionally, our methods identified episodes of decreased additional HRV during a participant's sleep. If actual measured HRV is well below expected HRV levels, this can happen, but clearly, such a phenomenon cannot be due to *conscious* psychological stress during a participant's sleep. Having said that, for methods in which the inverse regression model was calculated based on data acquired during waking periods, these episodes of decreased additional HRV may have meaning, given that an association between low sleeping HRV and preceding stress has been reported (e.g., Hall et al., 2004; Brosschot et al., 2007). This relationship has been suggested to reflect unconscious stress-related cognition (Brosschot, 2010; Brosschot et al., 2010). Given that nocturnal HRV was not the primary focus of this paper, we have chosen not to include such episodes in our analyses.

## Bias

Given that there was no experimental manipulation in this study, experimenter bias toward participants was minimized. Given that all analyses were automatized through scripts, and the outcome variables were the number of episodes of reduced additional HRV that were detected by those scripts, experimenter bias and subjectivity were minimized.

## Study Size

Following the work reported by Brown et al. (2018), we continue to explore and refine our methods to detect episodes of reduced additional HRV. Our choice of sample size reflects this exploratory nature (also see the Discussion).

## Quantitative Variables

The handling of quantitative variables was described in detail under Data sources/management.

## Statistical Methods

The current paper's objective was to evaluate the efficacy of the six methods described above in detecting episodes of reduced additional HRV. To this end, we have compared these methods in three ways, following the strategy introduced in Brown et al. (2018). Two of these comparisons served to demonstrate the variation in number of episodes of reduced additional HRV that was identified by each method. Firstly, to explore the variation in numbers of identified episodes of reduced additional HRV, we compared the number of episodes each method detected by using repeated-measures analyses of variance (ANOVAs) with test day and the different additional HRV estimation method (e.g., first 10 min of every hour, etc.) as within-subjects factors. We then computed Pearson correlations between the numbers of episodes that were detected in three 24 h periods, to test the temporal reliability of these methods. These two analyses have been performed for both datasets analyzed here.

In our newly acquired data, participants reported once an hour whether they had been stressed or worried during the past hour. We then performed a vital analysis, in which the onsets of participants' self-reported episodes of psychological stress and worry were compared to the onsets of the episodes of reduced additional HRV that were identified by each of the methods. The first two analyses charted the distribution of the number of physiological episodes of reduced additional HRV that the different methods identified, as well as the temporal reliability of each method, but this key comparison revealed true episodes of reduced additional HRV by demonstrating to what extent these *physiological* events matched up with *psychological* events of stress and worry. We have therefore computed, separately for every method and for every available hour worth of data, the percentage of participants that had matches between episodes of reduced additional HRV and self-reported stress and worry episodes. We then calculated the average percentage of such matches within a given method. These three comparisons are expected to assess the quality of each of the methods discussed here and to ascertain which of these methods seems to be the best alternative to the laboratory calibration method presented by Verkuil et al. (2016).

# RESULTS

## Reanalysis of Versluis and Colleagues Data

As there was no calibration phase in the study by Versluis et al. (2018), we utilized four methods to identify episodes of additional HRV: we used the first 10 min of every hour, the full dataset of 24 h, the method that used a combination of the first 10 min of three consecutive hours, and the method that used activity type bins. Some methods identified more reduced additional HRV-episodes than others, as presented in **Table 1**.

**TABLE 1 |** Mean number of additional HRV episodes (*SD*) identified in three test days of 24 h each.

| | First 10 min of every hour | Full 24 h of first test day | First 10 min of 3 h | Activity type bins |
|---|---|---|---|---|
| T1 | 9.6 (2.6) | 16.4 (2.1) | 12.4 (1.1) | 5.2 (3.3) |
| T2 | 7.8 (3.7) | 14.6 (3.1) | 12.8 (2.9) | 11.0 (6.1) |
| T3 | 6.8 (1.9) | 13.6 (2.0) | 10.4 (1.1) | 14.0 (2.5) |
| $\bar{X}$ | 8.1 | 14.9 | 11.9 | 10.1 |

*Calibration time period was also included in detection periods. Every method detected additional HRV episodes in all five analyzed participants.*

A repeated-measures ANOVA with test day and estimation method as within-subjects factors revealed a significant difference between methods, $F(3, 12) = 25.5$, $p < 0.0005$, $\eta_p^2 = 0.72$. This effect suggested that the method that used the full dataset identified the largest mean number of episodes of reduced additional HRV (14.9), while the methods that used the first 10 min of every hour identified the lowest number of episodes (8.1). There was no reliable effect of test day, $F(2, 8) = 0.14$, $p = 0.87$, $\eta_p^2 = 0.03$, but test day and method interacted, $F(6, 24) = 10.0$, $p < 0.0005$, $\eta_p^2 = 0.72$. Three of the four methods investigated here seem to identify relatively robust numbers of additional HRV episodes over time, but this interaction seems to be driven by the outlying observation in the first test day for the method that used activity class to detect reduced additional HRV episodes (5.2 episodes in the first session vs. 11.0 and 14.0 episodes in the second and third sessions, respectively). This may be an artifact of the low power of the analyzed sample, as there is no theoretical reason to assume this method would identify a lower number of additional HRV episodes in one of the three 24 h periods that were analyzed. Furthermore, pairwise comparisons suggested that the only significant differences in identified additional HRV episodes were those between the first and second test day for the activity class method, $t_4 = 3.7$, $p = 0.02$ and between the first and third times series for the activity class method, $t_4 = 4.5$, $p = 0.01$. All other differences were not significant (all $p$s $> 0.06$).

To further explore the reliability of the methods over time, we computed Pearson correlations between the average number of episodes detected over time, separately for every method. The largest correlation observed was the one between the number of episodes detected in the second and third test day for the method that used a combination of 10 min from three consecutive hours, $r = -0.97$, $p = 0.008$, $CI_{95} = [-0.99, -0.61]$. The correlation between the first and second test day for the method that used activity type was also large, $r = 0.88$, $p = 0.051$, $CI_{95} = [-0.01, 0.99]$. The lack of statistically robust correlations is likely due to the low power of the analyzed sample: we therefore urge the reader to interpret these correlations as effect sizes without reliance on the associated $p$-values; excepting the activity class method, all methods seem to identify numerically relatively similar numbers of additional HRV episodes over time. Note that the method that used the full dataset to identify episodes of reduced HRV, which was associated with a good match between episodes of self-reported stress and worry and method-identified episodes of additional HRV in other work (Brown et al., 2018), also

appears to provide a stable estimate of additional HRV reduction episodes over time (all *r*s between 0.70 and 0.80). Unfortunately, the crucial test, in which self-reported psychological stress and worry episodes are related to method-identified physiological episodes of reduced additional HRV, could not be performed for the Versluis et al. (2018) dataset, as there was no hourly self-reported stress or worry data available for these participants. This was a powerful motivation to test additional participants.

### New Data to Test All Methods

We tested six new participants to further evaluate all six methods and to corroborate findings reported elsewhere (Brown et al., 2018). We first compared the numbers of episodes of reduced additional HRV that were identified by every method. Given that we could only analyze two of the three days' worth of data for the method that used the data of day $n$ to detect episodes of reduced additional HRV in day $n + 1$ (because there is no data before day 1, only days 2 and 3 could be analyzed with this method), we have evaluated that method separately. We therefore first performed a repeated-measures ANOVA with test day and estimation methods as within-subjects factors, which suggested that the various methods identified different numbers of episodes of reduced additional HRV, $F(5, 20) = 6.6$, $p = 0.001$, $\eta_p^2 = 0.62$.

As can be seen in **Table 2**, Verkuil et al. (2016) lab calibration method detected the lowest number of episodes (3.6), while the method that used the full dataset identified the largest number of episodes (11.4). Interestingly, pairwise comparisons revealed no significant differences whatsoever, suggesting that the different methods each identified reliable numbers of episodes of reduced additional HRV over time. Furthermore, it is interesting to note that, once again, the method that used the full dataset identified the largest number of episodes overall. There was no reliable effect of test day on number of episodes detected, nor did method and test day reliably interact (*p*s of 0.29 and 0.49, respectively).

We then computed Pearson correlations between numbers of episodes detected over time, again, separately for every method: we have, once more, treated these correlations as effect sizes. The largest correlation was for the numbers of episodes detected in test days 1 (6.1 episodes) and 3 (6.8 episodes) for the method that used bins of activity type, $r = 0.95$, $p = 0.01$, $CI_{95} = [0.61, 0.99]$. The majority of correlations (11 out of 15) exceeded $r = 0.40$. Clearly, these correlations are based on a low-powered dataset. Furthermore, these low correlations are not necessarily indicative of poor temporal reliability of the methods evaluated here, as participants could simply have experienced different numbers of episodes of reduced additional HRV on the various days they were tested on.

We performed another repeated measures ANOVA like the one described above, but we now incorporated the final method, which used the data from day $n$ to detect episodes of reduced additional HRV in day $n + 1$. Because this method only allowed us to analyze data from the second and third days of testing, we have only incorporated these two test days for every method analyzed. This analysis revealed a reliable difference in number of identified episodes of reduced additional HRV; even taking just the second and third test day for every participant into account, the lab calibration method by Verkuil et al. (2016) still identified the lowest number of episodes (3.5), while the method that used the data of day $n$ to detect episodes in day $n$ - 1 identified the largest number of episodes (11.2; 9.4 episodes during the second day and 13.0 during the third), $F(5, 20) = 6.7$, $p < 0.0005$, $\eta_p^2 = 0.63$. It is interesting that the latter method identified marginally more episodes than the method that used the full dataset (10.9). There was no effect of test day, nor did method and test day interact (*p*s 0.59 and 0.41, respectively).

## Associations With Worries and Stress

The crucial test for any of these methods is to see how well the physiological episodes of reduced additional HRV they detected correspond with participants' self-reported episodes of stress and worrying. After all, the detected physiological events are only of real interest if they actually coincide with—so, represent—known episodes of worrying or stress: only then do they actually signify reductions in *additional* HRV, as opposed to possibly random physiological events in the data that may not have a clear psychological cause. Participants reported and average of 2.8 ($SD = 3.2$) episodes of worrying and/or stress.

To this end, we first calculated the percentage of participants with at least one match between an episode of method-identified reduced HRV and an episode of self-reported worrying or stress in their data. These percentages were calculated separately for every method. As presented in the first row of **Table 3**, certain methods were characterized by considerably higher match percentages than others.

For example, the method that utilized the full dataset was associated with an 85.7% match between participants' self-reported stress and worrying episodes and the episodes of reduced additional HRV identified by this method; this method was also found to be very promising in other work (Brown et al., 2018). Interestingly, the method that used the first 10 min of three consecutive hours to detect episodes of reduced additional

**TABLE 2 |** Mean number of additional HRV episodes (SD) identified in three test day of 24 h each.

| | Verkuil et al. lab calibration | First 10 min of every hour | Full dataset (3 × 24 h) | First 10 min of 3 h | Activity type bins | Extended lab calibration |
|---|---|---|---|---|---|---|
| T1 | 3.8 (3.1) | 6.4 (2.7) | 12.4 (3.9) | 8.4 (3.2) | 6.2 (5.5) | 4.4 (4.2) |
| T2 | 3.8 (3.6) | 6.2 (2.1) | 11.5 (2.2) | 6.7 (3.7) | 5.0 (2.6) | 4.5 (4.2) |
| T3 | 3.2 (1.6) | 6.2 (3.2) | 10.3 (2.6) | 7.2 (3.7) | 6.8 (4.3) | 3.7 (3.2) |
| $\overline{X}$ | *3.6* | *6.3* | *11.4* | *7.4* | *6.0* | *4.2* |

*Calibration time period is also included in detection periods. Every method detected additional HRV episodes in all five analyzed participants. The bottom row contains the number of identified episodes averaged over test day.*

**TABLE 3 |** Matches between method-identified episodes of reduced additional HRV and self-reported episodes of stress and worry (expressed as percentages).

| | Verkuil et al. lab calibration (%) | First 10 min of every hour (%) | Full dataset (3 × 24 h) (%) | First 10 min of 3 h (%) | Activity type bins (%) | Extended lab calibration (%) | Next day detection (%) |
|---|---|---|---|---|---|---|---|
| Participants with ≥ 1 match | 42.9 | 78.6 | 85.7 | 85.7 | 78.6 | 42.9 | 60.0 |
| Mean percentage of matches | 50.9 | 65.5 | 70.1 | 73.6 | 59.8 | 53.0 | 68.7 |

*The first row presents percentages of participants with at least one match between method-identified episodes of reduced additional HRV and self-reported episodes of stress and worry. The second row presents, for this subset of participants, the percentage of average matches between method-identified episodes of reduced additional HRV and self-reported episodes of stress and worry.*

HRV was associated with an identical percentage of matches. The Verkuil et al. (2016) lab calibration method was associated with a match of 42.9%. This would suggest that the methods that used all available data for a given test session and that used a combination of 10 min of three consecutive hours' worth of data are more sensitive in detecting actual episodes of reduced additional HRV than Verkuil et al. (2016) lab calibration method, which we considered to be a golden standard. Indeed, earlier work (Brown et al., 2018) had identified the method that used a combination of 10 min of three consecutive hours to be a good "runner-up" to the method that used the full dataset.

The percentages reported above merely indicate how many participants had at least one match between method-detected episodes of reduced additional HRV and self-reported stress or worry episodes. A crucial next step is to compute the percentage of matches between method-detected episodes of reduced additional HRV and self-reported stress or worry episodes *within* the participants with at least one such match. Those percentages reveal how well, on average, each method was able to identify physiological episodes of reduced additional HRV that match and therefore represent self-reported episodes of stress or worry (provided that a participant had at least one such match). These percentages are listed in the second row of **Table 3**. Once more, the methods that used the full dataset and a combination of 10 min of three consecutive hours appear the most promising, with an average match between method-identified episodes of reduced additional HRV and self-reported stress worry of 70.1 and 73.6%, respectively. Although the method that used the data of day $n$ to identify episodes of additional HRV reduction in day $n + 1$ performed relatively well, with a matching percentage of 68.7%, this method was only able to detect matches in 60.0% of all tested participants. The extended calibration method performed similarly to the Verkuil et al. (2016) lab calibration method, which is not surprising, given that it is based on that method. Taken together, all of the results above suggest that using the full dataset to create an inverse regression model, which will then be used to detect episodes of reduced additional HRV, or using a combination of the first 10 min of three consecutive hours' worth of data to create such an inverse regression model appear to be the most promising methods. These two methods were associated with the best overall match between additional HRV physiology and self-reported stress and worry in the highest number of participants. Interestingly, all of the methods evaluated here identified more physiological reduced additional HRV episodes than that participants reported episodes of stress or worrying. The methods identified more episodes, on average, than participants reported in 85.2% of cases.

## DISCUSSION

The goal of the current paper was to evaluate several methods that can be used to identify episodes of reduced additional HRV and to identify the optimal method. As in previous work (Brown et al., 2018), we found that using every data point available in a given dataset appears to be a very promising method. Interestingly, the method that was identified to be a "runner-up" in our earlier study, slightly outperformed the method that used the full dataset in the current experiment: using the first 10 min of three consecutive hours to detect episodes of reduced additional HRV also led to a very good match between such physiological episodes and self-reported episodes of psychological stress and worry. Finally, the method that used the first 10 min of every available hour to detect episodes of reduced additional HRV also seemed to perform well enough to be considered an interesting option. The advantage of all three of these methods is that they do not rely on a calibration phase in the laboratory, which facilitates the identification of episodes of reduced additional HRV for researchers who do not have access to the calibration procedure introduced by Verkuil et al. (2016).

Two new methods were evaluated here: introducing an additional class of activity during the calibration phase, and using the data from day $n$ to detect episodes of reduced additional HRV in day $n + 1$. These two methods did not perform as well as the three methods described above: the extended calibration method performed similarly to the Verkuil et al. (2016) calibration method; the method that used data from day $n$ to detect episodes in day $n + 1$ seemed the more promising of the two new methods, and it may therefore warrant further exploration. One problem with this method is that it requires the acquisition of at least two sessions' worth of data, while episodes of reduced additional HRV are detected in only one of those sessions. Of course, one could devise ways to utilize the data of the "lost" session, for example by using the data of the last available test session to create an inverse regression model and to then use the resulting model parameters to detect episodes of reduced additional HRV in the first available test session. Clearly, this would complicate the method, as one would no longer be using a predictive model to detect episodes, but one would be using a kind of retroactive "prediction." For this reason, and given the method's overall unpromising level of performance, we have not explored this method beyond simply analyzing the data from the second and third test sessions in this paper.

One issue that we have commented on before (Brown et al., 2018), but that also characterizes the data of the six participants we have tested here, is the discrepancy in numbers of self-reported episodes of psychological worry and stress and method-identified physiological episodes of reduced additional HRV. The numerous methods invariably identify (occasionally, considerably) larger numbers of episodes of reduced additional HRV than that participants report episodes of stress and worry. For example, on average, the method that used the full dataset identified 11.4 episodes of reduced additional HRV, averaged over three test sessions, while participants reported 2.8 episodes of psychological stress or worry, averaged over three test sessions. It seems implausible that this effect is caused by technological glitches in the cell phones that were used to record participants' responses to questionnaires about their current psychological state. This leaves two explanations: either participants underreported episodes of stress and worry for any number of reasons (social expectations, shame, forgetfulness, etc.), or the episodes of reduced additional HRV that were detected by the various methods but that were not matched by self-reported stress and worrying episodes were due, at least in part, to unconscious stress. The latter option is corroborated by other work from our lab (Brosschot, 2010; Brosschot et al., 2010).

Another explanation could be that positive psychosocial events evoked these yet unexplained reductions in HRV. Indeed, previous work has shown that such events can also lead to increases in heart rate and therefore to concomitant reductions in HRV (see, e.g., Jacob et al., 1999). However, *sustained* increases in heart rate have been shown to be associated with events of negative valence only (Brosschot and Thayer, 2003). Given that the duration of our additional HRV reduction detection episodes was 7.5 min, and given that Brosschot and Thayer showed that heart rate already starts to decrease (and therefore, HRV starts to increase) after 5 min of being presented with a positively-valenced stimulus, we do not believe that additional HRV reductions due to positive psychosocial events provide an alternative explanation of our findings.

We also acknowledge that there are many factors that are known to affect HRV (Fattison et al., 2016; Sammito and Böckelmann, 2016), such as various cardiopulmonary diseases and metabolic diseases like diabetes mellitus, as well as lifestyle habits like alcohol consumption and smoking, plus external factors like heat. Of course, even respiration itself affects HRV. It is impossible to control for every possible confound in a study, but we believe that by excluding smokers and participants who used antihypertensive or cardiological medications, we have excluded some major confounds. Our participants were also relatively young students who typically were in good physical shape. Generally speaking, it is difficult to study the relationship between HRV and metabolic demands, because the increased heart rate that accompanies intense physical exercise complicates the delineation of the different non-neural mechanisms such as respiration, which are thought to underlie HRV changes under such intense circumstances (Casadei et al., 1995, 1996; Cottin et al., 2004, 2006; Michael et al., 2017). Furthermore, chronic anxiety is associated with increased autonomic tone (for a review, see Curtis and O'Keefe, 2002), and adverse childhood events can also affect HRV (Aimie-Salleh et al., 2018; Bakema et al., 2020). This may lead to baseline differences in HRV, but the current study was not designed to address such factors. Generally speaking, we believe that additional HRV decreases as measured with our methods can be interpreted to reflect psychosocial stress; of course, there might be other, unmeasured, sources of stress as well as long-term physiological baseline differences that our methods cannot currently detect. Having said that, now that our methods to estimate reductions in additional HRV have been evaluated twice, it would be very interesting to use them to further explore the role of these kinds of factors that are known to affect HRV. In future work, we intend to combine the methods described in the current paper with between-subjects designs, which will allow us to assess baseline differences in HRV, as well as potential underlying causes of such differences.

Currently, there is debate about whether or not methods that estimate HRV, like the RMSSD method that we used in this and previous papers, should be corrected for heart rate (for a review, see de Geus et al., 2018). This is an important matter, because as the interval between two heart beats increases, so does the variability of this interval (de Geus et al.), making heart rate a potential confound. We have chosen not to correct HRV for heart rate because there is strong empirical evidence to suggest that such corrections are not necessary (e.g., Thayer et al., 2020) or even do more harm than good, by removing variance that is due to autonomic or neuropsychological processes (de Geus et al., 2018).

Our findings have clear clinical relevance: episodes of reduced additional HRV cannot be sensed by people, but they do represent periods of either psychological or physical health (or both, of course). Therefore, methods to optimally and objectively detect such episodes can be of great use to clinicians as well as to end users both by signaling epochs during which interventions are most desirable (or even required) and by teaching end users what triggers evoke such epochs. One challenge for future work will thus be to further develop ways to probe participants' reduced additional HRV periods, and to assess all possible causes of these reductions. If these are indeed strongly related to bouts of (mindless) stress and worry, probing end users (e.g., patients suffering from stress-related (psychological or somatic) pathology) on their smartphones might become a new strategy to promote learning about and dealing with stress. One could easily not only make people more aware of their physiological stress level, but also provide them with a range of interventions that can be applied in order to directly change the stress level (i.e., breathing exercises, cognitive techniques).

We are aware that the analyses reported here have been performed on relatively low numbers of participants. However, given that we have now demonstrated, in three separate datasets, that using every available data point to detect episodes of reduced additional HRV appears to be a very accurate method to do so, and given the relative computational straightforwardness of that method, we feel confident that this method is promising and will lead to accurate results. It is particularly interesting that this method requires no special calibration phase: every researcher who has HRV and movement data will be able to analyze that data

using this method. Hopefully, further applications of this method by researchers in this field will further corroborate the validity of this method. We hope that this new method will lead to fruitful insights into reductions in additional HRV which, in turn, may lead to a better understanding of this powerful predictor of cardiovascular disease.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Leiden University Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

SB tested participants and created analysis scripts. AV tested participants. All authors contributed to the article and approved the submitted version.

## FUNDING

SB was supported by a "Top"-grant of the Netherlands Organisation for Health Research and Development (ZON-MW) awarded to JB (Grant No. 40-00812-98-11029); a grant from the Den Dulk-Moermans Fonds awarded to JB (6503/21-6-16); and a research grant from the Netherlands Organization for Scientific Research (NWO Veni Grant) awarded to BV (Grant No. 4451-14-013).

## ACKNOWLEDGMENTS

We would like to thank Paul Siegwardt for his assistance in testing participants.

## REFERENCES

Aimie-Salleh, N., Malarvili, M. B., and Philip, A. C. (2018). "The effect of adverse childhood experience on heart rate variability and salivary cortisol," in *Proceedings of the Seventh International Conference on Advances in Computing, Electronics and Communication* Kuala Lumpur.

Alwan, A. (2011). "Foreword," in *Global Atlas on Cardiovascular Disease Prevention and Control* (p. VI), eds S. Mendis, P. Puska, and B. Norrving (Geneva: WHO Press).

Bakema, M. J., van Zuiden, M., Collard, D., Zantvoord, J. B., de Rooij, S. R., Elsenburg, L. K., et al. (2020). Associations between child maltreatment, autonomic regulation, and adverse cardiovascular outcome in urban population: the HELIUS study. *Front. Psychiatry* 11:69. doi: 10.3389/fpsyt.2020.00069

Berntsen, G. G., Quigley, K. S., Norman, G. J., and Lozano, D. L. (2017). "Cardiovascular psychophysiology," in *Handbook of Psychophysiology*, 4th Edn, eds J. T. Cacioppo, L. G. Tassinary, and G. G. Berntson (Cambridge: Cambridge University Press), 183–216.

Blix, A. S., Stromme, S. B., and Ursin, H. (1974). Additional heart rate—An indicator of psychological activation. *Aerospace Med.* 45, 1219–1222.

Bosma, H., Peter, R., Siegrist, J., and Marmot, M. (1998). Two alternative job stress models and the risk of coronary heart disease. *Am. J. Public Health* 88, 68–74. doi: 10.2105/ajph.88.1.68

Brosschot, J. F. (2010). Markers of chronic stress: prolonged physiological activation and (un)conscious perseverative cognition. *Neurosci. Biobehav. Rev.* 35, 46–50. doi: 10.1016/j.neubiorev.2010.01.004

Brosschot, J. F., and Thayer, J. F. (2003). Heart rate response is longer after negative emotions than after positive emotions. *Int. J. Psychophysiol.* 50, 181–187. doi: 10.1016/S0167-8760(03)00146-6

Brosschot, J. F., van Dijk, E., and Thayer, J. F. (2007). Daily worry is related to low heart rate variability during waking and the subsequent nocturnal sleep period. *Int. J. Psychophysiol.* 63, 39–47. doi: 10.1016/j.ijpsycho.2006.07.016

Brosschot, J. F., Verkuil, B., and Thayer, J. F. (2010). Conscious and unconscious perseverative cognition: is a large part of prolonged physiological activity due to unconscious stress? *J. Psychos. Res.* 69, 407–416. doi: 10.1016/j.jpsychores.2010.02.002

Brown, S. B. R. E., Brosschot, J. F., Versluis, A., Thayer, J. F., and Verkuil, B. (2018). New methods to optimally detect episodes of non-metabolic heart rate variability reduction as an indicator of psychological stress in everyday life. *Int. J. Psychophysiol.* 131, 30–36.

Casadei, B., Cochrane, S., Johnston, J., Conway, J., and Sleight, P. (1995). Pitfalls in the interpretation of spectral analysis of the heart rate variability during exercise in humans. *Acta Physiol. Scand.* 153, 125–131. doi: 10.1111/j.1748-1716.1995.tb09843.x

Casadei, B., Moon, J., Johnston, J., Caiazza, A., and Sleight, P. (1996). Is respiratory sinus arrhythmia a good index of cardiac vagal tone in exercise? *J. Appl. Physiol.* 81, 556–564. doi: 10.1152/jappl.1996.81.2.556

Cottin, F., Durbin, F., and Papelier, Y. (2004). Heart rate variability during cycloergometric exercise or judo wrestling eliciting the same heart rate level. *Eur. J. Appl. Physiol.* 91, 177–184. doi: 10.1007/s00421-003-0969-1

Cottin, F., Lepretre, P. M., Lopes, P., Papelier, Y., Medigue, C., and Billat, V. (2006). Assessment of ventilatory thresholds from heart rate variability in well-trained subjects during cycling. *Int. J. Sports Med.* 27, 959–967. doi: 10.1055/s-2006-923849

Curtis, B. M., and O'Keefe, J. H. Jr. (2002). Autonomic tone as a cardiovascular risk factor: the dangers of chronic fight or flight. *Mayo Clin. Proc.* 77, 45–54. doi: 10.4065/77.1.45

de Geus, E. J. C., Gianaros, P. J., Brindle, R. C., Jennings, J. R., and Berntson, G. G. (2018). Should heart rate variability be "corrected" for heart rate? Biological, quantitative, and interpretative considerations. *Psychophysiology* 56:e13287. doi: 10.1111/psyp.13287

Ebner-Priemer, U. W., Welch, S. S., Grossman, P., Reisch, T., Linehan, M. M., and Bohus, M. (2007). Psychophysiological ambulatory assessment of affective dysregulation in borderline personality disorder. *Psychiatry Res.* 150, 265–275. doi: 10.1016/j.psychres.2006.04.014

Fattison, J., Oswald, V., and Lalonde, F. (2016). Influence diagram of physiological and environmental factors affecting heart rate variability: an extended literature overview. *Heart Int.* 11, e32–e40. doi: 10.5301/heartint.5000232

Hall, M., Vasko, R., Buysse, R., Ombao, H., Chen, Q., Cashmere, J. D., Kupfer, D., and Thayer, J. F. (2004). Acute stress affects heart rate variability during sleep. *Psychosom. Med.* 66, 56–62. doi: 10.1097/01.psy.0000106884.58744.09

Hillebrand, S., Gast, K. B., de Mutsert, R., Swenne, C. A., Jukema, J. W., Middeldorp, S., et al. (2013). Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace* 15, 742–749. doi: 10.1093/europace/eus341

Jacob, R. G., Thayer, J. F., Manuck, S. B., Muldoon, M. F., Tamres, L. K., Williams, D. M., et al. (1999). Ambulatory blood pressure responses and the circumplex model of mood: a 4-day study. *Psychos. Med.* 61, 319–333. doi: 10.1097/00006842-199905000-00011

Jarczok, M. N., Koenig, J., Wittling, A., Fischer, J. E., and Thayer, J. F. (2019). First evaluation of an index of low vagally-mediated heart rate variability as a marker of health risks in human adults: proof of concepts. *J. Clin. Med.* 8:1940. doi: 10.3390/jcm8111940

Kivimäki, M., Virtanen, M., Elovainio, M., Kouvonen, A., Väänänen, A., and Vahtera, J. (2006). Work stress in the etiology of coronary heart disease—a meta-analysis. *Scand. J. Work Environ. Health* 32, 431–442.

Lazarus, R. S. (1993). From psychological stress to the emotions: a history of changing outlooks. *Annu. Rev. Psychol.* 44, 1–22. doi: 10.1146/annurev.ps.44.020193.000245

Lugade, V., Fortune, E., Morrow, M., and Kaufman, K. (2014). Validity of using tri-axial accelerometers to measure human movement - part I: posture and movement detection. *Med. Eng. Phys.* 36, 659–669. doi: 10.1016/j.medengphy.2014.02.006

Matthews, K. A., and Gump, B. B. (2002). Chronic work stress and marital dissolution increase risk of posttrial mortality in men from the multiple risk factor intervention trial. *Arch. Intern. Med.* 62, 309–315. doi: 10.1001/archinte.162.3.309

McEwen, B. S. (2001). From molecules to mind. Stress, individual differences, and the social environment. *Ann. N.Y. Acad. Sci.* 935, 42–49. doi: 10.1111/j.1749-6632.2001.tb03469.x

Michael, S., Graham, K. S., and Davis, G. M. (2017). Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals—A review. *Front. Physiol.* 8:301. doi: 10.3389/fphys.2017.00301

Myrtek, M., and Brügner, G. (1996). Perception of emotions in everyday life: studies with patients and normals. *Biol. Psychol.* 42, 147–165. doi: 10.1016/0301-0511(95)05152-x

Myrtek, M. (2004). *Heart and Emotion: Ambulatory Monitoring Studies in Everyday Life*. Ashland, TN: Hogrefe Publishing.

Myrtek, M., Aschenbrenner, F., and Brügner, G. (2005). Emotions in everyday life: an ambulatory monitoring study with female students. *Biol. Psychol.* 68, 237–255. doi: 10.1016/j.biopsycho.2004.06.001

Orth-Gomér, K., Wamala, S. P., Horsten, M., Schenck-Gustafsson, K., Schneiderman, N., and Mittleman, M. A. (2000). Marital stress worsens prognosis in women with coronary heart disease. *JAMA* 284, 3008–3014. doi: 10.1001/jama.284.23.3008

Pieper, S., Brosschot, J. F., van der Leeden, R., and Thayer, J. (2010). Prolonged cardiac effects of momentary assessed stressful events and worry episodes. *Psychos. Med.* 72, 570–577. doi: 10.1097/psy.0b013e3181dbc0e9

Pieper, S., Brosschot, J. F., van der Leeden, R., and Thayer, J. F. (2007). Cardiac effects of momentary assessed worry episodes and stressful events. *Psychos. Med.* 69, 901–909. doi: 10.1097/psy.0b013e31815a9230

Prill, T., and Fahrenberg, J. (2007). New methods in ambulatory blood pressure monitoring: interactive monitoring and detection of posture and movement patterns. *Behav. Res. Methods* 39, 390–398. doi: 10.3758/BF03193008

Rennie, K. L., Hemingway, H., Kumari, M., Brunner, E., Malik, M., and Marmot, M. (2003). Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. *Am. J. Epidemiol.* 158, 135–143. doi: 10.1093/aje/kwg120

Rosengren, A., Hawken, S., Ounpuu, S., Sliwa, K., Zubaid, M., Almahmeed, W. A., et al. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 364, 953–962. doi: 10.1016/S0140-6736(04)17019-0

Sammito, S., and Böckelmann, I. (2016). Factors influencing heart rate variability. *Int. Cardiovasc. Forum J.* 6, 18–22. doi: 10.17987/icfj.v6i0.242

Sowder, E., Gevirtz, R. N., and Shapiro, W. (2010). Restoration of vagal tone: a possible mechanism for functional abdominal pain. *Appl. Psychophysiol. Biofeedback* 35, 199–206. doi: 10.1007/s10484-010-9128-8

Stewart, J. M., Montgomery, L. D., Glover, J. L., and Medow, M. S. (2007). Changes in regional blood volume and blood flow during static handgrip. *Am. J. Physiol. Heart Circ. Physiol.* 292, H215–H223.

Task Force of The European Society of Cardiology and The North American Society of Pacing, and Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Thayer, J. F., and Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biol. Psychol.* 74, 224–242. doi: 10.1016/j.biopsycho.2005.11.013

Thayer, J. F., Williams, D. P., and Tarvainen, M. P. (2020). Properties of three common time domain indices of heart rate variability: effects of detrending. *Psychos. Med.* 82:e1798.

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010). The relationship of heart rate variability, risk factors and cardiovascular disease. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Verkuil, B., Brosschot, J. F., Tollenaar, M. S., Lane, R. D., and Thayer, J. F. (2016). Prolonged non-metabolic heart rate variability reduction as a physiological marker of psychological stress in daily life. *Ann. Behav. Med.* 50, 704–714. doi: 10.1007/S12160-016-9795-7

Versluis, A., Verkuil, B., Spinhoven, P., and Brosschot, J. F. (2018). Feasibility and effectiveness of a worry-reduction training using the smartphone: a pilot randomized controlled trial. *Br. J. Guid. Counsel.* 48, 227–239. doi: 10.1080/03069885.2017.1421310

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 13 November 2020
doi: 10.3389/fnins.2020.582145

# The Role of Heart Rate Variability in the Future of Remote Digital Biomarkers

*Andrew P. Owens[1,2]* on behalf of the RADAR-AD Consortium*

*[1] Department of Old Age Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [2] The Remote Assessment of Disease and Relapse – Alzheimer's Disease (RADAR-AD) Consortium, London, United Kingdom*

Heart rate variability (HRV) offers insights into humoral, neural and neurovisceral processes in health and disorders of brain, body and behavior but has yet to be fully potentiated in the digital age. Remote measurement technologies (RMTs), such as, smartphones, wearable sensors or home-based devices, can passively capture HRV as a nested parameter of neurovisceral integration and health during everyday life, providing insights across different contexts, such as activities of daily living, therapeutic interventions and behavioral tasks, to compliment ongoing clinical care. Many RMTs measure HRV, even consumer wearables and smartphones, which can be deployed as wearable sensors or digital cameras using photoplethysmography. RMTs that measure HRV provide the opportunity to identify digital biomarkers indicative of changes in health or disease status in disorders where neurovisceral processes are compromised. RMT-based HRV therefore has potential as an adjunct digital biomarker in neurovisceral digital phenotyping that can add continuously updated, objective and relevant data to existing clinical methodologies, aiding the evolution of current "diagnose and treat" care models to a more proactive and holistic approach that pairs established markers with advances in remote digital technology.

**Keywords: autonomic nervous system, digital biomarkers, heart rate variability, homeostasis, neurovisceral integration, remote measurement technologies**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Luca Carnevali,*
*University of Parma, Italy*
*Michele Orini,*
*University College London,*
*United Kingdom*

****Correspondence:***
*Andrew P. Owens*
*andrew.owens@kcl.ac.uk*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *10 July 2020*
***Accepted:*** *28 October 2020*
***Published:*** *13 November 2020*

***Citation:***
*Owens AP (2020) The Role of Heart Rate Variability in the Future of Remote Digital Biomarkers. Front. Neurosci. 14:582145. doi: 10.3389/fnins.2020.582145*

## INTRODUCTION

Remote Measurement Technologies (RMTs) refers to, "any mobile technology that enables monitoring of a person's health status through a remote interface, with the data then either transmitted to a healthcare provider for review or to be used as a means of education for the user themselves" (Davis et al., 2014). Advances in healthcare devices, wearable sensors and smartphones have a potential role in how health assessment, monitoring and treatment will be conducted in the near future (Owens et al., 2020a). RMTs can remotely and passively index changes in health parameters, as well as providing contextual information to other health data, such as environment or what activity of daily living the wearer was engaged in during the epoch of data collection (Vegesna et al., 2017), offering a financially viable and easily deployable opportunity to accurately, objectively and continuously monitor changes in relevant domains (Owens et al., 2020b).

A "digital phenotype" describes the moment-to-moment quantification of the individual-level human phenotype *in situ* using data from personal digital devices (Onnela and Rauch, 2016). A "digital biomarker" refers to physiologic, pathologic or anatomic characteristics objectively measured and evaluated as an indicator of biologic processes, pathologic processes or biological response to therapeutic interventions collected by RMTs across various platforms of software and/or hardware (Coravos et al., 2019). RMTs can collect multiple datapoints during passive everyday wearing or active engagement in device-based tasks as the wearer goes about their normal routine. These datapoints can provide real-time status of health and disease, including symptom-severity and progression, stability and regression and treatment-responses (**Figure 1**). Deploying RMTs to remotely capture signals related to health and disease also offers the possibility of engaging those who would not ordinarily participate in research and empower users by giving them an engaged role in their own healthcare. RMTs can enhance care and assessment by providing highly powered data on relevant variables and equip the user with bespoke protocols that incorporate their lifestyle and clinical profiles. This can complement typical clinical scales and assessments that are often carried out months apart and can rely on subjective patient or carer recall on the day of testing. Ultimately, such technologies may provide a sea change from a "diagnose and treat" to a "predict and pre-empt" care model (Narayan and Manji, 2016).

## Heart Rate Variability

Homeostasis and allostasis are enabled and controlled by autonomic nervous system (ANS) efferent neurons that mediate the function of effector organs. ANS function is generally beyond conscious control and functionally, morphologically and chemically organized into two branches:

i. the parasympathetic nervous system (PNS) promotes vegetative activity, such as heart rate (HR) deceleration via the vagus nerve or increasing gut motility predominantly via acetylcholine (Ach) at the neuroeffector junction. The vagus nerve also maintains tonic inhibitory control of proinflammatory cytokines via Ach release into the reticuloendothelial system (spleen, gastrointestinal tract, heart, liver), mediating the inflammatory reflex through the cholinergic anti-inflammatory pathway (Dantzer and Kelley, 2007).
ii. the sympathetic nervous system (SNS) serves to upregulate effector organ function, such as raising blood pressure (BP) or increasing sudomotor activity, via the catecholamines, noradrenaline (NA), and adrenaline (Owens et al., 2017).

Cardiac tissue has inherent pacemaker properties and the ANS regulates the myocardium's contractile and electrical output via the vagus nerve and SNS (Spyer, 1994). The rate of pacemaker depolarization is increased by SNS activation and parasympathetic vagal flow promotes cardiac pacemaker cells to hyperpolarize and slow depolarization speed (Spyer, 1994). Respiratory sinus arrhythmia (RSA) refers to the increase in HR during inspiration and HR deceleration during expiration, which is the functional endpoint of cardioinhibitory vagal fibers stemming from the nucleus ambiguus (Neff et al., 2003). Heart rate variability (HRV) records these beat-to-beat variations of HR and the intervals between QRS complexes (RR intervals) of sinus depolarizations (Stein et al., 1994). HRV therefore describes vagal influences on the sinus node using non-invasive electrocardiographic (ECG) markers (Van Ravenswaaij-Arts et al., 1993).

The application of spectral analytical techniques to short or long-term neurocardiovascular changes is now widely utilized as a measure of cardiovagal activity. Power spectral analysis can be performed using parametric or non-parametric methodologies. The Fast Fourier transformation (FFT) non-parametric method is typified by discrete peaks of the frequency bands. FFT is a simple and quickly performed equation. The Autoregressive model results in a continuous spectrum of events. It is more complex than the FFT model and must be suitable to the experimental model (Ori et al., 1992). High frequency HRV (HF-HRV) is a measure of vagal efferent activity and is comparable to RSA. Low frequency HRV (LF-HRV) was originally believed to depict sympathetic cardiac influences (Malliani et al., 1991). However, LF-HRV as a purely sympathetic measure has been questioned (Goldstein et al., 2011), as more recent studies show LF-HRV may essentially provide information about sympathetic regulation of neurovascular mechanisms, such as vasomotor tone and baroreceptor activity (Moak et al., 2007; Goldstein et al., 2011; Rahman et al., 2011). LF-HRV may therefore provide information about sympathetic mechanisms but of baroreflex function and dysfunction rather than cardiac sympathetic nerve activity specifically. Therefore, how finely HRV represents ANS activity remains a matter of debate, and HRV metrics deficiency in capturing changes in sympathetic activity is a limitation of the approach, particularly considering the non-linear and non-reciprocal relationship between sympathetic and vagal activity (Boyett et al., 2019). Very low frequency (VLF) reflects long-term regulation mechanisms, such hormonal function (Theorell et al., 2007), the renin-angiotensin system (RAS) (Taylor et al., 1998) and thermoregulation (Fleisher et al., 1996; Taylor et al., 1998), although VLF's role is less clearly defied than LF-HRV and HF-HRV.

### Neural Correlates of Heart Rate Variability

Exercise-induced increases in LF-HRV are linked with metabolic activity in insula, cingulate and somatomotor regions (Critchley et al., 2003), and HF-HRV with basal ganglia and anterior temporal lobe function (Matthews et al., 2004; Lane et al., 2009). Limbic structures supply descending efferent drive to the hypothalamus and brainstem to modulate homeostatic and allostatic autonomic responses (Saper, 2002). Emotion-induced changes in HRV are associated with function in the insula, periaqueductal gray (PAG) and caudate nucleus (Lane et al., 2009). Bidirectional functional connectivity between the central autonomic network (CAN) structures of the medial prefrontal cortex (mPFC), insula, central nucleus of the amygdala, PAG and parabrachial region inform efferent autonomic outflow via structures, such as the stellate ganglia and vagus nerve to the sinoatrial node. Therefore, examining interbeat intervals via HRV

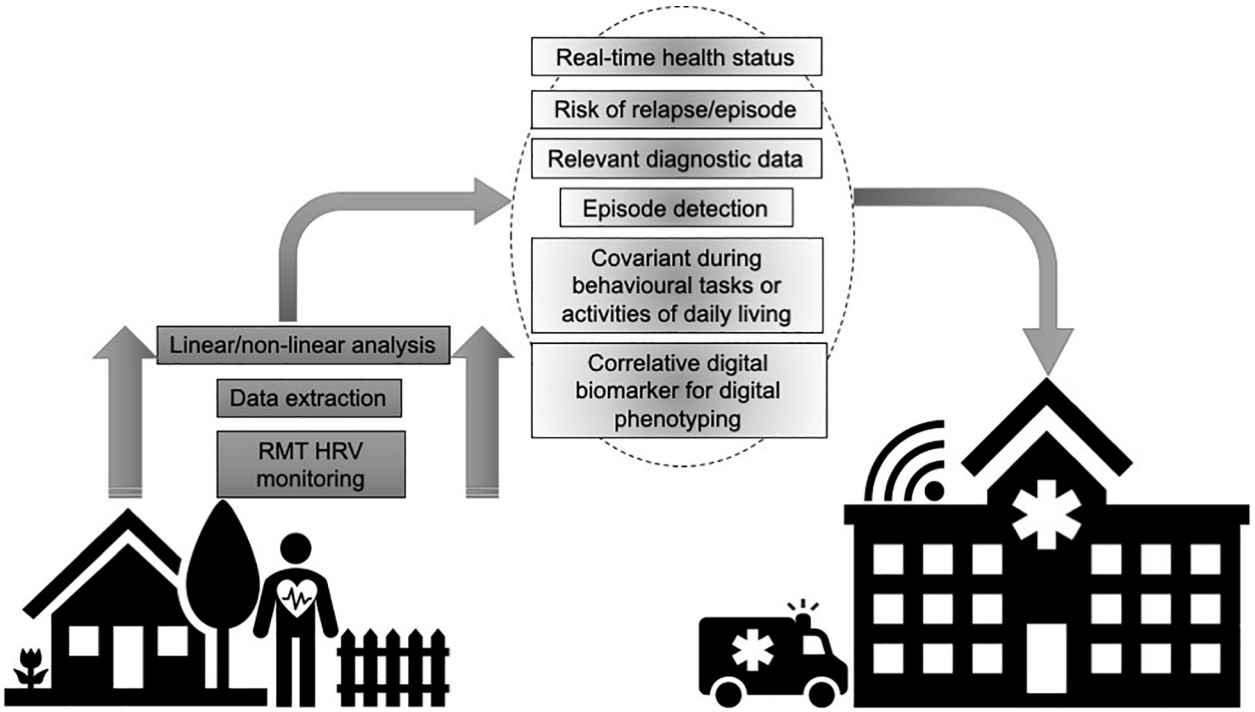



**FIGURE 1 |** Utilizing remote measurement technologies (RMTs) to remotely record heart rate variability (HRV) provides granular data that can index health and disease status, including symptom-severity and progression, stability and regression and treatment-responses.

provides functional endpoint insights into these areas of the brain, as supported by HRV data correlating with activity of brainstem and prefrontal areas (Lane et al., 2009).

## HEART RATE VARIABILITY AS A MEASURE OF NEUROVISCERAL INTEGRATION

### Neurovisceral Processes

"Interoception" is the term given to the transmission of afferent peripheral sensory information, which informs autonomic mediation of homeostasis, allostasis and contributes to psychological and behavioral processes (Owens et al., 2017, 2018a). Central autonomic networks within the spinal cord, brainstem and hypothalamus mediate autonomic efferent output to meet homeostatic and allostatic demands (Benarroch, 1993). Hemodynamic autonomic adjustments are informed by input from cortical, limbic forebrain and midbrain structures (Saper, 2002). Activity within the dorsal anterior cingulate cortex (ACC) (Critchley et al., 2003) and insula (Critchley et al., 2000) reflects engagement of sympathetic activity coupled to allostatic load. Therefore, the ANS can both influence and be influenced by brain processes via bottom-up interoceptive signaling ascending the neuraxis, or top-down brain signaling influencing efferent autonomic outflow, respectively (Owens et al., 2017, 2018a). These findings are enlightened by neuroimaging studies underlining how psychological and HRV are coupled. For example, empirical models of neurovisceral integration have evidenced vagal involvement, as indexed by HRV, in cognitive-affective regulatory processes (Thayer and Lane, 2000; Smith et al., 2017; Owens et al., 2018b).

### The Vagus and the Cholinergic Anti-inflammatory Pathway

Vagal nerve interoceptive function also has a central role in inflammatory processes via the cholinergic anti-inflammatory pathway. Peripheral proinflammatory mechanisms can be initiated through sympathetic innervation of lymphoid tissue, and anti-inflammatory processes can be promoted through vagal release of Ach or hypothalamic release of corticotrophin-releasing hormone (Epstein and Reichlin, 1993). The vagus nerve is comprised of 20% efferent parasympathetic fibers originating from the dorsal motor nucleus of the vagus, and 80% afferent sensory fibers that receive humoral and interoceptive feedback from the periphery before relaying these ascending signals to the neuraxis. Vagal tone and Ach inhibit proinflammatory cytokine release, such as interleukin-6 (IL-6) or tumor necrosis factor, but not anti-inflammatory cytokines, such as interleukin-10 (IL-10). The SNS is also involved in the inflammatory reflex, such as regulating cytokines via the hypothalamic pituitary-adrenal axis (Goehler et al., 2000). Inflammatory responses can be inducted by the nucleus ambiguous and dorsal motor nucleus of the vagus, which both receive input from the nucleus tractus solitarius (NTS). Medullary afferents to limbic structures, higher cortical areas and insula are implicated in "sickness

behavior" (Goehler et al., 2000), which is defined by anhedonia, anorexia, circadian disruption, fatigue, psychomotor retardation, and hyperalgesia (Kelley et al., 2003). Sickness behavior (Dantzer and Kelley, 2007) is an example of a neurovisceral feedback loop, in which ascending interoceptive information is received by the CAN, which then drives efferent physiological and behavioral changes to meet homeostatic and allostatic requirements.

As understanding has improved about the role of inflammation in not only neurodegeneration and related neuropsychiatric symptoms (Holmgren et al., 2014), but also psychiatric disorders in the young and middle-aged (Ramirez et al., 2017), the role of central and peripheral inflammation and how the peripheral immune state is communicated to the central nervous system (CNS) has become an increasingly attractive target for treatment and research. As with effector organ interoceptive processes, humoral interoceptive processing between the periphery and CNS are bidirectional and brain responses to immune-related interoceptive signals can influence behavioral, psychological and autonomically mediated processes (Wan et al., 1994; Kelley et al., 2003). Parallel humoral, neural and cellular interoceptive pathways communicate the homeostatic and allostatic state to the brain to elicit adaptations and recent studies have examined the relationship between HRV, physical and mental health and inflammatory markers (Halaris, 2017). HRV therefore offers a window into neurovisceral integrity in health and disorders of brain, body and behavior but has yet to be fully potentiated in the digital age.

## WHAT ROLE CAN REMOTELY CAPTURED HEART RATE VARIABILITY PLAY IN TELEMEDICINE?

It could be argued that the current COVID-19 pandemic and related social distancing guidelines have strengthened the case for RMT use in clinical care. Future outbreaks are conceivable and social isolation is likely to be recommended for high-risk groups even after social distancing restrictions are eased, with reduced clinic contact indefinitely suggested for such groups. Social isolation can lead to depression, anxiety, loneliness and hinder clinical care. Loneliness is a modern epidemic that predated COVID-19 and which older adults, who are most at-risk of infection from COVID-19, are particularly susceptible to due to retirement, being widowed, adult children moving away and ill-health causing functional decline and making social activities more difficult (Hacihasanoglu et al., 2012; Arslantaş et al., 2015). Loneliness is associated with the disruption of homeostatic and physiological processes, such as increased cardiovascular tone and cardiovascular responsiveness to stress (Cacioppo et al., 2014). Lonely individuals also evidence wide-ranging cognitive biases, such as increased attention to social threat (Spithoven et al., 2017) and are more likely to utilize dysfunctional emotion regulation strategies (which can be indexed by HRV when paired with contextual data) in social situations (Vanhalst et al., 2018), indicating that emotion regulation may be a key aspect in how social connections relate to mental health (Roberts and Burleson, 2013). This has increased the need for the employment of RMTs to be able to continue monitoring patients' health, symptom severity and functional status because the outbreak of COVID-19 has not affected the need effective treatment and diagnosis.

Remote measurement technologies that measure HRV provide the opportunity to identify digital biomarkers indicative of changes in health or disease status in disorders where neurovisceral processes are compromised, such as depression, epilepsy, substance abuse, neurodegeneration, dissociative disorders, and dysautonomia (Thaisetthawatkul et al., 2004; Halaris, 2013; Marhe and Franken, 2014; Eccles et al., 2015; Hyett et al., 2015; Owens et al., 2015). Current studies, such as the "Remote Assessment of Disease and Relapse – Central Nervous System" (RADAR-CNS)[1] (Polhemus et al., 2019) and "Remote Assessment of Disease and Relapse – Alzheimer's Disease" (RADAR-AD)[2] (Owens et al., 2020b) are using RMTs to actively and passively measure neurophysiological, motor, functional, cognitive and affective digital biomarkers remotely in disorders, such as Alzheimer's disease (AD), major depressive disorder, epilepsy, and multiple sclerosis. RMT-based HRV could provide additional insight and context into such studies, as it provides an easily deployable and scalable metric of health domains, such as inflammation, stress, emotion regulation, and sympathovagal function (Frazier et al., 2004). For example, in remitted depression cases with a history of suicidal ideation, reduced HRV (collected in a lab) and impulsivity significantly correlate to lower brain levels of tryptophan, which occurs in depression due to continuous low-level inflammation disrupting tryptophan metabolism via stimulation of indoleamine 2,3-dioxygenase (a key kynurenine pathway enzyme) (Myint, 2012). As discussed, the vagus is central to immune reactivity by tonic inhibition of proinflammatory cytokine release via the cholinergic anti-inflammatory pathway and HF-HRV is reduced during stress and recovery in depressed subjects (Schiweck et al., 2019). Therefore, simple monitoring and thresholding of such an individual's HRV could provide an adjunct "red flag" marker for risk of declining mental health to the patient's clinical team. Both frequency domain and time domain HRV data inversely relate to IL-6, HF-HRV correlates with many inflammatory markers and poorer HRV predicts C-reactive protein levels and white blood cell counts in healthy adults (Thayer and Fischer, 2009), providing a valuable surrogate measure of inflammation that can potentially be collected remotely over large periods of time and in correlation with other relevant clinical and digital signatures.

As a nested parameter of neurovisceral health, RMT-based HRV may provide insights into acute episodes that may be difficult to capture in clinic. In epilepsy, artificial neural networks have been combined with HRV frequency domain analysis to build an algorithm that can predict seizures with a sensitivity, specificity, and accuracy of 83.33%, 86.11%, and 84.72%, respectively, for complex partial episodes, and 88.66%, 90%, and 88.33%, respectively, for secondarily generalized seizures (Behbahani et al., 2014). Postictal HRV data significantly increases and can discriminate seizure laterality (Shimmura et al., 2019). Abnormal HRV profiles have been reported in epilepsy for

[1] https://www.radar-cns.org/
[2] https://www.radar-ad.org/

a quarter of a century (Frysinger et al., 1993) and remote HRV data may have a role in helping predict sudden unexpected death in epilepsy (SUDEP). Seizures can induce cardiac arrhythmias and SUDEP is the primary cause of premature mortality in epilepsy. In a Phase II study, Jeppesen et al. (2019) recently trialed RMT-based HRV to detect seizures in a hospital setting using the ePatch heart monitor, with positive results (93.1% sensitivity for all seizures, 90.5% for non-convulsive seizures). With the rapid progressive iterations of RMTs, such an approach could be adopted in a real-world setting, whereby, smartphone-based, home-based or wearable sensors could be deployed for people with epilepsy as they go about their daily lives, to passively collect HRV data that could be combined with other digital biomarkers and clinical profiles to predict acute episodes. RMT-based HRV can be paired with other biomarkers to create digital phenotypes (see **Figure 1**), for example, genetic mutations in voltage-gated ion channel genes (SCN5A and KCNH2) relate to long QT syndrome (Bagnall et al., 2016) and ion channel mutations may be expressed in both the heart and brain, therefore, genetic screening paired with remote HRV could be explored as a potential means of tracking disease status and risk in epilepsy.

Alzheimer's disease and dementia with Lewy bodies (DLB) are the first and second most common forms of dementia (Gascón-Bayarri et al., 2007; Aarsland et al., 2008), respectively, but distinguishing DLB from AD is challenging in the early mild cognitive impairment (MCI) stages and currently involves clinical examination and neuroimaging, such as DaTscan and $^{123}$I-metaiodobenzylguanidine ($^{123}$I-MIBG). DLB has poor prognosis and diagnosis can be complicated by its initial similar presentation to AD, yet early differentiation of DLB from AD is vital due to differing responses to medication and disease courses. Therefore, identifying cheaper yet reliable biomarkers that can differentiate AD and DLB are much-needed. Dysautonomia (autonomic dysfunction) and autonomic failure [particularly orthostatic hypotension (Freeman et al., 2011)] are common in DLB and may precede motor and neuropsychiatric symptoms (Iodice et al., 2011; Kaufmann et al., 2017) due to specific pathways of peripheral ganglia (such as postganglionic sympathetic lesions) or the CAN (such as brainstem, insula, and hypothalamus) being progressively damaged (Wakabayashi and Takahashi, 1997; Iwanaga et al., 1999; Benarroch et al., 2005). The impact of autonomic symptoms in DLB, Parkinson's disease (PD) and Parkinson's disease with dementia (PDD) causes significant functional decline (as indexed by activities of daily living) and quality of life (Allan et al., 2006) and present across cardiovascular (Freeman, 2008), genitourinary (Winge and Fowler, 2006), gastrointestinal (Pfeiffer, 2012), and thermoregulatory domains (Schestatsky et al., 2006). Dysautonomia and autonomic failure supports a diagnosis of DLB and the pattern of autonomic symptoms is similar to that of PD but generally more severe (Thaisetthawatkul et al., 2004; Lipp et al., 2009), though not as severe as multiple system atrophy (MSA) (Thaisetthawatkul et al., 2004; Lipp et al., 2009). $^{123}$I-MIBG is used to detect sympathetic noradrenergic denervation in DLB and distinguishes Lewy body disease [DLB (Odagiri et al., 2016), PD (Amino et al., 2006)] from non-Lewy body disease with autonomic failure (MSA). Recently, $^{123}$I-MIBG has been combined with single photon emission computed tomography (SPECT) (Niimi et al., 2017; Nuvoli et al., 2017) in Lewy body disease to compare neurological and autonomic pathology. However, SPECT is relatively time-consuming, expensive and arduous for patients who are often frail and reluctant to make hospital visits. Deploying RMT-based HRV in potential MCI-AD and MCI-DLB cases may describe endpoint markers of any noradrenergic denervation in MCI-DLB to aid differential diagnosis from MCI-AD, particularly when combined with energy expenditure, activities of daily living, motor/gait or accelerometery data.

Moreover, research has typically not found autonomic symptoms to occur in AD, yet very recent studies have found orthostatic hypotension can present in 42% of AD patients if head-up tilt table testing is used rather than standing tests or subjective self-report measures (Isik et al., 2019). In addition, compared to healthy controls, AD patients may have normal baseline autonomic function but produce divergent autonomic responses during tasks with higher cognitive load (Perpetuini et al., 2019). Therefore, the more nuanced (compared to MCI-DLB) autonomic perturbations that may occur in MCI-AD during instrumental or advanced activities of daily living that involve more cognitive load may be more detectable if RMT-based HRV data, as an index of stress, is contextualized with what activity of daily living the wearer is engaged in during any thresholded reductions in HRV.

Furthermore, capturing acute disease-related episodes, such as fluctuating cognition, seizures or falls, can be challenging in clinic and RMTs, including those that can remotely measure HRV, offer a "real-time" window into the mental and physical health and functional status of the patient. This can also provide relevant insights into treatment responses to therapeutic interventions, whilst negating potential "white coat syndrome," offering a more realistic and contextualized environment for data-collection and assessment. Moreover, remotely collected data offers the opportunity for greater confidentiality than a physical trip to a hospital, while removing the need for frail patients or carers to commute. RMT-based HRV therefore may have value as an adjunct digital biomarker in health and in neurovisceral digital phenotypes (Eccles et al., 2015), adding continuously updated and objective data on central and peripheral function to typical clinical methodologies.

## DEPLOYING REMOTE HEART RATE VARIABILITY

Wearables exist that provide long-term telemonitoring of HRV using low-power biosensors that employ methodologies to acquire ECG signals from on-body sensors (Pant and Krishnan, 2018). Although artifacts may be more common in comparison to Holter monitors in some RMTs that record HRV, this can be offset by benefits, such as longer battery life, superior comfort, higher user-acceptability/compliance (patients often do not want medical devices to be visible if they are worn in public) and the ability of RMTs to collect other relevant physiological

covariates, such as body temperature, respiration, and motor parameters (Akintola et al., 2016). Off-the-shelf consumer-grade sports watches equipped with HR sensor chest straps have been tested against 12-lead Holter monitors under extreme conditions (mountain running), providing highly comparative measures in time (effect size of <0.2) and frequency (no difference) domains (Caminal et al., 2018). Arrays also exists that not only provide ECG and electroencephalography (EEG) monitoring but also transcranial electrical stimulation (Ha et al., 2015).

If non-contact sensors are preferable, then due to the epidermis' translucency, subcutaneous changes in blood flow are measurable through remitted light that is detected using optical sensors (Stamatas et al., 2004). Photoplethysmography (PPG) uses reflected or transmitted light to non-invasively measure blood volume pulse (BVP) (Allen, 2007) and HRV acquired using PPG has high comparability to ECG-acquired signals in time and frequency domains (Lu et al., 2009). PPG has been used to measure HRV using Independent Component Analysis from color channel signals of digital footage captured by a standard digital single-lens reflex camera of participants' faces to find a significant ($p$ = 0.005) increase in LF/HF ratio during cognitive loading compared to resting baseline (McDuff et al., 2016). Recently, invisible near-infrared illumination has been used to capture PPG data for HRV analysis in darkness (Yu et al., 2018), though this has not yet been compared with ECG-derived HRV. Early PPG approaches to measure HRV using RMTs were susceptible to light and movements artifacts but as machine learning algorithms have improved, it is now possible for users to self-record using off-the-shelf smartphones with digital cameras to collect HRV data comparable to sensor data (Huang and Dung, 2016), though, again, this has not yet been field-tested against the gold-standard Holter monitoring.

Therefore, depending on the primary aims of a study, wearable or device-based RMT-based HRV collection can be routinely deployed and the selection of which means of data collection can be led by the primary outcomes of interest: If passive collection (i.e., not requiring the subject have an active role in data collection) are key requirements, then wearable sensors are preferable. If HRV is a covariate or secondary measure, then the convenience of camera-based PPG may be more suitable. The RMT selection process is challenging and technical experts should always be consulted, due to the speed with which technology is updated and the wealth of available options (**Figure 2**; Owens et al., 2020b). RMTs can assess a spectrum of motor, physiological or psychological parameters and are often suitable for up-scaling to larger cohorts after feasibility and pilot studies have been run. Guidance is available, such as the RADAR device-selection framework (Polhemus et al., 2019), which uses a Human-Centered Design strategy to build a three-stage iterative framework of preparation by exploring potential

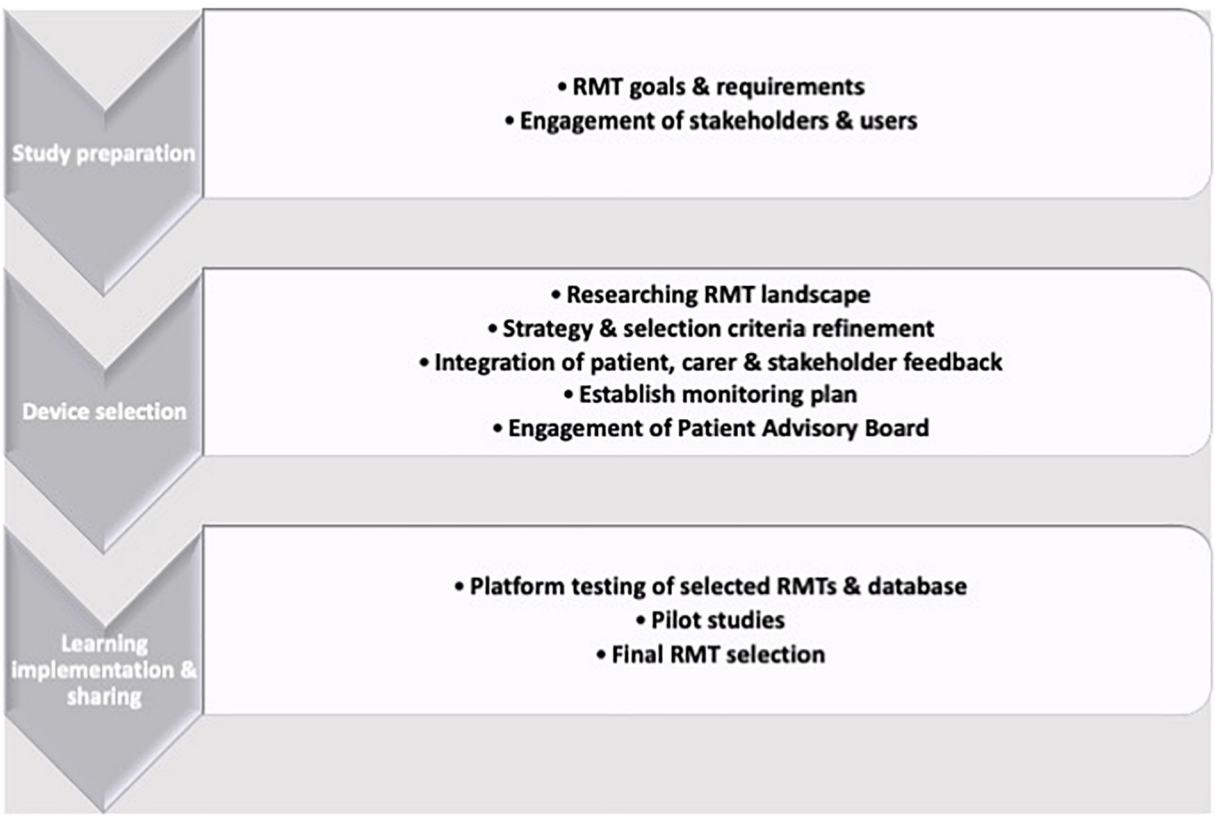



**FIGURE 2 |** An example of a remote measurement technology device selection framework.

approaches, RMT selection by exploration and choice refinement before learning from and acting on feedback and outcomes.

## POTENTIAL ISSUES AND BARRIERS

Data privacy and security remain substantial concerns for users, developers, clinicians, researchers, and regulators (Kaplan, 2018). Although patients with psychiatric and neurological disorders express enthusiasm for using RMTs for clinical and research purposes and RMTs are being increasingly used in dementia research (Czaja et al., 2017), only 17% of those age >80 years use smartphones (Anderson and Perrin, 2017). Moreover, after initial user enthusiasm and adherence, significant reduction in usage can occur if this is not monitored (Dorsey et al., 2017). Theories of adoption, such as the "Technology Acceptance Model" (Holden and Karsh, 2010) the "Unified Theory of Acceptance and Use of Technology" (Venkatesh et al., 2003) emphasize that developers must "know their customers." It is key that potential participants be part of the RMT selection process through workshops, patient advisory boards or feasibility studies to fully understand participants' perspectives. Such initiatives highlight the relevance of health-related factors, such as symptom intensity or severity, user-related factors, such as perceived utility, and technology-related factors, such as intrusiveness as important issues in RMT use for patients (Simblett et al., 2019). Clinicians and researchers have also raised ethical concerns about how to inform users of potential detectable downturns in physical and mental health and the effects such news may have on the user (Kaplan, 2018).

For widespread implementation, RMTs must be deployed to measure relevant, and sensitive variables. The wide variety of RMTs in the marketplace, makes selection challenging, particularly as manufacturers continually update their products, offering further challenges for planned deployment in existing healthcare systems. A further potential complication with many consumer devices is that they only provide aggregated rather than high-resolution raw data, complicating cross-device analysis and statistical analysis. Previous studies have indicated HRV could can provide some additional clinically relevant insight into health status (see What role can remotely captured heart rate variability play in telemedicine?), the advent of RMTs that capture indices of HRV offers the prospect of collecting relevant real-time data for clinical purposes. could therefore provide. This will require exploring the feasibility of deployment of RMT-based HRV as a meaningful clinical tool that enhances traditional methods and other digital biomarkers via robust piloting to standardize and define the most relevant temporal and spectral indices of HRV for the particular cohort and how artifacts or missing data can be mitigated.

## CONCLUSION

Many RMTs measure HRV, even consumer-grade wearables. HRV offers insights into neurovisceral processes in health and disorders of brain, body and behavior but has yet to be fully potentiated in the digital age. The use of RMTs to capture HRV and other CNS and ANS parameters can provide more detailed data across different contexts, such as activities of daily living or interventions and behavioral tasks. RMT-based HRV therefore has potential value as an adjunct digital biomarker in that has the potential to add continuously updated, objective and relevant data to typical clinical methodologies.

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

## FUNDING

The RADAR-AD project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No. 806999. This Joint Undertaking receives support from the European Union's Horizon 2020 Research and Innovation Programme and EFPIA and their associated partners.

## ACKNOWLEDGMENTS

This communication reflects the views of the RADAR-AD consortium and neither IMI nor the European Union and EFPIA are liable for any use that may be made of the information contained herein. The author would like to thank his colleges at KCL and RADAR-AD.

## REFERENCES

Aarsland, D., Rongve, A., Piepenstock Nore, S., Skogseth, R., Skulstad, S., Ehrt, U., et al. (2008). Frequency and case identification of dementia with Lewy bodies using the revised consensus criteria. *Dement Geriatr. Cogn. Disord.* 26, 445-452. doi: 10.1159/000165917

Akintola, A. A., van de Pol, V., Bimmel, D., Maan, A. C., and van Heemst, D. (2016). Comparative analysis of the equivital EQ02 lifemonitor with holter ambulatory ECG device for continuous measurement of ECG, heart rate, and heart rate variability: a validation study for precision and accuracy. *Front. Physiol.* 7:391. doi: 10.3389/fphys.2016.00391

Allan, L., McKeith, I., Ballard, C., and Kenny, R. A. (2006). The prevalence of autonomic symptoms in dementia and their association with physical activity, activities of daily living and quality of life. *Dement Geriatr. Cogn. Disord.* 22, 230-237. doi: 10.1159/000094971

Allen, J. (2007). Photoplethysmography and its application in clinical physiological measurement. *Physiol. Meas.* 28, R1-R39. doi: 10.1088/0967-3334/28/3/R01

Amino, T., Orimo, S., Itoh, Y., Takahashi, A., Uchihara, T., and Mizusawa, H. (2006). Profound cardiac sympathetic denervation occurs in parkinson disease. *Brain Pathol.* 15, 29-34. doi: 10.1111/j.1750-3639.2005.tb00097.x

Anderson, M., and Perrin, A. (2017). *Tech Adoption Climbs Among Older Adults*. Washington, DC: Pew Research Center.

Arslantaş, H., Adana, F., AbacigİI ErgİN, F., Kayar, D., and Acar, G. (2015). Loneliness in elderly people, associated factors and its correlation with quality of life: a field study from Western Turkey. *Iran J. Public Health* 44, 43-50.

Bagnall, R. D., Crompton, D. E., Petrovski, S., Lam, L., Cutmore, C., Garry, S. I., et al. (2016). Exome-based analysis of cardiac arrhythmia, respiratory control, and epilepsy genes in sudden unexpected death in epilepsy. *Ann. Neurol.* 79, 522-534. doi: 10.1002/ana.24596

Behbahani, S., Jafarnia Dabanloo, N., Motie Nasrabadi, A., Teixeira, C. A., and Dourado, A. (2014). A new algorithm for detection of epileptic seizures based on HRV signal. *J. Exp. Theor. Artif. Intell.* 26, 251-265. doi: 10.1080/0952813X.2013.861874

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin. Proc.* 68, 988-1001. doi: 10.1016/S0025-6196(12)62272-1

Benarroch, E. E., Schmeichel, A. M., Low, P. A., Boeve, B. F., Sandroni, P., and Parisi, J. E. (2005). Involvement of medullary regions controlling sympathetic output in Lewy body disease. *Brain* 128(Pt. 2), 338-344. doi: 10.1093/brain/awh376

Boyett, M., Wang, Y., and D'Souza, A. (2019). CrossTalk opposing view: heart rate variability as a measure of cardiac autonomic responsiveness is fundamentally flawed. *J. Physiol.* 597, 2599-2601. doi: 10.1113/JP277501

Cacioppo, S., Capitanio, J. P., and Cacioppo, J. T. (2014). Toward a neurology of loneliness. *Psychol. Bull.* 140, 1464-1504. doi: 10.1037/a0037618

Caminal, P., Sola, F., Gomis, P., Guasch, E., Perera, A., Soriano, N., et al. (2018). Validity of the Polar V800 monitor for measuring heart rate variability in mountain running route conditions. *Eur. J. Appl. Physiol.* 118, 669-677. doi: 10.1007/s00421-018-3808-0

Coravos, A., Khozin, S., and Mandl, K. D. (2019). Developing and adopting safe and effective digital biomarkers to improve patient outcomes. *NPJ Digit Med.* 2:14. doi: 10.1038/s41746-019-0090-4

Critchley, H. D., Corfield, D. R., Chandler, M. P., Mathias, C. J., and Dolan, R. J. (2000). Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. *J. Physiol.* 523(Pt. 1), 259-270. doi: 10.1111/j.1469-7793.2000.t01-1-00259.x

Critchley, H. D., Mathias, C. J., Josephs, O., O'Doherty, J., Zanini, S., Dewar, B. K., et al. (2003). Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain* 126, 2139-2152. doi: 10.1093/brain/awg216

Czaja, S., Gold, M., Bain, L. J., Hendrix, J. A., and Carrillo, M. C. (2017). Potential roles of digital technologies in clinical trials. *Alzheimer Dement.* 13, 1075-1076. doi: 10.1016/j.jalz.2017.08.001

Dantzer, R., and Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain Behav. Immun.* 21, 153-160. doi: 10.1016/j.bbi.2006.09.006

Davis, M. M., Freeman, M., Kaye, J., Vuckovic, N., and Buckley, D. I. (2014). A systematic review of clinician and staff views on the acceptability of incorporating remote monitoring technology into primary care. *Telemed. J.E Health* 20, 428-438. doi: 10.1089/tmj.2013.0166

Dorsey, E. R., Chan, Y. F., Mcconnell, M. V., Shaw, S. Y., Trister, A. D., and Friend, S. H. (2017). The use of smartphones for health research. *Acad. Med.* 92, 157–160. doi: 10.1097/acm.0000000000001205

Eccles, J. A., Owens, A. P., Mathias, C. J., Umeda, S., and Critchley, H. D. (2015). Neurovisceral phenotypes in the expression of psychiatric symptoms. *Front. Neurosci.* 9:4. doi: 10.3389/fnins.2015.00004

Epstein, F. H., and Reichlin, S. (1993). Neuroendocrine-Immune Interactions. *New Engl. J. Med.* 73, 1049-1061. doi: 10.1056/NEJM199310213291708

Fleisher, L. A., Frank, S. M., Sessler, D. I., Cheng, C., Matsukawa, T., and Vannier, C. A. (1996). Thermoregulation and heart rate variability. *Clin. Sci.* 90, 97-103. doi: 10.1042/cs0900097

Frazier, T. W., Strauss, M. E., and Steinhauer, S. R. (2004). Respiratory sinus arrhythmia as an index of emotional response in young adults. *Psychophysiology* 41, 75-83. doi: 10.1046/j.1469-8986.2003.00131.x

Freeman, R. (2008). Clinical practice. Neurogenic orthostatic hypotension. *N. Engl. J. Med.* 358, 615-624. doi: 10.1056/NEJMcp074189

Freeman, R., Wieling, W., Axelrod, F. B., Benditt, D. G., Benarroch, E., Biaggioni, I., et al. (2011). Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Auton. Neurosci. Basic Clin.* 21, 69-72. doi: 10.1016/j.autneu.2011.02.004

Frysinger, R. C., Engel, J., and Harper, R. M. (1993). Interictal heart rate patterns in partial seizure disorders. *Neurology* 43, 2136-2139. doi: 10.1212/WNL.43.10.2136

Gascón-Bayarri, J., Reñé, R., Del Barrio, J. L., De Pedro-Cuesta, J., Ramón, J. M., Manubens, J. M., et al. (2007). Prevalence of dementia subtypes in El Prat de Llobregat, Catalonia, Spain: The PRATICON study. *Neuroepidemiology* 28, 224-234. doi: 10.1159/000108597

Goehler, L. E., Gaykema, R. P. A., Hansen, M. K., Anderson, K., Maier, S. F., and Watkins, L. R. (2000). Vagal immune-to-brain communication: a visceral chemosensory pathway. *Auton. Neurosci. Basic Clin.* 85, 49-59. doi: 10.1016/S1566-0702(00)00219-8

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255-1261. doi: 10.1113/expphysiol.2010.056259

Ha, U., Lee, Y., Kim, H., Roh, T., Bae, J., Kim, C., et al. (2015). "A Wearable EEG-HEG-HRV Multimodal System with Simultaneous Monitoring of tES for Mental Health Management," in *Proceedings of the IEEE Transactions on Biomedical Circuits and Systems*, Piscataway, NJ.

Hacihasanoğlu, R., Yildirim, A., and Karakurt, P. (2012). Loneliness in elderly individuals, level of dependence in activities of daily living (ADL) and influential factors. *Arch. Gerontol. Geriatr.* 54, 61-66. doi: 10.1016/j.archger.2011.03.011

Halaris, A. (2013). Inflammation. Heart Disease, and Depression. *Curr. Psychiatry Rep.* 15:400. doi: 10.1007/s11920-013-0400-5

Halaris, A. (2017). Inflammation-associated co-morbidity between depression and cardiovascular disease. *Curr. Top. Behav. Neurosci.* 31, 45-70. doi: 10.1007/7854_2016_28

Holden, R. J., and Karsh, B. T. (2010). The Technology Acceptance Model: Its past and its future in health care. *J. Biomed. Inform.* 43, 159-172. doi: 10.1016/j.jbi.2009.07.002

Holmgren, S., Hjorth, E., Schultzberg, M., Lärksäter, M., Frenkel, D., Tysen-Bäckström, A. C., et al. (2014). Neuropsychiatric symptoms in dementia-A role for neuroinflammation? *Brain Res. Bull.* 108, 88-93. doi: 10.1016/j.brainresbull.2014.09.003

Huang, R. Y., and Dung, L. R. (2016). Measurement of heart rate variability using off-the-shelf smart phones. *Biomed. Eng. Online.* 15:11. doi: 10.1186/s12938-016-0127-8

Hyett, M. P., Breakspear, M. J., Friston, K. J., Guo, C. G., and Parker, G. B. (2015). Disrupted effective connectivity of cortical systems supporting attention and interoception in melancholia. *JAMA Psychiatry* 72, 350-358. doi: 10.1001/jamapsychiatry.2014.2490

Iodice, V., Low, D. A., Vichayanrat, E., and Mathias, C. J. (2011). Cardiovascular autonomic dysfunction in MSA and Parkinson's disease: Similarities and differences. *J. Neurol. Sci.* 310, 133-138. doi: 10.1016/j.jns.2011.07.014

Isik, A. T., Kocyigit, S. E., Smith, L., and Aydin, A. E. (2019). Comparison of the prevalence of orthostatic hypotension between older patients with Alzheimer's Disease, Lewy body dementia, and without dementia. *Exp Gerontol.* 124:110628. doi: 10.1016/j.exger.2019.06.001

Iwanaga, K., Wakabayashi, K., Yoshimoto, M., Tomita, I., Satoh, H., Takashima, H., et al. (1999). Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. *Neurology* 52, 1269-1271. doi: 10.1212/WNL.52.6.1269

Jeppesen, J., Fuglsang-Frederiksen, A., Johansen, P., Christensen, J., Wüstenhagen, S., Tankisi, H., et al. (2019). Seizure detection based on heart rate variability using a wearable electrocardiography device. *Epilepsia* 60, 2105-2113. doi: 10.1111/epi.16343

Kaplan, M. (2018). Happy with a 20% chance of sadness. *Nature* 563, 20-22. doi: 10.1038/d41586-018-07181-8

Kaufmann, H., Norcliffe-Kaufmann, L., Palma, J. A., Biaggioni, I., Low, P. A., Singer, W., et al. (2017). Natural history of pure autonomic failure: a united states prospective cohort. *Ann Neurol* 81, 287-297. doi: 10.1002/ana.24877

Kelley, K. W., Bluthé, R. M., Dantzer, R., Zhou, J. H., Shen, W. H., Johnson, R. W., et al. (2003). Cytokine-induced sickness behavior. *Brain Behav. Immun.* 29, 247-264. doi: 10.1016/S0889-1591(02)00077-6

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., and Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage* 44, 213-222. doi: 10.1016/j.neuroimage.2008.07.056

Lipp, A., Sandroni, P., Ahlskog, J. E., Fealey, R. D., Kimpinski, K., Iodice, V., et al. (2009). Prospective differentiation of multiple system atrophy from Parkinson

disease, with and without autonomic failure. *Arch. Neurol.* 66, 742-750. doi: 10.1001/archneurol.2009.71

Lu, G., Yang, F., Taylor, J. A., and Stein, J. F. (2009). A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J. Med. Eng. Technol.* 33, 634-641. doi: 10.3109/03091900903150998

Malliani, A., Pagani, M., Lombardi, F., and Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84, 482-492. doi: 10.1161/01.CIR.84.2.482

Marhe, R., and Franken, I. (2014). Error-related brain activity as a biomarker for cocaine relapse. *Neuropsychopharmacology* 39:241. doi: 10.1038/npp.2013.245

Matthews, S. C., Paulus, M. P., Simmons, A. N., Nelesen, R. A., and Dimsdale, J. E. (2004). Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *Neuroimage* 22, 1151-1156. doi: 10.1016/j.neuroimage.2004.03.005

McDuff, D. J., Hernandez, J., Gontarek, S., and Picard, R. W. (2016). "COGCAM: Contact-free measurement of cognitive stress during computer tasks with a digital camera," in *Proceedings of the Conference on Human Factors in Computing Systems*, New York, NY.

Moak, J. P., Goldstein, D. S., Eldadah, B. A., Saleem, A., Holmes, C., Pechnik, S., et al. (2007). Supine low-frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Hear Rhythm.* 4, 1522-1529. doi: 10.1016/j.hrthm.2007.07.019

Myint, A. M. (2012). Kynurenines: from the perspective of major psychiatric disorders. *FEBS J.* 279, 1375-1385. doi: 10.1111/j.1742-4658.2012.08551.x

Narayan, V. A., and Manji, H. K. (2016). Moving from "diagnose and treat" to "predict and pre-empt" in neuropsychiatric disorders. *Nat. Rev. Drug Discov.* 15, 71-72. doi: 10.1038/nrd.2015.20

Neff, R. A., Wang, J., Baxi, S., Evans, C., and Mendelowitz, D. (2003). Respiratory sinus arrhythmia: Endogenous activation of nicotinic receptors mediates respiratory modulation of brainstem cardioinhibitory parasympathetic neurons. *Circ. Res.* 93, 565-572. doi: 10.1161/01.RES.0000090361.45027.5B

Niimi, Y., Ito, S., Murate, K., Hirota, S., Hikichi, C., Ishikawa, T., et al. (2017). Usefulness of combining 123I-FP-CIT-SPECT striatal asymmetry index and cardiac 123I-metaiodobenzylguanidine scintigraphy examinations for diagnosis of parkinsonisms. *J. Neurol. Sci.* 377, 174-178. doi: 10.1016/j.jns.2017.04.026

Nuvoli, S., Spanu, A., Piras, M. R., Nieddu, A., Mulas, A., Rocchitta, G., et al. (2017). 123I-ioflupane brain SPECT and 123I-MIBG cardiac planar scintigraphy combined use in uncertain parkinsonian disorders. *Medicine* 96:e6967. doi: 10.1097/md.0000000000006967

Odagiri, H., Baba, T., Nishio, Y., Iizuka, O., Matsuda, M., Inoue, K., et al. (2016). On the utility of MIBG SPECT/CT in evaluating cardiac sympathetic dysfunction in lewy body diseases. *PLoS One* 11:e0152746. doi: 10.1371/journal.pone.0152746

Onnela, J. P., and Rauch, S. L. (2016). Harnessing smartphone-based digital phenotyping to enhance behavioral and mental health. *Neuropsychopharmacology* 41, 1691-1696. doi: 10.1038/npp.2016.7

Ori, Z., Monir, G., Weiss, J., Sayhouni, X., and Singer, D. H. (1992). Heart rate variability: frequency domain analysis. *Cardiol. Clin.* 10, 499-537. doi: 10.1016/S0733-8651(18)30231-5

Owens, A. P., Allen, M., Ondobaka, S., and Friston, K. J. (2018a). Interoceptive inference: From computational neuroscience to clinic. *Neurosci. Biobehav. Rev.* 90, 174-183. doi: 10.1016/j.neubiorev.2018.04.017

Owens, A. P., Ballard, C., Beigi, M., Kalafatis, C., Brooker, H., Lavelle, G., et al. (2020a). Implementing remote memory clinics to enhance clinical care during and after COVID-19. *Front. Psychiatry*. 11:579934. doi: 10.3389/fpsyt.2020.579934

Owens, A. P., David, A. S., Low, D. A., Mathias, C. J., and Sierra-Siegert, M. (2015). Abnormal cardiovascular sympathetic and parasympathetic responses to physical and emotional stimuli in depersonalization disorder. *Front. Neurosci.* 9:89. doi: 10.3389/fnins.2015.00089

Owens, A. P., Friston, K. J., Low, D. A., Mathias, C. J., and Critchley, H. D. (2018b). Investigating the relationship between cardiac interoception and autonomic cardiac control using a predictive coding framework. *Auton. Neurosci. Basic Clin.* 210, 65-71. doi: 10.1016/j.autneu.2018.01.001

Owens, A. P., Hinds, C., Manyakov, N. V., Stavropoulos, T. G., Lavelle, G., Gove, D., et al. (2020b). Selecting remote measurement technologies to optimise assessment of function in early Alzheimer's disease: a case study. *Front. Psychiatry* (in press). doi: 10.3389/fpsyt.2020.582207

Owens, A. P., Low, D. A., Iodice, V., Mathias, C. J., and Critchley, H. D. (2017). "Emotion and the autonomic nervous system – a two-way street: insights from affective, autonomic and dissociative disorders," in *Reference Module in Neuroscience and Biobehavioral Psychology*, ed. J. Stein (Elsevier), 1–15. doi: 10.1016/B978-0-12-809324-501799-5

Pant, J. K., and Krishnan, S. (2018). Robust QRS detection for HRV estimation from compressively sensed ECG measurements for remote health-monitoring systems. *Physiol. Meas.* 39:035002. doi: 10.1088/1361-6579/aaa3c9

Perpetuini, D., Cardone, D., Chiarelli, A. M., Filippini, C., Croce, P., Zappasodi, F., et al. (2019). Autonomic impairment in Alzheimer's disease is revealed by complexity analysis of functional thermal imaging signals during cognitive tasks. *Physiol. Meas.* 40:034002. doi: 10.1088/1361-6579/ab057d

Pfeiffer, R. F. (2012). Gastrointestinal dysfunction in Parkinson's disease. *Clin. Neurosci.* 5, 136-146. doi: 10.1201/b12948-26

Polhemus, A. M., Novak, J., Ferrão, J., Simblett, S., Radaelli, M., Locatelli, P., et al. (2019). Human-centered design strategies for device selection in mHealth programs: a novel framework and case study (Preprint). *JMIR Mhealth Uhealth* 8:e16043. doi: 10.2196/preprints.16043

Rahman, F., Pechnik, S., Gross, D., Sewell, L. T., and Goldstein, D. S. (2011). Low frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Clin. Auton. Res.* 21, 133-141. doi: 10.1007/s10286-010-0098-y

Ramirez, K., Fornaguera-Trìas, J., and Sheridan, J. F. (2017). Stress-induced microglia activation and monocyte trafficking to the brain underlie the development of anxiety and depression. *Curr. Top. Behav. Neurosci.* 31, 155-172. doi: 10.1007/7854_2016_25

Roberts, N. A., and Burleson, M. H. (2013). Processes linking cultural ingroup bonds and mental health: the roles of social connection and emotion regulation. *Front. Psychol.* 4:52. doi: 10.3389/fpsyg.2013.00052

Saper, C. B. (2002). The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu. Rev. Neurosci.* 25, 433-469. doi: 10.1146/annurev.neuro.25.032502.111311

Schestatsky, P., Valls-Solé, J., Ehlers, J. A., Rieder, C. R. M., and Gomes, I. (2006). Hyperhidrosis in Parkinson's disease. *Mov. Disord.* 21, 1744-1748. doi: 10.1002/mds.21006

Schiweck, C., Piette, D., Berckmans, D., Claes, S., and Vrieze, E. (2019). Heart rate and high frequency heart rate variability during stress as biomarker for clinical depression. A systematic review. *Psychol. Med.* 49, 200-211. doi: 10.1017/S0033291718001988

Shimmura, M., Uehara, T., Ogata, K., Shigeto, H., Maeda, T., Sakata, A., et al. (2019). Higher postictal parasympathetic activity following greater ictal heart rate increase in right- than left-sided seizures. *Epilepsy Behav.* 97, 161-168. doi: 10.1016/j.yebeh.2019.05.026

Simblett, S., Matcham, F., Siddi, S., Bulgari, V., di San Pietro, C. B., López, J. H., et al. (2019). Barriers to and facilitators of engagement with mHealth technology for remote measurement and management of depression: qualitative analysis. *JMIR Mhealth Uhealth.* 7:e11325. doi: 10.2196/11325

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274-296. doi: 10.1016/j.neubiorev.2017.02.003

Spithoven, A. W. M., Bijttebier, P., and Goossens, L. (2017). It is all in their mind: A review on information processing bias in lonely individuals. *Clin. Psychol. Rev.* 58, 97-114. doi: 10.1016/j.cpr.2017.10.003

Spyer, K. M. (1994). Annual review prize lecture. Central nervous mechanisms contributing to cardiovascular control. *J. Physiol.* 474, 1-19. doi: 10.1113/jphysiol.1994.sp019997

Stamatas, G. N., Zmudzka, B. Z., Kollias, N., and Beer, J. Z. (2004). Non-invasive measurements of skin pigmentation in situ. *Pigment Cell Res.* 17, 618-626. doi: 10.1111/j.1600-0749.2004.00204.x

Stein, P. K., Bosner, M. S., Kleiger, R. E., and Conger, B. M. (1994). Heart rate variability: a measure of cardiac autonomic tone. *Am. Heart J.* 127, 1376-1381. doi: 10.1016/0002-8703(94)90059-0

Taylor, J. A., Carr, D. L., Myers, C. W., and Eckberg, D. L. (1998). Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation* 98, 547-555. doi: 10.1161/01.CIR.98.6.547

Thaisetthawatkul, P., Boeve, B. F., Benarroch, E. E., Sandroni, P., Ferman, T. J., Petersen, R., et al. (2004). Autonomic dysfunction in dementia with Lewy bodies. *Neurology* 62, 1804–1809. doi: 10.1212/01.WNL.0000125192.69777.6D

Thayer, J. F., and Fischer, J. E. (2009). Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. *J. Intern. Med.* 265, 439-447. doi: 10.1111/j.1365-2796.2008.02023.x

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201-216. doi: 10.1016/S0165-0327(00)00338-4

Theorell, T., Liljeholm-Johansson, Y., Björk, H., and Ericson, M. (2007). Saliva testosterone and heart rate variability in the professional symphony orchestra after "public faintings" of an orchestra member. *Psychoneuroendocrinology* 32, 660-668. doi: 10.1016/j.psyneuen.2007.04.006

Van Ravenswaaij-Arts, C. M. A., Kollee, L. A. A., Hopman, J. C. W., Stoelinga, G. B. A., and Van Geijn, H. P. (1993). Heart rate variability. *Ann. Int. Med.* 93, 1043-1065. doi: 10.7326/0003-4819-118-6-199303150-00008

Vanhalst, J., Luyckx, K., Van Petegem, S., and Soenens, B. (2018). The detrimental effects of adolescents' chronic loneliness on motivation and emotion regulation in social situations. *J. Youth Adolesc.* 47, 162-176. doi: 10.1007/s10964-017-0686-4

Vegesna, A., Tran, M., Angelaccio, M., and Arcona, S. (2017). Remote patient monitoring via non-invasive digital technologies: a systematic review. *Telemed. J.E Health* 23, 3-17. doi: 10.1089/tmj.2016.0051

Venkatesh, V., Morris, M. G., Davis, G. B., and Davis, F. D. (2003). User acceptance of information technology: toward a unified view. *Manag. Inf. Syst.* 27, 425-478. doi: 10.2307/30036540

Wakabayashi, K., and Takahashi, H. (1997). Neuropathology of autonomic nervous system in parkinson's disease. *Eur. Neurol.* 38(Suppl. 2), 2-7. doi: 10.1159/000113469

Wan, W., Wetmore, L., Sorensen, C. M., Greenberg, A. H., and Nance, D. M. (1994). Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. *Brain Res. Bull.* 34, 7-14. doi: 10.1016/0361-9230(94)90179-1

Winge, K., and Fowler, C. J. (2006). Bladder dysfunction in Parkinsonism: mechanism, prevalence, symptoms, and management. *Mov. Disord.* 21, 737-745. doi: 10.1002/mds.20867

Yu, X., Paul, M., Antink, C. H., Venema, B., Blazek, V., Bollheimer, C., et al. (2018). "Non-Contact Remote Measurement of Heart Rate Variability using Near-Infrared Photoplethysmography Imaging," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, New Orleans, LA.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 13 November 2020
doi: 10.3389/fnins.2020.588813

# Cardiorespiratory Response to Moderate Hypercapnia in Female College Students Expressing Behaviorally Inhibited Temperament

*Paul F. Martino[1,2], Daniel P. Miller[3], Justin R. Miller[2], Michael T. Allen[4], Denise R. Cook-Snyder[1,2], Justin D. Handy[5] and Richard J. Servatius[6,7]**

[1] Biology Department, Carthage College, Kenosha, WI, United States, [2] Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, United States, [3] Neuroscience Department, Carthage College, Kenosha, WI, United States, [4] School of Psychological Sciences, College of Education and Behavioral Sciences, University of Northern Colorado, Greeley, CO, United States, [5] Naval Submarine Medical Research Laboratory, Groton, CT, United States, [6] United States Department of Veterans Affairs, Syracuse VA Medical Center, Syracuse, NY, United States, [7] Department of Psychiatry, State University of New York Upstate Medical University, Syracuse, NY, United States

OPEN ACCESS

**Edited by:**
Julian F. Thayer,
The Ohio State University,
United States

**Reviewed by:**
Joao Paulo Jacob Sabino,
Federal University of Piauí, Brazil
Luciane H. Gargaglioni,
São Paulo State University, Brazil

***Correspondence:**
Richard J. Servatius
richard.servatius@va.gov

**Specialty section:**
This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience

**Received:** 29 July 2020
**Accepted:** 26 October 2020
**Published:** 13 November 2020

**Citation:**
Martino PF, Miller DP, Miller JR, Allen MT, Cook-Snyder DR, Handy JD and Servatius RJ (2020) Cardiorespiratory Response to Moderate Hypercapnia in Female College Students Expressing Behaviorally Inhibited Temperament. Front. Neurosci. 14:588813. doi: 10.3389/fnins.2020.588813

Behaviorally inhibited (BI) temperament is marked by heightened behavioral sensitivity to environmental threats. The degree to which threat sensitivity is reflected in cardiorespiratory responses has been relatively unexplored. Female college students were exposed to modest hypercapnia (7.0% $CO_2$) or ambient air (AA) while engaging in a computerized task with cued reinforcement features. All physiological variables except for blood pressure were processed in 4 min epochs corresponding to pre-exposure, exposure, and post-exposure. Primary respiratory measures were respiratory frequency ($f_b$), tidal volume ($V_T$), and minute ventilation ($V_E$). Electrocardiograms (ECGs) were processed using ARTiiFACT software with resultant heart rate variability (HRV) measures in the frequency domain and time domain. Consistent with the literature, modest hypercapnia increased $V_T$, $F_b$, and $V_E$. No differences in respiratory parameters were detected between BI and non-behaviorally inhibited individuals (NI). For HRV in the time domain, RMSSD and NN50 values increased during $CO_2$ inhalation which then returned to pre-exposure levels after $CO_2$ cessation. Hypercapnia increased high frequency (HF) power which then recovered. BI exhibited reduced low frequency (LF) power during the pre-exposure period. For NI, LF power reduced over the subsequent phases ameliorating differences between BI and NI. Hypercapnia improved the task performance of BI. This is the largest study of female reactivity to hypercapnia and associated HRV to date. In general, hypercapnia increased time domain HRV and HF power, suggesting a strong vagal influence. Those expressing BI exhibited similar respiratory and HRV reactivity to NI despite inherently reduced LF power. Although 7% $CO_2$ represents a mild challenge to the respiratory and cardiovascular systems, it is nonetheless sufficient to explore inherent difference in stress reactivity in those vulnerable to develop anxiety disorders.

**Keywords: diatheses, temperament, stress, anxiety, heart rate variability, SDNN, vagal activity**

# INTRODUCTION

Anxiety disorders and posttraumatic stress disorder (PTSD) are best understood as a dynamic interaction of aversive experiences in those with inherent vulnerabilities to develop anxious states (Barlow, 2000; Chasiropoulou et al., 2019). A learning diathesis model accentuates individual differences in associative learning as a final common path to pervasive avoidance (Servatius et al., 2008; Beck et al., 2010; Allen et al., 2019), a core feature of anxiety disorders (Mellick et al., 2019), and PTSD (O'Donnell et al., 2007). The process by which avoidance develops may be through the individual differences in perceived aversiveness, the associativity of aversive experiences/ideations or both.

## Behaviorally Inhibited Temperament as a Vulnerability

Behaviorally inhibited (BI) temperament is one such vulnerability. BI is defined as a personality disposition marked by extreme withdrawal in the face of novel non-social or social challenges (Gladstone and Parker, 2005), with numerous studies linking BI to anxiety disorders (Biederman et al., 2001; Gladstone et al., 2005) and PTSD (Myers et al., 2012a,b; Servatius et al., 2017; Handy et al., 2018). Those individuals with BI temperament display associative learning biases, evident as facilitated acquisition of the classically conditioned eyeblink responses in adolescents (Holloway et al., 2012; Caulfield et al., 2015), adults (Allen et al., 2014, 2016), active duty military (Handy et al., 2018), and veterans (Myers et al., 2012a). The psychophysiological basis of associative learning biases in BI individuals is unclear. One line of reasoning focuses on attentional processes as a predisposing factor in learning (Mcauley et al., 2009). Those with BI temperament have difficulty disengaging attention from novel stimuli or stimuli associated with threat or distress (Pérez-Edgar et al., 2010; Ricart et al., 2011; Fox and Pine, 2012; Holloway et al., 2012; White et al., 2017).

## HRV as a Physiological Source of Vulnerability

A common substrate for hypervigilance and associative learning biases is the autonomic nervous system (Friedman, 2007), composed of the sympathetic and parasympathetic branches. Heart rate variability (HRV) is a means of assessing contributions of these two branches through decomposition of successive beat-to-beat variations in heart rate (peak R wave to R wave within the QRS complexes of electrocardiograms) (Berntson et al., 1997). HRV is partitioned in time and frequency domains. For the frequency domain, accepted bands are low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.4 Hz), the latter corresponding to the vagal influences on respiration (i.e., respiratory sinus arrhythmia). In the time domain, three measures are commonly evaluated: the standard deviation of normal to normal (NN) intervals (SDNN), root mean square of successive differences (RMSSD) between normal heartbeats, and the number of pairs of successive NNs that differ by more than 50 ms (NN50). Decreased HRV is evident as reduced LF power and increased HF power in the frequency domain and greater SDNN, RMSSD, and NN50 in the time domain. In general, better sustained attention or vigilance (Luque-Casado et al., 2016) and facilitated associative learning (Tapp et al., 1997) is associated with decreased HRV.

Further, temperaments with inhibitory features have inherently low HRV. For example, individuals expressing harm avoidance display reduced low frequency power (Puttonen et al., 2008; Huang et al., 2013). Moreover, distressed (Type D) personality, a combination of negative affect and social inhibition linked to cardiovascular disease, has been reported to have low HRV (Martin et al., 2010; Bibbey et al., 2015). Both harm avoidance (Allen et al., 2017) and Type D personality (Servatius et al., 2017; Allen et al., 2018; Handy et al., 2018) are highly correlated with BI temperament. Thus, it is reasonable to expect BI to have inherently reduced HRV or alternatively greater reductions of HRV from exposure to challenges.

## Experimental Induced Hypercapnia as a Challenge to HRV

Experimentally induced hypercapnia has a long history as a potent psychophysiological challenge. Panic-like responses are elicited by high concentrations of $CO_2$ delivered in bolus (e.g., 35% $CO_2$) (Van Den Hout et al., 1987; Perna et al., 2003) or lower concentrations (e.g., 5% $CO_2$) delivered over extended exposure (e.g., 20 min) (Gorman et al., 1988; Bystritsky et al., 2000; Valenca et al., 2002). Such challenge parameters are quite demanding and may narrow the range in which to observe differences in those vulnerable to anxiety disorders.

Lower levels of $CO_2$ delivered over shorter durations are more tolerable and produce gradations in demands placed on the system to adjust to hypercapnia from simple increases in tidal volume ($V_t$) (Brown et al., 2014) to increased $V_t$ accompanied by elevated HR (Tzeng et al., 2007). Even at mild levels of hypercapnia, the attendant acidosis drives deeper breathing through central chemoreceptors (Brown and Howden, 2008a). In terms of HRV, mild hypercapnia increases HF power (Brown et al., 2007, 2014; Tzeng et al., 2007). Although HF HRV is closely linked to vagal activity and respiratory sinus arrhythmia (RSA) in general, increased HF power secondary to hypercapnia may be dissociated from RSA (Brown et al., 2014). To date, time domain measures of HRV have been unaffected by mild hypercapnia (Brown et al., 2007). Thus, mild hypercapnia is potent enough to affect both respiratory parameters and HRV, particularly in the frequency domain.

## The Present Study

The present study examined respiratory and cardiovascular reactivity to mild hypercapnia as a function of BI temperament. Hypercapnia was expected to increase HF power. The Adult Measure of Behavioral Inhibition (AMBI) (Gladstone and Parker, 2005) was used to classify individuals as either BI or non-inhibited (NI). Those expressing BI were expected to exhibit reduced HRV. We further expected BI individuals to express more reactivity to mild hypercapnia. After a baseline period, participants engaged in a computerized task that requires attention; those expressing BI tend to perform better than NI

on the task. Onset and terminations of the computer task were contemporaneous with introduction of hypercapnia and its return to ambient air (AA). The computerized task was used as a behavioral indicant of stress reactivity inasmuch as attention to hypercapnic challenge could be manifest as poorer performance on the task through divided attention. While male BI participants tend to perform better than male NI participants, BI females tend to be similar to NI females (Sheynin et al., 2014). Further, females are largely underrepresented in the study of mild hypercapnia and HRV (Brown et al., 2007; Tzeng et al., 2007). Therefore, we restricted this initial study to female participants.

# MATERIALS AND METHODS

## Participants and Recruitment

Undergraduate volunteers were recruited at Carthage College, Kenosha, WI, United States. Ninety-nine females with an age range of 18–22 years voluntarily completed the study. Participants with a self-reported history of psychiatric illnesses, neurological disorders, cardiac disorders, or respiratory illnesses were excluded from the study. Participants were asked to refrain from drinking alcohol or chewing, smoking, or vaping any tobacco products for 24 h prior to the start of the study. Additionally, participants were asked to refrain from eating, exercising, or drinking caffeinated beverages for 2 h prior to the study. All eligible participants completed an informed consent agreement upon arrival for their scheduled study appointments and were given the opportunity to ask questions before initiating study procedures. Participants were not compensated for their participation, but received partial credit toward academic research requirements. The study was approved by the Institutional Review Board of Carthage College (IRB Approval #: 1133068-4) in compliance with all applicable Federal regulations governing protection of human subjects.

## Experimental Design

Participants were randomly assigned to receive either room ambient air (AA groups) through the breathing apparatus or 7% $CO_2$ (7% $CO_2$ groups). There were three phases: Baseline, Exposure, and Recovery. The Exposure and Recovery phases were 4 min each. The Baseline period was 15 min, but only the last 4 min were evaluated to equate with the other two phases for all physiological parameters. The exposure groups were characterized as NI or BI, as elaborated below. Thus, a 2 × 2 design was constructed: AA-NI ($N$ = 29), AA-BI ($N$ = 16), 7% $CO_2$- NI ($N$ = 32), and 7% $CO_2$-BI ($N$ = 21). During informed consent, participants were advised that exposure to hypercapnia was a possibility, but subjects were otherwise blind to assignment. With respect to behavioral inhibition, investigators were blind to the condition.

## Scales

All participants completed the Adult Measure of Behavioral Inhibition (AMBI) which consists of 16 items probing aspects of BI temperament (Gladstone and Parker, 2005; Gladstone et al., 2005). Items assess the degree to which participants exhibit inhibited or avoidant behaviors in new or unfamiliar social and non-social situations on a 3-point Likert-type scale. Possible scores range from 0 to 32 with higher scores indicating higher levels of BI. Consistent with previously established methodology (Allen et al., 2014; Servatius et al., 2017; Handy et al., 2018), individuals were classified as NI or BI based on a cut score of 15.5.

## Computerized Task

Participants were presented with a computerized game to measure attention and performance during modest hypercapnia. The task was adapted from a spaceship game in which a participant defends a base from incoming spaceships which drop bombs, which has been more completely described elsewhere (Sheynin et al., 2015). The software was programmed in SuperCard version 3.7.1 (Solutions Etcetera, Pollock Pines, CA, United States) and presented on a Macintosh iMac computer. The keyboard was masked except for three keys labeled FIRE, LEFT, and RIGHT, which the subject could use to enter responses. Briefly, a participant began with 325 points which could increase or decrease based on performance. The game began with exposure and lasted 8 min.

## Gas Preparation, Delivery, Measurement

The custom system for gas preparation and delivery employed in the present study was previously described (Miller et al., 2018). Ambient air (AA) was mixed with medical grade $CO_2$ using a 13.5 L respirometer/spirometer (P-1300, Warren E. Collins, Inc., Braintree, MA, United States) to achieve a concentration of 7% $CO_2$. This was verified through capnography using an $O_2$CAP O2 and $CO_2$ analyzer (Oxigraf, 07-7021). Once the gases were mixed, the mixture was transferred from the respirometer/spirometer to 300 g weather balloons, which could store up to 1600 L of gases for at least 1 h without any change in the concentrations of gases.

## Experimental Procedures

Data collection occurred during one of two 1-h time slots at 1400 and 1500 h 5 days a week (Monday–Friday). All participants were seated in the upright position in front of a computer desk which displayed a computerized task described above. Upon signing informed consent, blood pressure measurements were taken while seated upright with both arms resting on the deck in front of them, using a Leader fully automated blood pressure monitor (BP-3AG1-1PLDR, Cardinal Health). Subjects were outfitted with a face mask system that covered both the mouth and the nose and was connected to a two-way non-rebreathing valve and pneumotach (Model 3813, Hans Rudolph, Shawnee, KS, United States). Participants were also instrumented for ECG collection. Baseline acclimation to the apparatus was 15 min. During the acclimation period participants completed the demographic data and scales. At the end of the baseline period, the computerized task began. The task period was 8-min. During the first 4-min task period participants were exposed to AA or 7% $CO_2$. During the last 4-min task period all participants were exposed to AA. Upon the task completion a second blood pressure measure was obtained.

## Respiration and ECG Collection and Processing

Airflow as minute ventilation ($V_E$; L/min), breathing frequency (fb; breaths/min), and tidal volume ($V_T$; L) were all measured with an airflow transducer and digital data acquisition module (Bio Pac MP36 and MP150, Goleta, CA, United States).

Three-lead electrocardiogram (ECG) was collected at 100 Hz with BIOPAC models (ECG 100C and MEC 110C) and digital data acquisition module (MP150). ECGs were extracted for the 4 min prior to start of task, 4 min of exposure (7% $CO_2$ or AA), and 4 min of recovery. Subjects without complete records for the all three 4-min epochs were excluded from analysis. ECG was processed with ARTiiFACT software (Kaufmann et al., 2011). Interbeat intervals were computed from identified and visualized R waves. Artifacts were identified through Berntson detection and cubic spline correction. HRV parameters were computed with standard values of 4 Hz interpolation rate and 50% window overlap with standard frequency bands 0.04, 0.15, and 0.4 parameters. The very low frequency band (0.04–0.15 Hz) was otherwise ignored. Consistent with the literature, the normalized value of power (nu) as well as the natural log (ln) of raw power of LF and HF were analyzed to interpret power changes attributable to a particular band (Quintana and Heathers, 2014; Quintana et al., 2016). nuLF and nuHF are complementary, thus analysis of one is sufficient to understand power has changed with experimental conditions, but still not *specific* to either band. Analysis of the raw power is necessary to understand the nature of changes in nuLF/nuHF (Heathers, 2014).

## Analytic Approach

All statistical analyses were performed in IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, United States) and R (R Core Team, 2020, Vienna, Austria). Respiratory measures and HRV measures were subjected to separate 2 (Exposure; AA vs. $CO_2$) × 2 (Temperament; NI vs. BI) × 3 [Phase; Baseline (B), Exposure (E), and Recovery (R)] mixed analyses of variance (ANOVAs) with $p < 0.05$. Blood pressure measured prior to baseline recording and after recovery was analyzed with Exposure × Temperament × Time (pre vs. post) mixed ANOVA. Behavioral performance was analyzed with Exposure × Temperament multivariate ANOVA.

# RESULTS

## Respiratory Parameters

Breathing frequency increased in all participants during the Exposure phase, which corresponded with the start of the computerized task. Breathing frequency increased from 14 breathes/min during baseline to 16–17 breathes/min during the Exposure and Recovery phases (See **Table 1**). The main effect of Phase was significant, $F(2,178) = 87.3$; $\eta_p^2 = 0.58$, $p < 0.001$. As expected, hypercapnia specifically increased $V_T$, $V_E$, and $V_I$. Exposure × Phase interactions were appear for both $V_T$, $F(2,178) = 23.6$; $\eta_p^2 = 0.21$, and $V_E$, $F(2,178) = 35.8$; $\eta_p^2 = 0.29$, all $p$'s $< 0.002$ (See **Table 1**). In addition, the time of inspiration ($T_I$) and expiration ($T_E$) were analyzed. Hypercapnia induced shorter $V_I$ without altering $V_E$. For the $V_i$, an Exposure × Phase interaction, $F(2,178) = 6.6$, $\eta_p^2 = 0.07$. Although hypercapnia induced quicker inhalation with greater depth, there were no main effects or interactions with BI.

## HR and HRV

Analysis of HR only yielded a significant main effect of Phase, $F(2,178) = 11.5$, $\eta_p^2 = 0.115$. HR generally decreased from Baseline to the Exposure phase. Accordingly, average RR intervals generally increased from the Baseline to the Exposure phase (See **Table 1**). The exposure phase was coincident with the start of the computerized task, thus general phases changes in HR and RR intervals may reflect attention to the task.

For the time domain, Exposure × Phase interactions were evident for SDNN, $F(2,178) = 4.1$, $p = 0.02$, $\eta_p^2 = 0.04$, RMSSD, $F(2,178) = 6.5$, $p = 0.002$, $\eta_p^2 = 0.07$, and NN50, $F(2,178) = 9.7$, $p < 0.001$, $\eta_p^2 = 0.10$ (see **Figure 1**). For each,

**TABLE 1 |** Respiratory and cardiovascular parameters over experiment epochs within ambient air (AA) and 7% $CO_2$ conditions in BI and NI participants.

| | Baseline | Exposure | Recovery |
|---|---|---|---|
| **AA-NI** | | | |
| HR | 77.8 ± 2.1 | 75.5 ± 2.1 | 77.9 ± 2.1 |
| Mean RR | 786.2 ± 20.6 | 809.9 ± 21.2 | 784.0 ± 20.6 |
| Breathing frequency (b/min) | 13.8 ± 0.7 | 16.0 ± 0.7* | 16.6 ± 0.7 |
| $V_T$ (L) | 0.9 ± 0.04 | 0.8 ± 0.04 | 0.7 ± 0.03 |
| $V_E$ (L/min) | 11.3 ± 0.5 | 11.7 ± 0.5 | 11.5 ± 0.4 |
| $T_I$ (s) | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.4 ± 0.1 |
| $T_E$ (s) | 1.1 ± 0.1 | 1.0 ± 0.1 | 0.9 ± 0.1 |
| **AA-BI** | | | |
| HR | 78.4 ± 2.7 | 75.4 ± 2.7 | 77.8 ± 2.7 |
| Mean RR | 777.6 ± 25.9 | 808.3 ± 26.7 | 782.6 ± 25.9 |
| Breathing frequency (b/min) | 14.2 ± 0.9 | 17.3 ± 0.8* | 17.2 ± 0.8 |
| $V_T$ (L) | 0.8 ± 0.06 | 0.7 ± 0.05 | 0.6 ± 0.04 |
| $V_E$ (L/min) | 10.6 ± 0.6 | 10.9 ± 0.6 | 10.7 ± 0.5 |
| $T_I$ (s) | 2.7 ± 0.1 | 1.8 ± 0.1* | 2.1 ± 0.1* |
| $T_E$ (s) | 1.3 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 |
| **7% $CO_2$-NI** | | | |
| HR | 81.0 ± 2.0 | 79.6 ± 2.0 | 81.9 ± 2.0 |
| Mean RR | 750.6 ± 19.5 | 765.6 ± 20.1 | 745.6 ± 19.5 |
| Breathing frequency (b/min) | 13.9 ± 0.7 | 16.9 ± 0.6* | 17.3 ± 0.6 |
| $V_T$ (L) | 0.9 ± 0.05 | 1.0 ± 0.04* | 0.8 ± 0.03 |
| $V_E$ (L/min) | 11.2 ± 0.4 | 15.4 ± 0.5* | 13.3 ± 0.3 |
| $T_I$ (s) | 2.8 ± 0.2 | 2.3 ± 0.1 | 2.4 ± 0.1 |
| $T_E$ (s) | 1.0 ± 0.2 | 0.8 ± 0.1 | 1.0 ± 0.1 |
| **7% $CO_2$-BI** | | | |
| HR | 80.8 ± 2.5 | 80.5 ± 2.5 | 81.3 ± 2.5 |
| Mean RR | 764.0 ± 24.5 | 765.1 ± 25.3 | 756.4 ± 24.5 |
| Breathing frequency (b/min) | 14.1 ± 0.8 | 16.5 ± 0.8* | 17.5 ± 0.8 |
| $V_T$ (L) | 0.9 ± 0.06 | 1.0 ± 0.05* | 0.8 ± 0.04 |
| $V_E$ (L/min) | 11.1 ± = 0.5 | 15.9 ± 0.6* | 13.5 ± 0.4 |
| $T_I$ (s) | 2.5 ± 0.2 | 1.8 ± 0.1* | 2.0 ± 0.1* |
| $T_E$ (s) | 1.0 ± 0.2 | 0.9 ± 0.1 | 0.8 ± 0.1 |

** indicates significant change from Baseline, $p < 0.05$.*

HRV increased during hypercapnia whereas increases were not evident in those given AA. For SSDN, the AA group reduced vagal tone across the experimental phases. There were no main effects or interactions with BI.

For the frequency domain, normalized frequencies (nuLF or nuHF) yielded significant interactions of Exposure × Phase, $F(2,178) = 3.5$, $p = 0.03$; $\eta_p^2 = 0.04$ (See **Figure 2**, left panel) and BI × Phase, $F(2,178) = 3.8$, $p = 0.02$; $\eta_p^2 = 0.04$, (See **Figure 2**, right panel). Interpretation of normalized power requires analysis of the individual lnLF and lnHF power. An analysis of lnHF power indicated an Exposure × Phase interaction, $F(2,178) = 6.4$, $p = 0.002$; $\eta_p^2 = 0.07$ (See **Figure 3**, left panel), without significant effects related to BI. This supports the interpretation that differences in normalized power attributable to $CO_2$ exposure reflected an increase in HF power. Whereas an analysis of lnLF power showed a BI × Phase interaction, $F(2,178) = 3.4$, $p = 0.03$; $\eta_p^2 = 0.034$, without effects of Exposure (See **Figure 3**, right panel). This supports the interpretation that BI × Phase interaction in normalized power was due to decreased lnLF.

## Blood Pressure

Blood pressure, measured before donning the mask and after its removal, did not change as a function of Exposure, BI or time, all $p$'s $> 0.35$. Mean arterial pressure before the experiment was $92.4 \pm 0.9$ mmHg and $91.9 \pm 1.4$ mmHg after.

## Behavior

There were significant BI × Exposure interactions for shots fired, $F(1,81) = 4.04$, $\eta_p^2 = 0.04$, $p = 0.048$, and total points scored, $F(1,81) = 4.63$, $\eta_p^2 = 0.54$, $p = 0.034$. No significant effects were noted for screen movement. For NI individuals, exposure to $CO_2$ tended to inhibit performance, whereas BI individuals exposed to $CO_2$ performed better (See **Figure 4**).

# DISCUSSION

The current protocol examined respiratory and cardiovascular adaptations to moderate hypercapnia in female BI and NI undergraduates while performing an engaging computerized task.

## Task-Related Adjustments

The task, a space-based game, was coincidental with the exposure period. Therefore, changes in respiratory and cardiovascular parameters apparent in both AA and 7% $CO_2$ groups are interpreted as adaptations to attending to the task and task performance. Such general changes were apparent as a modest bradycardia and increased respiratory rate. It is against this background that effects from hypercapnia are evaluated.

## Hypercapnia Induces Modest Respiratory Adjustments

Overall, hypercapnia induced modest but robust adjustments in respiratory parameters measured. Hypercapnia increased $V_t$ and increased $V_E$, compared to the participant's baseline and relative to those given AA. Ti also decreased during 7% $CO_2$ exposure. The pattern of adjustments in respiration parameters are similar to the work of others (Brown et al., 2007; Tzeng et al., 2007), although the magnitude of changes in $V_T$ appear less which may reflect differences in the composition of the participant pool.

## Hypercapnia and HRV

Recording of ECG concomitant with respiratory parameters afforded the opportunity to assess the impact of hypercapnia on HRV. The recording parameters were non-optimal (100 Hz sampling frequency over successive 4-min epochs), thus these results need to be understood under this light. Consistent with others, hypercapnia increased nuHF relative to their baseline and the AA group (Brown et al., 2014). Inasmuch as increased nuHF may be a function of increased HF or lowered LF, the individual frequencies were assessed (Laborde et al., 2017). A corresponding interaction showing increased lnHF was apparent, supporting the interpretation that hypercapnia increased HF power. Increased HF power in response to hypercapnia is consistent with the work of others (Brown et al., 2007, 2014). Further, hypercapnia induced increases in HRV in the time domain. An increase in vagal power was apparent with lnSDNN, lnRMSSD, and NN50, with a recovery evident in NN50. The increases were apparent with and without respiration rates included as a covariate in the analyses. The increase in HRV was apparent in the presence of an engaging computerized task that was contemporaneous, albeit not exclusive to hypercapnia. Previous work assessing time domain HRV in moderate hypercapnia have not found differences (Brown et al., 2007, 2014). One potential difference may be the body position of individuals during exposure. In previous studies individuals were supine during exposure; in this study, individuals were seated. Inasmuch as time domain measures of HRV are considerably less in the seated compared to supine position (Young and Leicht, 2011), seated exposure may provide a greater dynamic range to observe increases from hypercapnia.

Hypercapnia did not affect HR. A dissociation between HR and HF HRV in hypercapnia has previously been observed (Brown et al., 2007, 2014) with modest exposure to hypercapnia. Analysis of HRs segregated into inspiratory and expiratory beats found very subtle differences between AA and 5% $CO_2$ inhalation (Brown et al., 2007). Although we did not assess RSA, our exposure represents a similarly mild challenge. Further, there are dissociations between HR, HF HRV and respiratory sinus arrhythmia (RSA) during hypercapnia at low levels of exposure (Tzeng et al., 2007; Brown et al., 2014), suggesting a dissociation between vagal tone and HF HRV.

One potential mechanism for increased HF power is acidosis secondary to hypercapnia (Brown and Howden, 2008b). Peripheral chemoreceptors (the carotid bodies and secondarily the aortic bodies) along with central chemoreceptors are sensitive to changes in the blood pH/$CO_2$ tension. Evidence suggests that pontomedullary nuclei (medial and lateral parabrachial nucleus, retrotrapezoid nucleus/parafacial area, the medullary raphe, and the pre-Bötzinger complex) are sensitive to pH/$CO_2$ tension and signal the phrenic nerve to increase minute ventilation in order to expel excess $CO_2$ (Guyenet et al., 2013). The pH/$CO_2$ central

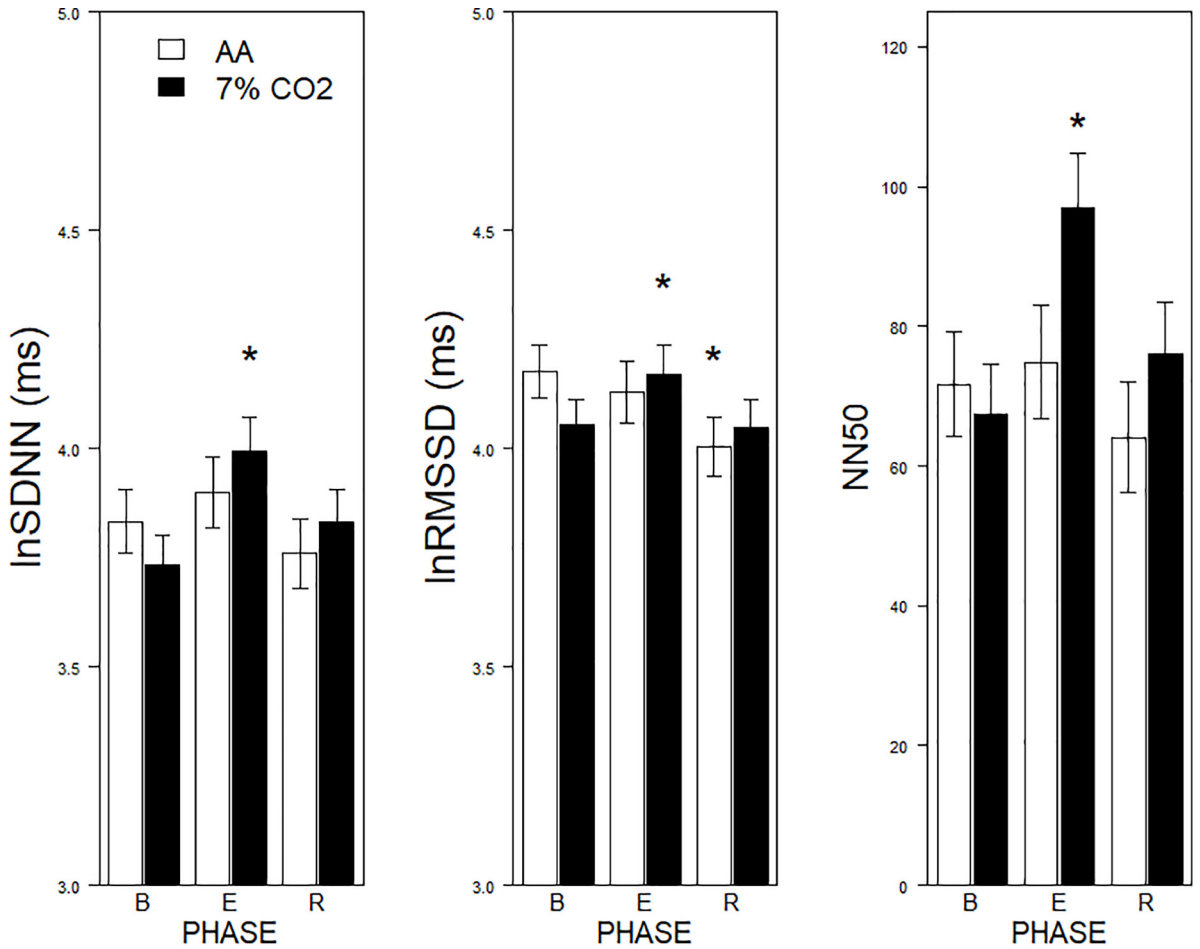



**FIGURE 1 |** Effects of 7% $CO_2$ on measures of Time Domain HRV across the three experimental phases. Three measures of the Time Domain in HRV are depicted from **left to right panels**, SDNN, RMSSSD, and NN50, respectively. The legend for all three panels is contained in the **left panel**. For all three measures, * indicates a significant change from baseline, $p < 0.05$. For NN50, * additionally indicates a difference in recovery, $p < 0.05$.

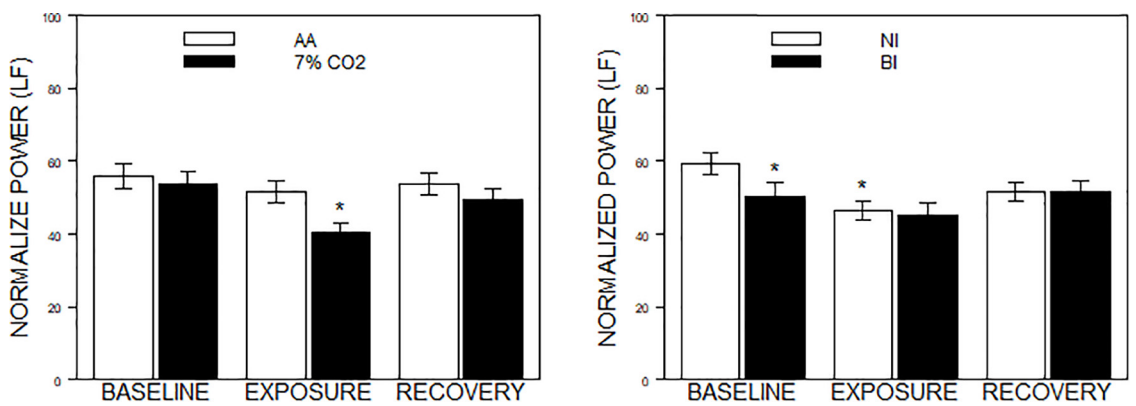



**FIGURE 2 |** Normalized power in the frequency domain as a function of 7% $CO_2$ exposure and temperament. The panels depict measures of normalized power from the HF and LF domains across the three experimental phases. Normalized LF was used for the analysis, but note that LF and HF are reciprocal. The legend for each panel is contained within the panel. The **left panel** depicts the Exposure × Phase interaction in which normalized LF power decreased during 7% $CO_2$ exposure which recovered with reintroduction of AA. * indicate $p < 0.05$ compared to both baseline values and AA during Exposure. The **right panel** depicts the BI × Epoch interaction. * indicate differences relative to the baseline phase, $p < 0.05$. Normalized LF was initially lower in BI than NI. Over participation in the experiment (e.g., computerized task), LF reduced in NI to the levels of BI.

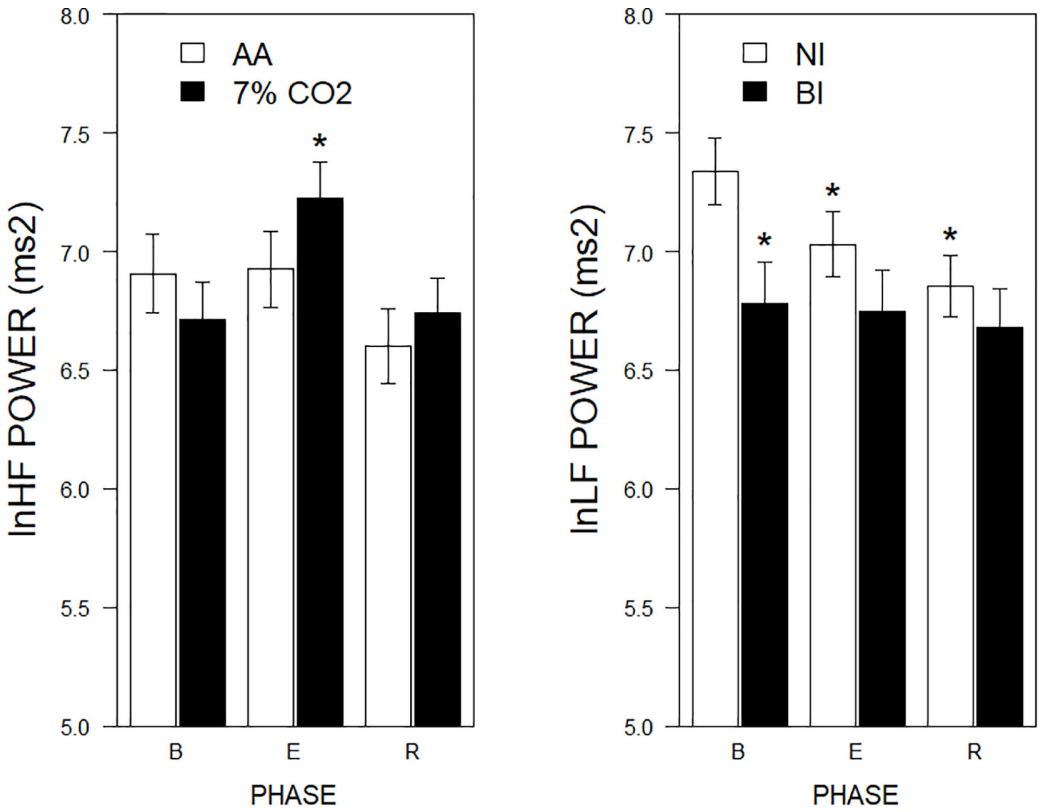



**FIGURE 3 |** lnLF and lnHF power across experimental phases as a function of 7% $CO_2$ exposure and temperament. The **right panel** depicts lnLF power, whereas the **left panel** depicts lnHF power. The legend for each panel is contained within the panel. In the left panel, lnHF power increased in 7% $CO_2$ relative to baseline and recovery epochs. lnHF power did not appreciably change in AA over experimental epochs. This pattern is reminiscent of **left panel** of **Figure 2**. In the **right panel**, lnLF power of BI group was initially lower during baseline than NI. LF power of NI reduced over experimental epochs to be similar to that of BI, which did not change from baseline. This pattern is reminiscent of **right panel** of **Figure 2**. * indicate a significant change from the respective baseline phase, $p < 0.05$.

chemoreceptors drive the increase in activity of the pre-Bötzinger complex (the proposed rhythm generator) eventually stimulating the phrenic nerve, which then drives diaphragm activity, and this increased diaphragm drive ultimately increases breathing. Increased HF power may be secondary to phrenic drive.

Thus, the current protocol and procedures of hypercapnia of 7% $CO_2$ delivered over a 4-min period represented a mild physiological challenge.

## BI Temperament

A primary goal of the study was to determine whether BI temperament differed in HRV either as a constituent process and/or as a response to challenge compared to NI individuals. As a constituent process, time domain measures of HRV did not differ between BI and NI individuals. A difference was evident in terms of lnLF power, with BI individuals displaying lower LF power. The difference between BI and NI diminished over experimental phases. Hypercapnia drove LF power lower in NI, however, hypercapnia did not diminish LF in BI suggesting LF power may have been at nadir in BI individuals. An alternative explanation for the lower baseline LF power is BI individuals are more responsive to the laboratory setting, apparatus or instructions therein (a "white coat" reaction). Arguing against this interpretation, HR and BP, two responses normally sensitive to laboratory reactivity, were essentially poolable between NI and BI individuals.

The hypercapnia challenge did not differentially affect the physiological responses of BI participants. The respiratory effects of hypercapnia were no different in BI than NI individuals. Further, there were no exposure-specific differences in HRV in the time or frequency domains. Thus, the inherently low LF power did not manifest in further changes in response to hypercapnia. Note that there was no evidence of panic or panic-like behavioral or physiological responses to hypercapnia in any participant.

A computerized game was presented as a foreground task contemporaneous with $CO_2$ exposure. Participants knew that exposure to $CO_2$ was possible, but did not know when or if they would be exposed. BI individuals could have perceived hypercapnia as stressful, as more of a challenge than NI individuals. If so, one would expect performance on the foreground task to suffer more than NI performance inasmuch as attention would be divided between the hypercapnia and attendant adjustments and the game. Indeed, hypercapnia mildly degraded NI performance. However, performance of BI individuals exposed to hypercapnia was *better* than

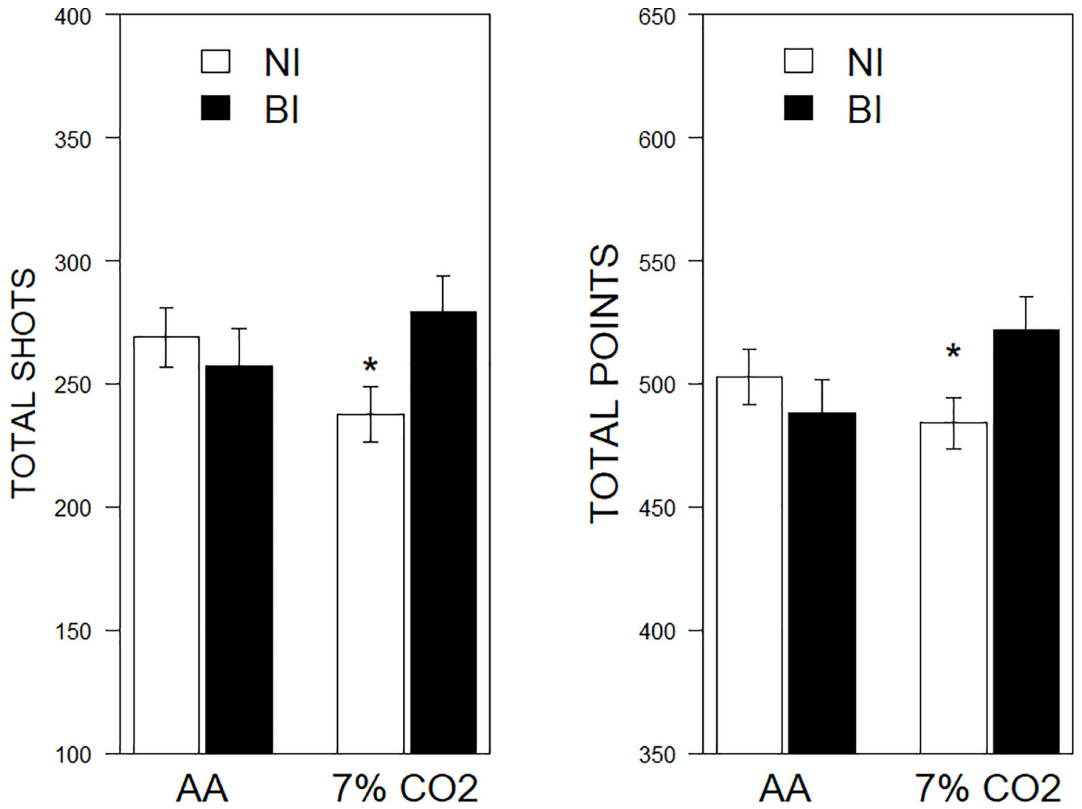



**FIGURE 4 |** Performance on a computerized game as a function of 7% $CO_2$ or AA. The legend is contained within the panels. In the **left panel**, total shots fired are depicted. NI exposed to 7% $CO_2$ fired fewer shots than NI given AA and BI exposed to 7% $CO_2$. The **right panel** depicts total points scored. Similarly, NI exposed to 7% $CO_2$ scored fewer points than NI given AA and BI exposed to 7% $CO_2$. * indicate a significant change from the respective AA group, $p < 0.05$.

hypercapnia-exposed NI participants. Performance was not significantly related to any of the HRV parameters examined.

The enhanced performance of BI individuals is reminiscent of learning performances of BI and animal models of BI. In eyeblink conditioning, the unconditional reflex of BI is of similar magnitude to NI, but learning is facilitated (Holloway et al., 2012; Handy et al., 2018). In avoidance motivated by shock, response to the shock *per se* is similar between models of BI and NI, but avoidance is facilitated (Servatius et al., 2008). While primary physiological responses to challenges may be similar between BI and NI, the motivational properties to alter behavior largely distinguish BI and NI and by extension vulnerability to develop anxiety disorders. In the face of challenges, BI individuals marshal behavioral and by extension experiential resources. Displacement and focused attention, as exhibited by BI individuals, may be fundamental to anxiety vulnerability. Inherently low LF power may provide the physiological basis for biased performance under modest challenge.

## Limitations

There are several limitations to consider in evaluating the results from the current study. As stated earlier, the parameters for assessing HRV were not optimal. It is recommended that epochs for LF power assessments are 5 min and sampling rate for ECG is 200 Hz (Quintana and Heathers, 2014; Laborde et al., 2017). The finding of inherently low LF power in BI should be replicated with more optimal parameters. The HF component and the time domain measures are well within recommendations. Accordingly, the observed increased HF during hypercapnia is consistent with that in the literature regarding the impact of moderate hypercapnia on HRV (Brown et al., 2007, 2014). It should also be noted that we restricted the study to female participants. Females are generally an understudied population in psychophysiology. In the literature cited concerning hypercapnia and HRV, females comprise about 25% of those samples with numbers too small to know whether males and females differ in reactivity to hypercapnia. A study with male participants to directly compare and extend the findings is warranted. We also did not record respiration as an independent channel precluding an analysis of RSA. Similarly, blood gases were also not monitored. Both of these measures were challenging within the small undergraduate college environment with the resources available.

## Summary

In summary, mild hypercapnia induces increases in HF power consistent with the work of others. However, we also observed increased HRV in the time domain in response to

7% $CO_2$ particularly with the measures of RMSSD and NN50. A primary purpose was to evaluate HRV in BI as well as in response to the 7% $CO_2$ challenge. A trait-like reduction in LF power was observed in BI females. Moreover, BI females displayed enhanced reactivity to 7% $CO_2$ with improved performance on the computerized task. However, the respiratory responses to hypercapnia as well as HRV reactivity were similar between BI and NI. To the degree which inherently lower LF power contributes to vulnerability needs to be elaborated. These data contribute to an understanding of vulnerability to develop anxiety disorders in keeping with a learning diathesis model.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Carthage College Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

RS, PM, and DM performed the design of the experiment. PM, DM, JM, and DC-S were responsible for IRB interactions and data collection. PM, DM, and JM accomplished the instrumentation. PM, DM, JM, and DC-S accomplished the data collation. RS, JH, and MA accomplished the data analyses. All authors contributed to manuscript preparation and editing.

## FUNDING

This work was supported by the Stress and Motivated Behavior Institute through funding from the Department of Defense, United States Army Combat Capabilities Development Command (CCDC).

## REFERENCES

Allen, M. T., Handy, J. D., Blankenship, M. R., and Servatius, R. J. (2018). The distressed (Type D) personality factor of social inhibition, but not negative affectivity, enhances eyeblink conditioning. *Behav. Brain Res.* 345, 93–103. doi: 10.1016/j.bbr.2018.02.035

Allen, M. T., Jameson, M. M., and Myers, C. E. (2017). Beyond behavioral inhibition: a computer avatar task designed to assess behavioral inhibition extends to harm avoidance. *Front. Psychol.* 8:1560. doi: 10.3389/fpsyg.2017.01560

Allen, M. T., Myers, C. E., Beck, K. D., Pang, K. C. H., and Servatius, R. J. (2019). Inhibited personality temperaments translated through enhanced avoidance and associative learning increase vulnerability for PTSD. *Front. Psychol.* 10:496.

Allen, M. T., Myers, C. E., and Servatius, R. J. (2014). Avoidance prone individuals self reporting behavioral inhibition exhibit facilitated acquisition and altered extinction of conditioned eyeblinks with partial reinforcement schedules. *Front. Behav. Neurosci.* 8:347.

Allen, M. T., Myers, C. E., and Servatius, R. J. (2016). Uncertainty of trial timing enhances acquisition of conditioned eyeblinks in anxiety vulnerable individuals. *Behav. Brain Res.* 304, 86–91. doi: 10.1016/j.bbr.2016.02.007

Barlow, D. H. (2000). Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. *Am. Psychol.* 55, 1247–1263. doi: 10.1037/0003-066x.55.11.1247

Beck, K. D., Jiao, X., Pang, K. C., and Servatius, R. J. (2010). Vulnerability factors in anxiety determined through differences in active-avoidance behavior. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 852–860. doi: 10.1016/j.pnpbp.2010.03.036

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Bibbey, A., Carroll, D., Ginty, A. T., and Phillips, A. C. (2015). Cardiovascular and cortisol reactions to acute psychological stress under conditions of high versus low social evaluative threat: associations with the type D personality construct. *Psychosom. Med.* 77, 599–608. doi: 10.1097/psy.0000000000000194

Biederman, J., Hirshfeld-Becker, D. R., Rosenbaum, J. F., Herot, C., Friedman, D., Snidman, N., et al. (2001). Further evidence of association between behavioral inhibition and social anxiety in children. *Am. J. Psychiatry* 158, 1673–1679. doi: 10.1176/appi.ajp.158.10.1673

Brown, S. J., and Howden, R. (2008a). "The effects of a respiratory acidosis on human heart rate variability," in *Integration in Respiratory Control: Advances in Experimental Medicine and Biology*, Vol. 605, eds M. J. Poulin, and R. J. A. Wilson, (New York, NY: Springer), 361–365. doi: 10.1007/978-0-387-73693-8_63

Brown, S. J., Barnes, M. J., and Mundel, T. (2014). Effects of hypoxia and hypercapnia on human HRV and respiratory sinus arrhythmia. *Acta Physiol. Hung.* 101, 263–272. doi: 10.1556/aphysiol.101.2014.3.1

Brown, S. J., and Howden, R. (2008b). The effects of a respiratory acidosis on human heart rate variability. *Adv. Exp. Med. Biol.* 605, 361–365.

Brown, S. J., Mundel, T., and Brown, J. A. (2007). Cardiac vagal control and respiratory sinus arrhythmia during hypercapnia in humans. *J. Physiol. Sci.* 57, 337–342. doi: 10.2170/physiolsci.rp009407

Bystritsky, A., Craske, M., Maidenberg, E., Vapnik, T., and Shapiro, D. (2000). Autonomic reactivity of panic patients during a CO2 inhalation procedure. *Depress Anxiety* 11, 15–26. doi: 10.1002/(sici)1520-6394(2000)11:1<15::aid-da3>3.0.co;2-w

Caulfield, M. D., Vanmeenen, K. M., and Servatius, R. J. (2015). Facilitated acquisition of standard but not long delay classical eyeblink conditioning in behaviorally inhibited adolescents. *Behav. Brain Res.* 278, 476–481. doi: 10.1016/j.bbr.2014.10.027

Chasiropoulou, C., Siouti, N., Mougiakos, T., and Dimitrakopoulos, S. (2019). The diathesis-stress model in the emergence of major psychiatric disorders during military service. *Psychiatriki* 30, 291–298. doi: 10.22365/jpsych.2019.304.291

Fox, N. A., and Pine, D. S. (2012). Temperament and the emergence of anxiety disorders. *J. Am. Acad. Child Adoles. Psychiatry* 51:125. doi: 10.1016/j.jaac.2011.10.006

Friedman, B. H. (2007). An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone. *Biol. Psychol.* 74, 185–199. doi: 10.1016/j.biopsycho.2005.08.009

Gladstone, G., and Parker, G. (2005). Measuring a behaviorally inhibited temperament style: development and initial validation of new self-report measures. *Psychiatry Res.* 135, 133–143. doi: 10.1016/j.psychres.2005.03.005

Gladstone, G. L., Parker, G. B., Mitchell, P. B., Wilhelm, K. A., and Malhi, G. S. (2005). Relationship between self-reported childhood behavioral inhibition and lifetime anxiety disorders in a clinical sample. *Depress Anxiety* 22, 103–113. doi: 10.1002/da.20082

Gorman, J. M., Fyer, M. R., Goetz, R., Askanazi, J., Liebowitz, M. R., Fyer, A. J., et al. (1988). Ventilatory physiology of patients with panic disorder. *Arch. Gen. Psychiatry* 45, 31–39. doi: 10.1001/archpsyc.1988.01800250035006

Guyenet, P. G., Abbott, S. B., and Stornetta, R. L. (2013). The respiratory chemoreception conundrum: light at the end of the tunnel? *Brain Res.* 1511, 126–137. doi: 10.1016/j.brainres.2012.10.028

Handy, J. D., Avcu, P., Ko, N., Ortiz, A., Doria, M. J., and Servatius, R. J. (2018). Facilitated acquisition of the classically conditioned eyeblink response in active duty military expressing posttraumatic stress disorder symptoms. *Behav. Brain Res.* 339, 106–113. doi: 10.1016/j.bbr.2017.11.014

Heathers, J. A. (2014). Everything Hertz: methodological issues in short-term frequency-domain HRV. *Front. Physiol.* 5:177.

Holloway, J. L., Trivedi, P., Myers, C. E., and Servatius, R. J. (2012). Enhanced conditioned eyeblink response acquisition and proactive interference in anxiety vulnerable individuals. *Front. Behav. Neurosci.* 6:76.

Huang, W.-L., Chang, L.-R., Kuo, T. B., Lin, Y.-H., Chen, Y.-Z., and Yang, C. C. (2013). Gender differences in personality and heart-rate variability. *Psychiatry Res.* 209, 652–657. doi: 10.1016/j.psychres.2013.01.031

Kaufmann, T., Sutterlin, S., Schulz, S. M., and Vogele, C. (2011). ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. *Behav. Res. Methods* 43, 1161–1170. doi: 10.3758/s13428-011-0107-7

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213.

Luque-Casado, A., Perales, J. C., Cardenas, D., and Sanabria, D. (2016). Heart rate variability and cognitive processing: the autonomic response to task demands. *Biol. Psychol.* 113, 83–90. doi: 10.1016/j.biopsycho.2015.11.013

Martin, L. A., Doster, J. A., Critelli, J. W., Lambert, P. L., Purdum, M., Powers, C., et al. (2010). Ethnicity and Type D personality as predictors of heart rate variability. *Int. J. Psychophysiol.* 76, 118–121. doi: 10.1016/j.ijpsycho.2010.03.001

Mcauley, J., Stewart, A., Webber, E., Cromwell, H., Servatius, R., and Pang, K. (2009). Wistar–Kyoto rats as an animal model of anxiety vulnerability: support for a hypervigilance hypothesis. *Behav. Brain Res.* 204, 162–168. doi: 10.1016/j.bbr.2009.05.036

Mellick, W. H., Mills, J. A., Kroska, E. B., Calarge, C. A., Sharp, C., and Dindo, L. N. (2019). Experiential avoidance predicts persistence of major depressive disorder and generalized anxiety disorder in late adolescence. *J. Clin. Psychiatry* 80:18m12265.

Miller, J. R., Cook-Snyder, D., Buchholz, A., Evenhouse, A., Nicosia, T., Grove, A., et al. (2018). "Developing a low budget system to mix, store, and deliver enhanced respiratory gases for human research in liberal arts college setting," in *Proceedings of the 1st Wisconsin Space Conference*, Appleton, WI. Available online at: https://wsgc.carthage.edu/ojs/index.php/wsc/article/view/240

Myers, C. E., Vanmeenen, K. M., Mcauley, J. D., Beck, K. D., Pang, K. C., and Servatius, R. J. (2012a). Behaviorally inhibited temperament is associated with severity of post-traumatic stress disorder symptoms and faster eyeblink conditioning in veterans. *Stress* 15, 31–44. doi: 10.3109/10253890.2011.578184

Myers, C. E., Vanmeenen, K. M., and Servatius, R. J. (2012b). Behavioral inhibition and PTSD symptoms in veterans. *Psychiatry Res.* 196, 271–276. doi: 10.1016/j.psychres.2011.11.015

O'Donnell, M. L., Elliott, P., Lau, W., and Creamer, M. (2007). PTSD symptom trajectories: from early to chronic response. *Behav. Res. Ther.* 45, 601–606. doi: 10.1016/j.brat.2006.03.015

Pérez-Edgar, K., Bar-Haim, Y., Mcdermott, J. M., Chronis-Tuscano, A., Pine, D. S., and Fox, N. A. (2010). Attention biases to threat and behavioral inhibition in early childhood shape adolescent social withdrawal. *Emotion* 10:349. doi: 10.1037/a0018486

Perna, G., Romano, P., Caldirola, D., Cucchi, M., and Bellodi, L. (2003). Anxiety sensitivity and 35% CO2 reactivity in patients with panic disorder. *J. Psychosom. Res.* 54, 573–577. doi: 10.1016/s0022-3999(02)00468-3

Puttonen, S., Elovainio, M., Kivimäki, M., Koskinen, T., Pulkki-Råback, L., Viikari, J. S., et al. (2008). Temperament, health-related behaviors, and autonomic cardiac regulation: the cardiovascular risk in young Finns study. *Biol. Psychol.* 78, 204–210. doi: 10.1016/j.biopsycho.2008.03.003

Quintana, D. S., Alvares, G. A., and Heathers, J. A. (2016). Guidelines for Reporting Articles on Psychiatry and Heart rate variability (GRAPH): recommendations to advance research communication. *Transl. Psychiatry* 6:e803. doi: 10.1038/tp.2016.73

Quintana, D. S., and Heathers, J. A. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front. Psychol.* 5:805.

Ricart, T. M., De Niear, M. A., Jiao, X., Pang, K. C., Beck, K. D., and Servatius, R. J. (2011). Deficient proactive interference of eyeblink conditioning in Wistar-Kyoto rats. *Behav. Brain Res.* 216, 59–65. doi: 10.1016/j.bbr.2010.07.005

Servatius, R. J., Handy, J. D., Doria, M. J., Myers, C. E., Marx, C. E., Lipsky, R., et al. (2017). Stress-related mental health symptoms in coast guard: incidence, vulnerability, and neurocognitive performance. *Front. Psychol.* 8:1513.

Servatius, R. J., Jiao, X., Beck, K. D., Pang, K. C., and Minor, T. R. (2008). Rapid avoidance acquisition in Wistar-Kyoto rats. *Behav. Brain Res.* 192, 191–197. doi: 10.1016/j.bbr.2008.04.006

Sheynin, J., Beck, K. D., Pang, K. C., Servatius, R. J., Shikari, S., Ostovich, J., et al. (2014). Behaviourally inhibited temperament and female sex, two vulnerability factors for anxiety disorders, facilitate conditioned avoidance (also) in humans. *Behav. Proc.* 103, 228–235. doi: 10.1016/j.beproc.2014.01.003

Sheynin, J., Moustafa, A. A., Beck, K. D., Servatius, R. J., and Myers, C. E. (2015). Testing the role of reward and punishment sensitivity in avoidance behavior: a computational modeling approach. *Behav. Brain Res.* 283, 121–138. doi: 10.1016/j.bbr.2015.01.033

Tapp, W., Servatius, R., Hunt, J., and Powell, D. A. (1997). Vagal activity predicts eyeblink conditioning in human subjects. *Neuroreport* 8, 1203–1207. doi: 10.1097/00001756-199703240-00029

Tzeng, Y. C., Larsen, P. D., and Galletly, D. C. (2007). Effects of hypercapnia and hypoxemia on respiratory sinus arrhythmia in conscious humans during spontaneous respiration. *Am. J. Physiol. Heart Circulat. Physiol.* 292, H2397–H2407.

Valenca, A. M., Nardi, A. E., Nascimento, I., Zin, W. A., and Versiani, M. (2002). Respiratory panic disorder subtype and sensitivity to the carbon dioxide challenge test. *Braz. J. Med. Biol. Res.* 35, 783–788. doi: 10.1590/s0100-879x2002000700004

Van Den Hout, M. A., Griez, E., Van Der Molen, G. M., and Lousberg, H. (1987). Pulmonary carbon dioxide and panic-arousing sensations after 35% carbon dioxide inhalation: hypercapnia/hyperoxia versus hypercapnia/normoxia. *J. Behav. Ther. Exp. Psychiatry* 18, 19–23. doi: 10.1016/0005-7916(87)90067-x

White, L. K., Degnan, K. A., Henderson, H. A., Pérez-Edgar, K., Walker, O. L., Shechner, T., et al. (2017). Developmental relations among behavioral inhibition, anxiety, and attention biases to threat and positive information. *Child Dev.* 88, 141–155. doi: 10.1111/cdev.12696

Young, F. L., and Leicht, A. S. (2011). Short-term stability of resting heart rate variability: influence of position and gender. *Appl. Physiol. Nutr. Metab.* 36, 210–218. doi: 10.1139/h10-103

**Disclaimer:** The views expressed in this article reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of Veterans Affairs, Department of the Navy, Department of Defense, nor the United States Government. RS and JH are employees of the United States Government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. §101 defines a United States Government work as a work prepared by a military service member or employee of the United States Government as part of that person's official duties.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 19 November 2020
doi: 10.3389/fnins.2020.594880

# A Critical Review of Ultra-Short-Term Heart Rate Variability Norms Research

*Fred Shaffer[1]*, Zachary M. Meehan[2] and Christopher L. Zerr[3]*

*[1] Center for Applied Psychophysiology, Truman State University, Kirksville, MO, United States, [2] Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, United States, [3] Department of Psychological and Brain Sciences, Washington University in St. Louis, St. Louis, MO, United States*

Heart rate variability (HRV) is the fluctuation in time between successive heartbeats and is defined by interbeat intervals. Researchers have shown that short-term (∼5-min) and long-term (≥24-h) HRV measurements are associated with adaptability, health, mobilization, and use of limited regulatory resources, and performance. Long-term HRV recordings predict health outcomes heart attack, stroke, and all-cause mortality. Despite the prognostic value of long-term HRV assessment, it has not been broadly integrated into mainstream medical care or personal health monitoring. Although short-term HRV measurement does not require ambulatory monitoring and the cost of long-term assessment, it is underutilized in medical care. Among the diverse reasons for the slow adoption of short-term HRV measurement is its prohibitive time cost (∼5 min). Researchers have addressed this issue by investigating the criterion validity of ultra-short-term (UST) HRV measurements of less than 5-min duration compared with short-term recordings. The criterion validity of a method indicates that a novel measurement procedure produces comparable results to a currently validated measurement tool. We evaluated 28 studies that reported UST HRV features with a minimum of 20 participants; of these 17 did not investigate criterion validity and 8 primarily used correlational and/or group difference criteria. The correlational and group difference criteria were insufficient because they did not control for measurement bias. Only three studies used a limits of agreement (LOA) criterion that specified *a priori* an acceptable difference between novel and validated values in absolute units. Whereas the selection of rigorous criterion validity methods is essential, researchers also need to address such issues as acceptable measurement bias and control of artifacts. UST measurements are proxies of proxies. They seek to replace short-term values which, in turn, attempt to estimate long-term metrics. Further adoption of UST HRV measurements requires compelling evidence that these metrics can forecast real-world health or performance outcomes. Furthermore, a single false heartbeat can dramatically alter HRV metrics. UST measurement solutions must automatically edit artifactual interbeat interval values otherwise HRV measurements will be invalid. These are the formidable challenges that must be addressed before HRV monitoring can be accepted for widespread use in medicine and personal health care.

**Keywords: biofeedback, Bland–Altman limits of agreement, criterion validity, heart rate variability, norms, Pearson product-moment correlation coefficient, predictive validity, reliability**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Hidenori Koyama,*
*Hyogo College of Medicine, Japan*
*Dorota Zyśko,*
*Wrocław Medical University, Poland*

****Correspondence:***
*Fred Shaffer*
*fredricshaffer@gmail.com*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *14 August 2020*
***Accepted:*** *15 October 2020*
***Published:*** *19 November 2020*

***Citation:***
*Shaffer F, Meehan ZM and Zerr CL (2020) A Critical Review of Ultra-Short-Term Heart Rate Variability Norms Research. Front. Neurosci. 14:594880. doi: 10.3389/fnins.2020.594880*

## INTRODUCTION

The purpose of this review article is to critically examine the criteria used in studies of ultra-short-term (UST) heart rate variability (HRV) and to identify challenges of criterion, concurrent, and predictive validity, and measurement artifacts.

Section "Heart Rate Variability" explains HRV from the perspectives of the neurovisceral integration mode and vagal tank theory. We underscore that HRV metrics are associated with regulatory capacity and health, providing an indication of how HRV predicts health crises such as fetal distress before the appearance of symptoms or mortality. Further, these metrics describe the correlation between low HRV, disease, and mortality.

Section "Length of the HRV Recording Period" describes long-term, short-term, and UST HRV recordings, and it emphasizes that long-term measurements best predict health outcomes, and provides a description of time domain, frequency domain, and non-linear metrics. We explain that short-term measurements poorly correlate with long-term values, and stress that we cannot use long-term and short-term norms interchangeably. We caution that short-term measurements are proxies of long-term measurements and that their predictive validity is uncertain. Finally, we characterize UST measurements as proxies of proxies and call for research into their predictive validity.

Section "Why Is There Interest in UST HRV Measurements?" discusses the reasons for the limited use in HRV measurements in medicine, the challenges to their integration into routine medical care, the opportunity created by wearable products for consumer HRV monitoring, and the research required before the widespread adoption of HRV metrics in fitness and wellness applications.

Section "Criterion Validity Ensures Measurement Integrity" explains criterion validity, which can be established using the concurrent and predictive validity approaches. These approaches depend on a high-quality criterion that is relevant, reliable, and valid.

Section "UST HRV Research" provides an overview of 28 studies that have reported UST HRV features. We argue that comparison approaches using correlational coefficients, coefficients of determination or regression, and group mean or median comparisons approaches cannot establish criterion validity because they do not control for measurement bias, which is the difference between novel and validated measurements. Section "Correlation Coefficients" explains that although correlation coefficients can identify potential surrogates, they cannot establish criterion validity. Correlations show association but cannot establish equivalence. A proxy measurement can be perfectly correlated with a reference standard measurement while falling outside an acceptable range (e.g., ±10% of the reference standard's range). Section "Coefficient of Determination or Regression" argues that neither method is appropriate for demonstrating equivalence. The coefficient of determination shares the same limitations as correlation coefficients and use of regression for this purpose violates its underlying statistical assumptions. Section "Group Mean or Median Comparisons" challenges the claim that two methods are comparable if they yield a non-significant group mean or median difference because this does not ensure validity and can be confounded by insufficient statistical power. Lastly, Section "Limits of Agreement (LOA) Solutions" describes how this approach establishes criterion validity when accuracy standards are specified *a priori*.

Section "UST HRV Studies Reporting Limits of Agreement Solutions" summarizes four studies that have reported LOA and compares findings from three reports (Esco and Flatt, 2014; Munoz et al., 2015; Shaffer et al., 2019) that utilized LOA as a selection criterion for valid UST measurements. Finally, Section "Practical Recommendations" outlines four steps for determining the shortest period that can estimate a 300-s measurement.

## HEART RATE VARIABILITY

Heart rate and HRV are calculated from the time intervals between successive heartbeats and HRV is associated with executive function, regulatory capacity, and health (Thayer and Lane, 2000; Byrd et al., 2015; Laborde et al., 2017; Mather and Thayer, 2018). *Heart rate*, the number of heart beats per minute (bpm), is an UST (<5 min) metric that is widely used in medicine, performance, and daily fitness assessment using wearables. HRV is the organized fluctuation of time intervals between successive heartbeats defined as interbeat intervals (Shaffer and Ginsberg, 2017; Lehrer et al., 2020). The complexity of a healthy heart rhythm is critical to the maintenance of homeostasis because it provides the flexibility to cope with an uncertain and changing environment (Beckers et al., 2006). "A healthy heart is not a metronome" (Shaffer et al., 2014). From the perspective of the neurovisceral integration model (Thayer and Lane, 2000), increased HRV is associated with improved executive function and may strengthen descending medial prefrontal cortex regulation of emotion (Mather and Thayer, 2018). Laborde et al. (2018) have proposed the *vagal tank theory* as an integrative model of *cardiac vagal control* or vagus nerve regulation of heart rate. Cardiac vagal control indexes how efficiently we mobilize and utilize limited self-regulatory resources during resting, reactivity, and recovery conditions (Laborde et al., 2017). HRV metrics are important because they are associated with regulatory capacity, health, and performance (Shaffer et al., 2014) and can predict morbidity and mortality.

A decline in HRV can signal dangerous health changes and low HRV values are associated with an increased risk of illness and death. HRV reductions precede heart rate changes in conditions of fetal distress (Hon and Lee, 1963) and sensory disturbances in diabetic autonomic neuropathy (Ewing et al., 1976). Low HRV correlates with anxiety (Cohen and Benjamin, 2006), asthma (Kazuma et al., 1997; Lehrer et al., 2004), cardiac arrhythmia, chronic obstructive pulmonary disease (Giardino et al., 2004), depression (Agelink et al., 2002), functional gastrointestinal disorders (Gevirtz, 2013), hypertension, inflammation, myocardial infarction (Bigger et al., 1992; Carney et al., 2007; Berntson et al., 2008), post-traumatic stress disorder (Shah et al., 2013), and sudden infant death (Hon and Lee, 1963). Low HRV also correlates with all-cause mortality (Tsuji et al., 1994; Dekker et al., 1997). For example, low

power in the very-low-frequency (VLF) band (0.0033–0.04 Hz) more strongly predicted all-cause mortality (higher *Z*-scores and relative risk) than low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.4 Hz) bands, and is associated with arrhythmic death (Bigger et al., 1992).

## LENGTH OF THE HRV RECORDING PERIOD

Heart rate variability recording periods range from under 1 min to over 24 h. *Long-term recordings* (≥24 h) constitute the reference standard for clinical evaluation due to their *predictive validity*, which is the ability to predict future outcomes (Hoenig et al., 2001). For example, 24-h measurements of the standard deviation (SD) of the interbeat intervals of normal sinus beats (SDNN) predict cardiac risk (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). Acute myocardial infarction patients with SDNN values under 50 ms are unhealthy, between 50 and 100 ms have compromised health, and over 100 ms are healthy (Kleiger et al., 1987). Acute myocardial infarction patients with SDNN values over 100 ms have been reported to have a 5.3 lower mortality risk at a 31-month mean follow-up than those under 50 ms.

While long-term, short-term (∼5 min), and UST (<5 min) recordings calculate HRV metrics using the same mathematical formulas, they are not interchangeable, reflect different underlying physiological processes, and achieve different predictive powers. HRV in long-term recordings may be attributed to changes in the circadian rhythm, fluctuations in core body temperature and the renin–angiotensin system, and the sleep cycle (Bonaduce et al., 1994; Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). Long-term recordings monitor cardiorespiratory regulation across diverse situations, physical workloads, and anticipatory central nervous system (CNS) reactions to environmental stimuli. These extended recording periods reveal the sympathetic nervous system (SNS) component of HRV (Grant et al., 2011; Shaffer and Ginsberg, 2017). HRV in short-term recordings is produced by four interdependent sources that operate on a briefer time scale and are defined by: (1) the complex interaction between the sympathetic and parasympathetic branches; (2) respiration-mediated increases and decreases in heart rate via the vagus nerve, termed respiratory sinus arrhythmia (RSA); (3) the baroreceptor reflex that regulates blood pressure using negative feedback; and (4) rhythmic adjustments in blood vessel diameter (Shaffer and Ginsberg, 2017). Short-term values correlate poorly with their long-term counterparts (Fei et al., 1996). Basic research is needed to identify the major HRV generators in UST recordings.

Although long-term, short-term, and UST HRV recordings are characterized using the same time-domain, frequency-domain, and non-linear indices, they differ in predictive power. *Time-domain* metrics calculate the amount of variability in a series of interbeat intervals. *Frequency-domain* measurements compute absolute or relative power distribution across four bands: *ultra-low-frequency* (ULF; ≤0.003 Hz), VLF (0.0033–0.04 Hz), LF (0.004–0.15 Hz), and HF (0.15–0.40 Hz). *Non-linear* indicators measure the interbeat interval time series' unpredictability (Stein and Reddy, 2005; **Table 1**). ST recordings achieve lower predictive power than long-term recordings (Bigger et al., 1989; Nolan et al., 1998; Kleiger et al., 2005). To summarize, long-term recordings represent the reference standard for predicting health outcomes. For this reason, long-term and short-term norms cannot be used interchangeably. Short-term values are proxies of long-term values with unknown predictive validity; therefore, UST measurements are proxies of proxies. Basic research is also needed to determine the predictive validity of UST recordings.

## WHY IS THERE INTEREST IN UST HRV MEASUREMENTS?

There is a potential role for UST HRV measurements in medical assessment, research involving brief (e.g., <30 s) experimental tasks, and personal wellness assessment once researchers validate their accuracy and predictive power. Despite the availability of short-term normative HRV values for adults (Umetani et al., 1998; Nunan et al., 2010) and elite athletes (Berkoff et al., 2007), HRV is not widely used in medical assessment outside of cardiology and obstetrics. For example, nurses do not routinely monitor HRV as a vital sign during general practice visits. Short-term HRV assessment's time cost is one of many barriers to its integration in routine medical practice: "...a 5-min HRV assessment is prohibitively long when compared with routine office or home measurements of blood glucose, blood pressure, core body temperature, heart rate, oxygen saturation, and weight" (Shaffer et al., 2019, p. 215). If researchers were to validate the accuracy and predictive power of UST HRV measurements, and provide age- and sex-related normative values, manufacturers could add this modality to widely used instruments like electrocardiographs and pulse oximeters.

Research studies in diverse areas (e.g., clinical and social psychology) may involve brief experimental tasks that require UST HRV measurements. For example, short-term HRV monitoring would be inappropriate for a 30-s task designed to induce frustration. As with medical applications, researchers need to validate the accuracy and meaning of UST HRV measurements.

Consumers increasingly monitor their physiology using dedicated tracking devices and smartwatches that incorporate electrocardiographic (ECG) and photoplethysmographic (PPG) sensors of heart rate and HRV. ECG sensors detect the R-spike and PPG sensors identify the peak of the pulse wave to determine when a heartbeat has occurred (Shaffer et al., 2014). The ECG method is more accurate than PPG during paced breathing (Jan et al., 2019) and when increased sympathetic tone results in vasoconstriction in monitored fingers (Giardino et al., 2002; Schafer and Vagedes, 2013). UST measurements are ideal for these ambulatory fitness and wellness applications if investigators can demonstrate their accuracy

**TABLE 1 |** Short-Term HRV metrics adapted from Shaffer and Ginsberg (2017) and Shaffer et al. (2019).

| HRV metrics | Units | Description |
|---|---|---|
| **Time domain** | | |
| Heart rate | 1/min | Average heart rate |
| HRV triangular index (HTI) | | Integral of the density of the RR interval histogram divided by its height; together, HTI and RMSSD can distinguish between normal rhythms and arrhythmias |
| NN | ms | Average of NN intervals |
| NN50 | count | Number of successive RR intervals that differ by more than 50 ms |
| pNN50 | % | Percentage of successive RR intervals that differ by more than 50 ms; associated with HF absolute power and RMSSD |
| RMSSD | ms | Root mean square of successive RR interval differences; estimates vagal contributions to HRV |
| SDNN | ms | Standard deviation of NN intervals; strongly associated with ULF, VLF, LF, and total power; vagally-mediated RSA is primary source, especially with slow, paced breathing during ST recording |
| TINN | | Baseline width of the RR interval histogram |
| **Frequency domain** | | |
| VLF | $ms^2$ | Absolute power of the very-low-frequency band (0.0033–0.04 Hz) |
| LF | $ms^2$ | Absolute power of the low-frequency power (0.04–0.15 Hz) |
| LFnu | nu | Relative power of the low-frequency band in normal units |
| HF | $ms^2$ | High-frequency power (0.15–0.4 Hz) |
| HFnu | nu | Relative power of the high-frequency band in normal units |
| LF/HF | % | Ratio of LF-to-HF absolute power |
| Total | $ms^2$ | Sum of absolute power in the VLF, LF, and HF bands in ST recordings |
| **Non-linear** | | |
| ApEn | | Approximate entropy, which measures the regularity and complexity of a time series; small values mean signal predictability |
| $D_2$ | | Correlation dimension, which estimates the minimum number of variables required to construct a model of system dynamics; more variables mean greater time series complexity |
| DET | % | Recurrence plot analysis determinism |
| DFα1 | | Detrended fluctuation analysis, which describes short-term fluctuations; reflects the baroreceptor reflex |
| DFα2 | | Detrended fluctuation analysis, which describes long-term fluctuations; reflects regulation of interbeat interval fluctuation |
| REC | % | Recurrence rate |
| SampEn | | Sample entropy, which measures the regularity and complexity of a time series; like ApEn, small values mean signal predictability |
| SD1 | ms | Poincaré plot standard deviation perpendicular to the line of identity; measures ST HRV and is associated with baroreflex sensitivity (BRS) |
| SD2 | ms | Poincaré plot standard deviation along the line of identity; measures ST and LT HRV and is associated with LF absolute power and BRS |
| ShanEn | | Shannon entropy; measures the average information in a time series; higher values indicate greater uncertainty and irregularity |

*Credit: Center for Applied Psychophysiology. Baroreflex sensitivity (BRS), the change in interbeat interval length per unit change in BP and HF absolute power; normal units (nu) are determined by dividing frequency band absolute power by the summed the absolute power of the LF and HF bands; frequency domain, measurements that compute absolute or relative power distribution across four bands: ultra-low-frequency (ULF; ≤0.003 Hz), very-low-frequency (VLF; 0.0033–0.04 Hz), low-frequency (LF; 0.004–0.15 Hz), and high-frequency (HF; 0.15–0.40 Hz); non-linear, indicators that measure the interbeat interval time series' unpredictability; short-term, measurements ∼ 5 min; time domain, metrics that calculate the amount of variability in a series of interbeat intervals.*

under non-stationary and stationary conditions, their predictive validity, and normative values.

## CRITERION VALIDITY ENSURES MEASUREMENT INTEGRITY

*Criterion validity* confirms that test scores accurately estimate scores of validated measures or metrics and depends on the identification of a high-quality criterion (Gulliksen, 1987). Researchers use concurrent and predictive validity approaches to provide evidence of criterion validity. In the *concurrent* approach, investigators obtain test and criterion scores simultaneously (Price, 2018). The UST HRV studies reviewed in this article illustrate this strategy. Here, the test scores are UST and the criterion scores are short-term HRV values. In the *predictive* approach, researchers obtain test scores to estimate future outcomes or performance. The success of both strategies depends on the existence of a *high-quality criterion*, which is relevant, valid, and reliable (Price, 2018). *Relevant* means that we can objectively assess the criterion (e.g., SDNN). *Validity* means that the criterion (e.g., 5-min SDNN) accurately measures the metric of interest (e.g., SDNN). Finally, *reliability* means that criterion scores (e.g., 5-min SDNN values) obtained from the same individuals under identical conditions are consistent. Although valid measures are always reliable, reliable measures are not valid unless they accurately assess a given construct (e.g., SDNN).

**TABLE 2 |** Studies that reported UST HRV measurements and their primary criterion validity criteria.

| **Did Not Investigate UST Criterion Validity** | |
|---|---|
| Arza et al. (2005) | Pandey et al. (2016) |
| Choi and Gutierrez-Osuna (2009) | Papousek et al. (2010) |
| De Rivecourt et al. (2008) | Pereira et al. (2017) |
| Hjortskov et al. (2004) | Schubert et al. (2009) |
| Kim et al. (2008) | Sun et al. (2010) |
| Kwon et al. (2016) | Wang et al. (2009) |
| Li et al. (2009) | Wijsman et al. (2011) |
| Mayya et al. (2015) | Xu et al. (2015) |
| Nardelli et al. (2018) | |
| **Correlational and/or Group Difference UST Criterion Validity Criteria** | |
| Baek et al. (2015) | Munoz et al. (2015) |
| Brisinda et al. (2015) | Nussinovitch et al. (2011) |
| Esco and Flatt (2014) | Salahuddin et al. (2007) |
| Li et al. (2009) | Schroeder et al. (2004) |
| McNames and Aboy (2006) | Thong et al. (2003) |
| **Limits of Agreement UST Criterion Validity Criterion** | |
| Esco and Flatt (2014) | |
| Munoz et al. (2015) | |
| Shaffer et al. (2019) | |

*Credit: Center for Applied Psychophysiology. Correlational criterion, two methods are equivalent if their values are correlated; concurrent validity, a novel measurement procedure produces comparable results to an already validated measurement tool; HRV, heart rate variability; group difference criterion, two methods are comparable if they yield a non-significant group mean or median difference; limits of agreement criterion, two methods are equivalent if there is an acceptable a priori difference between their values in absolute units; UST, ultra-short-term (<5 min).*

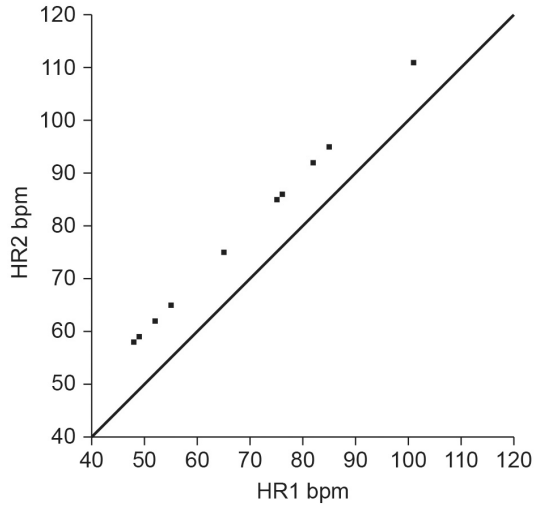



**FIGURE 1 |** Hypothetical scatterplot of UST and ST heart rates (bpm) depicting a perfect correlation ($r$ = 1), but no agreement (points do not fall along the line of equality where $y = x$). Credit: Center for Applied Psychophysiology.

## UST HRV RESEARCH

We evaluated 28 studies that reported UST HRV features with a minimum of 20 participants (**Table 2**). Seventeen studies did not investigate criterion validity. Eight studies primarily used correlational and/or group difference criteria to demonstrate the criterion validity of UST (test scores) with respect to short-term values (criterion scores; Thong et al., 2003; Schroeder et al., 2004; McNames and Aboy, 2006; Salahuddin et al., 2007; Li et al., 2009; Nussinovitch et al., 2011; Baek et al., 2015; Brisinda et al., 2015). Correlation coefficients, the coefficient of determination or regression, and group mean or median comparisons are insufficient to establish criterion validity because they do not control for *measurement bias*—the difference between UST and short-term measurements.

### Correlation Coefficients

Although correlation analysis can help researchers identify potential surrogates, they cannot measure criterion validity (Pecchia et al., 2018). Many researchers make the mistake of applying a correlation coefficient, typically Pearson's $r$, to conclude that two methods are sufficiently comparable or in agreement. The Pearson $r$ quantifies the direction, magnitude, and probability of a linear relationship between two continuous variables, $x$ and $y$. The magnitude of the Pearson $r$ ranges from −1 to +1 (Devore, 2016). A correlation coefficient, however, is merely a measure of association and does not provide evidence that one method agrees with or is comparable to another method (Altman and Bland, 1983). In fact, it is possible for two methods to have a perfect correlation of $r$ = 1 but no agreement or comparability between the measurements (Watson and Petrie, 2010). For example, consider the situation where Method A and Method B both measure heart rate, but only Method A does this accurately. If Method B yields readings that are consistently 10 bpm higher than Method A, they would be perfectly correlated ($r$ = 1) but their measurements would disagree by 10 bpm (**Figure 1**).

The American National Standards Institute criterion (ANSI/AAMI, 2002) for heart rate accuracy is the larger of ±10% of all values or ±5 bpm. If we set the allowable heart rate difference at ±10% of Method A's range, Method B would report heart rates far beyond acceptable measurements as shown by a Bland–Altman plot (**Figure 2**).

Additionally, a significant correlation between two different methods "is generally useless because two methods designed to measure the same quantity will rarely be uncorrelated" (Choudhary and Nagaraja, 2005, p. 218). For these reasons, researchers conclude that a "correlation coefficient ... is of no practical use in the statistical analysis of comparison data" (Westgard and Hunt, 1973, p. 53).

### Coefficient of Determination or Regression

Some method comparison studies use the coefficient of determination ($r^2$) or simple regression analysis to claim two methods are comparable via intercepts or slopes (Bland and Altman, 2003). The *coefficient of determination* estimates the percentage of variability of variable $y$ that can predicted by $x$.

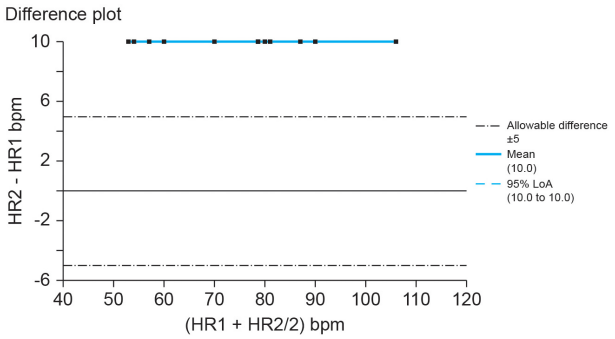



**FIGURE 2 |** Hypothetical Bland–Altman difference plot of UST and ST heart rates (bpm). Credit: Center for Applied Psychophysiology. The line at 0 represents the line of equality or $y = x$ (the diagonal line from **Figure 1**). When measures achieve absolute agreement, they will all fall along that line at 0.

Denoted as $r^2$, the coefficient of determination is identical to the square of the Pearson $r$ coefficient. For example, a Pearson $r$ coefficient of 0.50 corresponds to an $r^2$ value of 0.25, meaning that 25% of the variability in $y$ is accounted for by variability in $x$. The magnitude of $r^2$ ranges from −1 to +1. *Simple regression analysis* estimates a straight line with a slope ($B_1$) and height at which the line crosses the vertical axis ($B_0$) to predict the value of $y$, given $x$ (Devore, 2016). These measures are also inappropriate for demonstrating agreement. The coefficient of determination estimates the proportion of variance that Method A and Method B share but present the same pitfalls as the correlation coefficient (Zaki et al., 2012). In addition, the coefficient of determination calculates how well a regression equation or model fits the observed data. This is problematic for method comparison studies as measurements from each method are dependent variables, each possessing their own measurement error. Linear regression models make an implicit assumption that some portion of the variance in a dependent variable (Y) is being explained by variance in an independent variable (X). Therefore, a simple linear regression assumes that the procedure measures X without error. This method is not appropriate when comparing two dependent measures and may produce a biased regression coefficient (Altman and Bland, 1983; Hays, 1991). If regression is used, both variables should be treated as possessing measurement error. In these cases, Deming regression (parametric) or Passing–Bablok regression (non-parametric) are more appropriate alternatives (Giavarina, 2015).

Deming regression (Deming, 1943) is a type of total least squares regression that accounts for measurement error in both X and Y variables, as opposed to ordinary least squares regression which merely accounts for error in the dependent variable. Deming regression assumes that errors are independent and normally distributed, but the procedure is sensitive to outliers. Passing–Bablok regression (Passing and Bablok, 1983, 1984) is a robust non-parametric rank method that also accounts for error in both X and Y and produces an unbiased slope estimate by calculating the median of all possible slopes (Linnet, 1993). Passing–Bablok regression is less sensitive to outliers and does not have assumptions about the distribution of errors, but it does require that the two variables measured do not significantly deviate from linearity (Passing and Bablok, 1983).

## Group Mean or Median Comparisons

Another statistical approach misused in method comparison studies is to claim that two methods are comparable if they yield a non-significant group mean or median difference via parametric or non-parametric tests. For example, a *two-sample t-test* is a parametric statistic that evaluates whether the difference between pairs of normally-distributed scores can be explained by chance. A *Kruskal–Wallis test* is a non-parametric procedure that determines whether samples were obtained from a single distribution (Devore, 2016). There are several issues with such an approach. First, the goal of comparing two different methods of measurement is not to have an equivalent overall group agreement (mean or median), but rather that the methods appropriately agree across individual observations. Such logic would imply that having greater measurement error would be more favorable because it decreases the probability of finding a significant difference (Altman and Bland, 1983). Non-significant group differences do not indicate whether two methods agree or have acceptable bias. Second, significance is related to the power and sample size of the study (Zaki et al., 2012), and so a non-significant mean or median difference between two methods could be the result of an underpowered study or one without a large enough sample. Third, because many HRV measures are non-normally distributed, some studies inappropriately use a parametric $t$-test or ANOVA on data that have not been log-transformed or fail to use a non-parametric test instead (Pecchia et al., 2018).

## Limits of Agreement (LOA) Solutions

To overcome the aforementioned issues with analyzing agreement between methods, the authors recommend the use of LOA in Bland–Altman plots (Altman and Bland, 1983; Bland and Altman, 1986). An important caveat is that Bland–Altman plots and LOA do not indicate whether or not the agreement between measures is sufficient. The researcher must decide *a priori* the extent to which two measures must agree for them to be comparable. Although there are industry standards for the accuracy of blood pressure and heart rate measurement (ANSI/AAMI, 2002, 2008), there are no comparable standards for HRV short-term measurements such as SDNN. The degree of precision may depend upon the specific question being asked and may vary by discipline (Giavarina, 2015).

Bland–Altman plots are a graphical approach to assessing the extent to which two methods agree with each other by plotting the difference between the two methods (Method A – Method B) on the $y$-axis against the mean of the two methods ([Method A + Method B]/2) on the $x$-axis. If the two methods agree completely, the mean difference ($\bar{d}$) between them will be zero, and all the points on the Bland–Altman plot would fall along a line of $y = 0$. Because perfect agreement between two methods rarely occurs, the distance between an ideal $\bar{d}$ of zero and the observed $\bar{d}$ is an index of bias. The greater the bias—the distance of $\bar{d}$ from zero—between the two methods,

the less the two measures tend to agree. Assuming that the differences are normally distributed, the SD of the differences can then be multiplied by 1.96 and added/subtracted from the mean difference $\bar{d}$. This calculation produces a lower LOA ($\bar{d} - 1.96s$) and an upper LOA ($\bar{d} + 1.96s$), representing the range where 95% of the differences should fall; the lower LOA represents the 2.5th percentile and the upper LOA represents the 97.5th percentile.

Researchers should construct confidence intervals and statistically determine whether the disagreement between the two methods falls within the LOA. They should construct 95% confidence intervals around the mean difference and the lower/upper LOA to take variability into account (Hamilton and Stamey, 2007; Ludbrook, 2010). Next, they should perform a statistical analysis to determine whether the differences between the two methods fall within the appropriate LOA (Giavarina, 2015). Finally, they should follow with an equality test ($H_0$: $\mu$difference = 0) such as the Student's *t*-test. Bland–Altman plots do not require the raw measurements from the two methods to be normally distributed, but the *differences* between the two methods should be normally distributed. Researchers should take appropriate steps if the differences are not normally distributed or the differences are proportional to the size of the measurement (e.g., greater differences between the two methods as the measurements get larger). They can logarithmically transform the raw data or the ratios or percentages ([Method A – Method B]/Mean%) before constructing a Bland–Altman plot. This transformation can provide superior results to plotting a simple difference between the methods against the average

**TABLE 3 |** UST studies that reported limits of agreement adapted from Shaffer and Ginsberg (2017).

| Study, date | *N* | Method | Position | Conditions | UST (s) | HRV metrics | UST criteria |
|---|---|---|---|---|---|---|---|
| Baek et al., 2015 | 467 249 men 218 women | PPG | Sitting | Baseline | 10–270 | HR, pNN50, RMSSD, SDNN, VLF, LF, HF, LF/HF, Total, LFnu, HFnu | Pearson *r* and non-significant Kruskal–Wallis |
| Esco and Flatt, 2014 | 23 men | ECG | Supine | Pre/post-exercise | 10, 30, 60 | RMSSD | ICC and Bland–Altman |
| Munoz et al., 2015 | 3,387 1658 men 1729 women | Portapres® | Supine | Baseline | 10, 30, 120 | RMSSD, SDNN | ICC, Pearson *r*, and Bland–Altman |
| Shaffer et al. (2019) | 38 20 men 18 women | ECG | Sitting | Baseline | 10, 20, 30, 60, 90, 120, 180, 240 | **Table 1** | $r \geq 0.90$ and Bland–Altman LOA ± 5% of the range |

*Credit: Center for Applied Psychophysiology. $D_2$ (also CD), correlation dimension, which estimates the minimum number of variables required to construct a model of a studied system; DFA α1, detrended fluctuation analysis, which describes short-term fluctuations; DFA α2, detrended fluctuation analysis, which describes long-term fluctuations; ECG, electrocardiogram; HF $ms^2$, absolute power of the high frequency band; HF nu, relative power of the high frequency band in normal units; HF peak, highest amplitude frequency in the HF band; HF%, HF power as a percentage of total power; HR, heart rate; HTI, HRV triangular index or integral of the density of the NN interval histogram divided by its height; limits of agreement, criterion that two methods are equivalent if there is an acceptable a priori difference between their values in absolute units; LF $ms^2$, absolute power of the low frequency band; LF nu, relative power of the low frequency band in normal units; LF peak, highest amplitude frequency in the LF band; LF%, LF power as a percentage of total power; LF/HF, ratio of LF-to-HF power; NN interval, time between adjacent normal heartbeats; nu, normal units calculated by dividing the absolute power for a specific frequency band by the summed absolute power of the LF and HF bands; pNN50, percentage of successive interbeat intervals that differ by more than 50 ms; RMSSD, root mean square of successive R–R interval differences; R–R interval, time between all adjacent heartbeats; SampEn, sample entropy, which measures signal regularity and complexity; SD1, Poincaré plot standard deviation perpendicular to the line of identity; SD2, Poincaré plot standard deviation along the line of identity; SD1/SD2, ratio of SD1 to SD2 that measures the unpredictability of the R–R time series and autonomic balance under appropriate monitoring conditions; SDNN, standard deviation of NN intervals; TINN, triangular interpolation of the R–R interval histogram or baseline width of the RR interval histogram; total power, sum of power ($ms^2$) in VLF, LF, and HF bands; UST, ultra-short-term (<5 min).*

**TABLE 4 |** Minimum time period required to estimate 5-min HRV metrics adapted from Shaffer et al. (2019).

| Minimum UST period | HRV metric |
|---|---|
| 10 s | HR |
| 60 s | pNN50, NN50, RMSSD, SDNN |
| 90 s | TINN, LF absolute power, SD1, and SD2 |
| 120 s | HRV triangular index, DFA α1 |
| 180 s | LFnu, HF absolute power, HFnu, LF/HF power, DFA α2, DET, SampEn |
| 240 s | ShanEn |

*DFA α1, detrended fluctuation analysis, which describes short-term fluctuations; DFA α2, detrended fluctuation analysis, which describes long-term fluctuations; ECG, electrocardiogram; HF $ms^2$, absolute power of the high frequency band; HF nu, relative power of the high frequency band in normal units; HF peak, highest amplitude frequency in the HF band; HF%, HF power as a percentage of total power; HR, heart rate; HTI, HRV triangular index or integral of the density of the NN interval histogram divided by its height; limits of agreement, criterion that two methods are equivalent if there is an acceptable a priori difference between their values in absolute units; LF $ms^2$, absolute power of the low frequency band; LF nu, relative power of the low frequency band in normal units; LF peak, highest amplitude frequency in the LF band; LF%, LF power as a percentage of total power; LF/HF, ratio of LF-to-HF power; NN interval, time between adjacent normal heartbeats; nu, normal units calculated by dividing the absolute power for a specific frequency band by the summed absolute power of the LF and HF bands; pNN50, percentage of successive interbeat intervals that differ by more than 50 ms; RMSSD, root mean square of successive R–R interval differences; R–R interval, time between all adjacent heartbeats; SampEn, sample entropy, which measures signal regularity and complexity; SD1, Poincaré plot standard deviation perpendicular to the line of identity; SD2, Poincaré plot standard deviation along the line of identity; SD1/SD2, ratio of SD1 to SD2 that measures the unpredictability of the R–R time series and autonomic balance under appropriate monitoring conditions; SDNN, standard deviation of NN intervals; TINN, triangular interpolation of the R–R interval histogram or baseline width of the RR interval histogram; total power, sum of power ($ms^2$) in VLF, LF, and HF bands; UST, ultra-short-term (<5 min).*

(Giavarina, 2015; Hoffman, 2015). In addition to assessing agreement, Bland–Altman plots can also be used to detect outliers (Watson and Petrie, 2010).

## UST HRV STUDIES THAT REPORT LIMITS OF AGREEMENT SOLUTIONS

Of the 28 UST HRV studies that we reviewed, four reported LOA plots whether used as a selection criterion or not (Esco and Flatt, 2014; Baek et al., 2015; Munoz et al., 2015; Shaffer et al., 2019) (**Table 3**).

Baek et al. (2015) obtained resting PPG measurements from 467 healthy participants (249 men and 218 women; aged 8–69 years). They compared 10-, 20-, 30-, 60-, 90-, 180-, 210-, 240-, and 270-s values with 300-s measurements. Their criteria for selecting the shortest UST period were a significant Pearson *r* and non-significant ($p > 0.05$) Kruskal–Wallis statistic. Although they illustrated their results with Bland–Altman plots (mean difference ± 1.96 *SD*), the authors did not use them to draw conclusions.

Esco and Flatt (2014) acquired ECG measurements from 23 male collegiate athletes (aged 19–21 years) for 10 min while supine before a treadmill test and for 30 min post-exercise. They analyzed the last 5 min of each rest period and compared log-transformed 10-, 30-, and 60-s with 300-s root mean square of the successive differences (RMSSD) values. They compared intra-class correlations (ICCs) and Bland–Altman plots (mean difference ± 1.96 *SD*) across the three UST periods and concluded that that 60 s yielded the largest ICC and most stringent LOA. Whereas the ICC test identified 60 s as a potential surrogate, a Bland–Altman plot confirmed its criterion validity with respect to 300-s RMSSD measurements.

Munoz et al. (2015) recorded beat-to-beat middle finger pressure using a Portapres® device from 3387 participants (1660 men and 1727 women; aged 44–63 years) in the Prevention of Renal and Vascular End-Stage Disease study. They obtained recordings over a 15-min period while resting in the supine position. The authors analyzed the last 4–5 min of data that exhibited a stationarity pattern and compared the log-transformed 10-, 30-, and 120-s with 300-s RMSSD and SDNN values. They compared ICC, Pearson *r* values, and Bland–Altman plots across the three UST periods. The authors concluded that a minimum of 10 s was required to measure RMSSD and 30 s to calculate SDNN.

Shaffer et al. (2019) obtained 5-min EEG recordings from 38 healthy undergraduates (20 men and 18 women; aged 18–23 years) while sitting upright under resting conditions with their eyes open. They acquired 10-, 20-, 30-, 60-, 90-, 120-, 180-, and 240-s epochs from the 5-min recordings. Following manual removal of artifacts, they calculated the time domain, frequency domain, and non-linear HRV metrics outlined in **Table 1**. The authors identified potential surrogates using a Pearson *r* with a conservative criterion ($r \geq 0.90$). They applied Bland–Altman's LOA technique using an allowable difference of ±5% of the range of the 5-min value and a Student's *t*-test to confirm the equality of UST and ST values. The results of LOA analyses are summarized in **Table 4**. These findings were consistent with Esco and Flatt (2014) who also reported that a time interval of 60 s was required to estimate 5-min RMSSD. However, the finding that a 60-s sample is required to measure RMSSD and SDNN was inconsistent with the study by Munoz et al. (2015) who reported minimum periods of 10 and 30 s, respectively. This disagreement may have been due to the more stringent LOA requirement (±5% of the range of the 5-min measurement) and smaller sample in the Shaffer et al. (2019) study.

## PRACTICAL RECOMMENDATIONS

Recommendations for analyses of data from method-comparison studies differ. As previously mentioned, correlation/regression analyses quantify the degree of association between variables but do not denote agreement (Bland and Altman, 1986). As such, we recommend using LoA solutions to assess whether two methods produce comparable results. Although oft-cited guidelines recommend correlation/regression analyses in addition to the LoA solutions (Dewitte et al., 2002), most researchers incorrectly consider them to be supplemental (Dewitte et al., 2002; Bunce, 2009). Although correlation/regression analyses may answer certain questions that are relevant in method-comparison studies (e.g., whether two measures are *not* associated), there is a strong argument against their inclusion in favor of only reporting the LoA and their respective confidence intervals (Bland and Altman, 1986; Bunce, 2009). Prior to conducting method-comparison studies, researchers should consider whether conducting correlation/regression analyses is appropriate.

Assuming that researchers obtain 10-s, 20-s, 30-s, 60-s, 90-s, 120-s, and 180-s RMSSD values and want to determine the shortest period that can estimate a 300-s RMSSD measurement, they should consider the following steps:

(1) Determine whether the RMSSD measurements are normally distributed. If not, use a logarithmic transformation like log(e) or the natural log (ln).
(2) Determine *a priori* the largest acceptable difference between 30-s and 300-s RMSSD values.
(3) Prepare difference plots like Bland–Altman using a 95% confidence interval and then conduct an equality test (e.g., Student's *t*-test) to confirm that the 30-s and 300-s RMSSD values are identical.
(4) If the 30-s RMSSD measurement passes the equality test, then a suitable surrogate has been found. If it fails the test, perform the same analysis with the 60-s measurement, and so on.

## CONCLUSION

Eight of the 11 HRV criterion validity studies we reviewed used correlational and/or group difference criteria that did not control for measurement bias. Because these criteria do not require a maximum acceptable difference (e.g., 5 bpm), they

could yield an UST heart rate value that was 10 bpm higher or lower than its 5-min counterpart. Therefore, minimum recording length prescriptions from studies that used these criteria (Thong et al., 2003; Schroeder et al., 2004; McNames and Aboy, 2006; Salahuddin et al., 2007; Li et al., 2009; Nussinovitch et al., 2011; Brisinda et al., 2015) should be treated with caution and confirmed by studies that use a LOA criterion and confirmative equality tests. As Fleming and DeMets (1996) succinctly stated, "A correlate does not a surrogate make" (p. 605).

The routine use of UST HRV measurements in medicine, performance, and personal fitness assessment awaits advances in six key areas. First, HRV monitoring with automatic artifact correction needs to be added to existing hardware (e.g., activity trackers, pulse oximeters, and smartwatches). Second, researchers should identify the short-term HRV metrics (e.g., RMSSD) most strongly associated with health and performance outcomes. Third, researchers should determine the minimum UST time periods required to estimate these short-term HRV features with respect to age and sex. We recommend a LOA criterion based on the *a priori* determination of the largest acceptable difference between UST and short-term values confirmed by an equality test. Fourth, researchers should demonstrate that UST HRV metrics themselves can forecast real-world health or performance outcomes. UST measurements are proxies of proxies. They seek to replace short-term values, which, in turn, attempt to estimate reference standard long-term metrics. This criterion validity requirement is the most intractable and may prove insurmountable. Fifth, researchers should establish UST HRV norms stratified by age and sex. Sixth, researchers and manufacturers need to educate healthcare professionals and the public about what HRV means, its importance to their health and performance, how it should be measured, and the strategies that can increase it. These six breakthroughs are necessary before HRV monitoring can be more widely used in medicine, performance, and personal health care.

## AUTHOR CONTRIBUTIONS

FS reviewed the literature, wrote the initial manuscript, and made subsequent revisions following feedback and editorial suggestions for all drafts from ZM and CZ. ZM reviewed the literature, created and managed the UST literature database, and summarized and critiqued the UST studies. CZ reviewed the method agreement literature and wrote the methodological critique section. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by Shawn and Jacqui Bergman Fund.

## REFERENCES


Agelink, M., Boz, C., Ullrich, H., and Andrich, J. (2002). Relationship between major depression and heart rate variability. Clinical consequences and implications for anti-depressive treatment. *Psychiatry Res.* 113, 139–149.

Altman, D. G., and Bland, J. M. (1983). Measurement in medicine: the analysis of method comparison studies. *J. R. Stat. Soc. Series B* 32, 307–317.

ANSI/AAMI (2002). *Cardiac Monitors, Heart Rate Meters, and Alarms*. Arlington, TX: American National Standards Institute, Inc.

ANSI/AAMI (2008). *Manual, Electronic, or Automated Sphygmomanometers*. Available online at:http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Manual,+electronic,+or+automated+sphygmomanometers#0\nhttp://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Manual,+Electronic,+Or+Automated+Sphygmomanometers#0(accessed August 13, 2020).

Arza, A., Garzón, J. M., Hermando, A., Aguiló, J., and Bailon, R. (2005). "Towards an objective measurement of emotional stress: preliminary analysis based on heart rate variability," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, (Milano: IEEE Engineering in Medicine and Biology Society), 3331–3334. doi: 10.1109/EMBC.2015.7319105

Baek, H. J., Cho, C. H., Cho, J., and Woo, J. M. (2015). Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability. *Telemed. J. E Health* 21, 404–414. doi: 10.1089/tmj.2014.0104

Beckers, F., Verheyden, B., and Aubert, A. E. (2006). Aging and nonlinear heart rate control in a healthy population. *Am. J. Physiol. Heart Circ. Physiol.* 290, H2560–H2570.

Berkoff, D. J., Cairns, C. B., Sanchez, L. D., and Moorman, C. T. (2007). Heart rate variability in elite American track-and-field athletes. *J. Strength Cond. Res.* 21, 227–231. doi: 10.1519/R-20135.1

Berntson, G. G., Norman, G. J., Hawley, L. C., and Cacioppo, J. T. (2008). Cardiac autonomic balance versus regulatory capacity. *Psychophysiology* 45, 643–652.

Bigger, J. T. Jr., Albrecht, P., Steinman, R. C., Rolnitzky, L. M., Fleiss, J. L., and Cohen, R. J. (1989). Comparison of time- and frequency domain-based measures of cardiac parasympathetic activity in Holter recordings after myocardial infarction. *Am. J. Cardiol.* 64, 536–538.

Bigger, J. T. Jr., Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., and Rottman, J. N. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 85, 164–171. doi: 10.1161/01.CIR.85.1.164

Bland, J. M., and Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 327, 307–310. doi: 10.1016/S0140-6736(86)90837-8

Bland, J. M., and Altman, D. G. (2003). Applying the right statistics: analyses of measurement studies. *Ultrasound Obstet. Gynecol.* 22, 85–93.

Bonaduce, D., Petretta, M., Morgano, G., Villari, B., Binachi, V., Conforti, G., et al. (1994). Left ventricular remodelling in the year after myocardial infarction: an echocardiographic, haemodynamic, and radionuclide angiographic study. *Coron. Artery Dis.* 5, 155–162. doi: 10.1097/00019501-199402000-00009

Brisinda, D., Venuti, A., Cataldi, C., Efremov, K., Intorno, E., and Fenici, R. (2015). Real-time imaging of stress-induced cardiac autonomic adaptation during realistic force-on-force police scenarios. *J. Police Crim. Psychol.* 30, 71–86.

Bunce, C. (2009). Correlation, agreement, and Bland-Altman analysis: statistical analysis of method comparison studies. *Am. J. Ophthalmol.* 148, 4–6. doi: 10.1016/j.ajo.2008.09.032

Byrd, D. L., Reuther, E. T., McNamara, J. P. H., DeLucca, T. L., and Berg, W. K. (2015). Age differences in high frequency phasic heart rate variability and performance response to increased executive function load in three executive function tasks. *Front. Psychol.* 5:1470. doi: 10.3389/fpsyg.2014.01470

Carney, R. M., Freedland, K. E., Stein, P. K., Miller, G. E., Steinmeyer, B., Rich, M. W., et al. (2007). Heart rate variability and markers of inflammation and coagulation in depressed patients with coronary heart disease. *J. Psychosom. Res.* 62, 463–467. doi: 10.1016/j.jpsychores.2006.12.004

Choi, J., and Gutierrez-Osuna, R. (2009). "Using heart rate monitors to detect mental stress," in *Proceedings of the Sixth International Workshop on Wearable and Implantable Body Sensor Networks, 2009*, Berkeley, CA, 219–223.

Choudhary, P. K., and Nagaraja, H. N. (2005). "Measuring agreement in method comparison studies: a review," in *Advances in Ranking and Selection, Multiple Comparisons, and Reliability: Methodology and Applications*,

eds N. Balakrishnan, N. Kannan, and H. N. Nagaraja (Boston, MA: Birkhauser), 215–244.

Cohen, H., and Benjamin, J. (2006). Power spectrum analysis and cardiovascular morbidity in anxiety disorders. *Auton. Neurosci.* 128, 1–8. doi: 10.1016/j.autneu.2005.06.007

De Rivecourt, M., Kuperus, M., Post, W., and Mulder, B. (2008). Cardiovascular and eye activity measures as indices for momentary changes in mental effort during simulated flight. *Ergonomics* 51, 1295–1319. doi: 10.1080/00140130802120267

Dekker, J. M., Schouten, E. G., Klootwijk, P., Pool, J., Swenne, C. A., and Kromhout, D. (1997). Heart rate variability from short electrocardiographic recordings predicts mortality from all causes in middle-aged and elderly men. The Zutphen Study. *Am. J. Epidemiol.* 145, 899–908.

Deming, W. E. (1943). *Statistical Adjustment of Data*. New York, NY: John Wiley & Sons.

Devore, J. L. (2016). *Probability and Statistics for Engineering and the Sciences*. Boston, MA: Cengage Learning.

Dewitte, K., Fierens, C., Stöckl, D., and Thienpont, L. M. (2002). Application of the Bland-Altman plot for the interpretation of method-comparison studies: a critical investigation of its practice. *Clin. Chem.* 48, 799–801.

Esco, M. R., and Flatt, A. A. (2014). Ultra-short-term heart rate variability indexes at rest and post-exercise in athletes: evaluating the agreement with accepted recommendations. *J. Sports Sci. Med.* 13, 535–541.

Ewing, D. J., Campbell, I. W., and Clarke, B. F. (1976). Mortality in diabetic autonomic neuropathy. *Lancet* 1, 601–603. doi: 10.1016/S0140-6736(76)90413-X

Fei, L., Copie, X., Malik, M., and Camm, A. J. (1996). Short- and long-term assessment of heart rate variability for risk stratification after acute myocardial infarction. *Am. J. Cardiol.* 77, 681–684.

Fleming, T. R., and DeMets, D. L. (1996). Surrogate end points in clinical trials: are we being misled? *Ann. Intern. Med.* 125, 605–613.

Gevirtz, R. (2013). The promise of heart rate variability biofeedback: evidence-based applications. *Biofeedback* 41, 110–120.

Giardino, N. D., Lehrer, P. M., and Edelberg, R. (2002). Comparison of finger plethysmograph to ECG in the measurement of heart rate variability. *Psychophysiology* 39, 246–253. doi: 10.1111/1469-8986.3920246

Giardino, N. D., Chan, L., and Borson, S. (2004). Combined heart rate variability and pulse oximetry biofeedback for chronic obstructive pulmonary disease: a feasibility study. *Appl. Psychophysiol. Biofeedback* 29, 121–133. doi: 10.1023/B:APBI.0000026638.64386.89

Giavarina, D. (2015). Understanding Bland-Altman analysis. *Biochem. Med.* 25, 141–151.

Grant, C. C., van Rensburg, D. C., Strydom, N., and Viljoen, M. (2011). Importance of tachogram length and period of recording during noninvasive investigation of the autonomic nervous system. *Ann. Noninvasive Electrocardiol.* 16, 131–139. doi: 10.1111/j.1542-474X.2011.00422.x

Gulliksen, H. (1987). *Theory of Mental Tests*. Hillsdale, NJ: Erlbaum.

Hamilton, C., and Stamey, J. (2007). Using Bland–Altman to assess agreement between two medical devices: don't forget the confidence intervals! *J. Clin. Monit. Comput.* 21, 331–333.

Hays, W. L. (1991). *Statistics*, 5th Edn. Fort Worth, TX: Harcourt Brace College Publishers.

Hjortskov, N., Rissén, D., Blangsted, A. K., Fallentin, L., Lundberg, U., and Sorgaard, K. (2004). The effect of mental stress on heart rate variability and blood pressure during computer work. *Eur. J. Appl. Physiol.* 92, 84–89. doi: 10.1007/s00421-004-1055-z

Hoenig, H., Hoff, J., McIntyre, L., and Branch, L. G. (2001). The self-reported functional measure: predictive validity for health care utilization in multiple sclerosis and spinal cord injury. *Arch. Phys. Med. Rehabil.* 82, 613–618. doi: 10.1053/apmr.2001.20832

Hoffman, J. I. E. (2015). *Biostatistics for Medical and Biomedical Practitioners*. London: Academic Press.

Hon, E. H., and Lee, S. T. (1963). Electronic evaluation of the fetal heart rate. VIII. Patterns preceding fetal death, further observations. *Am. J. Obstet. Gynecol.* 87, 814–826.

Jan, H. Y., Chen, M. F., Fu, T. C., Lin, W. C., Tsai, C. L., and Lin, K. P. (2019). Evaluation of coherence between ECG and PPG derived parameters on heart rate variability and respiration in healthy volunteers with/without controlled breathing. *J. Med. Biol. Eng.* 39, 783–795.

Kazuma, N., Otsuka, K., Matuoska, I., and Murata, M. (1997). Heart rate variability during 24 hours in asthmatic children. *Chronobiol. Int.* 14, 597–606. doi: 10.3109/07420529709001450

Kim, D., Seo, Y., Cho, J., and Cho, C.-H. (2008). "Detection of subjects with higher self-reporting stress scores using heart rate variability patterns during the day," in *Proceedings of the 30th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, Vancouver, BC, 682–685.

Kleiger, R. E., Miller, J. P., Bigger, J. T. Jr., and Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 59, 256–262.

Kleiger, R. E., Stein, P. K., and Bigger, J. T. Jr. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474X.2005.10101.x

Kwon, S., Lee, D., Kim, J., Lee, Y., Kang, S., Seo, S., et al. (2016). Sinabro: a smartphone-integrated opportunistic electrocardiogram monitoring system. *Sensors* 16:361. doi: 10.3390/s16030361

Laborde, S., Mosley, E., and Mertgen, A. (2018). Vagal tank theory: the three Rs of cardiac vagal control functioning–resting, reactivity, and recovery. *Front. Neursci.* 12:458. doi: 10.3389/fnins.2018.00458

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lehrer, P., Kaur, K., Sharma, A., Shah, K., Huseby, R., Bhavsar, J., et al. (2020). Heart rate variability biofeedback improves emotional and physical health and performance: a systematic review and meta analysis. *Appl. Psychophysiol. Biofeedback* 45, 109–129. doi: 10.1007/s10484-020-09466-z

Lehrer, P. M., Vaschillo, E., Vaschillo, B., Lu, S.-E., Scardella, A., Siddique, M., et al. (2004). Biofeedback treatment for asthma. *Chest* 126, 352–361. doi: 10.1378/chest.126.2.352

Li, Z., Snieder, H., Su, S., Ding, X., Thayer, J. F., Treiber, F. A., et al. (2009). A longitudinal study in youth of heart rate variability at rest and in response to stress. *Int. J. Psychophysiol.* 73, 212–217. doi: 10.1016/j.ijpsycho.2009.03.002

Linnet, K. (1993). Evaluation of regression procedures for method comparison studies. *Clin. Chem.* 39, 424–432.

Ludbrook, J. (2010). Confidence in Altman-Bland plots: a critical review of the method of differences. *Clin. Exp. Pharmacol. Physiol.* 37, 143–149.

Mather, M., and Thayer, J. (2018). How heart rate variability affects emotion regulation brain networks. *Curr. Opin. Behav. Sci.* 19, 98–104.

Mayya, S., Jilla, V., Tiwari, V. N., Nayak, M. M., and Narayanan, R. (2015). "Continuous monitoring of stress on smartphone using heart rate variability," in *Proceedings of the IEEE 15th International Conference on Bioinformatics and Bioengineering (BIBE)*, Belgrade, doi: 10.1109/BIBE.2015.7367627

McNames, J., and Aboy, M. (2006). Reliability and accuracy of heart rate variability metrics versus ECG segment duration. *Med. Biol. Eng. Comput.* 44, 747–756. doi: 10.1007/s11517-006-0097-2

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., et al. (2015). Validity of (ultra-) short recordings for heart rate variability measurements. *PLoS One* 10:e0138921. doi: 10.1371/journal.pone.0138921

Nardelli, M., Greco, A., Bolea, J., Valenza, G., Scilingo, E. P., and Bailón, R. (2018). Reliability of lagged Poincaré Plot parameters in ultra-short heart rate variability series: application on affective sounds. *IEEE J. Biomed. Health Inform.* 22, 741–749. doi: 10.1109/JBHI.2017.2694999

Nolan, J., Batin, P. D., Andrews, R., Lindsay, S. J., Brooksby, P., Mullen, M., et al. (1998). Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). *Circulation* 98, 1510–1516.

Nunan, D., Sandercock, G. R. H., and Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing Clin. Electrophysiol.* 33, 1407–1417.

Nussinovitch, U., Elishkevitz, K. P., Nussinovitch, M., Segev, S., Volovitz, B., and Nussinovitch, N. (2011). Reliability of ultra-short ECG indices for heart rate variability. *Ann. Noninvasive Electrocardiol.* 16, 117–122. doi: 10.1111/j.1542-474X.2011.00417.x

Pandey, P., Lee, E. K., and Pompili, D. (2016). A distributed computing framework for real-time detection of stress and of its propagation in a team. *IEEE J. Biomed. Health Inform.* 20, 1502–1512. doi: 10.1109/JBHI.2015.2477342

Papousek, I., Nauschnegg, K., Paechter, M., Lackner, H. K., Goswami, N., and Schulter, G. (2010). Trait and state positive affect and cardiovascular recovery from experimental academic stress. *Biol. Psychol.* 83, 108–115. doi: 10.1016/j.biopsycho.2009.11.008

Passing, H., and Bablok, W. (1983). A new biometrical procedure for testing the equality of measurements from two different analytical methods: application of linear regression procedures for method comparison studies in Clinical Chemistry, Part I. *J. Clin. Chem. Clin. Biochem.* 21, 709–720.

Passing, H., and Bablok, W. (1984). Comparison of several regression procedures for method comparison studies and determination of sample sizes: application of linear regression procedures for method comparison studies in clinical chemistry, part II. *J. Clin. Chem. Clin. Biochem.* 22, 431–445.

Pecchia, L., Castaldo, R., Montesinos, L., and Melillo, P. (2018). Are ultra-short heart rate variability features good surrogates of short-term ones? state-of-the-art review and recommendations. *Healthc. Technol. Lett.* 5, 94–100. doi: 10.1049/htl.2017.0090

Pereira, T., Almeida, P. R., Cunha, J. P., and Aguiar, A. (2017). Heart rate variability metrics for fine-grained stress level assessment. *Comput. Methods Programs Biomed.* 148, 71–80. doi: 10.1016/j.cmpb.2017.06.01

Price, L. R. (2018). *Psychometric Methods: Theory into Practice*. New York, NY: The Guilford Press.

Salahuddin, L., Cho, J., Jeong, M. G., and Kim, D. (2007). "Ultra-short-term analysis of heart rate variability for monitoring mental stress in mobile settings," in *Proceedings of the IEEE Engineering in Medicine and Biology Society*, Lyon, 4656–4659.

Schafer, A., and Vagedes, J. (2013). How accurate is pulse rate variability as an estimate of heart rate variability? A review on studies comparing photoplethysmographic technology with an electrocardiogram. *Int. J. Cardiol.* 166, 15–29. doi: 10.1016/j.ijcard.2012.03.119

Schroeder, E. B., Whitsel, E. A., Evans, G. W., Prineas, R. J., Chambless, L. E., and Heiss, G. (2004). Repeatability of heart rate variability measures. *J. Electrocardiol.* 37, 163–172. doi: 10.1016/j.jelectrocard.2004.04.004

Schubert, C., Lambertz, M., Nelesen, R., Bardwell, W., Cho, J.-B., and Dimsdale, J. E. (2009). Effects of stress on heart rate complexity—a comparison between short-term and chronic stress. *Biol. Psychol.* 80, 325–332. doi: 10.1016/j.biopsycho.2008.11.005

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability (HRV) metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shaffer, F., McCraty, R., and Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front. Psychol.* 5:1040. doi: 10.3389/fpsyg.2014.01040

Shaffer, F., Shearman, S., Meehan, Z., Gravett, N., and Urban, H. (2019). "The promise of ultra-short-term (UST) heart rate variability measurements: a comparison of Pearson product-moment correlation coefficient and limits of agreement (LoA) concurrent validity criteria," in *Physiological Recording Technology and Applications in Biofeedback and Neurofeedback*, eds D. Moss and F. Shaffer (Oakbrook Terrace, IL: Association for Applied Psychophysiology and Biofeedback), 214–220.

Shah, A. J., Lampert, R., Goldberg, J., Veledar, E., Bremner, J. D., and Vaccarino, V. (2013). Posttraumatic stress disorder and impaired autonomic modulation in male twins. *Biol. Psychiatry* 73, 1103–1110. doi: 10.1016/j.biopsych.2013.01.019

Stein, P. K., and Reddy, A. (2005). Non-linear heart rate variability and risk stratification in cardiovascular disease. *Indian Pacing Electrophysiol. J.* 5, 210–220.

Sun, F.-T., Kuo, C., Cheng, H.-T., Buthpitiya, S., Collins, P., and Griss, M. (2010). "Activity-aware mental stress detection using physiological sensors," in *Proceedings of the International Conference on Mobile Computing, Applications, and Services*, Santa Clara, CA, 211–230. doi: 10.1007/978-3-642-29336-8_12

Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216.

Thong, T., Li, K., McNames, J., Aboy, M., and Goldstein, B. (2003). "Accuracy of ultra-short heart rate variability measures," in *Proceedings of the 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, Cancun, 2424–2427. doi: 10.1109/IEMBS.2003.1280405

Tsuji, H., Venditti, F. J. Jr., Manders, E. S., Evans, J. C., Larson, M. G., Feldman, C. L., et al. (1994). Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. *Circulation* 90, 878–883. doi: 10.1161/01.CIR.90.2.878

Umetani, K., Singer, D. H., McCraty, R., and Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. Coll. Cardiol.* 31, 593–601.

Wang, X., Ding, X., Su, S., Li, Z., Riese, H., Thayer, J. F., et al. (2009). Genetic influences on heart rate variability at rest and during stress. *Psychophysiology* 46, 458–465. doi: 10.1111/j.1469-8986.2009.00793.x

Watson, P. F., and Petrie, A. (2010). Method agreement analysis: a review of correct methodology. *Theriogenology* 73, 1167–1179.

Westgard, J. O., and Hunt, M. R. (1973). Use and interpretation of common statistical tests in method-comparison studies. *Clin. Chem.* 19, 49–57.

Wijsman, J., Grundlehner, B., Liu, H., Hermens, H., and Penders, J. (2011). "Towards mental stress detection using wearable physiological sensors," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, Boston, MA, 1798–1801.

Xu, Q., Nwe, T. L., and Guan, C. (2015). Cluster-based analysis for personalized stress evaluation using physiological signals. *IEEE J. Biomed. Health Inform.* 19, 275–281.

Zaki, R., Bulgiba, A., Ismail, R., and Ismail, N. A. (2012). Statistical methods used to test for agreement of medical instruments measuring continuous variables in method comparison studies: a systematic review. *PLoS One* 7:e37908. doi: 10.1371/journal.pone.0037908

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 25 November 2020
doi: 10.3389/fnins.2020.575589

# Probing Neurovisceral Integration via Functional Near-Infrared Spectroscopy and Heart Rate Variability

*Emma E. Condy[1], Bruce H. Friedman[2]* and Amir Gandjbakhche[1]*

*[1] National Institute of Child Health and Human Development, Bethesda, MD, United States, [2] Department of Psychology, Virginia Tech, Blacksburg, VA, United States*

The neurovisceral integration model (NVM) proposes that an organism's ability to flexibly adapt to its environment is related to biological flexibility within the central autonomic network (CAN). One important aspect of this flexibility is behavioral inhibition (Thayer and Friedman, 2002). During a behavioral inhibition task, the CAN, which comprises a series of feedback loops, must be able to integrate information and react to these inputs flexibly to facilitate optimal performance. The functioning of the CAN is shown to be associated with respiratory sinus arrhythmia (RSA), as the vagus nerve is part of this feedback system. Although the NVM has been examined through neural imaging and RSA, only a few studies have examined these measures simultaneously during the neuroimaging procedure. Furthermore, these studies were done at rest or used tasks that were not targeted at processes associated with the NVM, such as behavioral inhibition and cognitive flexibility. For this reason, the present study assessed RSA and neural activation in the pre-frontal cortex simultaneously while participants completed a behavior inhibition task. RSA and functional near-infrared spectroscopy were collected in 38 adults, and resting levels of pre-frontal activation were negatively related to RSA, but pre-frontal activation during the behavior inhibition task was not. The negative relationship between RSA and oxygenated hemoglobin is consistent with previous functional magnetic resonance imaging work examining the NVM at baseline and should be further studied. Additional research investigating how this relationship may change based on task demands or environmental contexts would help clarify the applicability of the model.

**Keywords: respiratory sinus arrhythmia, behavioral inhibition, neurovisceral integration, functional near-infrared spectroscopy, central autonomic network**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer, The Ohio State University, United States*

***Reviewed by:***
*Riccardo Pernice, University of Palermo, Italy*
*Alessandro Tonacci, Italian National Research Council, Italy*

****Correspondence:***
*Bruce H. Friedman*
*bhfriedm@vt.edu*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *23 June 2020*
***Accepted:*** *19 October 2020*
***Published:*** *25 November 2020*

***Citation:***
*Condy EE, Friedman BH and Gandjbakhche A (2020) Probing Neurovisceral Integration via Functional Near-Infrared Spectroscopy and Heart Rate Variability. Front. Neurosci. 14:575589. doi: 10.3389/fnins.2020.575589*

## INTRODUCTION

The principal role of the autonomic nervous system (ANS) is to maintain the body's internal environment by distributing and integrating specific signals to target organs (Janig and Habler, 2000). The ANS is traditionally divided into two branches: the parasympathetic and sympathetic, which have anatomical, functional, and neurochemical

distinctions[1]. The neurovisceral integration model (NVM) proposes that these systems are part of the central autonomic network (CAN) (Thayer and Lane, 2000), which operates through a series of feedback loops within the central nervous system. These circuits include both central and peripheral inputs and outputs and subsequently influence several behavioral and physiological processes. Importantly, measurements of the peripheral outputs of this system can be used to index the functionality of feedback loops in the CAN. Cortical structures in the CAN that are highlighted in the NVM include the medial pre-frontal cortex (mPFC), anterior cingulate cortex, and the insula (Benarroch, 1993).

The NVM proposes that cortical structures such as the mPFC institute tonic inhibition on activation of the amygdala, resulting in heightened CAN output (Thayer and Lane, 2009). This view is based on evidence from animal and human neuroimaging studies, which have established structural interconnections, and pharmacological studies in which both branches of the ANS are blocked and show increased activation compared with the normal state, indicating that the system is under the tonic inhibitory influence (Thayer and Lane, 2009; Thayer et al., 2012). One of the outputs of the CAN is the $10^{th}$ cranial, or vagus nerve, which provides parasympathetic input to the heart. This vagal activation can be indexed through heart rate variability (HRV), which is commonly used to provide information about the functionality of the CAN.

The variation from beat-to-beat in heart rate, known as HRV, is controlled by sympathetic and parasympathetic nervous system inputs to the sinoatrial node of the heart (Task Force, 1996). Differences in the temporal dynamics of the primary neurotransmitters involved in sympathetic and parasympathetic activation (norepinephrine and acetylcholine, respectively) allow fast changes in interbeat interval (IBI) length to be attributed to vagal control (Saul, 1990). Respiratory sinus arrhythmia (RSA) refers specifically to changes in the lengths of IBIs as a result of respiration (Berntson et al., 1993), which can be quantified through changes within the high-frequency band (0.12–0.40 Hz), known as high-frequency heart rate variability (HF-HRV) (Allen et al., 2007), or through a time-based metric using the root mean square successive difference (RMSSD) (Berntson et al., 1997; Laborde et al., 2017).

In the NVM, high baseline RSA and increased RSA reactivity are viewed as indicative of increased flexibility within the CAN (Friedman, 2007). Flexibility within this biological system is manifested through greater cognitive and behavioral flexibility, as demonstrated by skills such as better emotion regulation and inhibitory capacities (Thayer et al., 2009). The present study aimed to further investigate the tenets of the NVM through simultaneous measurement of RSA and cortical activation. Although previous studies have looked at RSA in relation to brain activity, there are many limitations in this literature, such as small sample sizes and flawed methodology. In the present study, pre-frontal cortex activity was assessed via functional near-infrared spectroscopy (fNIRS) in concert with RSA during a baseline condition and a cognitive task. Although functional imaging has been used to examine this model during resting state in previous studies, the present study investigated whether the tenets of the NVM are upheld across multiple contexts using a more ecologically valid neural metric to do so. As such, the present study can be viewed as a "proof of concept" investigation; i.e., it is the implementation of a method to demonstrate its feasibility (Schmidt, 2006).

## Human Neuroimaging and the Neurovisceral Integration Model

Much of the literature supporting the NVM and the relationship between RSA and the CAN is based on animal models and blockade studies. However, there have been several human neuroimaging studies examining the theory. These studies were reviewed in two functional magnetic resonance imaging (fMRI) meta-analyses, which concluded that a number of areas associated with the NVM were activated in conjunction with heightened HF-HRV (Thayer et al., 2012; Beissner et al., 2013). Specifically, HF-HRV was associated with the activation of regions of the mPFC (Thayer et al., 2012).

Furthermore, Beissner et al. (2013) note that many of the structures associated with HF-HRV in their meta-analysis are part of the *default mode network*, a network of brain regions that are active when an individual is not engaged in a task and is instead focused on internal events (see Raichle, 2015 for a review). Some of these regions overlap with the CAN, such as the ventromedial pre-frontal cortex and dorsomedial pre-frontal cortex, posterior cingulate cortex, and hippocampal formation (Buckner et al., 2008). Indeed, a recent review paper on the NVM has implicated networks such as the executive control and default mode networks in the hierarchical structure of neurovisceral integration (Smith et al., 2017). However, the majority of studies included in these meta-analyses had limited sample sizes (Beissner et al., 2013, sample size median = 12, range = 4–41; Thayer et al., 2012, sample size median = 15, range = 6–93), used dissimilar methods (e.g., some baseline and others task-based measures), and incorporated HF-HRV measurements that were taken at a different time than the neuroimaging data. These are all significant limitations, but the last point is particularly concerning. It was assumed in these studies that the participants' state during an independent baseline HF-HRV measurement would be comparable with their state during the neuroimaging data collection. Simultaneous collection of HF-HRV and neuroimaging data is necessary to avoid such assumptions, which cannot be unequivocally made. Regardless, both meta-analyses indicated that baseline HF-HRV values correlate with neural activity in areas implicated in the CAN.

These limitations were noted and addressed in subsequent research aimed at improving the examination of the NVM. In two studies that had significantly larger samples, associations were found in similar brain regions (left insula, right hippocampus, and right superior temporal gyrus) to these meta-analyses (Allen et al., 2015; Jennings et al., 2015). However, HF-HRV data were

[1]A third enteric branch, which innervates the gastrointestinal organs, is sometimes also distinguished (see Janig and Habler, 2000).

still collected outside of the scanner, approximately 2 weeks before neuroimaging. Furthermore, these studies used resting-state data, whereas many of the studies in the meta-analyses used task reactivity. These authors argued that the relationship between RSA and neural activation would be inverse during the resting state because many previous studies examined RSA reactivity in relation to neural activation. The logic behind this argument is that because higher resting HF-HRV is associated with larger reductions in task HF-HRV (a negative correlation) and larger reductions in task HF-HRV are associated with less neural activation (a positive correlation), then the transitive property suggests that higher resting HF-HRV is associated with less neural activation (Allen et al., 2015). Their results supported this hypothesis; activation of multiple structures implicated in the NVM (e.g., insula, amygdala, pre-frontal cortex) was negatively related to HF-HRV.

Other studies have improved the co-examination of RSA and fMRI by using time series analyses that look at the two signals simultaneously. Through comparison of sliding-window calculations of both resting fMRI and HRV, greater functional *connectivity* between the dorsal anterior cingulate cortex/amygdala and the mPFC, as well as the insula, was associated with heightened HF-HRV (Chang et al., 2013). Although these results are compelling, the use of connectivity analyses (vs. regional activation) as the dependent variable may have missed other regions implicated by the NVM. Using a similar method, positive correlations between HF-HRV and several areas associated with the NVM including the amygdala, right dorsal mPFC, and right dorsal lateral PFC, as well as negative correlations with the left posterior insula and right medial temporal gyrus during a motor task, have been found (Gianaros et al., 2004). In the same study, during a series of memory tasks, HF-HRV was positively correlated with the left insula and amygdala–hippocampal complex and right ventromedial PFC and cerebellum. These findings support the NVM by examining CAN activation and RSA simultaneously, but they do not probe the model in terms of simple regional activation at rest; they either used functional connectivity metrics or did not include resting state. Extending this method to include resting-state measurement and a task that taps into the psychological constructs implicated in the NVM, such as behavioral inhibition, will better elucidate how the CAN functions, particularly when being actively recruited.

One way to test behavioral inhibition is the go/no-go (GNG) task, which was developed for this purpose (Donders, 1969). The GNG has been used across a variety of studies to assess how behavioral inhibition relates to other functions. GNG performance is attenuated in many disorders characterized by inhibition deficits, such as attention deficit–hyperactivity disorder (Dillo et al., 2010), obsessive–compulsive disorder (Lee et al., 2009), and autism spectrum disorder (Uzefovsky et al., 2016). The task consists of two types of stimuli: the "go" stimulus, which indicates that the subject should complete the behavioral response (e.g., a button press), and the "no-go" stimulus, which indicates that the subject should not complete the behavioral response (e.g., no response). By prompting a prepotent response through a series of "go" trials, the subject is then challenged to inhibit the response behavior during the "no-go" stimulus. Its wide use in the neuroimaging literature provides extensive information about the neural correlates of behavioral inhibition, which is implicated in the NVM. Meta-analyses on fMRI studies of the GNG show increased activity in frontal cortical areas during "no-go" trials, such as the bilateral mPFC (Watanabe et al., 2002), the pre-supplementary motor area (Mostofsky et al., 2003), and the right inferior parietal lobule (Swick et al., 2011). The mPFC is one of the structures in the CAN, making the GNG task an appropriate paradigm for the present study. However, due to temporal and ecological limitations of fMRI technology, we incorporated a different imaging modality (i.e., fNIRS) to assess inhibition in the GNG task.

## Functional Near-Infrared Spectroscopy

fNIRS is an optical imaging technique that projects infrared light (650–1,000 nm) from a source diode into tissue and measures the backscatter of this light using detectors. Based on the amount of backscatter, the concentration of oxygenated hemoglobin ($O_2Hb$) and deoxygenated hemoglobin (HHb) can be determined because of their different optical properties (Fox and Raichle, 1986; Ferrari and Quaresima, 2012). Not only does fNIRS have better temporal resolution than fMRI, but there is also less concern about motion artifact interfering with the signal, affording greater ecological validity and task flexibility (Irani et al., 2007; Lloyd-Fox et al., 2010). These are important considerations in the context of the NVM, in which anxiety and stress can be triggered by neuroimaging approaches such as fMRI (Eatough et al., 2009; Lueken et al., 2012). These states are associated with the activation of CAN structures, which confounds the external validity of conclusions from such studies. Additionally, the NVM has yet to be evaluated in populations with compliance issues in other neuroimaging environments, such as children and those with neurodevelopmental disorders (Yerys et al., 2009), making the use of fNIRS advantageous. For these reasons, fNIRS is a valuable technique for collecting neuroimaging data from the cortex during task-based studies of cognition and behavior that can be applied to the NVM.

Similar to fMRI findings, fNIRS studies have shown increased pre-frontal $O_2Hb$ during the GNG task compared with rest (Anderson et al., 2014) and increased $O_2Hb$ during "no-go" trials compared with "go" trials in lateral pre-frontal locations (Herrmann et al., 2005). However, these studies simply looked at neural activation between trial types. When actual responses to the GNG task are considered, successful behavioral inhibition during "no-go" trials predicts lower $O_2Hb$ in the mPFC but not in lateral locations (Rodrigo et al., 2014). Together, these findings indicate that increased activation in frontal lateral areas (such as the inferior frontal gyrus) may be associated with the presentation of the "no-go" condition or behavioral inhibition opportunity itself, but that successful behavioral inhibition appears to specifically be associated with decreased activity in the mPFC. This distinction may account for discrepancies across the fMRI GNG studies. Furthermore, the NVM posits that the mPFC is integral in the top–down, tonic inhibition of the CAN. Previous work suggests this may be reflected by a negative relationship between activation of the PFC and RSA at rest (Allen et al., 2015).

However, when the mPFC is recruited during an active behavior inhibition task, the relationship between its activation and RSA may differ. The present study aimed to incorporate simultaneous fNIRS and RSA measurements throughout a baseline period, as well as during the GNG task, to assess their relationship as described in the NVM across various contexts. We hypothesized that RSA and neural activation in areas contained in the CAN, namely the mPFC, will be related at rest and during the GNG task.

# MATERIALS AND METHODS

## Participants

Participants were recruited through the healthy volunteer database at the National Institutes of Health in Bethesda, MD, and were compensated for participation in the study. Exclusionary criteria included: past or present vascular disease, skin disease, or any history of head injury, cardiovascular disease, or congenital heart condition, seizure, or stroke. A total of 45 participants were brought to the lab to participate in the study. Participants completed a health history questionnaire that screened for psychiatric diagnoses; one indicated a current psychiatric diagnosis and was removed from the sample. Furthermore, due to data loss in either the fNIRS or electrocardiogram (ECG) signals, 38 participants were retained for analyses in the present paper. The final sample consisted of 38 healthy adults (age $M$ = 37.18, $SD$ = 14.67). The majority of participants were right-handed (32; 84.2%), three were left-handed, and three were ambidextrous as measured on the Edinburgh Handedness Inventory (Oldfield, 1971). These participants were retained in the analyses because we did not expect a lateralization effect for pre-frontal activation at rest or during the task. The present study was part of an ongoing research protocol that was approved by the National Institute of Child Health and Human Development's Institutional Review Board (NCT01212029). All participants underwent the informed consent procedure as approved by the Institutional Review Board before participating in the study.

## Measures

### Electrocardiogram

ECG was collected through the BioPac MP160 system, outfitted with an ECG amplifier (ECG100C). The ECG was sampled at 1,000 Hz with an amplifier gain of 1,000, a low pass filter set at 35 Hz, and high pass filters set at 0.5 Hz. The signal was recorded through AcqKnowledge 4.4 software (BioPac Systems Inc.). A Lead II ECG configuration was used, which requires Ag-AgCl spot electrodes to be placed underneath the left collar bone on the chest and underneath the rib cage on the right side. RSA was then derived from this signal through Kubios HRV software (Tarvainen et al., 2014) using the time-domain RMSSD metric. RMSSD is shown to reflect cardiac vagal tone (Laborde et al., 2017) and can be derived from short recording periods such as the 30-s windows used in the present study (Munoz et al., 2015).

### Functional Near-Infrared Spectroscopy

Neural activity was measured through a continuous wave fNIRS device (fNIR Devices LLC), which emits light at two wavelengths (730 and 850 nm) and samples at a rate of 2 Hz. The use of two infrared light wavelengths allows both oxy- and deoxy-hemoglobin levels to be measured, in which they produce differential amounts of backscatter to be picked up by the detectors. The system uses 4 sources and 10 detectors spaced 2.5 cm from another, creating a 16-channel silicone headband placed across the forehead of participants to measure pre-frontal activity. The headband was centered at Fpz. Data from the device were collected through COBI Studio software (Ayaz et al., 2011).

## Procedure

The protocol for this study was approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development's Institutional Review Board. Participants were instructed to abstain from alcohol for 24 h, caffeine for 6 h, and vigorous exercise for 2 h before their data collection session. All participants were scheduled for their session to begin between the hours of 8:15–11:30 am. Upon entering the lab, the subject was provided a copy of the informed consent and reviewed it with the researcher. Once subjects provided consent, they were outfitted with ECG electrodes and a respiration monitor. The ECG and respiration signal were then examined to ensure that the physiological equipment was properly and securely applied. Once this was completed, the subject filled out the series of behavioral scales. These questionnaires took approximately 15 min to complete, after which the fNIRS headband was applied, and signals were checked to ensure that the headband had been properly applied. The physiological and neural imaging portion of the session then began. Participants were positioned in front of a computer equipped with E-Prime 2 Stimulus Presentation software (Schneider et al., 2002), which presented all instructions and tasks for the remainder of the data collection session.

### Baseline

After completing the health history questionnaire and behavioral scales and application of the fNIRS headband, a 6:30 min recording period began. During this time, participants were instructed to sit quietly while watching a mildly stimulating video (Coral Sea Dreaming: Plankton Productions & MJL Network, 2014) presented on a computer monitor, consistent with "vanilla" baseline guidelines (Jennings et al., 1992). Such baselines are common in psychophysiology because they maintain minimal engagement and create more uniform conditions across participants than a traditional baseline. During this time, physiological and neural imaging data were collected.

### Cognitive Task

Participants then completed two versions of a GNG task: the simple GNG and the emotional GNG, the order of which was counterbalanced across participants. Only data from the Simple GNG task are analyzed for the purposes of this study. The emotional GNG was not included due to issues with performance on the task for a number of participants (e.g., participants had difficulty understanding or remembering the emotional GNG directions). The simple GNG paradigm uses basic stimuli (e.g., numbers, letters, shapes) to examine behavioral inhibition

abilities. In the present study, a GNG paradigm using letter stimuli was presented to participants through E-Prime 2. The timing and proportion standards for both the simple GNG and emotional GNG in this study were modeled after (Schulz et al., 2007), in which both the simple and emotional GNG task were used. Each block consisted of 192 total trials, which were comprised of 75% "go" stimuli (the letter "Y") and 25% "no-go" stimuli (the letter "X"), resulting in 144 "go" and 48 "no-go" stimuli. Each stimulus was presented for 500 ms, followed by a pseudorandom interstimulus interval of 1,500 ± 250 ms. The interstimulus interval jitter is used to reduce the effect of stimulus anticipation of the subject's reaction time. With these timing parameters, the task was approximately 6 min and 24 s long. The use of a block design best served the study's aim, in which it allowed for a sliding window analysis to be conducted throughout the task procedure to examine the relationship between the fNIRS and RSA metrics. Although this design does not provide a pure period of active behavioral inhibition, it draws upon behavior inhibition resources during the task period compared with baseline. On-screen instructions informed the participants that they would be presented with a series of letters. They were instructed to press the space bar when they saw the letter "Y" and not to press any buttons when they saw the letter "X." If they understood these instructions, they were instructed to press the space bar, which initiated six task practice trials. The task began upon completion of these trials.

## Data Processing

### Pre-processing Electrocardiogram

All ECG data were preprocessed using AcqKnowledge 4.4 software. First, event markers sent from the EPrime script were identified in the ECG file, and their times were recorded for use in calculating the sliding window parameters. Next, the "Find Cycle" function was used to automatically detect and mark R-spikes in the ECG signal. These marks were then visually inspected to ensure that aberrations in the signal were not mistakenly marked as R-spikes and that R-spikes were not missed by the algorithm. The "Find Cycle" function was used again to locate these visually inspected marks and then calculate the time between each R-spike pair (i.e., the IBI) in milliseconds. These values were then saved as a text file that contained two columns: time and IBI length (millisecond).

### Pre-processing Functional Near-Infrared Spectroscopy

Imaging data were preprocessed by the subject through HOMER2 software to remove motion artifact and physiological noise. The parameters used within each function are defined in **Table 1**. First, event markers sent from the EPrime script in each file were located and their times recorded for use in the sliding window parameter calculations. The raw light intensity files were converted to the HOMER2 file format (.nirs). The raw light intensity was converted to optical density (function: *hmrIntensity2OD*), and bad channels were removed (function: *enPruneChannels*, dRange = 500–4,000, SNRthresh = 2, SDrange = 0–45). A wavelet transform was used to correct for motion artifact (function: *hmrMotionCorrectWavelet*) using the default interquartile range (0.1), as this is optimal for motion correction (Brigadoi et al., 2014). Any remaining motion artifact was then removed through the motion artifact detection tool (function: *hmrMotionArtifact*, tMotion = 0.5, tMask = 2.0, STDEVthresh = 20, AMPthresh = 0.5). The signal was then bandpass filtered (function: *hmrBandpassFilt*, hpf = 0.010, lpf = 0.50) to remove baseline drift and physiological noise. Finally, the optical density signal was converted to hemoglobin concentration (function: *hmrOD2Conc*) by applying the Modified Beer–Lambert Law. Depending on the wavelengths of light that are used, the Modified Beer–Lambert Law allows for the calculation of oxygenated and deoxygenated hemoglobin concentration (micrometer) in a highly scattering medium, such as biological tissues, by accounting for scattering losses and a longer optical path length because of scattering. The $O_2Hb$ values were then saved as text files for each subject. Finally, the $O_2Hb$ time series for each subject was z-scored by channel.

### Post-processing Electrocardiogram and Functional Near-Infrared Spectroscopy

Event markers from each subject's ECG and fNIRS data files were used to calculate the parameters for sliding window epochs to be used for subsequent analyses. The window length was set to 30 s with 7 s of overlap with adjacent windows, resulting in a 46.67% overlap between adjacent windows. For each condition, this resulted in 16 windows, for a total of a 6:15 min condition period. This was done to generate start and stop times for each window within each condition for both the ECG and fNIRS signals. The window values from the ECG signal were then used

**TABLE 1 |** Homer2 fNIRS pre-processing parameter definitions.

| Function name | Parameter name | Definition |
|---|---|---|
| *enPruneChannels* | dRange | Allowable optical density (OD) range |
| | SNRthresh | Minimum signal-to-noise ratio allowable |
| | SDrange | Maximum standard deviation allowable |
| *hmrMotionArtifact* | tMotion | Time range to check for a motion artifact (seconds) |
| | tMask | Amount of time (seconds) surrounding a detected motion artifact where data are to be removed |
| | STDEVthresh | Threshold for a change in signal standard deviation within the tMotion period to be marked as motion artifact |
| | AMPthresh | Threshold for a change in signal amplitude within the tMotion period to be marked as motion artifact |
| *hmrBandpassFilt* | hpf | High pass filter cutoff (Hz) |
| | lpf | Low pass filter cutoff (Hz) |

to compute RMSSD for each 30 s window (Takahashi et al., 2017) in Kubios HRV software from the IBI files previously mentioned. The corresponding window values from the fNIRS signal markers were then used to compute the mean of the standardized $O_2Hb$ signals within each window for each channel. The corresponding RMSSD and $O_2Hb$ values were then used to complete subsequent analyses for each subject.

## Analyses

RMSSD and $O_2Hb$ concentrations were quantified over the baseline period through sliding window analysis. Doing this allowed a series of RMSSD and $O_2Hb$ values to be generated at multiple time points over the baseline period so that the correspondence between the RMSSD and neural values could be tracked over the baseline time course for each subject. These analyses were modeled after those conducted by Chang et al. (2013), who performed similar analyses to compare RMSSD and fMRI signals. These values were standardized and entered in a subject-level general linear model to determine whether RMSSD predicted $O_2Hb$ concentration. This was done for each subject at each channel, providing 16-channel parameter estimates per subject per condition to be used for statistical analyses in subsequent steps. Running these models for each channel for each subject yielded a 38 (subject) by 16 (fNIRS 0channels) matrix of standardized parameter estimates (β) for each condition. A series of one-sample *t*-tests were then conducted to determine whether the relationship between RMSSD and $O_2Hb$ was different than 0 across the 16 channels. If a test was significant, this would indicate that the relationship between RMSSD and activation at that channel was not equal to 0 and would support the hypothesis that activation at that specific region of the pre-frontal cortex was related to RMSSD. These processes and statistical tests were conducted through R software (R Core Team, 2017).

# RESULTS

## Baseline

A series of non-parametric one-sample *t*-tests were conducted using standardized β weights from the subject-level general linear models where RMSSD predicted $O_2Hb$ during the baseline condition as the dependent variable. At $p < 0.05$, increased RMSSD predicted lower $O_2Hb$ levels at channel 1 ($V = 60$, $p = 0.030$), channel 10 ($V = 179$, $p = 0.008$), and channel 15 ($V = 107$, $p = 0.049$) during baseline. The channel configuration across the PFC can be seen in **Figure 1**. The resulting statistics from the series of tests for the baseline condition are summarized in **Table 2**. None of these tests were significant after using the Holm–Bonferroni method of sequentially rejective *t*-tests.

## Go/No-Go Task

First, to verify that the GNG task had the intended effect of increasing pre-frontal activation, the average $O_2Hb$ concentrations over the baseline and GNG task period were compared. Although channel-level activation may show an

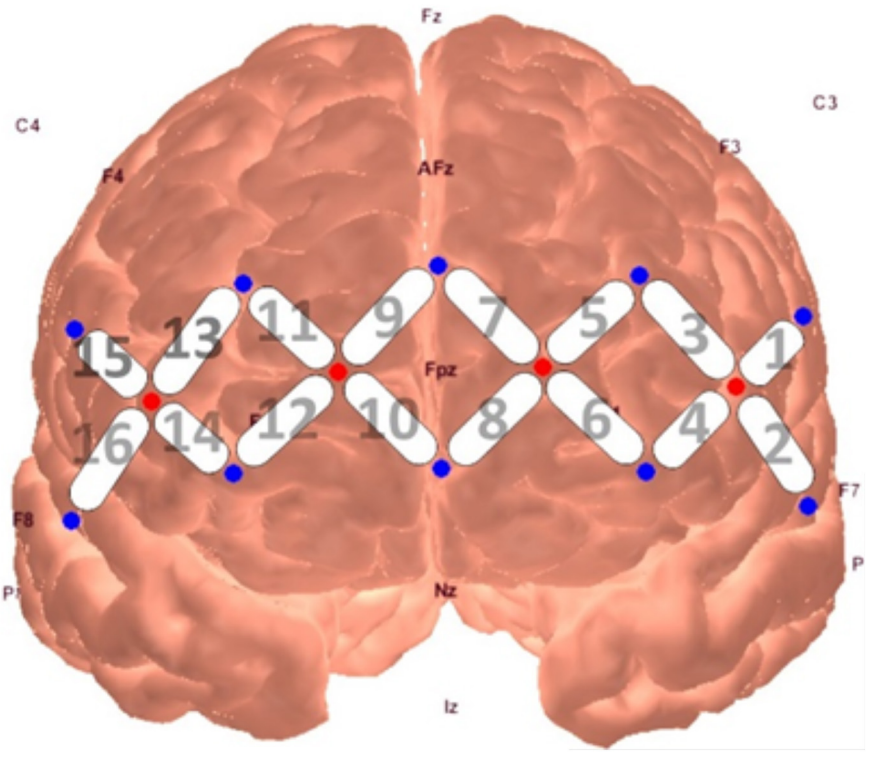



**FIGURE 1 |** fNIRS probe placement and channel locations rendered in AtlasViewer software (Aasted et al., 2015).

**TABLE 2 |** One sample *t*-tests of standardized regression coefficients of RMSSD predicting $O_2$Hb during baseline and the GNG task.

| | Baseline | | | | | | | Go/No-Go | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Channel** | ***M*** | ***SD*** | ***V*** | ***n*** | ***p*** | ***d*** | **95% CI$_d$** | ***M*** | ***SD*** | ***V*** | ***n*** | ***p*** | ***d*** | **95% CI$_d$** |
| 1 | −0.14 | 0.24 | 60 | 22 | 0.030* | −0.58 | [−1.03, −0.13] | 0.01 | 0.29 | 104 | 20 | 0.985 | 0.03 | [−0.41, 0.47] |
| 2 | −0.05 | 0.22 | 204 | 33 | 0.177 | −0.23 | [−0.58, 0.12] | 0.02 | 0.23 | 231 | 30 | 0.984 | 0.09 | [−0.27, 0.45] |
| 3 | −0.04 | 0.22 | 219 | 32 | 0.410 | −0.18 | [−0.53, 0.17] | −0.01 | 0.28 | 235 | 30 | 0.968 | −0.04 | [−0.40, 0.32] |
| 4 | −0.06 | 0.21 | 184 | 32 | 0.139 | −0.29 | [−0.64, 0.06] | 0.03 | 0.26 | 254 | 30 | 0.670 | 0.12 | [−0.24, 0.48] |
| 5 | −0.01 | 0.20 | 217 | 28 | 0.762 | −0.05 | [−0.42, 0.32] | −0.02 | 0.21 | 120 | 25 | 0.263 | −0.1 | [−0.49, 0.29] |
| 6 | −0.04 | 0.25 | 302 | 37 | 0.464 | −0.16 | [−0.48, 0.16] | 0.00 | 0.24 | 296 | 34 | 0.987 | 0 | [−0.34, 0.34] |
| 7 | −0.03 | 0.22 | 319 | 37 | 0.633 | −0.14 | [−0.46, 0.18] | −0.03 | 0.23 | 257 | 34 | 0.499 | −0.13 | [−0.47, 0.21] |
| 8 | −0.06 | 0.23 | 238 | 36 | 0.139 | −0.26 | [−0.59, 0.07] | −0.02 | 0.24 | 263 | 33 | 0.764 | −0.08 | [−0.42, 0.26] |
| 9 | −0.07 | 0.26 | 219 | 36 | 0.074 | −0.27 | [−0.60, 0.06] | 0.00 | 0.25 | 303 | 34 | 0.933 | 0 | [−0.34, 0.34] |
| 10 | −0.12 | 0.27 | 179 | 37 | 0.008* | −0.44 | [−0.78, −0.10] | 0.01 | 0.21 | 322 | 34 | 0.685 | 0.05 | [−0.29, 0.39] |
| 11 | −0.05 | 0.23 | 223 | 32 | 0.454 | −0.22 | [−0.57, 0.13] | −0.03 | 0.20 | 163 | 30 | 0.158 | −0.15 | [−0.51, 0.21] |
| 12 | −0.06 | 0.21 | 221 | 35 | 0.127 | −0.29 | [−0.63, 0.05] | 0.00 | 0.21 | 277 | 33 | 0.958 | 0 | [−0.34, 0.34] |
| 13 | −0.06 | 0.22 | 237 | 35 | 0.207 | −0.27 | [−0.61, 0.07] | 0.02 | 0.26 | 293 | 33 | 0.832 | 0.08 | [−0.26, 0.42] |
| 14 | −0.03 | 0.25 | 306 | 36 | 0.681 | −0.12 | [−0.45, 0.21] | 0.01 | 0.25 | 304 | 34 | 0.919 | 0.04 | [−0.30, 0.38] |
| 15 | −0.12 | 0.24 | 107 | 27 | 0.049* | −0.50 | [−0.90, −0.10] | 0.01 | 0.20 | 163 | 24 | 0.726 | 0.05 | [−0.35, 0.45] |
| 16 | −0.09 | 0.23 | 192 | 34 | 0.072 | −0.39 | [−0.74, −0.04] | 0.06 | 0.25 | 345 | 32 | 0.134 | 0.24 | [−0.11, 0.59] |

*The summarized statistical tests include uncorrected p-values Where, Cohen's* $d = \frac{M_i}{SD_i}$, $CI_d = d \pm (z_{crit} * SE_d)$, *and* $SE_d = \sqrt{\frac{1}{n_i} + \frac{d^2}{2*n_i}}$ *(Turner and Bernard, 2006).*
*$^*p < 0.05$.*

interaction effect across tasks, such analyses were outside the purview of the present study because the hypothesis was only directed at the relationship between pre-frontal activation and RSA. Thus, the mean $O_2$Hb level during baseline across all channels was averaged by the subject. The same was done for $O_2$Hb levels during the GNG task. This provided each subject with one $O_2$Hb measure that indicated global pre-frontal activation at baseline and during the GNG task. Three subjects were missing data during the GNG task and thus were not included in this analysis ($n = 35$). These values were compared using a paired samples *t*-test and indicated that the mean ($M$) $O_2$Hb across the pre-frontal cortex was different between the baseline and GNG task [$t(34) = -6.45$, $p < 0.001$], such that $O_2$Hb was higher during the GNG task ($M = 0.011$, $SD = 0.023$) than baseline ($M = -0.024$, $SD = 0.025$). These results indicate that the GNG task did elicit greater pre-frontal activation than during a resting baseline.

To examine whether the relationship between pre-frontal activation and RMSSD seen during baseline held during a behavioral inhibition task, the same set of analyses as described previously were conducted for the standardized β weights derived from the GNG task. None of the tests were significant at the $p < 0.05$ level, indicating that RMSSD did not predict pre-frontal activation during the GNG task. The results of these tests are summarized in **Table 2**.

## DISCUSSION

Consistent with the NVM, the results of this study support the hypothesis that RSA is related to pre-frontal activation at rest. Although these findings were not maintained when corrected for multiple comparisons, the relationship between baseline RSA and pre-frontal activation in this study was consistent with previous NVM studies. For example, standardized beta weights were significantly different than 0 (at $p = 0.05$) at various sites across the PFC during baseline, which reflects a predictive relationship between RSA and PFC activation. Notably, this was observed at channel 10, which is located over the right mPFC, one of the CAN structures implicated in the NVM (Gianaros et al., 2004; Thayer et al., 2012). The non-significant relationship between RSA and $O_2$Hb during the GNG task conflicted with previous meta-analyses that included task reactivity (Thayer et al., 2012; Beissner et al., 2013). However, our results mirror previous findings that have also found an inverse relationship between cortical activation and RSA during baseline (Allen et al., 2015; Jennings et al., 2015) and are counter to that found in prior meta-analyses of neuroimaging studies focused on the NVM (Thayer et al., 2012). In the present study, the previous literature was expanded through simultaneous acquisition of RSA and cortical activation data, providing a methodological advance and yielding results consistent with similar to Allen et al. (2015) and Jennings et al. (2015), in which resting cortical and HRV data were collected on separate occasions. Together, these studies suggest that the argument presented in previous meta-analyses of neuroimaging studies focused on the NVM (i.e., that RSA reactivity is positively related to activation of CAN structures during tasks; Thayer et al., 2012) are not representative of the model during rest. It is important to establish the relationship between these variables during a resting state because (1) the NVM is largely centered on resting RSA and its relation to CAN function, and (2) doing so will provide context for how this relationship is altered during reactivity to tasks with varying demands.

Previous studies examining RSA and cortical activation have been conducted at rest or have taken a task-based approach, but few have looked at the metrics simultaneously across both conditions. Of those that have measured RSA and cortical activation simultaneously during tasks (e.g., the n-back task, a speech stressor, a handgrip task, a working memory task), none have used a task involving behavioral inhibition or cognitive flexibility (Gianaros et al., 2004; Thayer et al., 2012; Jennings et al., 2015), although a few have done so using RSA measurements taken outside of the neuroimaging data collection session (Matthews et al., 2004; Neumann et al., 2006; Jennings et al., 2015). The present study used a task that is relevant to the cognitive and behavioral processes implicated in the NVM to determine whether CAN activity (i.e., pre-frontal activation) was still indexed by RSA in this context. The predictive relationship of RSA on mPFC activation during inhibition was not supported in the present study, although a negative relationship was detected at baseline. It is possible that although the relationship is negative at rest, this transitions to a positive relationship during cognitive tasks, as seen in the previous literature, but the behavioral inhibition task used here was not challenging enough to elicit these results. The discrepancy in these findings indicates the need for further investigation of how the NVM applies across varying contexts, as suggested by Jennings et al. (2015) and Smith et al. (2017). It is possible that when CAN structures are recruited for another task, as is the case with the PFC during the GNG, the relationship between these structures and RSA becomes irregular. Understanding this relationship has implications for how behavioral inhibition and flexibility can be assessed through these biological measures, with implications for identifying and tracking inhibition and flexibility deficits.

## Limitations

Although the present study adds to the literature on neuroimaging and the NVM, some limitations should be acknowledged. First, our final sample size (38 individuals) was smaller than the target number recruited for the study (45) due to data loss related to technical problems. Although this sample size is comparable with similar studies (e.g., Chang et al., 2013), we initially aimed for a larger sample to optimize the fNIRS signal-to-noise ratio (Cui et al., 2011). The analyses did not retain statistical significance after Holm–Bonferroni correction for multiple comparisons was applied, although they did at the uncorrected level and were consistent with findings from previous studies. The sole reliance on *p*-values, and consequently, the correcting of *p*-values, is cautioned by the American Statistical Association (Wasserstein and Lazar, 2016). However, the present findings provide preliminary evidence that is incompatible with the null hypothesis that the relation between RMSSD and $O_2Hb$ is non-existent (e.g., equal to 0), specifically in the mPFC, a finding consistent with previous neuroimaging literature evaluating the NVM. For this reason, continuing to investigate the NVM using fNIRS thorough replications of the present study with increased sample sizes and additional cognitive tasks is warranted.

Additionally, neuroimaging using fNIRS is subject to inherent limitations, which should also be considered in the present study. Although fNIRS has increased spatial resolution to other central nervous system measures, such as electroencephalogram, the spatial resolution is limited compared with fMRI (Irani et al., 2007). fNIRS channels measure cortical activation on the order of centimeters over the cortex, imposing a relative limit on the specificity of activation to certain cortical regions. Additionally, fNIRS can only penetrate approximately 1 cm into the cerebral cortex, limiting which neural structures can be interrogated with this neuroimaging modality. For that reason, subcortical structures in the CAN could not be targeted in the present study. It was with this limitation in mind that hypotheses were specifically written regarding the mPFC, allowing for a component of the CAN to be assessed while taking advantage of the flexibilities afforded by an fNIRS study (e.g., measuring brain activation and RSA while sitting upright, as is more common in resting-state psychophysiological assessment).

Further, the present analyses were largely based on previous fMRI studies that examined the NVM through traditional fMRI analysis procedures (Chang et al., 2013). Although these methods are standard in the field, recent work suggests that there are more advanced statistical approaches that can be applied to imaging data. A majority of the fMRI literature utilizes a summary statistic approach, wherein subject-level regressions are conducted at the voxel level, and the summary statistics (i.e., β estimates) resulting from these analyses are used to conduct group-level analyses (Monti, 2011). The problem with this approach is that it does not account for individual variances from the subject-level equations when conducting group-level analyses. More recently, fMRI researchers have been advocating for the use of more comprehensive statistical approaches, such as the "sufficient-summary-statistic approach" (Dowding and Haufe, 2018) or the use of hierarchical/multilevel mixed linear models (Chen et al., 2013). In the present study, the analyses were intentionally modeled after previous fMRI research because multiple hypotheses and corresponding research design elements were already being introduced (e.g., the use of fNIRS, adding multiple conditions to examine research questions examining the NVM). However, more robust modeling approaches should be considered when examining these research questions moving forward.

## Future Directions

In addition to the advanced statistical modeling approaches mentioned earlier, several other methodologies should be used, and other research questions pursued in relation to our findings. First, PFC activation in relation to RSA should be assessed across additional cognitive tasks related to behavioral inhibition and cognitive flexibility. For example, in addition to replicating the present study with more complex versions of the GNG task, various versions of the Stroop Color–Word task could be used to examine inhibition of cognitive interference (Scarpina and Tagini, 2017) and other task-switching or set-shifting paradigms (Dajani and Uddin, 2015). Although a relationship between RSA and pre-frontal activation during the GNG task was not found in the present study, it is possible that this could be due to task demands. A simple version of the GNG was used, which may not have required the mobilization of many cognitive resources. With a task requiring a higher cognitive load, there may be different results. For example, Gianaros et al. (2004)

noted the use of increasingly complex working memory tasks in their design, resulting in differences in the task-related findings. The present study used a simple GNG design, as GNG tasks with increased demands have been critiqued for drawing on cognitive functions beyond behavior inhibition alone, which are not as relevant to the NVM. However, assessing the relationship between PFC activation and RSA across various aspects of cognitive flexibility and at varying levels of a cognitive load will help validate whether the biological principles behind the NVM generalize across contexts. Such investigations could reveal whether these metrics differentially relate to various components of cognitive flexibility and have important implications for how these biological metrics could be utilized for individuals with deficits in cognitive flexibility.

## CONCLUSION

The present study examined pre-frontal activation during rest and a behavioral inhibition task through fNIRS, used simultaneous assessment of neural and parasympathetic output to improve upon prior evaluations of the NVM, and attempted to relate task performance, neuroimaging, and RSA measures during behavioral inhibition to deficits in other areas related to behavioral and cognitive flexibility. The present findings not only inform theoretical aspects of the NVM but also speak to broader applications of the model to other domains of functioning. From a methodological perspective, the present study provides valuable information regarding the use of fNIRS in conjunction with RSA to evaluate the NVM. The findings using fNIRS are consistent with previous studies that have used fMRI to investigate research questions surrounding the model and behavior inhibition. Furthermore, the present study indicates that fNIRS is a viable alternative to fMRI, which is comparatively more cumbersome and expensive, in assessing these research questions. From a theoretical perspective, the present study provides information about the NVM beyond the previous literature through assessment at baseline and during behavior inhibition.

Specifically, the findings indicate that the relationship between RSA and pre-frontal activation proposed in the model is likely to vary depending on environmental demands. These findings indicate the importance of assessing the NVM across multiple contexts moving forward and, consequently, provide insight for how these measures may be applied in addressing behavioral flexibility deficits. Incorporating these considerations into future studies will be imperative in assessing the practicability of using the biological markers proposed in the NVM as potential therapeutic targets or evaluation tools for these deficits.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Institute of Child Health and Human Development Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

EC, BF, and AG were responsible for developing the theoretical background, analytical plan, and drafting the manuscript. EC was responsible for data collection and data analysis. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This project was funded by the Intramural Research Program (IRP) of the National Institute of Child Health and Human Development (Project No. 1ZIAHD008882-10). The Virginia Tech Open Access Subvention Fund provided support for article processing charges.

## REFERENCES

Aasted, C. M., Yucel, M. A., Cooper, R. J., Dubb, J., Tsuzuki, D., Becerra, L., et al. (2015). Anatomical guidance for functional near-infrared spectroscopy: atlas viewer tutorial. *Neurophotonics* 2:020801. doi: 10.1117/1.NPh.2.2.020801

Allen, B., Jennings, J. R., Gianaros, P. J., Thayer, J. F., and Manuck, S. B. (2015). Resting high-frequency heart rate variability is related to resting brain perfusion. *Psychophysiology* 52, 277–287. doi: 10.1111/psyp.12321

Allen, J. J., Chambers, A. S., and Towers, D. N. (2007). The many metrics of cardiac chronotropy: a pragmatic primer and a brief comparison of metrics. *Biol. Psychol.* 74, 243–262. doi: 10.1016/j.biopsycho.2006.08.005

Anderson, A. A., Smith, E., Chernomordik, V., Ardeshirpour, Y., Chowdhry, F., Thurm, A., et al. (2014). Prefrontal cortex hemodynamics and age: a pilot study using functional near infrared spectroscopy in children. *Front. Neurosci.* 8:393. doi: 10.3389/fnins.2014.00393

Ayaz, H., Shewokis, P. A., Curtin, A., Izzetoglu, M., Izzetoglu, K., and Onaral, B. (2011). Using MazeSuite and functional near infrared spectroscopy to study learning in spatial navigation. *J. Vis. Exp.* 56:3443. doi: 10.3791/3443

Beissner, F., Meissner, K., Bar, K. J., and Napadow, V. (2013). The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. *J. Neurosci.* 33, 10503–10511. doi: 10.1523/JNEUROSCI.1103-13.2013

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo. Clin. Proc.* 68, 988–1001. doi: 10.1016/s0025-6196(12)62272-1

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Cacioppo, J. T., and Quigley, K. S. (1993). Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology* 30, 183–196. doi: 10.1111/j.1469-8986.1993.tb01731.x

Brigadoi, S., Ceccherini, L., Cutini, S., Scarpa, F., Scatturin, P., Selb, J., et al. (2014). Motion artifacts in functional near-infrared spectroscopy: a comparison of motion correction techniques applied to real cognitive data. *Neuroimage* 85(Pt 1), 181–191. doi: 10.1016/j.neuroimage.2013.04.082

Buckner, R. L., Andrews-Hanna, J. R., and Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann. N Y Acad. Sci.* 1124, 1–38. doi: 10.1196/annals.1440.011

Chang, C., Metzger, C. D., Glover, G. H., Duyn, J. H., Heinze, H. J., and Walter, M. (2013). Association between heart rate variability and fluctuations in resting-state functional connectivity. *Neuroimage* 68, 93–104. doi: 10.1016/j.neuroimage.2012.11.038

Chen, G., Saad, Z. S., Britton, J. C., Pine, D. S., and Cox, R. W. (2013). Linear mixed-effects modeling approach to FMRI group analysis. *Neuroimage* 73, 176–190. doi: 10.1016/j.neuroimage.2013.01.047

Cui, X., Bray, S., Bryant, D. M., Glover, G. H., and Reiss, A. L. (2011). A quantitative comparison of NIRS and fMRI across multiple cognitive tasks. *Neuroimage* 54, 2808–2821. doi: 10.1016/j.neuroimage.2010.10.069

Dajani, D. R., and Uddin, L. Q. (2015). Demystifying cognitive flexibility: Implications for clinical and developmental neuroscience. *Trends Neurosci.* 38, 571–578. doi: 10.1016/j.tins.2015.07.003

Dillo, W., Goke, A., Prox-Vagedes, V., Szycik, G. R., Roy, M., Donnerstag, F., et al. (2010). Neuronal correlates of ADHD in adults with evidence for compensation strategies–a functional MRI study with a Go/No-Go paradigm. *Ger. Med. Sci.* 8:Doc09. doi: 10.3205/000098

Donders, F. C. (1969). On the speed of mental processes. *Acta. Psychol.* 30, 412–431. doi: 10.1016/0001-6918(69)90065-1

Dowding, I., and Haufe, S. (2018). Powerful Statistical Inference for Nested Data Using Sufficient Summary Statistics. *Front. Hum. Neurosci.* 12:103. doi: 10.3389/fnhum.2018.00103

Eatough, E. M., Shirtcliff, E. A., Hanson, J. L., and Pollak, S. D. (2009). Hormonal reactivity to MRI scanning in adolescents. *Psychoneuroendocrinology* 34, 1242–1246. doi: 10.1016/j.psyneuen.2009.03.006

Ferrari, M., and Quaresima, V. (2012). A brief review on the history of human functional near-infrared spectroscopy (fNIRS) development and fields of application. *Neuroimage* 63, 921–935. doi: 10.1016/j.neuroimage.2012.03.049

Fox, P. T., and Raichle, M. E. (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc. Natl. Acad. Sci.* 83, 1140–1144. doi: 10.1073/pnas.83.4.1140

Friedman, B. H. (2007). An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biol. Psychol.* 74, 185–199. doi: 10.1016/j.biopsycho.2005.08.009

Gianaros, P. J., Van Der Veen, F. M., and Jennings, J. R. (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: Implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology* 41, 521–530. doi: 10.1111/1469-8986.2004.00179.x

Herrmann, M. J., Plichta, M. M., Ehlis, A. C., and Fallgatter, A. J. (2005). Optical topography during a Go-NoGo task assessed with multi-channel near-infrared spectroscopy. *Behav. Brain Res.* 160, 135–140. doi: 10.1016/j.bbr.2004.11.032

Irani, F., Platek, S. M., Bunce, S., Ruocco, A. C., and Chute, D. (2007). Functional near infrared spectroscopy (fNIRS): an emerging neuroimaging technology with important applications for the study of brain disorders. *Clin. Neuropsychol.* 21, 9–37. doi: 10.1080/13854040600910018

Janig, W., and Habler, H. J. (2000). Specificity in the organization of the autonomic nervous system: a basis for precise neural regulation of homeostatic and protective body functions. *Prog. Brain Res.* 122, 351–367. doi: 10.1016/s0079-6123(08)62150-0

Jennings, J. R., Allen, B., Gianaros, P. J., Thayer, J. F., and Manuck, S. B. (2015). Focusing neurovisceral integration: cognition, heart rate variability, and cerebral blood flow. *Psychophysiology* 52, 214–224. doi: 10.1111/psyp.12319

Jennings, J. R., Kamarck, T., Stewart, C., Eddy, M., and Johnson, P. (1992). Alternate cardiovascular baseline assessment techniques: vanilla or resting baseline. *Psychophysiology* 29, 742–750. doi: 10.1111/j.1469-8986.1992.tb02052.x

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research–recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lee, H. J., Yost, B. P., and Telch, M. J. (2009). Differential performance on the go/no-go task as a function of the autogenous-reactive taxonomy of obsessions: findings from a non-treatment seeking sample. *Behav. Res. Ther.* 47, 294–300. doi: 10.1016/j.brat.2009.01.002

Lloyd-Fox, S., Blasi, A., and Elwell, C. E. (2010). Illuminating the developing brain: the past, present and future of functional near infrared spectroscopy. *Neurosci. Biobehav. Rev.* 34, 269–284. doi: 10.1016/j.neubiorev.2009.07.008

Lueken, U., Muehlhan, M., Evens, R., Wittchen, H. U., and Kirschbaum, C. (2012). Within and between session changes in subjective and neuroendocrine stress parameters during magnetic resonance imaging: A controlled scanner training study. *Psychoneuroendocrinology* 37, 1299–1308. doi: 10.1016/j.psyneuen.2012.01.003

Matthews, S. C., Paulus, M. P., Simmons, A. N., Nelesen, R. A., and Dimsdale, J. E. (2004). Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *Neuroimage* 22, 1151–1156. doi: 10.1016/j.neuroimage.2004.03.005

Monti, M. M. (2011). Statistical Analysis of fMRI Time-Series: A Critical Review of the GLM Approach. *Front. Hum. Neurosci.* 5:28. doi: 10.3389/fnhum.2011.00028

Mostofsky, S. H., Schafer, J. G., Abrams, M. T., Goldberg, M. C., Flower, A. A., Boyce, A., et al. (2003). fMRI evidence that the neural basis of response inhibition is task-dependent. *Brain Res. Cogn. Brain. Res.* 17, 419–430. doi: 10.1016/s0926-6410(03)00144-7

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., et al. (2015). Validity of (Ultra-)Short Recordings for Heart Rate Variability Measurements. *PLoS One* 10:e0138921. doi: 10.1371/journal.pone.0138921

Neumann, S. A., Brown, S. M., Ferrell, R. E., Flory, J. D., Manuck, S. B., and Hariri, A. R. (2006). Human choline transporter gene variation is associated with corticolimbic reactivity and autonomic-cholinergic function. *Biol. Psychiatry.* 60, 1155–1162. doi: 10.1016/j.biopsych.2006.03.059

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Raichle, M. E. (2015). The brain's default mode network. *Annu. Rev. Neurosci.* 38, 433–447. doi: 10.1146/annurev-neuro-071013-014030

Rodrigo, A. H., Domenico, S. I., Ayaz, H., Gulrajani, S., Lam, J., and Ruocco, A. C. (2014). Differentiating functions of the lateral and medial prefrontal cortex in motor response inhibition. *Neuroimage* 85(Pt 1), 423–431. doi: 10.1016/j.neuroimage.2013.01.059

Saul, J. P. (1990). Beat-to-Beat Variations of Heart-Rate Reflect Modulation of Cardiac Autonomic Outflow. *News Physiol. Sci.* 5, 32–37. doi: 10.1152/physiologyonline.1990.5.1.32

Scarpina, F., and Tagini, S. (2017). The Stroop Color and Word Test. *Front. Psychol.* 8:557. doi: 10.3389/fpsyg.2017.00557

Schmidt, B. (2006). Proof of principle studies. *Epilep. Res.* 68, 48–52. doi: 10.1016/j.eplepsyres.2005.09.019

Schneider, W., Eschman, A., and Zuccolotto, A. (2002). *E-Prime User's Guide*. Pittsburgh: Psychology Software Tools Inc.

Schulz, K. P., Fan, J., Magidina, O., Marks, D. J., Hahn, B., and Halperin, J. M. (2007). Does the emotional go/no-go task really measure behavioral inhibition? Convergence with measures on a non-emotional analog. *Arch. Clin. Neuropsychol.* 22, 151–160. doi: 10.1016/j.acn.2006.12.001

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Swick, D., Ashley, V., and Turken, U. (2011). Are the neural correlates of stopping and not going identical? Quantitative meta-analysis of two response inhibition tasks. *Neuroimage* 56, 1655–1665. doi: 10.1016/j.neuroimage.2011.02.070

Takahashi, N., Kuriyama, A., Kanazawa, H., Takahashi, Y., and Nakayama, T. (2017). Validity of spectral analysis based on heart rate variability from 1-minute or less ECG recordings. *Pacing Clin. Electrophysiol.* 40, 1004–1009. doi: 10.1111/pace.13138

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Task Force (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur. Heart J.* 17, 354–381.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Friedman, B. H. (2002). Stop that! inhibition, sensitization, and their neurovisceral concomitants. *Scand. J. Psychol.* 43, 123–130. doi: 10.1111/1467-9450.00277

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect Disord.* 61, 201–216. doi: 10.1016/s0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Turner, H. M. I., and Bernard, R. M. (2006). Calculating and synthesizing effect sizes. *Contempor. Issues Commun. Sci. Disord.* 33, 42–55. doi: 10.1044/cicsd_33_s_42

Uzefovsky, F., Allison, C., Smith, P., and Baron-Cohen, S. (2016). Brief Report: The Go/No-Go Task Online: Inhibitory Control Deficits in Autism in a Large Sample. *J. Autism. Dev. Disord.* 46, 2774–2779. doi: 10.1007/s10803-016-2788-3

Wasserstein, R. L., and Lazar, N. A. (2016). The ASA's Statement on p-Values: Context. *Process Purp. Am. Statist.* 70, 129–131. doi: 10.1080/00031305.2016.1154108

Watanabe, J., Sugiura, M., Sato, K., Sato, Y., Maeda, Y., Matsue, Y., et al. (2002). The human prefrontal and parietal association cortices are involved in NO-GO performances: an event-related fMRI study. *Neuroimage* 17, 1207–1216. doi: 10.1006/nimg.2002.1198

Yerys, B. E., Jankowski, K. F., Shook, D., Rosenberger, L. R., Barnes, K. A., Berl, M. M., et al. (2009). The fMRI success rate of children and adolescents: typical development, epilepsy, attention deficit/hyperactivity disorder, and autism spectrum disorders. *Hum. Brain Mapp.* 30, 3426–3435. doi: 10.1002/hbm.20767

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 02 December 2020
doi: 10.3389/fnins.2020.576308

# Modeling Stress-Recovery Status Through Heart Rate Changes Along a Cycling Grand Tour

*Anna Barrero[1,2*†], Anne Le Cunuder[3†], Guy Carrault[2,4], François Carré[5], Frédéric Schnell[5] and Solène Le Douairon Lahaye[1]*

[1] University of Rennes 2, M2S Laboratory, Rennes, France, [2] CHU Rennes, Inserm CIC 1414, Rennes, France, [3] University of Lyon, Ecole Normale Supérieure, CNRS UMR 5672, Lyon, France, [4] University of Rennes 1, Inserm, LTSI – UMR 1099, Rennes, France, [5] University of Rennes, CHU Rennes, Inserm, LTSI – UMR 1099, Rennes, France

**Background:** Heart rate (HR) and HR variability (HRV) indices are established tools to detect abnormal recovery status in athletes. A low HR and vagally mediated HRV index change between supine and standing positions reflected a maladaptive training stress-recovery status.

**Objectives:** Our study was focused on a female multistage cycling event. Its overall aim was twofold: (1) quantify the correlation between (a) the change in HR and HRV indices during an active orthostatic test and (b) subjective/objective fatigue, physical load, and training level indicators; and (2) formulate a model predicting the stress-recovery status as indexed by $\Delta\overline{RR}$ and $\Delta$LnRMSSD (defined as the difference between standing and supine mean RR intervals and LnRMSSD, respectively), based on subjective/objective fatigue indicators, physical load, and training levels.

**Methods:** Ten female cyclists traveled the route of the 2017 Tour de France, comprising 21 stages of 200 km on average. From 4 days before the beginning of the event itself, and until 1 day after its completion, every morning, each cyclist was subjected to HR and HRV measurements, first at rest in a supine position and then in a standing position. The correlation between HR and HRV indices and subjective/objective fatigue, physical load, and training level indicators was then computed. Finally, several multivariable linear models were tested to analyze the relationships between HR and HRV indices, fatigue, workload, and training level indicators.

**Results:** HR changes appeared as a reliable indicator of stress-recovery status. Fatigue, training level, and $\Delta\overline{RR}$ displayed a linear relationship. Among a large number of linear models tested, the best one to predict stress-recovery status was the following: $\Delta\overline{RR} = 1{,}249.37 + 12.32\dot{V}O_{2max} + 0.36\ \text{km}\cdot\text{week}^{-1} - 8.83\ HR_{max} - 5.8\ RPE - 28.41$ perceived fatigue with an adjusted $R^2 = 0.322$.

**Conclusion:** The proposed model can help to directly assess the adaptation status of an athlete from RR measurements and thus to anticipate a decrease in performance due to fatigue, particularly during a multistage endurance event.

**Keywords: females, cycling, endurance, mathematical model, performance, heart rate variability, stress-recovery status**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Dorota Zyśko, Wrocław Medical University, Poland*
*Antonio Roberto Zamunér, Catholic University of Maule, Chile*

***Correspondence:**
*Anna Barrero*
*annabarrero@gmail.com*

*† These authors have contributed equally to this work*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *25 June 2020*
***Accepted:*** *26 October 2020*
***Published:*** *02 December 2020*

***Citation:***
*Barrero A, Le Cunuder A, Carrault G, Carré F, Schnell F and Le Douairon Lahaye S (2020) Modeling Stress-Recovery Status Through Heart Rate Changes Along a Cycling Grand Tour. Front. Neurosci. 14:576308. doi: 10.3389/fnins.2020.576308*

## INTRODUCTION

Physical training must combine workloads and recovery periods (Bishop et al., 2008). An optimal match between these two parameters is requested to avoid fatigue accumulation and thus reach the best individual physical performance level. Conversely, in the event of an imbalance between the two parameters, a state of overreaching or overtraining with marked drop in performance may occur (Kuipers, 1998). The individual optimal balance between work and recovery is difficult to achieve, especially for highly trained athletes. Thus, having relevant indicators of the exercise stress-recovery status could be a real advantage for individual athlete's monitoring. The aim of this study was to provide a new tool for coaches to help them to assess the athlete's individual fatigue level.

Several tools have been proposed to assess the fatigue level in daily routine of athletes (Buchheit, 2014; Halson, 2014). The most used are those providing an indirect evaluation of heart rate (HR) control by the autonomic nervous system (ANS) as analysis of spontaneous RR interval duration or HR variability (HRV) temporal and spectral indices (Bellenger et al., 2016), although the spectral ones present some limits (Buchheit, 2014). The HRV analysis can be performed either statically or dynamically, reflecting the heart adaptation to a physiological stress (Buchheit, 2014). Training-induced fatigue causes a more or less weak response of the ANS to external stimuli. Specifically, under normal training conditions (stress/recovery balance), intense strenuous exercise results in a decrease in vagally mediated HRV indices, followed by cardiac parasympathetic reactivation, which takes place during the recovery period (24–48 h) during which the cardiovascular system plays an important role in restoring body's homeostasis (Stanley et al., 2013). In training with a stress/recovery imbalance, a more marked and prolonged alteration of only vagally mediated HRV indices is reported (Bosquet et al., 2008; Plews et al., 2012). These results seem to support a link between fatigue and mainly the parasympathetic nervous system. Thus, HRV analysis, and especially focusing on parasympathetic' activity, represents a noninvasive method to track and record training status, "exercise readiness," and post-exercise fatigue in athletes (Buchheit, 2014; Bellenger et al., 2016). Indeed, in the parasympathetic system presenting the more marked impact on the post-exercise recovery, the vagally mediated HRV index variation is able to evaluate the cardiac response (Michael et al., 2017): the root-mean-square difference of successive normal RR intervals (RMSSD or its natural log, LnRMSSD) in the time domain is the most recommended (Plews et al., 2012; Buchheit, 2014; Bellenger et al., 2016), since it represents pure parasympathetic HR modulation (Malik and Camm, 1993).

However, it seems that the correct interpretation of HR or HRV fluctuations during the training period requires the comparison of these markers with other objective signs of fatigue to detect the risk of overreaching or overtraining (Stanley et al., 2013; Buchheit, 2014). From nocturnal ANS activity recordings in swimmers, Chalencon et al. (2012) found strong relationships between resting high-frequency (HF) power of HRV and 400-m freestyle time-trial performance of the next morning (Chalencon et al., 2012). The higher the HF power values, the better the performance. Moreover, using the same protocol of ANS activity and performance recordings, the authors demonstrated that intensive training periods have a negative impact on both performance and HF due to fatigue (negative influence). In addition, modeling the effect of a 30-week training on swimmers' performance allowed for an accurate prediction of individual performance (Chalencon et al., 2015), supporting the relevance of a mathematical modeling of HRV in order to predict responses to training. To our knowledge, such mathematical analysis was only conducted over long training periods. Therefore, it appears relevant to also assess daily athlete's response on a shorter timescale, such as during a competitive event spreading over a few weeks (e.g., Grand Tours in cycling and World Football Cup) or during training camps, in which stress and fatigue affect day-to-day performances.

In a previous study on well-trained female cyclists, we have demonstrated that during a multistage event, HR and HRV indices evolved along the event in correlation with the daily physical load (Barrero et al., 2019). Briefly, we have observed a progressive increase of resting HR during the event and a progressive imbalance in the autonomic balance with an increase in the low-frequency (LF) power value that partly reflects the effects of sympathetic tone and a decrease in the HF and RMSSD values that reflects the parasympathetic effects. Our results also highlighted that variation in HR and HRV indices when changing from supine-to-standing position during an active orthostatic test is strongly correlated with the fatigue status (Barrero et al., 2019). The use of active orthostatic test has been recommended to detect fatigue in athletes, because of its ability to detect autonomic responses not observed with isolated supine or standing measures (Bosquet et al., 2008; Schmitt et al., 2015). This test explores the reactivities of sympathetic (excitation) and parasympathetic systems (withdrawal) in response to the position change (Taylor, 1994) and is the most widely used physiological maneuver for assessing neuro-vegetative and cardiovascular responsiveness. When standing from a supine position, the normal response is an increase in HR to maintain blood pressure (Tse et al., 2005). In well-trained athletes, with respect to supine rest values, after active stand-up, a marked increase of HR associated with a decrease in global HRV, HF, and RMSSD and with an increase of LF/HF ratio has been reported (Hynynen et al., 2008). These observations suggest that standing up normally induced mainly parasympathetic withdrawal. In overtrained athletes, an attenuation of parasympathetic and sympathetic activity during both supine and standing positions has been observed. Moreover, in these athletes, in response to stand-up position, the HR increases, and the decrease of total power, HF, LF, and RMSSD seemed lower than in non-overtrained athletes (Hynynen et al., 2008). Although a complex phenomenon (Schmitt et al., 2015), it is proposed that training induced-fatigue attenuates baroreflex response to change in position (Uusitalo et al., 2000). Thus, a low HR change between supine and standing positions reflects a maladaptive training stress-recovery status.

The multidimensional monitoring of recovery status has been underlined (Heidari et al., 2018), and it was

recommended to associate other markers with HR/HRV indices to more accurately detect the state of overtraining (Stanley et al., 2013; Buchheit, 2014). Because progression to overtraining syndrome appears to be associated with psychological states, the use of self-administered questionnaires on perceived physical and psychological well-being (WB) by athletes has been recommended (Saw et al., 2016). In this context, monitoring the evolution of several indices such as rate of perceived exertion (RPE), the WB, the sleep quality, and the delayed-onset muscle soreness (DOMS) during intensive periods of training has been proposed (Ouergui et al., 2020).

In our previous study, we observed a progressive decrease of HR and HRV responses induced by the active orthostatic test. These alterations were positively associated with the athlete's daily physical load and thus with fatigue (Barrero et al., 2019). However, the descriptive method we used to analyze the day-to-day HR and HRV indices did not allow to accurately predict the cyclist's individual adaptation ability in response to the exercise performed.

Thus, based on these previous data, the aim of the present study was first to investigate the individual correlation between the supine-standing difference of HR and HRV index values presented above, fatigue, physical load, and training level and second to formulate models relating two dependent HR variables ($\Delta\overline{RR}$ and $\Delta$LnRMSSD defined as the difference between standing and supine mean RR intervals and LnRMSSD, respectively) to subjective/objective fatigue, physical load, and training level indicators in order to investigate the parameters better predicting $\Delta\overline{RR}$ and $\Delta$LnRMSSD. The added value of the current study is to propose a model allowing coaches to understand the adaptation ability of athletes to a multistage endurance event and helping them to anticipate a decrease in performance due to fatigue.

The design of our study has been based on two assumptions.

First, a relation exists between ANS alterations, mainly the cardiac vagal tone and early fatigue detection in athletes. Indeed, the development of fatigue in athletes is considered as a continuum process with, one side, the voluntary and controlled fatigue and, the opposite side, the uncontrolled fatigue, so-called overtraining (Meeusen et al., 2013). All the states of fatigue in athletes classically associate the same more or less marked symptoms: decrease in physical performance, neuro-endocrinal abnormalities, and psychological trouble such as irritability. Thus, the early detection of fatigue in athletes by use of HR and HRV analyses could be in line with the biological behavioral model (Grossman and Taylor, 2007). In accordance with previously published data concerning this first hypothesis, we assumed that due to the repetition of the successive endurance stages, we would observe a lower change of HR and of HR vagally mediated HRV between supine and standing positions during the orthostatic test. Moreover, we also hypothesized that HR and time-domain HRV vagal indices would present the higher relation with fatigue parameter levels.

Second, based on previous research (Ouergui et al., 2020), we hypothesized that the most suitable prediction models for both $\Delta\overline{RR}$ and $\Delta$LnRMSSD should include parameters related on the one hand with physical performance, such as amount of training and level of performance, and on the other hand with levels of WB and of exertion feeling. We hypothesized that parameters of $\dot{V}O_{2max}$, WB, RPE, sleep quality, and DOMS could be included in the prediction model.

# MATERIALS AND METHODS

The first results of this prospective study have been previously published (Barrero et al., 2019).

This scientific project took place as part of the sports project "Donnons des elles au vélo J-1" aimed to promote women's cycling. This sport project included only 11 well-trained female cyclists, and of them, 10 participated in the whole study.

## Population

All were healthy, with no medical history of cardiovascular disease, and were not currently taking medication. At the time of this study, their weekly training mileage ranged from 100 to 250 km per week. All cyclists had a minimum of 2 years of competitive cycling experience. The characteristics of the athletes already presented (Barrero et al., 2019) are recalled in **Table 1**.

This study received the approval of our hospital ethics committee. After information was given, all participants provided written informed consent. The study was conducted in accordance with the "Good Clinical Practice" guidelines as laid down in the Declaration of Helsinki.

## Cycling Event

The characteristics of the multistage cycling event were previously described (Barrero et al., 2019). In brief, cyclists performed the 21 stages of men's 2017 Tour de France (TdF) 1 day before each stage of the official race. All event stages were performed without any spirit of competition or performance goal. The unique cyclists' objective was to complete 3,540 km of the TdF to promote women's cycling.

**TABLE 1 |** Anthropometric and physical performance characteristics of the cyclists (mean ± SD).

| Characteristic | Value |
|---|---|
| Age (years) | 31.7 ± 4.7 |
| Weight (kg) | 57.0 ± 6.3 |
| Height (m) | 1.60 ± 0.1 |
| BMI ($kg{\cdot}m^{-2}$) | 21.4 ± 1.9 |
| $\dot{V}O_{2max}$ ($ml{\cdot}min{\cdot}kg^{-1}$) | 53.6 ± 5.2 |
| Maximal power output (W) | 285.5 ± 19.2 |
| Relative maximal power output ($W\ kg^{-1}$) | 5.0 ± 0.5 |
| Maximal HR (bpm) | 185.7 ± 7.2 |
| Previous cycling experience (years) | 14.0 ± 8.9 |
| Current training level ($km{\cdot}week^{-1}$) | 187.5 ± 51.5 |

*BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen uptake; HR, heart rate.*

## Preliminary Testing

Each cyclist had a preparticipation medical evaluation with a clinical exam, a resting ECG (Mac 1600, GE Healthcare, Chicago, IL, United States) and an incremental maximal cardiopulmonary exercise test performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Netherlands) with continuous ECG and blood pressure monitoring and gas exchange analysis (Case system-Power cube, GE Healthcare, Chicago, IL, United States). The French Cycling Federation incremental exercise protocol was used. It started with a warm-up period (100 W for 5 min and 150 W for 1 min) followed by a step load-increase of 25 W $min^{-1}$ until exhaustion. This preliminary testing took place 1 week before the first stage.

## RR Interval Recording and Analysis

The RR interval recording and HRV analysis protocols used were previously described (Barrero et al., 2019). Baseline pre-TdF RR intervals were collected daily during 4 days before the event to obtain a basal HRV state. Then RR recordings were performed every day of the multistage event. All resting recordings were made in the morning fasting, right after awakening, before the cyclist gets up. In order to avoid mental activity and stress and thus to place the cyclists in an optimal physiological rest state, RR recordings were performed in spontaneous breathing (Bernardi et al., 2000).

Briefly, all RR interval samples were recorded with a portable HR monitor (Polar V800, Kempele, Finland) during the two successive phases of the test: 7 min in a supine position followed by 7 min in standing position as recommended (Bourdillon et al., 2017). Individual RR recorded data were downloaded via Polar FlowSync software for mac version 2.6.4 (Polar, Kempele, Finland) and exported for later analysis. The Kubios HRV Standard software version 3.0.0 2 (Biosignal Analysis and Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio, Finland) was used. For the HRV analysis, the last 5-min window for each position was used. All the ectopic beats were filtered with the artifact correction option of the software. A very low threshold was applied when needed (<5% of corrected beats). Both time and frequency domain HRV analysis were performed. The HRV spectrum is calculated with fast Fourier transform-based Welch's periodogram for spectral analysis. The RMSSD, which reflects cardiac vagal tone, was calculated. The HF (0.15–0.40 Hz) and LF (0.04–0.15 Hz) domains were analyzed. The HF band reflects cardiac vagal tone, while the LF band indicates both sympathetic and parasympathetic influences. RMSSD, HFnu, LFnu (normal units) absolute values, and their difference between supine and standing positions were calculated. The difference of the natural logarithm LnRMSSD between supine and standing positions was also studied. The normalized (or normalized unit) spectral indices are defined by the developers of the Kubios HRV Standard software v3.0.0 2 as HFnu = HF/(LF + HF) and LFnu = LF/(LF + HF) (Biosignal Analysis and Medical Imaging Group at the Department of Applied Physics, Kuopio, Finland) in accordance with the recommendations (No authors listed, 1996).

## Daily Collection and Analysis of Heart Rate and Workload

### Heart Rate and GPS Recording

Both HR and GPS data were continuously registered with the Polar V800 (Polar, Kempele, Finland) portable monitor during each stage.

### Workload Evaluation

The objective load of the daily individual exercise performed was estimated from the HR collected during each stage. For this, the recorded HR values were divided into five zones according to the percentages (i.e., 50–60, 60–70, 70–80, 80–90, and 90–100%) of the individual maximum HR obtained during the maximum effort test. Then, the individual daily internal workload was estimated using the training pulse score (TRIMP) method (Edwards, 1993). In this model proposed by Edwards, the quantification of the workload is derived from the duration of the exercise maintained in the five HR zones described above (Edwards, 1993).

The individual RPE of the 21 stages was evaluated with Borg CR-10 scale within 30 min after each stage (Borg, 1990). RPE was indicative of the subjective load of each stage.

## Perceived Fatigue Evaluation (Well-being Questionnaire)

Cyclists answered every morning, during breakfast, a questionnaire with four questions focusing on perceived general fatigue, sleep quality, DOMS, and stress level. Each question scored on a 7-point (with 1 and 7 representing poor and very good WB ratings, respectively) scale. Overall WB was determined by summing the four questions' scores (Hooper et al., 1995).

## Statistical Analysis

The first step of the statistical analysis concerned the first hypothesis of the study. We studied the evolution of HR and HRV indices throughout the cycling event in our previously published study (Barrero et al., 2019). Then, we investigated the correlation between individual HR (RR interval duration) and HRV indices, and subjective/objective fatigue, physical load, and training level indicators, in order to establish the best fatigue marker. We analyzed more precisely the RMSSD of RR interval duration in supine (RMSSDsup) and in standing (RMSSDsta) positions, the difference $\Delta$RMSSD = RMSSDsup−RMSSDsta and its natural logarithm ($\Delta$LnRMSSD), the LF and HF indices of RR time series, and the ratio LF/HF. We also computed the RR duration mean values in supine (MeanRRsup) and standing (MeanRRsta) positions. Finally, the difference of the mean time interval between two successive heart beats ($\Delta\overline{RR}$) between supine and standing positions was computed (White, 1980; Tse et al., 2005): $\Delta\overline{RR}$ = RRsupine−RRstanding.

We then calculated the correlation coefficient between each of these indices and subjective/objective fatigue, physical load, and training level indicators (see section "Materials and Methods").

The second step of the analysis concerned the second hypothesis of the study. To test this second hypothesis, we first had to characterize the influence of the cyclist's training level

on her adaptation all along the multistage event. To do this, the impact of each cycling stage was evaluated through the daily change observed on WB, with $\Delta WB = WB_{(staget)} - WB_{(staget-1)}$. A low daily ΔWB indicates that the cyclist was slightly impacted by the stage. Then, all daily ΔWB was averaged and plotted for each cyclist as a function of her weekly pre-TdF training load (km·week$^{-1}$).

The final step of the statistical analysis concerned the second hypothesis of the study. We aimed to test models to investigate which objective/subjective fatigue, physical load, and training level indicators best predict $\Delta\overline{RR}$ and ΔLnRMSSD. For this purpose, different multivariable linear models (LMMs) were tested in order to estimate which set of variables best explains the $\Delta\overline{RR}$ and ΔLnRMSSD.

For both, each model was a linear combination of up to eight parameters related to training and physical condition (km·week$^{-1}$, $\dot{V}O_{2max}$, $HR_{max}$) on the one hand and to the perceived difficulty of the cycling stage and its impact on the cyclist (RPE, DOMS, perceived fatigue, quality of sleep, and stress) on the other hand:

$\Delta\overline{RR} = f$ (km·week$^{-1}$, $\dot{V}O_{2max}$, $HR_{max}$, RPE, perceived fatigue, DOMS, quality of sleep, stress)
where $f$ is a linear function.

ΔLnRMSSD $= f$ (km·week$^{-1}$, $\dot{V}O_{2max}$, $HR_{max}$, RPE, perceived fatigue, DOMS, quality of sleep, stress)
where $f$ is a linear function.

The training level, assessed trough $\dot{V}O_{2max}$ and km·week$^{-1}$, clearly influences the fatigue accumulated during the event.

For each parameter, we thus tested all $2^8 = 256$ possible models, covering all possible combinations of the eight parameters. The predictive power of each model was estimated through its Akaike information criterion (AIC) and the highest adjusted $R^2$, which penalize for including extra fitting parameters (Akaike, 1974). The best model corresponds to the lowest AIC value. In particular, if two models have the same $R^2$, the one having the less parameters has the lowest AIC.

The statistical significance of each parameter was estimated through its $p$-value with a significant value stated at $p < 0.05$. The dispersion of our data did not allow us to evaluate nonlinear effects beyond LMMs.

All statistical analyses were performed with Python 3.7 software version.

# RESULTS

All participants cycled for 21 consecutive stages, including two resting days. They all successfully completed the whole circuit of the TdF 2017 (3,540 km) (Barrero et al., 2019).

## Effects of Cycling Event on Heart Rate and Heart Rate Variability Indices

The evolution of HR and HRV indices throughout the cycling event has been previously published (Barrero et al., 2019). To summarize, resting supine HR increased progressively in comparison with its basal value during the multistage event, and the HR value returned to its basal values after each rest day. On the other hand, standing HR values showed no significant evolution during the cycling event. All along the multistage event, we observed a progressive decrease in $\Delta\overline{RR}$. Indeed, compared with its initial value (day 0), a progressive decrease in $\Delta\overline{RR}$ was observed through successive stages. A small increase in $\Delta\overline{RR}$ was noted after each resting day (days 10, 17, and 24) (**Figure 1A**).

Regarding HRV, a progressive imbalance in the autonomic balance marked with a decrease in cardiac vagal activity, evaluated through RMSSD and HF, was noted all along the cycling event. The daily RMSSD standing–supine difference was lower than the basal value during the multistage event.

## Correlations Between Heart Rate and Heart Rate Variability Indices, Fatigue, Physical Load, and Training Levels Indicators

These correlations concern our first hypothesis. **Table 2** shows correlations between HRV and subjective/objective fatigue, physical load, and training level indicators. As expected, MeanRRsup, MeanRRsta, ΔRMSSD, ΔLnRMSSD, and $\Delta\overline{RR}$ were negatively correlated with workload. We also observed significant negative correlations between RMSSDsup and workload indicators.

No correlation was observed between RMSSDsta and RPE ($r = 0.19$, $p = 0.41$), RMSSDsta and TRIMPS ($r = 0.047$, $p = 0.84$), nor RMSSDsta and distance of the stages ($r = 0.21$, $p = 0.35$). Lastly, the LF, HF, and LF/HF indices were not correlated with workload indicators ($r < 0.15$, $p > 0.46$). The ΔRMSSD index showed significant negative correlation with TRIMPS ($r = -0.20$, $p = 0.02$), RPE ($r = -0.17$, $p = 0.03$), and KMS ($r = -0.43$, $p = 0.05$). We also computed the correlations of ΔLnRMSSD with TRIMPS ($r = -0.23$, $p = 0.009$), RPE ($r = -0.04$, $p = 0.12$), KMS ($r = -0.41$, $p = 0.06$), perceived fatigue ($r = -0.24$, $p = 0.002$), DOMS ($r = -0.22$, $p = 0.004$), quality of sleep ($r = -0.06$, $p = 0.447$), and stress ($r = -0.22$, $p = 0.004$).

The $\Delta\overline{RR}$ index showed significant correlation with workload markers with negative correlations with TRIMPS ($r = -0.22$, $p = 0.009$), RPE ($r = -0.26$, $p < 0.05$), daily stage distance ($r = -0.41$, $p = 0.07$), perceived fatigue ($r = -0.39$, $p < 0.001$), DOMS ($r = -0.40$, $p < 0.001$), quality of sleep ($r = -0.13$, $p = 0.107$), and stress ($r = -0.24$, $p = 0.001$) (**Table 2**). Otherwise, the overall $\Delta\overline{RR}$ decrease observed all along the multistage cycling event was correlated ($r = 0.41$, $p < 0.05$) with the increase of RPE (**Figure 1B**).

Correlations between $\Delta\overline{RR}$, ΔRMSSD or ΔLnRMSSD, and subjective/objective fatigue, physical load, and training level indicators are statically comparable (the $p$-value of the William's test we used to compare correlations is $>0.1$ for each indicator).

## Impact of Training Level

To test the second hypothesis of the study, we first had to characterize the influence of the cyclist's training level on her adaptation all along the multistage event, and we considered the

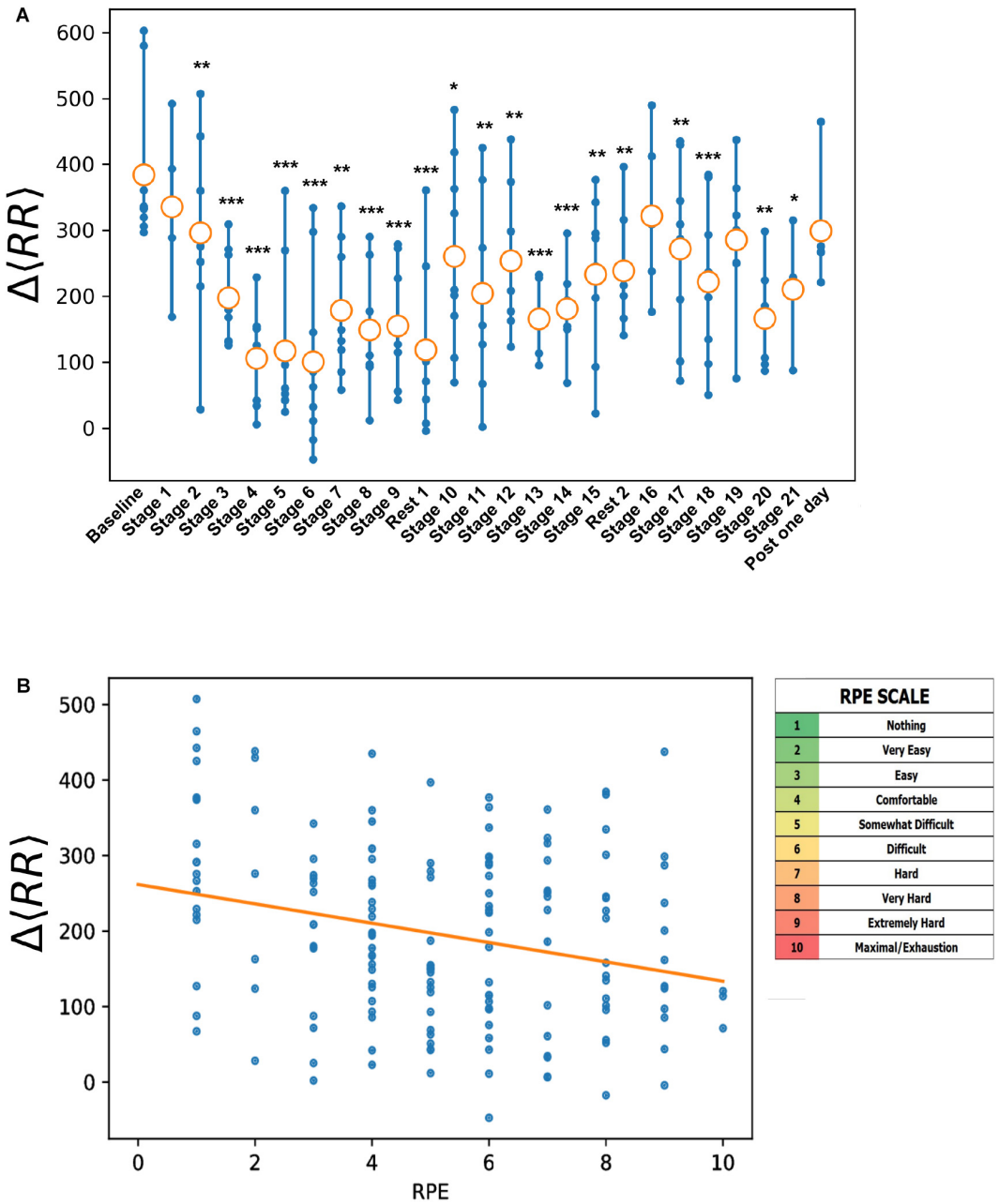



**FIGURE 1 |** Evolution of $\Delta\overline{RR}$ stage by stage **(A)** and with daily workload assessed through rate of perceived exertion (RPE) score on CR-10 Borg scale **(B)**. **(A)** $\Delta\overline{RR}$ of stage *n* reflect stress/fatigue induced by stage n–1. Statistical differences with baseline: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

change in mean WB on all the stages as a function of individual weekly training load. The results are shown in **Figure 2**. Overall, consequent to the accumulated fatigue, the feeling of WB gradually decreased all along the event. This decrease was less marked in the most trained cyclists. However, the relationship observed was not linear but curvilinear. Finally, we must emphasize the great individual variability of the evolution of WB for the same weekly training load (i.e., 100 or 200 km·week$^{-1}$ in **Figure 2**).

## Multivariable Linear Models

To test our second hypothesis, we had lastly to consider 256 models for both $\Delta\overline{RR}$ and ΔLnRMSSD. Among them, the four presenting the lowest AIC and the highest adjusted $R^2$ for $\Delta\overline{RR}$ and ΔLnRMSSD are shown in **Tables 3**, **4**, respectively. The fitting coefficients are reported with their *p*-value.

It must be underlined that the $\dot{V}O_{2max}$ value and the pre-TdF training load (km·week$^{-1}$) that were included in all models appeared to be the most relevant parameters to explain the

**TABLE 2 |** Correlation coefficients (and their *p*-values) between heart rate (RR) and heart rate variability indices and subjective/objective fatigue, physical load, and training levels indicators.

| | MeanRRsup | MeanRRsta | RMSSDsup | RMSSDsta | <ΔRR> | ΔRMSSD | ΔLn RMSSD | LF(nu) | HF(nu) | LF(ms²) | HF(ms²) | LF/HF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KMS | −0.44 ($p = 0.04$) | −0.34 ($p = 0.04$) | −0.35 ($p = 0.02$) | 0.21 ($p = 0.35$) | −0.41 ($p = 0.07$) | −0.43 ($p = 0.05$) | −0.41 ($p = 0.06$) | −0.07 ($p = 0.77$) | 0.07 ($p = 0.77$) | 0.15 ($p = 0.51$) | 0.13 ($p = 0.58$) | 0.09 ($p = 0.70$) |
| TRIMPS | −0.27 ($p = 0.001$) | −0.12 ($p = 0.175$) | −0.20 ($p = 0.019$) | −0.05 ($p = 0.532$) | −0.22 ($p = 0.009$) | −0.20 ($p = 0.020$) | −0.23 ($p = 0.005$) | 0.07 ($p = 0.415$) | −0.07 ($p = 0.408$) | −0.13 ($p = 0.112$) | −0.14 ($p = 0.100$) | 0.06 ($p = 0.465$) |
| RPE | −0.17 ($p = 0.033$) | −0.04 ($p = 0.574$) | −0.26 ($p = 0.001$) | −0.06 ($p = 0.444$) | −0.26 ($p = 0.001$) | −0.17 ($p = 0.029$) | −0.16 ($p = 0.042$) | −0.10 ($p = 0.200$) | −0.11 ($p = 0.150$) | 0.05 ($p = 0.545$) | −0.05 ($p = 0.538$) | −0.01 ($p = 0.900$) |
| Perceived fatigue | 0.10 ($p = 0.184$) | 0.20 ($p = 0.011$) | −0.17 ($p = 0.023$) | 0.21 ($p = 0.006$) | −0.39 ($p < 0.001$) | −0.01 ($p = 0.903$) | −0.24 ($p = 0.002$) | 0.11 ($p = 0.169$) | 0.13 ($p = 0.091$) | −0.02 ($p = 0.756$) | 0.02 ($p = 0.759$) | 0.03 ($p = 0.701$) |
| DOMS | 0.16 ($p = 0.032$) | 0.23 ($p = 0.003$) | −0.26 ($p = 0.001$) | 0.10 ($p = 0.195$) | −0.40 ($p < 0.001$) | 0.05 ($p = 0.500$) | −0.22 ($p = 0.004$) | 0.21 ($p = 0.005$) | 0.20 ($p = 0.009$) | −0.01 ($p = 0.906$) | 0.01 ($p = 0.904$) | −0.02 ($p = 0.797$) |
| Quality of sleep | 0.24 ($p = 0.002$) | 0.21 ($p = 0.007$) | −0.16 ($p = 0.042$) | −0.09 ($p = 0.259$) | −0.13 ($p = 0.107$) | 0.14 ($p = 0.080$) | −0.06 ($p = 0.447$) | 0.29 ($p = 0.000$) | 0.22 ($p = 0.005$) | −0.00 ($p = 0.988$) | 0.00 ($p = 0.993$) | −0.09 ($p = 0.248$) |
| Stress | 0.15 ($p = 0.056$) | −0.14 ($p = 0.069$) | −0.41 ($p = 0.000$) | −0.28 ($p = 0.000$) | −0.24 ($p = 0.001$) | −0.07 ($p = 0.392$) | 0.03 ($p = 0.696$) | −0.14 ($p = 0.063$) | −0.13 ($p = 0.097$) | −0.01 ($p = 0.872$) | 0.01 ($p = 0.879$) | −0.11 ($p = 0.154$) |

*KMS, km of the daily stage; TRIMPS, training impulse; RPE, rate of perceived exertion; DOMS, delayed-onset muscle soreness.*

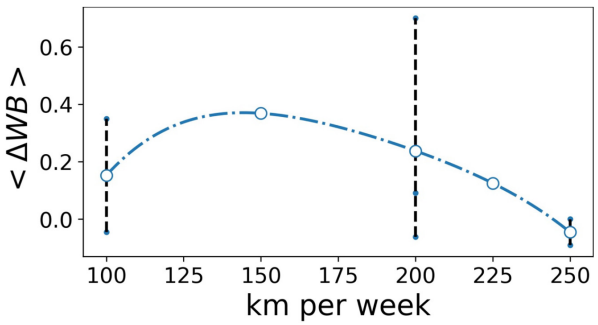



**FIGURE 2 |** Averaged change in well-being (<ΔWB>) as a function of weekly training load (km week$^{-1}$). Blue circles represent change in well-being averaged on cyclists covering the same distance per week. The small blue points and the dotted lines represent individual change in WB.

$\Delta\overline{RR}$ and the ΔLnRMSSD observed. To further quantify the importance of these indicators of the physical condition, we also included in **Tables 3**, **4** the best model, which does not include the $\dot{V}O_{2max}$ (model 5), the number of km·week$^{-1}$ (model 6), or none of them (model 7). Clearly, beyond the multistage event-induced fatigue, the physical condition is the key to account the $\Delta\overline{RR}$ and ΔLnRMSSD variations.

Specifically, the most relevant model to explain $\Delta\overline{RR}$ can be expressed as a multi-linear function:

$\Delta\overline{RR}$ = 1,249.37 + 12.32 $\dot{V}O_{2max}$ + 0.36 km·week$^{-1}$−8.83 $HR_{max}$−5.8 RPE−28.41 perceived fatigue

where the *p*-values of each coefficient are given in **Table 3**.

And the best model to predict ΔLnRMSSD is:

ΔLnRMSSD = 1.647 + 0.042 $\dot{V}O_{2max}$ + 0.002 km·week$^{-1}$−0.017 $HR_{max}$−0.019 RPE−0.100 perceived fatigue + 0.092 stress

where the *p*-values of each coefficient are given in **Table 4**.

Finally, to formulate a more accessible model for coaches and team managers, we looked for a linear relationship between $\Delta\overline{RR}$, a single indicator of fatigue and physiologic constants excluding the $\dot{V}O_{2max}$, which is not easily measurable. This model can be expressed as $\Delta\overline{RR}$ = −14.36 WB−2.06 $HR_{max}$ + 0.35 km·week$^{-1}$ + 689.62. The $R^2$ adjusted and AIC observed for this model were, respectively, 0.194 and 2033.

Concerning ΔLnRMSSD, as we can see from adjusted $R^2$, models predict less variance than for $\Delta\overline{RR}$ index (**Table 4**).

## DISCUSSION

Intense physical training exposes the athlete to the risk of overreaching or of overtraining, partly due to an imbalance between training and recovery (Bishop et al., 2008). The information from overreaching and overtraining markers, especially based on HR and HRV analyses, seems reinforced when it is associated with other fatigue parameters (Stanley et al., 2013; Buchheit, 2014). Our study aimed at modeling the relationship between the level of fatigue reported by well-trained female cyclists during a multistage cycling event, their physical load,

**TABLE 3 |** Linear coefficients, their corresponding *p*-values (in parentheses), adjusted $R^2$ coefficients, and Akaike information criterion (AIC) of the different linear multivariable mixed models tested to explain the difference between standing and supine RR duration of cyclists.

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
|---|---|---|---|---|---|---|---|
| $\dot{V}O_{2max}$ | 12.32** (<0.001) | 11.39** (<0.001) | 12.59** (<0.001) | 12.40** (<0.001) | | 11.78** (<0.001) | |
| Training load (km·week$^{-1}$) | 0.36 (0.021) | 0.36 (0.019) | 0.35 (0.023) | 0.36 (0.023) | 0.35 (0.033) | | |
| $HR_{max}$ | −8.83** (<0.001) | −8.13** (<0.001) | −9.02** (<0.001) | −8.79** (<0.001) | | −8.97** (<0.001) | −2.52 (0.075) |
| RPE | −5.90 (0.096) | −5.12 (0.16) | −5.99 (0.093) | −5.78 (0.156) | −4.71 (0.222) | −6.02 (0.094) | −5.94 (0.129) |
| Perceived fatigue | −28.41** (<0.001) | −24.55* (0.006) | −28.80** (<0.001) | −28.88 (<0.001) | −24.58* (0.009) | −29.62** (<0.001) | −19.32 (0.058) |
| DOMS | | −6.20 (0.365) | | | −19.13* (0.004) | | −19.33* (0.005) |
| Sleep quality | | | | 1.15 (0.85) | | | −10.81 ($p = 0.10$) |
| Stress | | | 2.23 (0.748) | | | | |
| Constant | 1,249.37** (<0.001) | 1,166.96** (<0.001) | 1,266.30** (<0.001) | 1,234.21** (<0.001) | 314.60** (<0.001) | 1,375.93** (<0.001) | 860.54** (<0.001) |
| Adjusted $R^2$ | 0.322 | 0.321 | 0.318 | 0.319 | 0.234 | 0.302 | 0.223 |
| AIC | 1,805 | 1,807 | 1,807 | 1,807 | 1,823 | 1,824 | 1,825 |

*Adjusted $R^2$ and AIC are used to estimate the predictive power of each model. These criteria are used instead of $R^2$ in order to penalize including extra fitting parameters. Training load corresponds to the weekly pre-TdF training load of the cyclists.*
*$\dot{V}O_{2max}$, maximal oxygen uptake; $HR_{max}$, maximal heart rate; RPE, rate of perceived exertion; WB, well-being; DOMS, delayed-onset muscle soreness.*
**p-value < $10^{-2}$, **p-value < $10^{-3}$.*

**TABLE 4 |** Linear coefficients, their corresponding *p*-values (in parentheses), adjusted $R^2$ coefficients, and Akaike information criterion (AIC) of the different linear multivariable mixed models tested to explain the difference between standing and supine LnRMSSD of cyclists.

| | R1″ | R2″ | R3″ | R4″ | R5″ | R6″ | R7″ |
|---|---|---|---|---|---|---|---|
| $\dot{V}O_{2max}$ | 0.042* (0.002) | 0.026* (0.004) | 0.024* (0.008) | 0.038* (0.009) | | 0.040* (0.003) | |
| Training load (km·week$^{-1}$) | 0.002 (0.010) | 0.002 (0.013) | 0.002 (0.011) | 0.002 (0.014) | 0.0017 (0.036) | | |
| $HR_{max}$ | −0.017 (0.094) | | | −0.014 (0.217) | | −0.018 (0.081) | |
| RPE | −0.019 (0.308) | −0.019 (0.303) | −0.013 (0.487) | −0.015 (0.421) | −0.016 (0.407) | −0.020 (0.297) | −0.017 (0.392) |
| Perceived fatigue | −0.100 (0.013) | −0.114* (0.005) | −0.081 (0.083) | −0.084 (0.072) | −0.075 (0.118) | −0.108* (0.009) | 0.085 (0.078) |
| DOMS | | | −0.044 (0.018) | −0.026 (0.464) | −0.059 (0.079) | | −0.054 (0.109) |
| Sleep quality | | | | | | | |
| Stress | 0.092 (0.012) | 0.074 (0.034) | 0.074 (0.033) | 0.089 (0.016) | 0.059 (0.092) | 0.095 (0.011) | 0.063 (0.078) |
| Constant | 1.647 (0.238) | −0.495 (0.388) | −0.427 (0.457) | 1.271 (0.393) | 0.982** (<0.001) | 2.365 (0.090) | 1.331** (<0.001) |
| Adjusted $R^2$ | 0.141 | 0.130 | 0.135 | 0.139 | 0.098 | 0.111 | 0.076 |
| AIC | 229.9 | 230.9 | 231.0 | 231.4 | 236.4 | 234.1 | 239.1 |

*Adjusted $R^2$ and AIC are used to estimate the predictive power of each model. These criteria are used instead of $R^2$ in order to penalize including extra fitting parameters. Training load corresponds to the weekly pre-TdF training load of the cyclists.*
*$\dot{V}O_{2max}$, maximal oxygen uptake; $HR_{max}$, maximal heart rate; RPE, rate of perceived exertion; WB, well-being; DOMS, delayed-onset muscle soreness.*
**p-value < $10^{-2}$, **p-value < $10^{-3}$.*

their training level, and the variations in HRV and HR rate indices in response to an active orthostatic test.

Regarding our first hypothesis, we noted a lower change of HR and of HR vagally mediated HRV between supine and standing positions during the orthostatic test. However, only HR and RMSSD, a time domain HRV index, were correlated with subjective/objective fatigue, physical load, and training level indicators. Lastly, the index $\Delta\overline{RR}$, defined as the difference between the average RR intervals measured in a supine position and then in a standing position, appeared a new indicator of stress/recovery status. These results confirmed our first hypothesis.

Regarding our second hypothesis, we have then demonstrated that $\Delta\overline{RR}$ and ΔLnRMSSD could be modeled as a linear function of training volume, $\dot{V}O_{2max}$ and fatigue level, assessed through the RPE and the WB questionnaire. Thus, the results observed confirmed only partly our second hypothesis, because DOMS and sleep quality previously proposed (Ouergui et al., 2020) did not provide major information to specify the stress-recovery status in the multistage of endurance event studied (**Table 3**). Regarding ΔLnRMSSD, models predict less variance than for $\Delta\overline{RR}$ index. This result underlines the interest of $\Delta\overline{RR}$ in stress-recovery status prediction.

## Respective Values of Heart Rate Variability Indices

From all the HRV indices we used, time domain's is the one that was the best correlated with workload and fatigue parameters (**Table 2**). Limits of spectral indices have been previously reported (Buchheit, 2014). Our results confirm also that the time-domain markers of parasympathetic effects seemed to be better adapted

to explore fatigue level (Buchheit, 2014; Fazackerley et al., 2019). This can be explained by the fact that parasympathetic nervous system is implied in self-regulation mechanisms, which are critical for adaptation (Laborde et al., 2017). Lastly, among these indices, the $\Delta\overline{RR}$ index was significantly correlated with subjective/objective fatigue, physical load, and training level indicators, as ΔRMSSD and ΔLnRMSSD. Previous reviews were focused on interests and limits of HR and HRV measures on monitoring training status (Buchheit, 2014; Bellenger et al., 2016). If Buchheit considers resting HRV (more precisely RMSSD) as the HR measure more sensitive to fatigue (Buchheit, 2014), Bellenger et al. (2016) underlined some limits of this ANS status analysis. Indeed, in their meta-analysis, Bellenger et al. (2016) have observed that overload training had little effect on resting HRV due to various effects on vagally mediated HRV indices. The authors explained that the disagreement between studies may be the result of methodological issues. In addition to these methodological aspects, HRV analysis appears as a complex process due to different fatigue-induced alterations of HRV pattern (Schmitt et al., 2015). Given these limits of resting HRV, it is interesting to bring out new tools in ANS status evaluation. Our study therefore outlines a new indicator of stress/recovery status, the $\Delta\overline{RR}$. Based on our results, a low $\Delta\overline{RR}$, translating a low HR change between supine and standing positions, could mean a lack of post-exercise recovery. This observation is in accordance with the increase cardiac sympathetic modulation during supine rest and attenuated baroreflex response to change position observed by Uusitalo et al. (2000) in heavily trained females. The decrease observed here seems explained by an increase in supine HR without modification of standing HR (Barrero et al., 2019). This observation may be due to a decrease in parasympathetic and/or an increase in sympathetic HR influence (Schmitt et al., 2015). To our knowledge, the precise cause of this observation, decrease in sensitivity, and/or density of sinus cell membrane receptors or other one has not been formally demonstrated (Bellenger et al., 2016).

## Mathematical Model

As specified in the *Introduction*, we tested eight parameters that quantify physical load, training level, and fatigue indicators. The $\dot{V}O_{2max}$ is widely used to assess both physical capacity and training level in endurance sports, but it is typically not repeatedly measured during a sporting season. Therefore, we included the training volume, summarized by the mean number of kilometers per week during the training period prior to the event. The chosen fatigue indicators reflect the internal load: RPE is commonly used to evaluate the perceived difficulty of an exercise and appeared strongly correlated to $\Delta\overline{RR}$, and the DOMS is specifically targeted at muscular fatigue; the perceived fatigue reflects general tiredness; quality of sleep and stress are associated with physical and mental fatigue.

We first studied the influence of the individual training level on the adaptation throughout the multistage cycling event. This training level depends on two main factors: the number of years of practice and the quality of training carried out during the weeks preceding the event. We noted no correlation between the number of years of practice and WB during the event. On the other hand (see **Figure 2**), except for the least trained cyclist, we observed a positive correlation between the training volume per week before the event and the adaptation all along the event. We therefore observed a predominant influence of recent training on the level of exercise tolerance during the multistage event.

Then, several multivariable models were tested. We showed that the indices $\Delta\overline{RR}$ and ΔLnRMSSD could be modeled linearly as a function of three main individual variables: training volume, $\dot{V}O_{2max}$, and fatigue assessed through RPE and WB questionnaire. These indices appear to be relevant indicators of the adaptation ability along multistage events. However, we have noticed that linear models based on $\Delta\overline{RR}$ have more predictive power than those based on ΔLnRMSSD. Moreover, the individual $\Delta\overline{RR}$ and ΔLnRMSSD appear to be reliable indicators of both athlete's training level and fatigue level. Indeed, we observed a positive correlation between $\Delta\overline{RR}$ (respectively, ΔLnRMSSD) and $\dot{V}O_{2max}$, which reflects the training level and a negative correlation between $\Delta\overline{RR}$ (respectively, ΔLnRMSSD) and fatigue, RPE, WB, and maximal HR. The positive correlation we observed with the individual fitness and recovery of altered autonomic regulation after prolonged exercise confirms previous observations (Hautala et al., 2001; Fazackerley et al., 2019). Finally, the negative correlation with maximal HR confirms that the latter decreases with chronic endurance training (Bailey and Davies, 1999).

## Practical Applications

A decrease in the value of HRV indices is a marker of weak adaptability of the cardiovascular system to stress conditions that it faces (Michael et al., 2017); for example, the supine vagally mediated HRV parameters (RMSSD, total spectral power, and HF but not LF/HF) were lower in athletes identified in the fatigue state compared with the nonfatigue one (Plews et al., 2012; Schmitt et al., 2015). The active orthostatic test, a well-described marked physiological stress, is recommended to study HRV in athletes (Uusitalo et al., 2000; Hynynen et al., 2008; Schmitt et al., 2015).

From our results, it seems that the higher is $\Delta\overline{RR}$, HR difference between supine and standing positions, the better is the cardiovascular adaptability to orthostatic stress. Conversely, a low $\Delta\overline{RR}$ means stress-recovery imbalance as described in the paragraph *Respective Values of Heart Rate Variability Indices*. The negative impact of a Grand Tour on physical performance, mood, and WB of competitive cyclists is well reported, and a study performed with professional male cyclists during the Vuelta a España has noted that changes in supine HRV were inversely correlated to the exercise level (Earnest et al., 2004; Lastella et al., 2015; Rodríguez-Marroyo et al., 2017). Our results confirmed this observation. The impact of the preintervention physiological status on HRV alteration has also been proposed (Lastella et al., 2015; Rodríguez-Marroyo et al., 2017). Our data also underlined the importance of the pre-event training level to explain the HR adaptability change during a cyclist's multistage event.

To our knowledge, it is the first time that the HR changes observed during an easy physiological test are modeled as a function of two physical parameters, training level and $\dot{V}O_{2max}$, and one psychological parameter, fatigue. The model

proposed allowed us to understand the ability to adapt to a repeated endurance exercise measuring the mean RR interval changes observed between supine and standing positions in well-trained female cyclists. If the measured $\Delta\overline{RR}$ is lower than predicted by the model, we could conclude that the imbalance of stress-recovery status is higher than perceived by the athlete in this context of a cyclist multistage event.

Thus, our findings support the use of $\Delta\overline{RR}$ monitoring to quantify training load, as $\Delta\overline{RR}$ can be directly predicted from fatigue and training level indicators. The use of $\Delta\overline{RR}$ monitoring can help coaches and athletes to make strategic decisions during a multistage long-duration event. It should be noted that the proposed model can be used with unsophisticated HR monitors (i.e., those recording only RR average values). This therefore makes it accessible to a majority of coaches and athletes, interested in the scientific approach of the training and performance monitoring. Finally, we also presented a model more accessible for coaches and team managers, connecting HR changes to a single indicator of fatigue. However, in accordance with its $R^2$ adjusted and AIC values, the robustness of this model appears low, and it should be used with caution.

### Study Limitations

This study presents three main limitations. First, the small population sample studied can reduce the predictive power of the proposed model. However, the daily evolution of HRV in female athletes has been scarcely studied, and the model we proposed seems to be easy to use to follow the individual training and performance level. Second, our study focuses on well-trained female cyclists, and the validity of our model deserves to be confirmed in other sports. Specific studies are also needed in endurance male athletes, because HRV gender's difference has been reported (Schäfer et al., 2015). Third, as some HRV indices are closely related and dependent on the individual's breathing frequency during recording (No authors listed, 1996), it could have been relevant to impose controlled breathing (Gregoire et al., 1996), although this induces a mental activity and stress (Bernardi et al., 2000). At least, monitoring of respiratory rate could have been considered.

## CONCLUSION

From our data on well-trained female cyclists, we introduced a new indicator of post-endurance exercise recovery, the $\Delta\overline{RR}$ based on the change of mean HR observed during an orthostatic active test. This index is influenced by the training level and by the $\dot{V}O_{2max}$ of the athlete. The proposed quantitative model can help to assess the adaptation ability of an athlete and thus to anticipate a decrease in endurance performance due to fatigue, particularly during a long-duration multistage cycling event. Investigating larger populations of athletes, included in other sports than cycling, represents an exciting perspective for future studies.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CHU Rennes, France. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

AB, SL, GC, FC, and FS performed the experimental conception and design. AB and SL performed the experiments. AB and ALC analyzed the data. AB, ALC, SL, FC, and FS written the manuscript. All authors read and approved the final manuscript.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the enthusiastic participation of the subjects in this study and the generous technical support of Johan Cassirame and Armel Cretual.

## REFERENCES

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Trans. Auto. Control* 19, 716–723. doi: 10.1109/tac.1974.1100705

Bailey, D. M., and Davies, B. (1999). Decreased chronotropic drive as an adaptation to chronic exercise; possible mechanisms. *Int. J. Sports Med.* 20, 219–221. doi: 10.1055/s-2007-971120

Barrero, A., Schnell, F., Carrault, G., Kervio, G., Matelot, D., Carre, F., et al. (2019). Daily fatigue-recovery balance monitoring with heart rate variability in well-trained female cyclists on the Tour de France circuit. *PLoS One* 14:e0213472. doi: 10.1371/journal.pone.0213472

Bellenger, C. R., Fuller, J. T., Thomson, R. L., Davison, K., Robertson, E. Y., and Buckley, J. D. (2016). Monitoring athletic training status through autonomic heart rate regulation: a systematic review and meta-analysis. *Sports Med.* 10, 1461–1486. doi: 10.1007/s40279-016-0484-2

Bernardi, L., Wdowczyk-Szulc, J., Valenti, C., Castoldi, S., Passino, C., Spadacini, G., et al. (2000). Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability. *J Am Coll. Cardiol.* 35, 1462–1469. doi: 10.1016/s0735-1097(00)00595-7

Bishop, P. A., Jones, E., and Woods, A. K. (2008). Recovery from training: a brief review. *J. Strength Cond. Res.* 22, 1015–1024. doi: 10.1519/jsc.0b013e31816eb518

Borg, G. (1990). Psychophysical scaling with applications in physical work and the perception of exertion. *Scand. J. Work Environ. Health* 16(Suppl. 1), 55–58. doi: 10.5271/sjweh.1815

Bosquet, L., Merkari, S., Arvisais, D., and Aubert, A. E. (2008). Is heart rate a convenient tool to monitor over-reaching? A systematic review of the literature. *Br. J. Sports Med.* 42, 709–714. doi: 10.1136/bjsm.2007.042200

Bourdillon, N., Schmitt, L., Yazdani, S., Vesin, J. M., and Millet, G. P. (2017). Minimal window duration for accurate HRV recording in athletes. *Front. Neurosci.* 11:456. doi: 10.3389/fnins.2017.00456

Buchheit, M. (2014). Monitoring training status with HR measures: do all roads lead to Rome? *Front. Physiol.* 5:73. doi: 10.3389/fphys.2014.00073

Chalencon, S., Busso, T., Lacour, J. R., Garet, M., Pichot, V., Connes, P., et al. (2012). A model for the training effects in swimming demonstrates a strong relationship between parasympathetic activity, performance and index of fatigue. *PLoS One* 7:e52636. doi: 10.1371/journal.pone.005263

Chalencon, S., Pichot, V., Roche, F., Lacour, J. R., Garet, M., Connes, P., et al. (2015). Modeling of performance and ANS activity for predicting future responses to training. *Eur. J. Appl. Physiol.* 115, 589–596. doi: 10.1007/s00421-014-3035-2

Earnest, C. P., Jurca, R., Church, T. S., Chicharro, J. L., Hoyos, J., and Lucia, A. (2004). Relation between physical exertion and heart rate variability characteristics in professional cyclists during the Tour of Spain. *Br. J. Sports Med.* 38, 568–575. doi: 10.1136/bjsm.2003.005140

Edwards, S. (1993). "High performance training and racing," in *The Heart Rate Monitor Book*, ed. S. Edwards (Sacramento: Feet Fleet Press), 113–123.

Fazackerley, L. A., Fell, J. W., and Kitic, C. M. (2019). The effect of an ultra-endurance running race on heart rate variability. *Eur. J. Appl. Physiol.* 119, 2001–2009. doi: 10.1007/s00421-019-04187-6

Gregoire, J., Tuck, S., Yamamoto, Y., and Hughson, R. L. (1996). Heart rate variability at rest and exercise: influence of age, gender, and physical training. *Can. J. Appl. Physiol.* 21, 455–470. doi: 10.1139/h96-040

Grossman, P., and Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsycho.2005.11.014

Halson, S. L. (2014). Monitoring training load to understand fatigue in athletes. *Sports Med.* 44(Suppl. 2), S139–S147. doi: 10.1007/s40279-014-0253-z

Hautala, A., Tulppo, M. P., Mäkikallio, T. H., Laukkanen, R., Nissilä, S., and Huikuri, H. V. (2001). Changes in cardiac autonomic regulation after prolonged maximal exercise. *Clin. Physiol.* 21, 238–245. doi: 10.1046/j.1365-2281.2001.00309.x

Heidari, J., Beckmann, J., Bertollo, M., Brink, M., Kallus, W., Robazza, C., et al. (2018). Multidimensional monitoring of recovery status and implications for performance. *Int. J. Sports Physiol. Perform.* 15, 1–24. doi: 10.1123/ijspp.2017-0669

Hooper, S. L., Mackinnon, L. T., Howard, A., Gordon, R. D., and Bachmann, A. W. (1995). Markers for monitoring overtraining and recovery. *Med. Sci. Sports Exerc.* 27, 106–112.

Hynynen, E., Uusitalo, A., Konttinen, N., and Rusko, H. (2008). Cardiac autonomic responses to standing up and cognitive task in overtrained athletes. *Int. J. Sports Med.* 29, 552–558. doi: 10.1055/s-2007-989286

Kuipers, H. (1998). Training and overtraining: an introduction. *Med. Sci. Sports Exerc.* 30, 1137–1139. doi: 10.1097/00005768-199807000-00018

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Physiol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lastella, M., Roach, G. D., Halson, S. L., Martin, D. T., West, N. P., and Sargent, C. (2015). The impact of a simulated grand tour on sleep, mood, and well-being of competitive cyclists. *J. Sports Med. Phys. Fitness* 55, 1555–1564.

Malik, M., and Camm, A. J. (1993). Components of heart rate variability–what they really mean and what we really measure. *Am. J. Cardiol.* 72, 821–822. doi: 10.1016/0002-9149(93)91070-x

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., et al. (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Med. Sci. Sports Exer.* 45, 186–205. doi: 10.1249/MSS.0b013e318279a10a

Michael, S., Graham, K. S., and Davis, G. M. (2017). Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals-a review. *Front. Physiol.* 8:301. doi: 10.3389/fphys.2017.00301

No authors listed (1996). Heart rate variability: standards of measurement physiological interpretatioin and clinical use. task Force of the European society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Ouergui, I., Ardigò, L. P., Selmi, O., Levitt, D. E., Chtourou, H., Bouassida, A., et al. (2020). Changes in perceived exertion, well-being, and recovery during specific judo training: impact of training period and exercise modality. *Front. Physiol.* 11:931. doi: 10.3389/fphys10.3389/fphys.2020.00931

Plews, D. J., Laursen, P. B., Kilding, E. A., and Buchheit, M. (2012). Heart rate variability in elite triathletes, is variation in variability the key to effective training? A case comparison. *Eur. J. Appl. Physiol.* 112, 3729–3741. doi: 10.1007/s00421-012-2354-4

Rodríguez-Marroyo, J. A., Villa, J. G., Pernía, R., and Foster, C. (2017). Decrement in professional cyclists' performance after a grand tour. *Int. J. Sports Physiol. Perform.* 12, 1348–1355. doi: 10.1123/ijspp.2016-0294

Saw, A. E., Main, L. C., and Gastin, P. B. (2016). Monitoring the athlete training response: subjective self-reported measures trump commonly used objective measures: a systematic review. *Br. J. Sports Med.* 50, 281–291. doi: 10.1136/bjsports-2015-094758

Schäfer, D., Gjerdalen, G. F., Solberg, E. E., Khokhlova, M., Badtieva, V., Herzig, D., et al. (2015). Sex differences in heart rate variability: a longitudinal study in international elite crosscountry skiers. *Eur. J. Appl. Physiol.* 115, 2107–2114. doi: 10.1007/s00421-015-3190-0

Schmitt, L., Regnard, J., Parmentier, A. L., Mauny, F., Mourot, L., Coulmy, N., et al. (2015). Typology of "Fatigue" by heart rate variability analysis in elite nordic-skiers. *Int. J. Sports Med.* 36, 999–1007. doi: 10.1055/s-0035-1548885

Stanley, J., Peake, J. M., and Buchheit, M. (2013). Cardiac parasympathetic reactivation following exercise: implications for training prescription. *Sports Med.* 43, 1259–1277. doi: 10.1007/s40279-013-0083-4

Taylor, A. A. (1994). Autonomic control of cardiovascular function: clinical evaluation in health and disease. *J. Clin. Pharmacol.* 34, 363–374. doi: 10.1002/j.1552-4604.1994.tb04976.x

Tse, H. F., Siu, C. W., Tsang, V., Yu, C., Park, E., Bornzin, G. A., et al. (2005). Blood pressure response to transition from supine to standing posture using an orthostatic response algorithm. *Pacing Clin. Electrophysiol.* 28, S242–S245. doi: 10.1111/j.1540-8159.2005.00054.x

Uusitalo, A. L., Uusitalo, A. J., and Rusko, H. K. (2000). Heart rate and blood pressure vari- ability during heavy training and overtraining in the female athlete. *Int. J. Sports Med.* 21, 45–53. doi: 10.1055/s-2000-8853

White, N. J. (1980). Heart-rate changes on standing in elderly patients with orthostatic hypotension. *Clin. Sci.* 58, 411–413. doi: 10.1042/cs0580411

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

HYPOTHESIS AND THEORY
published: 21 December 2020
doi: 10.3389/fnins.2020.609570

# Interpretation of Heart Rate Variability: The Art of Looking Through a Keyhole

*John M. Karemaker**

*Department of Medical Biology, Section Systems Physiology, Amsterdam University Medical Centers, Amsterdam, Netherlands*

The heart may be a mirror of the soul, but the human mind is more than its heart rate variability (HRV). Many techniques to quantify HRV promise to give a view of what is going on in the body or even the psyche of the subject under study. This "Hypothesis" paper gives, on the one hand, a critical view on the field of HRV-analysis and, on the other hand, points out a possible direction of future applications. In view of the inherent variability of HRV and the underlying processes, as lined out here, the best use may be found in serial analysis in a subject/patient, to find changes over time that may help in early discovery of developing pathology. Not every future possibility is bright and shining, though, as demonstrated in a fictional diary excerpt from a future subject, living in a society geared toward preventive medicine. Here implanted biochips watch over the health of the population and artificial intelligence (AI) analyses the massive data flow to support the diagnostic process.

**Keywords: baroreflex sensitivity, biochip, cuffless blood pressure, non-linear analysis, preventive medicine, reticular activating system, science fiction, sympathovagal balance**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Michal Javorka,*
*Comenius University, Slovakia*
*Roberto Maestri,*
*Clinical Scientific Institutes Maugeri (ICS Maugeri), Italy*

***Correspondence:**
*John M. Karemaker*
*j.m.karemaker@amsterdamumc.nl*
*orcid.org/0000-0003-0142-5425*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *23 September 2020*
***Accepted:*** *02 December 2020*
***Published:*** *21 December 2020*

***Citation:***
*Karemaker JM (2020) Interpretation of Heart Rate Variability: The Art of Looking Through a Keyhole. Front. Neurosci. 14:609570. doi: 10.3389/fnins.2020.609570*

## INTRODUCTION

### Diary Entries:

#### August 1, 2030, 10:30 a.m.

This morning I downloaded the data from my biochip to iCAIre, my home health system. It took longer than normal, which worried me, as always when the chip obviously had decided to do more measurements. A few days ago, iCAIre already made an appointment for me to see an eGP later today, we will have a videocall at 17:30 CET (Central European Time). The eGPs have their office in the United States, they came with the system that I chose. I could have checked in my preferences to have them contact my own GP directly, but hey, it is my health. I have looked at the files that were transmitted, but they did not make much sense to me: heart rate excursions, very large while I was asleep, same for blood pressure, oxygen- and $CO_2$-levels. Probably I was tossing and turning very much while asleep. Would that explain my headaches, sleepiness and higher than normal blood pressure during the day? This biochip-thing is getting on my nerves. At the time it seemed like a good idea to have it implanted, my health insurance company insisted on it, or else my premium would almost double. So much for freedom of choice in the era of preventive medicine.

#### August 1, 2030, 20:30

That was not a nice videocall at all. The woman on the other side was rather blunt: I am overweight, my blood glucose is not OK, and, worst of all, I have developed obstructive sleep apnea,

that is causing all this trouble during the night- and daytime. What now to do? See a dietician, I have to lose at least 20 kilos of my present 109. I always lived under the impression that my height of 1.85 m would allow me some more mass than average, but maybe she is right. In the meantime, I must make an appointment with my local GP whom they will contact to have me equipped with a device to prevent these apneic periods during sleep. I was reprimanded, because I should have checked in earlier, when the system started to bleep after every upload. In a way it was a lucky choice, this US system. It was more expensive than the regular ones, but it promised more privacy; the more common Asian systems are automatically coupled to the national registrar for preventive health care, my data are kept private as long as they ascertain that I am in the green zone. Which I, obviously, am not, at least not always. Now I risk to be exposed anyway. ICAIre will follow the developments closely, the operator said, not so much for my sake, but in order not to lose their license to sell and contract in the EU.

## Rationale

The title of the present Research Topic: *Horizon 2030: innovative applications of Heart Rate Variability* pushes the authors to, somehow, rise above their field and imagine a not-too-distant future where heart rate variability (HRV) might feature in a new role. The *Frontiers* journal is usually about science, but this invites now to venture the border between science and science fiction. Therefore, the present contribution is more a personal view rather than a systematic review of HRV interpretation. The paper's title is to stress this fact: looking through a keyhole one can only observe part of what is going on behind that door and much of the testimony of what happened there is interpretation, maybe helped by knowledge of the players and what the room looks like, but not an actual eyewitness report.

## HEART RATE VARIABILITY

The use of HRV has expanded into many areas of biomedical research and even into medical apps on smartphones that are supposed to help a person relax. Still, in accepted medical practice HRV is at best a side note in a Holter monitoring report (i.e., a 24 h ambulatory ECG-recording). Why the discrepancy? Might this change in the coming decade? What do we understand about HRV, and how and when can that knowledge be of help in medical practice?

In theory, the origin of HRV is not elusive: generally speaking, fast, beat-to-beat variations in heart rate are parasympathetic, vagally mediated. The slower changes, extending over many beats, are mainly due to sympathetic nervous influences. Heart rate changes over long periods (tens of seconds to minutes and hours) are due to humoral factors like adrenaline, vasopressin and angiotensin (Karemaker, 2017).

## SPECIFIC FREQUENCIES

Recognition of the underlying autonomic neural activities has awakened hope to understand the (central) nervous condition from heart rate variability. Initially, this was restricted mainly to measurement of respiratory variations (respiratory sinus arrhythmia, RSA), in search for a measure of vagal outflow. By maximal deep in- and expiration at a low frequency, it was held that vagal outflow was switched on and off, thus the peak-to-trough values of RSA would represent total vagal outflow. Decreases beyond those due to aging were (and are still) considered indicative of disease processes like diabetic neuropathy, as reviewed in Wieling et al. (1985). Later studies demonstrated the strict necessity of conditioning depth and frequency of respiration, as well as the level of exercise or sympathetic background activity, before making any inferences from RSA (Grossman et al., 1991).

Closer inspection of heart rate variations, aided by computer spectral analysis, had demonstrated the existence of slower than respiratory heart rate variations, in particular with around 10 s periodicity. These are also (mainly) due to vagal effects, but riding on slow sympathetically driven blood pressure waves (Sayers, 1971; Akselrod et al., 1981; DeBoer et al., 1987). Since the latter frequency, therefore, is related to sympathetic activity and the former, respiratory, to parasympathetic, the ratio between the powers in the two frequency bands LF/HF (LF, low frequency, i.e., 10 s rhythm, over HF, high frequency, respiratory rate) (Malliani et al., 1991; Camm et al., 1996) has developed into a number that is used to describe the balance in the central autonomic drive: sympatho-vagal balance. If we pause to think about this, attractive as it may be, it is obvious that this number comes with an intrinsic problem: both the fast and the slower heart rate variations are (mainly) vagally mediated and in most cases respiration depth and frequency are undefined. After administration of a vagolytic dose of atropine the time curve of heart rate is almost flat, only slow variations remaining. Likewise, during exercise the parasympathetic outflow is almost silenced, and no regular rhythms remain, neither around 0.1 Hz, nor respiratory. Still, no one would deny the existence of a strongly increased sympathetic state when heart rates go up to 150 beats per minute and higher.

## VARIABILITY COMES IN FLAVORS

In 1987 Kleiger and the multicenter post-infarction research group showed that decreased HRV in particular in the low- and very low-frequency area as calculated from 24 h ECG's could predict mortality in the years after a myocardial infarction (Kleiger et al., 1987). A few years later, Bigger et al. (1993) demonstrated in a subset of the same patient population, that even 5–15 min recordings gave the same results with the same predictive power. These studies inspired from that time onward an almost exponential rise in publications related to HRV, as demonstrated in **Figure 1** for the Core Journals in Web of Science™. Of the around 30,000 core publications mentioning HRV up to now, only some 1,500 (5%) have "heart failure" as keyword as well. Much of the work is not so much devoted to practical medical applications as it is to the development of analysis techniques to squeeze information out of the HRV-signal. In particular, the easy availability of digitized long heart rate recordings has inspired the mathematics- and physics-savvy

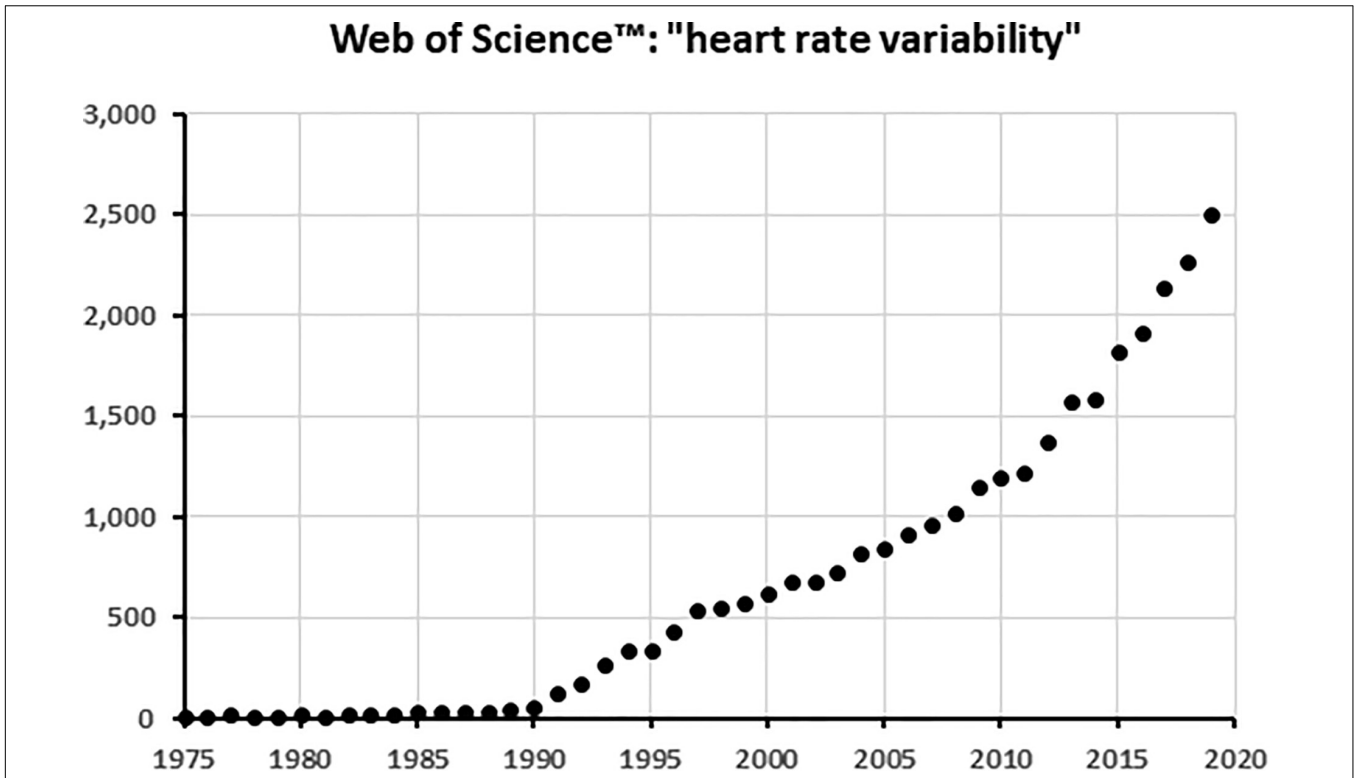



**FIGURE 1 |** The number of articles with "Heart rate variability" in the title per year, as found in the Web of Science core collection™ of scientific journals. When plotted on a logarithmic scale, the curve becomes an almost perfect straight line from 1996 onward, demonstrating its exponential increase.

community to devise more and more intricate techniques of non-linear analysis to discover hidden features in HRV beyond those detectable by simple statistics of the signal in the time domain or by frequency domain analysis.

The first attempts were exploiting the fractal properties of the heart rate signal: the "jumpiness" that repeats itself on any scale, from seconds to minutes to hours (Goldberger, 1990). A true fractal signal shows up as a straight line when frequency and power are plotted on logarithmic scales (power law). And indeed, heart rate signals do (more or less). This inspired hope that the slope of that fractal line, the fractal dimension, would tell something about the condition of the test subject (di Renzo et al., 1996; Goldberger, 1996, 1997; Otsuka et al., 1997). However, that did not turn out so well: Eckberg and colleagues showed that the fractal dimension could change remarkably from day to day without any obvious reason in perfectly healthy subjects (Tan et al., 2009). On the other hand, Churchill et al. (2016) demonstrated that the fractal properties of the blood oxygen level dependent (BOLD) signal in fMRI are suppressed to variable degrees depending on the attention level required. If the resting level of brain activity is reflected in HRV, variable outcomes of resting HRV fractal exponent may be the result of (not-so resting) brain activity, unknown to the observer.

The next push came from application of information theory: a purely regular sequence of heart intervals has, information-wise, low entropy. From that insight various descriptors for the predictability, or rather unpredictability, have been proposed. The first application came by Pincus, who coined the term "Approximate Entropy" (ApEn) to describe the irregularity in a series of biological events like successive heart periods (Pincus, 1991; Pincus and Goldberger, 1994). Together with Goldberger he wrote a tutorial for physiologists in 1994 where they explained how this parameter should be calculated and interpreted. ApEn led to a number of follow-up and/or improved parameters: for one, Sample Entropy, introduced by Moorman to produce computationally more stable numbers, which also have more consistent outcomes when repeated in the same subject on different days (Richman and Moorman, 2000). Costa and Goldberger took the entropy analysis still one step further when they introduced multiscale entropy (MSE), where entropy calculations are done on "coarse grained," time series i.e., resampled by packing more and more successive data points together to emphasize slower and slower trends in the signal (Costa et al., 2002, 2005).

The calculation of information entropy, applied to heart period series, has opened new doors to quantify the visual impression that one may have when looking at a beat-to-beat heart rate signal. It must be stressed, though, that there are thus far few practical applications. Moorman et al. devised a tool that has shown promise in neonatal intensive care units (Fairchild et al., 2013). Comparisons between more classical statistical- or frequency analysis derived- and entropy-derived measures do, however, not always favor the newer ones (Zhang et al., 2013). This may, partly, be due to the fact that in many applications,

entropy-measurements require rather long recordings before reaching a stable value. In critical situations this is a serious drawback. However, when the requirement of more time and more data points it is not an issue, entropy analysis may be a viable option to dig deeper in the complexities of the heart rate signal at hand. The examples found in the literature demonstrate that it may point from changes in mood to underlying pathology, present or in the making (Ho et al., 2011). Therefore, the "dystopic diary entries" in the beginning of this paper point to a possible application: follow a person's HRV over longer time to observe changes that might point to developing problems, like the diagnosis of obstructive sleep apnea during the night with its sequels in the daytime.

## NON-INVASIVE CONTINUOUS BLOOD PRESSURE

Heart rate should not be judged separately from the prevalent blood pressure (BP). The invention of a reliable method for non-invasive measurement of continuous BP by the Czech physiologist Jan Peňáz, further developed together with the Dutch biomedical engineer Karel Wesseling and his team (Penaz, 1973; Molhoek et al., 1984), has opened up a wide field of research by enabling riskless measurement in many test subjects under a large variety of circumstances. By the use of an inflatable finger cuff and concurrent measurement of the photoplethysmogram of the phalanx under study, a servo pressure loop keeps the pressure inside the cuff such that the vessels are continuously in a condition of almost-collapse. The only way to do that properly is by applying inside the cuff the same pressure as the one inside the vessels (give or take some pressure drop due to tissue pressure). This will reliably track intra-arterial pressure, if provisions inside the servo-machine are made to adjust for slow changes in the tissues of the compressed finger. This combination of ideas and engineering craftsmanship resulted in the successful introduction of the Finapres and the many varieties of the technique (Imholz et al., 1998). However, the cost of the necessary equipment has prevented its penetration into the consumer market; another drawback is its sensitivity to artefacts due to finger movement, to name but one.

Presently, cuffless blood pressure estimation is under way to fill that gap: by correlating upper arm BP, or some other calibrated pressure, to pulse wave velocity measurement. Pulse wave velocity in a vessel is related to its stiffness via the Moens–Korteweg equation: $PWV = \sqrt{(h.E/\rho.d)}$, where h is wall thickness, d its diameter, E Young's modulus (quantifying stiffness) and $\rho$ the density of the fluid. Stiffness is related to wall composition, to the level of blood pressure and (strongly) to age. The method calculates BP by applying the estimated biophysical properties of the vascular wall in that particular subject to the arrival time of the pressure wave at e.g. the wrist after, for instance, the R-wave of the ECG (as such, it might even lend itself to incorporation of a biochip as mentioned in the introduction). A review of the method and various algorithms and calibration methods used is given in Sharma et al. (2017). The results have been quite variable until now, the method is not used in clinical monitoring devices due to its unreliability (Moharram et al., 2020). This has not prevented the marketing of devices for instance for sleep recording[1] of blood pressure or 24 h continuous beat-to-beat estimation by the same principle, starting from one calibration with a traditional arm cuff. The issue has been picked up by the Institute of Electrical and Electronics Engineers (IEEE) that has developed its P1708-2014 – IEEE Standard for Wearable Cuffless Blood Pressure Measuring Devices. Even though that may sound like a precise way of testing some new device, the center piece remains the arm cuff, requiring two independent, trained observers who read the same sphygmomanometer using a Y-piece for their stethoscopes. In many hospitals and medical practices, doctors and nurses will prefer an automatic upper arm blood pressure measuring device. This works by the oscillometric principle and gives exact looking numbers for heart rate and systolic, diastolic (sometimes mean) to the nearest mmHg pressure value. The technique of accurate measurement by the hand-inflated arm cuff and stethoscope seems like a dying art.

In blood pressure measurement we seem to be stuck to a one century-old technique (Riva-Rocci cuff inflation, listening to the Korotkov sounds distal from the occluding point) expressing the pressure in a unit that no longer makes sense (mmHg) since mercury has long been banished from use due to its toxicity when spills are entering the environment. The various hypertension societies have successfully blocked the introduction of the International SI-unit of pressure, the Pascal (or rather the kilopascal in the case of BP), arguing that comparison to older literature and patient data will be hampered, which is a good point, but it postpones the shift forever and it makes for instance the understanding of flow resistance units so much more difficult.

## BAROREFLEX SENSITIVITY

The combined analysis of heart rate and continuous blood pressure adds another sought-after key figure to the non-invasive diagnostic and research arsenal, i.e., baroreflex sensitivity (BRS), the amount of heart period lengthening divided by the amount of causative blood pressure rise (ms/mmHg). BRS measurement, originally, was developed by imposing a fast blood pressure rise by the injection into the blood stream of a strongly vasoactive substance. At first this was mainly angiotensin (Smyth et al., 1969), later phenylephrine was predominantly used, when it became apparent that angiotensin has central effects on the vagal outflow to the heart. A thorough discussion of the use of bolus injections to measure BRS and the various drugs to do so is given in Eckberg and Sleight (1992).

Baroreflex sensitivity is the overall effect of a multi-step transduction process; at every step its effectiveness may be altered by factors external or internal to the reflex. It starts at the transformation of blood pressure changes to vessel wall extension, then to receptor activity to afferent nerve activity to nucleus tractus solitarii activity to efferent cardiac vagus nerve activity (and inhibition of sympathetic outflow) to sinoatrial node depolarization to atrial and then ventricular activation to arterial

[1] https://somnomedics.de/en/solutions/blood-pressure/somnotouch-nibp/

pressure pulse generation. Details of the various steps will be discussed below under the appropriate headings. This reflex loop is, of course, not only active when phenylephrine is injected, but continuously, from beat to beat. As a consequence, one may relate the changes in pressure to concomitant changes in heart period and expect to find a number related to the phenylephrine BRS. This, indeed proves to be the case: the correlation of the slower systolic pressure variations around 0.1 Hz to those in heart period gives numbers in the right order of magnitude (Robbe et al., 1987). The faster (HF-) variations give higher numbers, probably tainted by direct effects of the respiratory centers on vagal outflow to the heart (Frederiks et al., 2000). The numbers found are not perfect, they tend to come with a sizeable variability, but so does BRS by the phenylephrine method; the output via the vagus nerve is rather variable.

The variability of BRS comes to light by a technique that follows the baroreflex, as it were, from moment to moment while comparing sliding windows of 10 s blood pressure values to ensuing heart periods (xBRS). This gives very variable numbers over short periods, but the geometric averages correlate favorably to the BRS determined by vasoactive drug injections in the same experimental subjects (Wesseling et al., 2017). The same variability is found when short segments are searched in BP-HR recordings, to measure a valid baroreflex slope when sufficient correlation is detected [the "slopes-technique," cf. (di Rienzo et al., 1996)]. The inherent variability of beat-to-beat blood pressure control has led (Wessel et al., 2020) to take the position that these methods only look at variability ratios between blood pressure and heart rate. Probably that is exactly what the baroreflex should do: exchange blood pressure variability for heart rate variability. The former is the value to be kept between boundaries for proper functioning of the body, the latter is, within limits, less of a consequence (Karemaker and Wesseling, 2008).

## AFFERENTS THAT MAY ALTER HEART RATE

Considering all incoming nervous activity to the CNS, ultimately any afferent nerve may have an effect on HR. Not all nerves will have an equally strong or immediate effect, but, if the stimulus is sufficiently strong, this corollary will hold true. Importantly, visceral afferents from thoracic or abdominal organs can elicit large HR-effects, mainly by their ability to modulate outgoing vagal activity to the heart. This occurs at a fairly basal level, just above the spinal cord, in the medulla oblongata (Guyenet, 2006). There, the nucleus of the solitary tract (NTS, nucleus tractus solitarii) is receiving these inputs. Not all afferents, be they sensory (e.g., touch, pressure, and pain), proprioceptive (e.g., muscle and tendon) or visceral (e.g., stretch of the intestinal wall) evoke heart rate changes to the same extent, or even in the same direction, up or down. One way how the incoming afferent information may evoke these heart rate responses is by blocking the transfer from baroreceptor inputs to cardiac vagal efferent output. This has been shown in very elegant experiments by Iriuchijima (Iriuchijima and Kumada, 1964) who stimulated baroreceptor afferent nerves and concurrently afferents from muscles or skin, while looking at the reflexly induced cardiac vagal activity. In human subjects it also appeared to be the best explanation for the peripheral part of the "muscle-heart" reflex, where a single bout of muscular exercise can induce an immediate heart rate increase. The same effect was provoked by electrical stimulation of the nerve to have the (arm-) muscle contract, but not when the vagus nerves had been blocked by atropine (Hollander and Bouman, 1975).

Afferent information from the region of the head, incoming *via* the cranial nerves, is not special in this respect: this, too, may give rise to heart rate effects when activated, for this a descending pathway down the brain stem to the medullary level exists. Some specific afferent activity may even trigger early evolutionary mechanisms, in particular the diving response can be mentioned here in response to cold wetting of the face (Folkow et al., 1971). On the other hand, input from the "special senses" (taste, smell, sound, and sight) is so much intertwined with emotional cues from earlier ingrained memory trails that one cannot predict the autonomic effects. The same holds true for the C-fiber afferents that are specifically sensitive to stroking or caressing between subjects. For instance, depending on who seems to be doing the caressing to an infant, the heart rate will decrease more if this is done by the daily caregiver [although, unseen by the infant, the caressing was always done, in the experiment, by the same "stranger" (Aguirre et al., 2019)].

## CENTRAL MECHANISMS: WAKE-SLEEP AND ALERTNESS

Alertness and wake-sleep state are maintained by a dispersed system of nuclei extending from the lower medulla oblongata all the way to the upper part of the midbrain. These nuclei are interconnected like a network, hence its name: the reticular formation, i.e., the Network, also known as the ascending reticular activating system or ARAS. Its activity is a requirement for consciousness and to keep the higher centers of the brain awake [for a critical review of the concept see Berlucchi and Marzi (2019)]. The relation between ARAS and HRV is unexplored territory; for functional imaging the number of active neurons is relatively low and the medulla/brainstem system is difficult to lift out of the noise.

In view of the difficulties of investigating the brainstem and related structures, only few researchers in brain fMRI have made the central autonomic system their field of interest. Still, in the last one and a half decade or so good progress has been made, while tracing non-invasively in awake, healthy humans the path of sympathetic and parasympathetic control of the circulation. In the first place a group around Macefield and Henderson in Australia is to be mentioned who, in a clever experimental design, tracked the origin of simultaneously recorded peripheral sympathetic nerve activity to the level of the hypothalamus and to higher loci in the cortex (Macefield et al., 2013). A Canadian group around K. J. Shoemaker focused more on the cortical and directly subcortical centers involved in blood pressure and heart rate control (Cechetto and Shoemaker, 2009;

Ruiz Vargas et al., 2016). Finally, a group in Germany, combining researchers from various institutes must be mentioned (Gerlach et al., 2019; Manuel et al., 2020). In their last study they provoked hypotensive responses by enclosing the lower body of the test subjects in a box and applying suction to induce a situation that may be compared to hemorrhage (or prolonged standing). In an earlier study they looked at the path of baroreceptor information at blood pressure increases by injection of phenylephrine. From the combined analyses of these groups we may start to construct a model of the brain stem nuclei involved in control of the cardiovascular system, managing vagal, sympathetic and humoral outputs like the antidiuretic hormone. This, effectively confirms in awake humans the wealth of earlier animal studies relating brain stem nuclei to cardiovascular control. Moreover, some "higher" centers also join into this framework, making the link to what has become known as the Default Mode Network, i.e., the ongoing cortical activity, ranging around when the mind is wandering in a resting, but awake condition.

## FINAL COMMON AUTOMATIC PATHWAY: THE VAGUS NERVE AND SYMPATHETICS

The healthy nervous system is never silent. Even while meditating, trying to stop the thoughts from running around and "emptying the mind," techniques like EEG and fMRI will show that whatever the self may perceive as emptiness, nonetheless the central nervous system is active and not just in basic life support for respiration and blood pressure. Thereby, radiating out from this activity of the default mode network, vagal and sympathetic nervous outflow may still be modulated, leading to heart rate variability. The basic level of activity of either system is set by the demands of the moment (rest or activity), dictated by higher centers enforcing a perception of the present condition: flight or fight, stress or relaxation or something in between. In this personal review I will not go into the origins and specifics of the "polyvagal theory" (Porges, 2011) but see Grossman and Taylor (2007). One may take a practical view about this: if it works for psychotherapy to help patients understand and manage their innermost feelings, then the scientific merits of the theory are irrelevant.

## THE HEART AS RECIPIENT OF INCOMING OF INCOMING AUTONOMIC INFORMATION

Final output from the CNS to the heart comes from the nucleus ambiguous in the medulla oblongata as far as the vagal activity is concerned and from the sympathetic command neurons in the rostroventrolateral medulla. The axons of the latter descend to the sympathetic motor neurons in the intermediolateral columns at the lowest cervical and upper thoracic segments of the spinal cord. However, the simple pictures from the textbooks as to how the final, peripheral postganglionic fibers reach their target require an update. Close to the heart the autonomic fibers form intricate plexuses, together nicknamed the heart's "little brain" (Armour, 2008), like the elaborate autonomic nervous system that regulates all of intestinal function from within as reviewed in Karemaker (2017).

Nervous activity in this interwoven network of sympathetic and parasympathetic fibers will, finally, lead to heart rate variability, or rather to liberation of autonomic transmitters at the sinus node. Its effect is not exactly predetermined by the type (adrenergic or cholinergic) and amount, but dependent on the moment of arrival in the cycle of the sinus node and on the previous history of autonomic stimuli. In an earlier study (Karemaker, 2015). I showed that the time of arrival of a burst of vagal activity, although beat-by-beat exactly identical, can be such, that the ensuing heart rate is fairly irregular. On the other hand, if the same bursts were applied earlier or later, heart rate would be very stable. If the vagal bursts were diminished by one stimulus only, near the unstable region one would see much more effect on the ongoing heart period than in the stable regions.

## DISCUSSION: WHAT DOES HEART RATE VARIABILITY TELL?

Apart from heart rate variations due to some form of cardiac disease, which have been left out of the equation altogether in this review, one should maybe better ask "what does HRV not tell?" In the literature a wide range of conditions, healthy and diseased, has been explored, where HRV in some way would be different, at the group level, from a reference group. As I tried to demonstrate in the above, so many different circuits are involved in the stream of information reaching the motor neurons in the medulla and finally the heart, that it is next to impossible to retrace at any given moment what may have caused this particular output. Even quasi simple effects, like respiratory sinus arrhythmia, may turn out very differently in the same subject under slightly different circumstances.

Therefore, as hinted in the beginning, tracking a person's HRV over time may better tell a story where pathology is developing. We have demonstrated something along those lines when we looked at HRV in pregnant women who developed pre-eclampsia (Rang et al., 2004). In yet another study we have shown that those who go on to develop full blown rheumatoid arthritis have higher resting heart rates and lower parasympathetic activity expression levels on circulating monocytes than a control group (Koopman et al., 2016). This conclusion may seem at variance with fMRI imaging studies where definite relations between centrally induced states of stress or relaxation were found at loci that subsequently were labeled sympathetic or parasympathetic with changes in HRV-patterns (Guyenet, 2006; Thayer et al., 2012; Beissner et al., 2013; Churchill et al., 2016). However, that does not make the reverse true: the relation between some (changed) HRV and a specific central condition is ambiguous. The various physiological processes involved between a primary emotional condition and what, finally, results in HRV, or autonomic nervous outflow for that matter, are too numerous. They may all leave their fingerprints, making the

effect at the target organ uninterpretable. There are good reasons to mistrust the outcome of a lie detector test, as a combination of autonomic nervous responses to an emotional challenge. Therefore, this paper is intended to stress the importance of serial measurement in a subject if conclusions are to be based on autonomic measures like HRV. The measurements from an implanted biochip as mentioned in the beginning will make sense when they are followed over longer time periods, while measuring more than just one parameter. The day-to-day variability will be canceled out and deep learning algorithms may extract recurring features important for healthcare. The sheer amount of data that will be produced this way, obviously necessitates automatic data collection and artificial intelligence techniques to make sense of this. The present use of smartwatches is leading the way in this direction by applications like out of hospital detection of atrial fibrillation attacks or the cardiovascular status of chronic heart failure patients (Wang and Zhou, 2019; Wasserlauf et al., 2019). However, the diary entrances in the beginning of this paper may serve as warning signs, underlining the need for a discussion on "digital healthcare" that is gradually and silently taking control of our lives (Lupton, 2015).

## AUTHOR CONTRIBUTIONS

The author is the sole creator of this manuscript. All views expressed here are entirely his own responsibility.

## REFERENCES


Aguirre, M., Couderc, A., Epinat-Duclos, J., and Mascaro, O. (2019). Infants discriminate the source of social touch at stroking speeds eliciting maximal firing rates in CT-fibers. *Dev. Cognit. Neurosci.* 36:100639. doi: 10.1016/j.dcn.2019.100639

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C., and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213, 220–222. doi: 10.1126/science.6166045

Armour, J. A. (2008). Potential clinical relevance of the 'little brain' on the mammalian heart. *Exp. Physiol.* 93, 165–176. doi: 10.1113/expphysiol.2007.041178

Beissner, F., Meissner, K., Bär, K.-J., and Napadow, V. (2013). The Autonomic Brain: An Activation Likelihood Estimation Meta-Analysis for Central Processing of Autonomic Function. *J. Neurosci.* 33:10503. doi: 10.1523/jneurosci.1103-13.2013

Berlucchi, G., and Marzi, C. A. (2019). Neuropsychology of Consciousness: Some History and a Few New Trends. *Front. Psychol.* 10:50. doi: 10.3389/fpsyg.2019.00050

Bigger, J. T., Fleiss, J. L., Rolnitzky, L. M., and Steinman, R. C. (1993). The ability of several short-term measures of RR variability to predict mortality after myocardial infarction. *Circulation* 88, 927–934. doi: 10.1161/01.cir.88.3.927

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., et al. (1996). Heart Rate Variability - Standards of Measurement, Physiological Interpretation, and Clinical Use. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Cechetto, D. F., and Shoemaker, J. K. (2009). Functional neuroanatomy of autonomic regulation. *NeuroImage* 47, 795–803. doi: 10.1016/j.neuroimage.2009.05.024

Churchill, N. W., Spring, R., Grady, C., Cimprich, B., Askren, M. K., Reuter-Lorenz, P. A., et al. (2016). The suppression of scale-free fMRI brain dynamics across three different sources of effort: aging, task novelty and task difficulty. *Sci. Rep.* 6:30895. doi: 10.1038/srep30895

Costa, M., Goldberger, A. L., and Peng, C. K. (2002). Multiscale entropy analysis of complex physiologic time series. *Physical Rev. Lett.* 89:068102. doi: 10.1103/physrevlett.89.068102

Costa, M., Goldberger, A. L., and Peng, C. K. (2005). Multiscale entropy analysis of biological signals. *Physical Rev. E* 71:021906. doi: 10.1103/physreve.71.021906

DeBoer, R. W., Karemaker, J. M., and Strackee, J. (1987). Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. *Am. J. Physiol.* 253, H680–H689. doi: 10.1152/ajpheart.1987.253.3.H680

di Renzo, G. C., Montani, M., Fioriti, V., Clerici, G., Branconi, F., Pardini, A., et al. (1996). Fractal analysis: a new method for evaluating fetal heart rate variability. *J.Perinat.Med.* 24, 261–269. doi: 10.1515/jpme.1996.24.3.261

di Rienzo, M., Castiglioni, P., Parati, G., Mancia, G., and Pedotti, A. (1996). Baroreflex modulation of the cardiovascular system: new insights from the joint analysis of blood pressure and heart rate signals. *Technol. Health Care* 4, 121–128. doi: 10.3233/thc-1996-4113

Eckberg, D. L., and Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Fairchild, K. D., Schelonka, R. L., Kaufman, D. A., Carlo, W. A., Kattwinkel, J., Porcelli, P. J., et al. (2013). Septicemia mortality reduction in neonates in a heart rate characteristics monitoring trial. *Pediatr. Res.* 74, 570–575. doi: 10.1038/pr.2013.136

Folkow, B., Lisander, B., and ÖBerg, B. (1971). Aspects of the Cardiovascular Nervous Control in a Mammalian Diver (Myocastor Coypus). *Acta Physiol. Scandinavica* 82, 439–446. doi: 10.1111/j.1748-1716.1971.tb04987.x

Frederiks, J., Swenne, C. A., TenVoorde, B. J., Honzíkovaá, N., Levert, J. V., Maan, A. C., et al. (2000). The importance of high-frequency paced breathing in spectral baroreflex sensitivity assessment. *J. Hyperten.* 18, 1635–1644. doi: 10.1097/00004872-200018110-00015

Gerlach, D. A., Manuel, J., Hoff, A., Kronsbein, H., Hoffmann, F., Heusser, K., et al. (2019). Novel Approach to Elucidate Human Baroreflex Regulation at the Brainstem Level: Pharmacological Testing During fMRI. *Front. Neurosci.* 13:193. doi: 10.3389/fnins.2019.00193

Goldberger, A. L. (1990). Nonlinear dynamics, fractals and chaos: applications to cardiac electrophysiology. *Ann. Biomed. Eng.* 18, 195–198. doi: 10.1007/bf02368429

Goldberger, A. L. (1996). Non-linear dynamics for clinicians: chaos theory, fractals, and complexity at the bedside. *Lancet* 347, 1312–1314. doi: 10.1016/s0140-6736(96)90948-4

Goldberger, A. L. (1997). Fractal variability versus pathologic periodicity - complexity loss and stereotypy in disease. *Perspect. Biol. Med.* 40, 543–561. doi: 10.1353/pbm.1997.0063

Grossman, P., and Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsycho.2005.11.014

Grossman, P., Karemaker, J. M., and Wieling, W. (1991). Prediction of tonic parasympathetic cardiac control using respiratory sinus arrhythmia: the need for respiratory control. *Psychophysiology* 28, 201–216. doi: 10.1111/j.1469-8986.1991.tb00412.x

Guyenet, P. G. (2006). The sympathetic control of blood pressure. *Nat. Rev. Neurosci.* 7, 335–346. doi: 10.1038/nrn1902

Ho, Y. L., Lin, C., Lin, Y. H., and Lo, M. T. (2011). The Prognostic Value of Non-Linear Analysis of Heart Rate Variability in Patients with Congestive Heart FailureGÇöA Pilot Study of Multiscale Entropy. *PLoS One* 6:e18699. doi: 10.1371/journal.pone.0018699

Hollander, A. P., and Bouman, L. N. (1975). Cardiac acceleration in man elicited by a muscle-heart reflex. *J. Appl. Physiol.* 38, 272–278. doi: 10.1152/jappl.1975.38.2.272

Imholz, B. P. M., Wieling, W., van Montfrans, G. A., and Wesseling, K. H. (1998). Fifteen years experience with finger arterial pressure monitoring. *Cardiovascul. Res.* 38, 605–616. doi: 10.1016/s0008-6363(98)00067-4

Iriuchijima, J., and Kumada, M. (1964). Activity of single vagal fibers efferent to the heart. *Jap. J. Physiol.* 14, 479–487. doi: 10.2170/jjphysiol.14.479

Karemaker, J. M. (2015). How the vagus nerve produces beat-to-beat heart rate variability; experiments in rabbits to mimic in vivo vagal patterns. *J. Clin. Transl. Res.* 1, 190–204.

Karemaker, J. M. (2017). An introduction into autonomic nervous function. *Physiol. Measurement* 38, R89–R118. doi: 10.1088/1361-6579/aa6782

Karemaker, J. M., and Wesseling, K. H. (2008). Variability in cardiovascular control: the baroreflex reconsidered. *Cardiovascul. Engine.* 8, 23–29. doi: 10.1007/s10558-007-9046-4

Kleiger, R. E., Miller, J. P., Bigger, J. T. Jr., and Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 59, 256–262. doi: 10.1016/0002-9149(87)90795-8

Koopman, F. A., Tang, M. W., Vermeij, J., de Hair, M. J. I, Choi, Y., Vervoordeldonk, M. J., et al. (2016). Autonomic Dysfunction Precedes Development of Rheumatoid Arthritis: A Prospective Cohort Study. *EBioMedicine* 6, 231–237. doi: 10.1016/j.ebiom.2016.02.029

Lupton, D. (2015). Health promotion in the digital era: a critical commentary. *Health Promot. Int.* 30, 174–183. doi: 10.1093/heapro/dau091

Macefield, V. G., James, C., and Henderson, L. A. (2013). Identification of sites of sympathetic outflow at rest and during emotional arousal: Concurrent recordings of sympathetic nerve activity and fMRI of the brain. *Int. J. Psychophysiol.* 89, 451–459. doi: 10.1016/j.ijpsycho.2013.06.002

Malliani, A., Pagani, M., Lombardi, F., and Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84, 482–492. doi: 10.1161/01.cir.84.2.482

Manuel, J., Färber, N., Gerlach, D. A., Heusser, K., Jordan, J., Tank, J., et al. (2020). Deciphering the neural signature of human cardiovascular regulation. *Elife* 9:e55316. doi: 10.7554/eLife.55316

Moharram, M., Wilson, L., Williams, M., and Coffey, S. (2020). Beat-to-beat blood pressure measurement using a cuffless device does not accurately reflect invasive blood pressure. *Int. J. Cardiol. Hyperten.* 5:100030. doi: 10.1016/j.ijchy.2020.100030

Molhoek, G. P., Wesseling, K. H., Settels, J. J. M., Van Vollenhoven, E., Weeda, H. W. H., De Wit, B., et al. (1984). Evaluation of the Penaz servo-plethysmo-manometer for the continuous, non-invasive measurement of finger blood pressure. *Basic Res. Cardiol.* 79, 598–609. doi: 10.1007/bf01910489

Otsuka, K., Cornelissen, G., and Halberg, F. (1997). Age, gender and fractal scaling in heart rate variability. *Clin.Sci.* 93, 299–308. doi: 10.1042/cs0930299

Penaz, J. (1973). Photoelectric measurement of blood pressure, volume and flow in the finger, Dresden, in *10th International Conference on Medical and Biological Engineering -1973- Dresden*, (Dresden: ICMBE).

Pincus, S. M. (1991). Approximate entropy as a measure of system complexity. *Proc. Natl. Acad. Sci.* 88, 2297–2301. doi: 10.1073/pnas.88.6.2297

Pincus, S. M., and Goldberger, A. L. (1994). Physiological time-series analysis: what does regularity quantify?. *Am. J. Physiol.* 266, H1643–H1656. doi: 10.1152/ajpheart.1994.266.4.H1643

Porges, S. W. (2011). *The polyvagal theory: neurophysiological foundations of emotions, attachment, communication, and self-regulation (Norton Series on Interpersonal Neurobiology)*. New York, NY: WW Norton & Company.

Rang, S., Wolf, H., Montfrans, G. A., and Karemaker, J. (2004). Serial assessment of cardiovascular control shows early signs of developing pre-eclampsia. *J. Hyperten.* 22, 369–376. doi: 10.1097/00004872-200402000-00022

Richman, J. S., and Moorman, J. R. (2000). Physiological time-series analysis using approximate entropy and sample entropy. *Am. J. Physiol. Heart Circulatory Physiol.* 278, H2039–H2049.

Robbe, H. W., Mulder, L. J., Ruddel, H., Langewitz, W. A., Veldman, J. B., and Mulder, G. (1987). Assessment of baroreceptor reflex sensitivity by means of spectral analysis. *Hypertension* 10, 538–543. doi: 10.1161/01.hyp.10.5.538

Ruiz Vargas, E., Sörös, P., Shoemaker, J. K., and Hachinski, V. (2016). Human cerebral circuitry related to cardiac control: A neuroimaging meta-analysis. *Ann. Neurol.* 79, 709–716. doi: 10.1002/ana.24642

Sayers, B. M. (1971). The analysis of cardiac interbeat interval sequences and the effects of mental work load. *Proc. R. Soc. Med.* 64, 707–710. doi: 10.1177/003591577106400702

Sharma, M., Barbosa, K., Ho, V., Griggs, D., Ghirmai, T., Krishnan, S. K., et al. (2017). Cuff-less and continuous blood pressure monitoring: a methodological review. *Technologies* 5:21. doi: 10.3390/technologies5020021

Smyth, H. S., Sleight, P., and Pickering, G. W. (1969). Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ. Res.* 24, 109–121. doi: 10.1161/01.res.24.1.109

Tan, C. O., Cohen, M. A., Eckberg, D. L., and Taylor, J. A. (2009). Fractal properties of human heart period variability: physiological and methodological implications. *J. Physiol.* 587(Pt 15), 3929–3941. doi: 10.1113/jphysiol.2009.169219

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Wang, L., and Zhou, X. (2019). Detection of congestive heart failure based on LSTM-based deep network via short-term RR intervals. *Sensors* 19:1502. doi: 10.3390/s19071502

Wasserlauf, J., You, C., Patel, R., Valys, A., Albert, D., and Passman, R. (2019). Smartwatch performance for the detection and quantification of atrial fibrillation. *Circulat. Arrhythmia Electrophysiol.* 12:e006834. doi: 10.1161/circep.118.006834

Wessel, N., Gapelyuk, A., Kraemer, J. F., Berg, K., and Kurths, J. (2020). Spontaneous baroreflex sensitivity: sequence method at rest does not quantify causal interactions but rather determines the heart rate to blood pressure variability ratio. *Physiol. Meas.* 41:03LT01. doi: 10.1088/1361-6579/ab7edc

Wesseling, K. H., Karemaker, J. M., Castiglioni, P., Toader, E., Cividjian, A., Settels, J. J., et al. (2017). Validity and variability of xBRS: instantaneous cardiac baroreflex sensitivity. *Physiol. Rep.* 5:e13509. doi: 10.14814/phy2.13509

Wieling, W., Karemaker, J. M., Borst, C., and Dunning, A. J. (1985). Testing for autonomic neuropathy: heart rate response to forced breathing. *Clin. Physiol.* 5(Suppl. 5), 28–33.

Zhang, Y., de Peuter, O. R., Kamphuisen, P. W., and Karemaker, J. M. (2013). Search for HRV-parameters that detect a sympathetic shift in heart failure patients on β-blocker treatment. *Front. Physiol.* 4:81. doi: 10.3389/fphys.2013.00081

**Conflict of Interest:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 08 January 2021
doi: 10.3389/fphys.2020.614765

# The Impact of Functional Overreaching on Post-exercise Parasympathetic Reactivation in Runners

*Clint R. Bellenger[1,2]*, Rebecca L. Thomson[1], Kade Davison[1], Eileen Y. Robertson[2] and Jonathan D. Buckley[1]*

*[1] Alliance for Research in Exercise, Nutrition and Activity (ARENA), Allied Health and Human Performance Unit, University of South Australia, Adelaide, SA, Australia, [2] South Australian Sports Institute, Adelaide, SA, Australia*

While post-exercise heart rate (HR) variability (HRV) has been shown to increase in response to training leading to improvements in performance, the effect of training leading to decrements in performance (i.e., overreaching) on this parameter has been largely ignored. This study evaluated the effect of heavy training leading to performance decrements on sub-maximal post-exercise HRV. Running performance [5 km treadmill time-trial (5TTT)], post-exercise HRV [root-mean-square difference of successive normal R-R intervals (RMSSD)] and measures of subjective training tolerance (Daily Analysis of Life Demands for Athletes "worse than normal" scores) were assessed in 11 male runners following 1 week of light training (LT), 2 weeks of heavy training (HT) and a 10 day taper (T). Post-exercise RMSSD was assessed following 5 min of running exercise at an individualised speed eliciting 85% of peak HR. Time to complete 5TTT likely increased following HT (*ES* = 0.14 ± 0.03; $p < 0.001$), and then almost certainly decreased following T (*ES* = −0.30 ± 0.07; $p < 0.001$). Subjective training tolerance worsened after HT (*ES* = −2.54 ± 0.62; $p = 0.001$) and improved after T (*ES* = 2.16 ± 0.64; $p = 0.004$). In comparison to LT, post-exercise RMSSD likely increased at HT (*ES* = 0.65 ± 0.55; $p = 0.06$), and likely decreased at T (*ES* = −0.69 ± 0.45; $p = 0.02$). A moderate within-subject correlation was found between 5TTT and post-exercise RMSSD ($r = 0.47 \pm 0.36$; $p = 0.03$). Increased post-exercise RMSSD following HT demonstrated heightened post-exercise parasympathetic modulation in functionally overreached athletes. Heightened post-exercise RMSSD in this context appears paradoxical given this parameter also increases in response to improvements in performance. Thus, additional measures such as subjective training tolerance are required to interpret changes in post-exercise RMSSD.

**Keywords: heart rate, heart rate variability, athletic performance, autonomic nervous system, overload training**

OPEN ACCESS

***Edited by:***
*Mathias Baumert, University of Adelaide, Australia*

***Reviewed by:***
*Julien Louis, Liverpool John Moores University, United Kingdom*
*Chloe E. Taylor, Western Sydney University, Australia*

****Correspondence:***
*Clint R. Bellenger*
*clint.bellenger@unisa.edu.au*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

***Received:*** *07 October 2020*
***Accepted:*** *03 December 2020*
***Published:*** *08 January 2021*

***Citation:***
*Bellenger CR, Thomson RL, Davison K, Robertson EY and Buckley JD (2021) The Impact of Functional Overreaching on Post-exercise Parasympathetic Reactivation in Runners. Front. Physiol. 11:614765. doi: 10.3389/fphys.2020.614765*

## INTRODUCTION

The ability of coaches and sport science practitioners to readily and accurately predict athletic training status would assist in optimising training, since this information could be used to adjust training loads (Buchheit, 2014). Of particular importance in well-trained athletes, who often experience periods of high training stress, is the ability to detect the early onset of training-induced

fatigue (i.e., functional overreaching) (Meeusen et al., 2013). Accurate identification of functional overreaching may ensure that training load is reduced to facilitate recovery and supercompensatory performance improvement, before the accumulation of training-induced fatigue gives rise to the more severe conditions of non-functional overreaching and overtraining, which can lead to extended periods (i.e., weeks to months) of attenuated performance (Meeusen et al., 2013).

Numerous psychological and physiological markers of training status have been evaluated over the years, however, their validity and/or practicality has yet to be fully established (Borresen and Lambert, 2009; Meeusen et al., 2013). Psychological measures such as the Profile of Mood Status questionnaire (McNair et al., 1971) and the Daily Analysis of Life Demands for Athletes questionnaire (Rushall, 1990) have been investigated extensively, however, the subjective nature of these assessments means that they are susceptible to manipulation for competition or training gain (Urhausen and Kindermann, 2002), are influenced by age and cognitive development (Groslambert and Mahon, 2006) and may be unreliable as a result of their reliance on memory recall (Shephard, 2003). Similarly, physiological parameters, including sub-maximal blood lactate concentrations, hormones and neuromotor control of movement have been shown to be altered by heavy overload training (Jacobs, 1986; Lehmann et al., 1992; Fuller et al., 2017; Greenham et al., 2018; Bellenger et al., 2019), however, their practicality and/or validity remains to be established as a result of either variable results, invasive assessment techniques or need for specialised/expensive equipment (Urhausen and Kindermann, 2002; Borresen and Lambert, 2009; Greenham et al., 2018).

The assessment of autonomic nervous system function has become a popular tool for predicting athletic training status (Buchheit, 2014). This is because the ANS interacts with many physiological systems (Aubert et al., 2003), and the ANS's responsiveness to changes in training load may indicate the ability to adapt to an exercise stimulus (Borresen and Lambert, 2008). Specifically, research has focussed on predicting training status through autonomic heart rate (HR) regulation as it provides a simple, non-invasive measure of ANS function (Bosquet et al., 2008). Common measures of autonomic HR regulation include resting HR, submaximal HR, maximum HR, resting and post-exercise HR variability (HRV), HR Recovery (HRR) and HR acceleration (Borresen and Lambert, 2008; Buchheit, 2014; Bellenger et al., 2016a).

With regard specifically to post-exercise HRV, a review of the literature on autonomic HR regulation and athletic training showed that while a number of studies had investigated the effect of training leading to positive adaptations on post-exercise HRV (Bellenger et al., 2016a), only one study (Dupuy et al., 2013) had investigated the effect of overreaching training leading to negative adaptations on post-exercise HRV. This lack of research on the potential for post-exercise HRV to indicate negative training adaptation is surprising given that studies facilitating positive training adaptations showed increases in post-exercise parasympathetic modulation (Buchheit et al., 2008, 2010, 2011, 2012a,b), indicating the sensitivity of post-exercise HRV for detecting changes in training status in this context. Furthermore, the one study that has evaluated post-exercise HRV responses to overreaching training (Dupuy et al., 2013), did so after maximal exercise, which negates its practical application in athletes as it may be contraindicated to have an athlete exercise at maximal intensities if they are at risk of developing non-functional overreaching or overtraining, since this will only exacerbate the condition (Bellenger et al., 2016a). Additionally, given the aim of continuous monitoring of HR parameters is to predict training status (for which the gold standard assessment is maximal exercise performance), the assessment of any HR parameter during or following maximal performance is essentially redundant in practice since a measure of performance (i.e., time to complete a set distance, time to exhaustion, maximal aerobic power or speed) will also be measured (Bellenger et al., 2016a).

Consequently, the primary aim of this study was to evaluate the effect of heavy overload training leading to performance decrements, reflecting a state of functional overreaching, on sub-maximal post-exercise parasympathetic modulation. Additionally, Bellenger et al. (2016a) highlighted that previous studies assessing post-exercise HRV had done so during the final 3–5 min of a 5 min period of quiet rest (Buchheit et al., 2008, 2010, 2011, 2012a,b; Dupuy et al., 2013), and thus this study sought to examine whether the time-course of post-exercise parasympathetic modulation assessment could be reduced to aid practical application.

# MATERIALS AND METHODS

## Participants

Fifteen male runners or triathletes were recruited from clubs in Adelaide, South Australia. Participants were eligible for inclusion if they displayed no known signs or symptoms of cardiometabolic disease, were currently completing at least 40 km of running per week, self-reported as injury free in the 3 months prior to undertaking the study, and could complete a 5 km treadmill time trial (5TTT) in less than 23 min. The University of South Australia's Human Research Ethics Committee granted study approval and volunteers provided written informed consent prior to participating.

## Experimental Overview

In this study, individual specific running speeds corresponding to 65 and 85% of peak HR were used as the exercise stimulus prior to assessment of post-exercise HRV as a means of normalising the cardiovascular stress between individuals. This was ultimately performed to facilitate the assessment of a separate HR parameter (the maximal rate of heart rate increase, rHRI) at individual specific workloads, and results of this analysis have been published elsewhere (Bellenger et al., 2020). The current analysis therefore represents a secondary analysis of post-exercise HRV data from the study designed to evaluate rHRI that was unable to be reported in the aforementioned publication due to word limit restrictions. As such, the running performance and training-related variables assessed in the current study have also been published elsewhere (Bellenger et al., 2020). These same

performance and training-related variables were required in the present study to demonstrate the effectiveness of the training intervention and to evaluate the sensitivity of post-exercise HRV for tracking training status (via correlation analysis).

Two pre-study familiarisation sessions allowed for quantification of the aforementioned individual specific running speeds. As described in greater detail in Bellenger et al. (2020), the linear relationship between running speed and HR at three submaximal workloads was first determined. Using the peak HR obtained during a 5TTT, running speeds corresponding to 65 and 85% of peak HR were identified. This process was repeated during both familiarisation sessions and the calculated running speeds were averaged. These speeds were then fixed and utilised at each testing visit thereafter, such that running speeds were constant within individuals, but different between individuals, in order to elicit a similar cardiovascular stress across all participants.

Following familiarisation, participants had their post-exercise HRV and 5TTT performance assessed after 1 week of light training (LT; baseline), 2 weeks of heavy training (HT; overreached state) and 10 days of Tapering (T; recovered and adapted state). Assessments occurred the day after completion of each period's final training session. The impact of this training on daily resting HRV and markers of subjective training tolerance was also investigated.

## Post-exercise HRV Assessment and Calculation

To assess post-exercise HRV, participants ran for 10 min at the two speeds designed to elicit 65 and 85% of peak HR (5 min each) on a treadmill. At the cessation of this running task, participants sat upright in a chair for 5 min to allow for collection of post-exercise HR data.

RR interval data from this running task and associated recovery period were transferred to Polar Protrainer 5 software (Polar Electro Oy, Kempele, Finland) where artefacts or ectopic heart beats were removed using the manufacturer's automatic filtering process. Data were then exported to HRV analysis software (Kubios HRV Analysis, version 2.0 beta 1, The Biomedical Signals Analysis Group, University of Kuopio, Finland) where any remaining artefacts or ectopic heart beats were manually removed. The root-mean-square difference of successive normal R-R intervals (RMSSD) has been advocated as the preferred index of HRV for the monitoring of athletic training status (Plews et al., 2013a; Buchheit, 2014; Bellenger et al., 2016a), and the natural logarithm of this index was consequently analysed as a measure of vagal-related HR modulation. Minutes 3–5 of the recovery period (i.e., 3 min of recording; Ln $RMSSD_{min3-5}$) were analysed in accordance with the usual practice of evaluating post-exercise HRV between 3 and 5 min after exercise to allow for stationarity of the heart rate response (Buchheit et al., 2008, 2010, 2011, 2012a,b; Dupuy et al., 2013). In addition, minutes 3–4 (i.e. 2 min of recording; Ln $RMSSD_{min3-4}$) and minute 3 (i.e., 1 min of recording; Ln $RMSSD_{min3}$) were also assessed to determine if post-exercise HRV assessment may be shortened to facilitate greater practical application. Post-exercise RR interval, Ln RMSSD and Ln RMSSD: RR interval were presented as values collected during testing visits at the end of LT, HT and T.

## Running Performance Assessment

Post-exercise HR testing was followed by a 5TTT where the time taken to run 5 km on a motorised treadmill was recorded as the measure of exercise performance. As previously reported (Bellenger et al., 2020), participants chose their preferred starting speed during familiarisation which remained constant across visits. Participants were blinded to running time and speed, but were free to adjust the treadmill speed as desired to complete 5 km in the fastest time possible. Reliability of the 5TTT in a separate group of well-trained runners was determined to be excellent ($CV$ = 1.3%, Fuller et al., 2015).

## Resting Heart Rate Variability Assessment and Calculation

RR intervals during 3 min of quiet rest were recorded every morning at home upon wakening and after emptying the urinary bladder for assessment of resting HRV. A standing posture was adopted for this assessment based on literature demonstrating enhanced sensitivity of this posture over supine measures (Le Meur et al., 2013; Bellenger et al., 2016a,b).

Minutes 2 and 3 of the RR interval data from these morning-waking assessments were analysed in the same means as described previously for post-exercise HRV. Morning-waking RR interval, Ln RMSSD and Ln RMSSD: RR interval were analysed as a rolling 7 day average and presented as values on the final days of LT, HT, and T.

## Training Tolerance Assessment

As previously reported (Bellenger et al., 2020), subjective measures of training tolerance were determined throughout the training intervention via a Daily Analysis of Life Demands for Athletes (DALDA) questionnaire, which has been shown to detect perturbations in various parameters (e.g., diet, social/work life, sleep, fatigue, muscle soreness, etc.) resulting from periods of overload training in athletes (Halson et al., 2002; Bellenger et al., 2016b). The DALDA was scored on a three-point scale (worse than normal, normal, better than normal).

## Training Intervention

Peak HR determined during familiarisation was used to prescribe training intensities. LT required 7 days of running exercise for 30 min per day at 65–75% of peak HR, such that it would allow participants to be rested and recovered from any pre-study training before starting HT. During HT, participants were required to complete 14 days of running exercise for 66 min per day, with 36% of the training performed above 88% of peak HR, and was intended to induce substantial fatigue from which participants would not recover by the day after the final training session. Specific details of the HT interval program have been provided previously (Bellenger et al., 2016b). Following HT, participants completed 10 days of tapering, with rest on days 1 and 9. Seven of the eight training sessions during T required 30 min per

day at 65–75% of peak HR, with one interval session (four repeats of 3 min at 69–81% peak HR followed by 2 min at 88–92% peak HR) conducted on day seven to provide participants with variety in training during this training phase. HR data were recorded at 15 s intervals during training for determination of training load using Training Impulse (TRIMP) (Banister, 1991) (duration in minutes multiplied by% of peak HR).

## Statistical Analysis

Data were analysed using PASW Statistics 18.0 (SPSS, Chicago, IL, United States) and presented as mean ± SD, and effect sizes (ES) ± 90% confidence intervals. Data were log transformed before analysis to reduce bias from non-uniformity of error (Hopkins et al., 2009). Outcome measures were compared using repeated measures analysis of variance with Bonferroni *post hoc* comparison and statistical significance set at $p < 0.05$. Data were also analysed using magnitude-based inferences (Hopkins et al., 2009), with changes in variables after each training period analysed using a modified statistical spreadsheet (Hopkins, 2006), which calculated ES between time-points of interest using pooled standard deviation (Cohen, 2010). Threshold values for ES statistics were ≤ 0.2 (trivial), >0.2 (small), >0.6 (moderate), >1.2 (large), >2.0 (very large), and >4.0 (extremely large) (Hopkins et al., 2009). Probabilities to establish whether the true (unknown) differences were lower, similar, or higher than the smallest worthwhile change were also calculated. Chances of higher or lower differences were evaluated qualitatively as: <1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely; 25–75%, possibly; 75–95%, likely; 95–99%, very likely; and >99%, almost certain. If the chance of higher and lower differences was >5%, the true difference was assessed as unclear. Within-subject correlations between HR and performance variables across testing time-points were evaluated using univariate analysis of covariance (Bland and Altman, 1995), with r values evaluated as: 0.0–0.1, trivial; 0.1–0.3, small; 0.3–0.5, moderate; 0.5–0.7, large; 0.7–0.9, very large; 0.9–1.0, nearly perfect (Hopkins et al., 2009). Absolute agreement between Ln $RMSSD_{min3}$, Ln $RMSSD_{min3-4}$ and Ln $RMSSD_{min3-5}$ was determined through limits of agreement analysis (Bland and Altman, 2010), while relative agreement was determined using the intra-class correlation (ICC).

# RESULTS

As previously reported (Bellenger et al., 2020), 14 of the 15 recruited participants completed the study (age 35.8 ± 10.0 years; height 1.78 ± 0.09 m; body mass 77.3 ± 10.0 kg). The participant who did not complete was unable to tolerate the demands of HT and withdrew during this phase of the study. Three of the 14 completed participants were diagnosed as acutely fatigued, but not overreached, as they did not experience a decline in 5TTT performance that was greater than the natural variability in this test [i.e., CV = 1.3% (Fuller et al., 2015)] (Le Meur et al., 2014), and were excluded from further analysis so as not to attenuate analysis of the true effect of HT on variables of interest in those participants experiencing functional overreaching as recommended by Bellenger et al. (2016a). A sub-group analysis was not performed on these three participants given the small sample size. Thus, data for 11 participants were included for analysis (age 37.5 ± 8.2 years; body mass 78.5 ± 10.3 kg; self-reported weekly running distance 46.2 ± 16.8 km in the previous 6 months).

## Training Impulse, 5 km Treadmill Time-Trial and Peak Heart Rate

**Table 1** shows the average daily TRIMP, 5TTT performance and peak HR throughout the training intervention. As previously reported (Bellenger et al., 2020), daily TRIMP almost certainly increased (ES ± 90% confidence interval = 3.85 ± 0.77; $p < 0.001$) at HT, and was accompanied by a very likely increase in time taken to complete 5TTT (0.14 ± 0.03; $p < 0.001$) and an almost certain decrease in peak HR (−0.75 ± 0.24; $p = 0.001$). In comparison to HT, daily TRIMP almost certainly decreased at T (−5.78 ± 0.71; $p < 0.001$), while 5TTT almost certainly decreased (−0.30 ± 0.07; $p < 0.001$) and peak HR almost certainly increased (0.72 ± 0.22; $p < 0.001$).

## Training Tolerance

As previously reported (Bellenger et al., 2020), the number of "worse than normal" scores on the DALDA was 1.3 ± 1.0 at LT. DALDA "worse than normal" scores were almost certainly increased at HT (2.54 ± 0.62; $p = 0.001$), and then almost certainly decreased at T (−2.16 ± 0.64; $p = 0.004$; **Table 1**).

## Resting Heart Rate Variability

Standing values of RR interval, Ln RMSSD and Ln RMSSD: RR interval were 859 ± 94 ms, 3.40 ± 0.29 ms and 3.99 ± 0.47 units, respectively, at LT. Ln RMSSD likely increased at HT in comparison to LT (0.53 ± 0.47; $p = 0.04$), while changes at other time-points were assessed as possible or very likely trivial (≤0.31 ± 0.31; $p \geq 0.21$; **Figure 1A**). Changes in RR interval and Ln RMSSD: RR interval throughout the intervention were statistically significant (≤0.57 ± 0.22; $p \leq 0.006$), but were assessed as likely trivial to almost certainly trivial when contextualised by their respective SWCs.

**TABLE 1 |** Effect of training intervention on outcomes of interest.

| | LT | HT | T |
|---|---|---|---|
| **Daily TRIMP (AU)** | 2740 (301) | 5182 (890)* | 2028 (407)*# |
| **5TTT (min:s)** | 19 : 35 (2 : 21) | 19 : 57 (2 : 21)* | 19 : 09 (2 : 13)*# |
| **Peak HR (bpm)** | 184 (11) | 177 (9)* | 184 (8)# |
| **DALDA (AU)** | 1.3 (1.0) | 7.3 (3.4)* | 2.2 (2.1)# |

*Values are mean (standard deviation). AU, arbitrary units; bpm, beats per minute; DALDA; number of "worse than normal" scores on the Daily Analysis of Life Demands for Athletes questionnaire; HR, heart rate; HT, heavy training; LT, light training; min:s, minutes:seconds; TRIMP, training impulse; T, taper training; 5TTT, 5 km treadmill time-trial. *, significantly different to LT ($p < 0.05$); #, significantly different to HT ($p < 0.05$).*

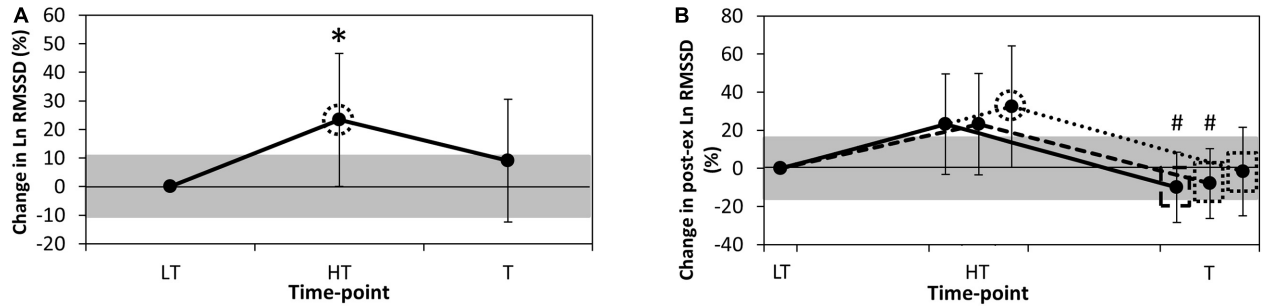



**FIGURE 1 |** Percentage change in **(A).** Resting Ln RMSSD and **(B).** Post-exercise Ln RMSSD from LT. Data are mean ± 90% confidence level. HT, heavy training; Ln RMSSD, natural logarithm of the root-mean-square difference of successive normal R-R intervals; LT, light training; T, taper training. Grey shaded areas represent the smallest worthwhile change for each variable. Continuous line **(B)**, analysis of post-exercise recording minutes 3–5; dashed line **(B)**, analysis of post-exercise recording minutes 3–4; dotted line **(B)**, analysis of post-exercise recording minute 3; dotted circle, likely chance of practically meaningful difference in value from LT; dotted rectangle, likely chance of practically meaningful difference in value from HT; dashed rectangle, very likely chance of practically meaningful difference in value from HT; *, significantly different to LT ($p < 0.05$); #, significantly different to HT ($p < 0.05$).

## Steady-State Heart Rate During Exercise

The mean running speed during assessment of post-exercise HR parameters was 9.2 ± 1.5 km/h (range 8.0–12.0 km/h) for the first stage, and 14.0 ± 2.2 km/h (range 11.5–18.5 km/h) for the second. At LT, steady-state HR was 84.51 ± 1.88% of peak HR, which almost certainly decreased at HT in comparison to LT (−1.43 ± 0.53; $p = 0.002$), and very likely remained decreased at T in comparison to LT (−0.95 ± 0.43; $p = 0.007$).

## Post-exercise Heart Rate Variability

Post-exercise Ln $RMSSD_{min3}$, Ln $RMSSD_{min3-4}$ and Ln $RMSSD_{min3-5}$ were 3.21 ± 0.36, 3.24 ± 0.35, and 3.22 ± 0.33 ms, respectively, at LT. Post-exercise Ln $RMSSD_{min3}$ likely increased at HT in comparison to LT (0.65 ± 0.55; $p = 0.06$), while Ln $RMSSD_{min3-4}$ and Ln $RMSSD_{min3-5}$ were possibly increased at this time-point (0.48 ± 0.47; $p = 0.11$ and 0.47 ± 0.46; $p = 0.10$, respectively). At T, all durations of post-exercise Ln RMSSD likely to very likely decreased in comparison to HT (−0.69 ± 0.45; $p = 0.02$, −0.64 ± 0.32; $p = 0.01$, and −0.71 ± 0.33; $p = 0.003$, respectively, **Figure 1B**). Differences in the response to training between post-exercise Ln $RMSSD_{min3}$, Ln $RMSSD_{min3-4}$ and Ln $RMSSD_{min3-5}$ were unclear (≤0.22 ± 0.72; $p \geq 0.24$).

Changes in all durations of post-exercise RR interval and Ln RMSSD: RR interval throughout the intervention were statistically significant (≤0.85 ± 0.33; $p \leq 0.006$), but were assessed as likely trivial to almost certainly trivial when contextualised by their respective SWCs.

## Agreement Between Measures of Post-exercise Heart Rate Variability

Differences between post-exercise Ln $RMSSD_{min3-5}$, Ln $RMSSD_{min3-4}$ and Ln $RMSSD_{min3}$ were likely trivial to almost certainly trivial (≤0.18 ± 0.22; $p \geq 0.22$; **Table 2**) across the testing timepoints. Limits of agreement analysis indicated that the precision of the difference between Ln $RMSSD_{min3}$ and each of Ln $RMSSD_{min3-5}$ and Ln $RMSSD_{min3-4}$ was greater than the coefficient of variation for this parameter (i.e., 15.7%, Al Haddad et al., 2011), such that a practically meaningful difference between these recording durations may be evident. ICCs were very large to almost perfect across LT, HT and T ($r \geq 0.87$).

## Correlations

Within-subject analysis (using LT, HT, and T) revealed a large inverse correlation between 5TTT performance and 5TTT peak HR ($r = -0.67$; $p < 0.001$). Moderate positive correlations were found between 5TTT and post-exercise Ln RMSSD at minute 3 ($r = 0.40$; $p = 0.06$), minutes 3–4 ($r = 0.47$; $p = 0.03$) and minutes 3–5 ($r = 0.49$; $p = 0.02$). Moderate to large positive correlations were also found between resting Ln RMSSD and post-exercise Ln RMSSD at minute 3 ($r = 0.45$; $p = 0.03$), minutes 3–4 ($r = 0.47$; $p = 0.02$) and minutes 3–5 ($r = 0.55$; $p = 0.01$).

# DISCUSSION

The primary finding in this study was heightened post-exercise parasympathetic modulation in functionally overreached athletes. Additionally, this heightened parasympathetic modulation was able to be detected over a period of time shorter than that previously assessed, which may aid the practical application of post-exercise HRV assessment for monitoring athletes.

The performance impairment induced by heavy overload training in the present study was subsequently followed by supercompensatory performance improvements after a period of taper. These changes in running performance were first accompanied by small to moderate increases in post-exercise parasympathetic modulation as assessed by Ln RMSSD, followed by moderate reductions in this parameter. Resultantly, the present study observed moderate positive within-subject correlations between running performance and post-exercise parasympathetic modulation, indicating that the heightened post-exercise parasympathetic modulation seen in the fatigued state explained at least some of the attenuated running performance at this timepoint. The finding of increased post-exercise parasympathetic modulation in a fatigued state may be considered paradoxical given that this parameter has also

**TABLE 2 |** Agreement between post-exercise heart rate variability assessments.

| Comparison | Variable | LT | HT | T |
|---|---|---|---|---|
| **Post-exercise Ln RMSSD$_{min3-5}$ vs. Ln RMSSD$_{min3-4}$ (ms)** | ICC | 0.99 | 0.99 | 0.99 |
| | Absolute bias (min3–4 − min3–5) | 0.02 | 0.02 | 0.04 |
| | % bias (min3–4 − min3–5) | 2.07 | 2.02 | 4.46 |
| | Absolute LOA | ±0.13 | ±0.19 | ±0.11 |
| | % LOA | ±13.08 | ±19.87 | ±11.65 |
| **Post-exercise Ln RMSSD$_{min3-4}$ vs. Ln RMSSD$_{min3}$ (ms)** | ICC | 0.91 | 0.95 | 0.94 |
| | Absolute bias (min3 − min3–4) | −0.03 | 0.05 | 0.04 |
| | % bias (min3 − min3–4) | −2.73 | 4.62 | 3.91 |
| | Absolute LOA | ±0.33 | ±0.33 | ±0.32 |
| | % LOA | ±35.66 | ±35.67 | ±35.27 |
| **Post-exercise Ln RMSSD$_{min3-5}$ vs. Ln RMSSD$_{min3}$ (ms)** | ICC | 0.87 | 0.91 | 0.91 |
| | Absolute bias (min3 − min3–5) | −0.01 | 0.07 | 0.08 |
| | % bias (min3 − min3–5)% | −0.71 | 6.74 | 8.55 |
| | Absolute LOA | ±0.38 | ±0.42 | ±0.37 |
| | % LOA | ±42.41 | ±46.71 | ±41.05 |

*HT, end of heavy training testing visit; ICC, intra class correlation; Ln RMSSD, natural logarithm of the root-mean-square difference of successive normal R-R intervals; LOA, limits of agreement; LT, end of light training testing visit; $_{min3-5}$, heart rate variability measurement performed on data collected during the third and fifth minutes of a 5 min recovery period; $_{min3-4}$, heart rate variability measurement performed on data collected during the third and fourth minutes of a 5 min recovery period; $_{min3}$, heart rate variability measurement performed on data collected during the third minute of a 5 min recovery period; ms, milliseconds; T, end of tapering testing visit.*

been shown to increase in athletes experiencing improvements in performance (Bellenger et al., 2016a). In the context of improved performance, increased post-exercise parasympathetic modulation was considered a positive adaptation to training since it indirectly indicated an enhanced ability to return to homeostasis following exercise. However, in the context of fatigue leading to attenuated performance, increased post-exercise parasympathetic modulation may be a consequence of the heightened *resting* parasympathetic modulation also shown in the present study and in a number of other recent studies (Le Meur et al., 2013; Bellenger et al., 2016b, 2017), ultimately indicating an overall parasympathetic "hyperactivity" in the autonomic regulation of HR by the ANS. While the exact mechanism by which the increased resting parasympathetic modulation occurs under fatigue is not fully known at present, it is hypothesised to limit the engagement of the sympathetic nervous system during exercise, which is supported by the reduction in peak HR observed in the present study and other overreaching studies (Achten and Jeukendrup, 2003; Bosquet et al., 2008; Le Meur et al., 2013, 2014; Bellenger et al., 2016a,b, 2017), likely attenuating cardiac output at maximal intensities and thereby decreasing exercise performance capacity (Le Meur et al., 2013).

Given that the post-HT testing visit occurred within 24 h of the final HT session, it may be speculated that the increase in post-exercise Ln RMSSD is an acute response to exercise, rather than a chronic response to HT. Greater resolution in the post-exercise Ln RMSSD data would be required to confirm or refute this speculation, however, the results of Bellenger et al. (2016b) suggest that increases in *resting* Ln RMSSD are the result of a cumulative, chronic impact of heavy overload training. Specifically, Bellenger et al. (2016b) used a rolling 7 day average of morning-waking Ln RMSSD assessments to show trivial (as denoted by ES) changes in resting Ln RMSSD on days 1–7 of HT, small changes on days 8–10 and finally moderate changes on days 11–14. Importantly, a similar cumulative pattern of change occurred in resting Ln RMSSD in the present study (data not reported), indicating that HT induced a chronic increase in resting Ln RMSSD. Since resting Ln RMSSD was correlated with post-exercise Ln RMSSD in the present study, it may be concluded that HT also induced a chronic increase in post-exercise Ln RMSSD.

Together, the paradoxical increases in resting and post-exercise parasympathetic modulation under conditions of fatigue further highlight the need for additional measures, such as the quantification of training load and an athlete's subjective tolerance of that training load, to contextualise these increases and aid appropriate interpretation. This study adds further support to the utilisation of the DALDA questionnaire in this context (Bellenger et al., 2016b, 2017).

The present study also informs on the methodology of post-exercise parasympathetic modulation assessment. Specifically, the assessment of this parameter during the third minute of post-exercise recovery elicited a similar response to assessments at minutes 3–4 and 3–5. While precision of bias (i.e., limit of agreement analysis) between the three analysis durations indicated that Ln RMSSD$_{min3}$ may give a practically different value to each of Ln RMSSD$_{min3-5}$ and Ln RMSSD$_{min3-4}$, the very large to nearly perfect ICCs indicated that the between-participant ordering of these values was consistent for each recording duration, such that the response to training was similar. Consequently, assessment of parasympathetic modulation in the third minute following exercise may be utilised to detect changes in training status, which may subtly reduce the time burden associated with using post-exercise HR kinetics for monitoring athletic training.

As a result of the training intervention being performed by the participants outside of the laboratory, post-exercise HRV assessments were conducted only at the scheduled laboratory visits following each phase of training. Given the natural variation in day-to-day measures of post-exercise RMSSD (%$CV$ = 15.7%, Al Haddad et al., 2011), training-induced changes in this parameter may be attenuated by a diminished signal-to-noise ratio. Consequently, it may be hypothesised that taking more than one measure per week, and calculating a weekly average of these measures could improve the signal-to-noise ratio, thereby increasing the sensitivity of post-exercise RMSSD assessment for detecting meaningful changes in training status. Utilising weekly averages in this context has previously been demonstrated in measures of resting HRV (Le Meur et al., 2013; Plews et al., 2013b; Bellenger et al., 2016b), where Plews et al. (2014) also showed that at least three measures per week were required. Thus, three or more assessments of post-exercise RMSSD in response to a standardised workload, perhaps performed as a warm-up prior to main training sets, may improve the sensitivity, and therefore the applicability, of post-exercise HRV for the practical monitoring of athletes in the field.

## CONCLUSION

The results of this study demonstrate heightened post-exercise parasympathetic modulation in functionally overreached athletes. However, this heightening appears paradoxical given the results of earlier research, and therefore emphasises the need for additional measures, such as subjective training tolerance, to allow for effective monitoring of athletic training. The present study also demonstrated that post-exercise parasympathetic modulation may be determined over a period of time shorter than that previously assessed, potentially enhancing the practical application of this HR parameter for athletic monitoring.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## ETHICS STATEMENT

This study involving human participants was reviewed and approved by the University of South Australia Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

CB conceived, designed the research project, conducted experiments, analysed data, and wrote the manuscript. CB, RT, KD, ER, and JB interpreted the data, drafted, and approved the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

This study was supported by Polar Electro Oy and the South Australian Sports Institute. Polar Electro Oy also provided the HR monitors and accelerometers for the study, and the South Australian Sports Institute provided facilities for data collection.

## ACKNOWLEDGMENTS

We thank Samuel Tebeck for his assistance in data extraction and entry.

## REFERENCES

Achten, J., and Jeukendrup, A. E. (2003). Heart rate monitoring: applications and limitations. *Sports Med.* 33, 517–538. doi: 10.2165/00007256-200333070-00004

Al Haddad, H., Laursen, P., Chollet, D., Ahmaidi, S., and Buchheit, M. (2011). Reliability of resting and postexercise heart rate measures. *Int. J. Sports Med.* 32, 598–605. doi: 10.1055/s-0031-1275356

Aubert, A. E., Seps, B., and Beckers, F. (2003). Heart rate variability in athletes. *Sports Med.* 33, 889–919. doi: 10.2165/00007256-200333120-00003

Banister, E. W. (1991). "Modeling elite athletic performance," in *Physiological Testing of the High Performance Athlete*, eds J. D. Macdougall, H. A. Wenger, and H. J. Green (Windsor, ON: Human Kinetics), 403–424.

Bellenger, C. R., Arnold, J. B., Buckley, J. D., Thewlis, D., and Fuller, J. T. (2019). Detrended fluctuation analysis detects altered coordination of running gait in athletes following a heavy period of training. *J. Sci. Med. Sport* 22, 294–299. doi: 10.1016/j.jsams.2018.09.002

Bellenger, C. R., Fuller, J. T., Thomson, R. L., Davison, K., Robertson, E. Y., and Buckley, J. D. (2016a). Monitoring athletic training status through autonomic heart rate regulation: a systematic review and meta-analysis. *Sports Med.* 46, 1461–1486. doi: 10.1007/s40279-016-0484-2

Bellenger, C. R., Karavirta, L., Thomson, R. L., Robertson, E. Y., Davison, K., and Buckley, J. (2016b). Contextualising parasympathetic hyperactivity in functionally overreached athletes with perceptions of training tolerance. *Int. J. Sports Physiol. Perform.* 11, 685–692. doi: 10.1123/ijspp.2015-0495

Bellenger, C. R., Thomson, R. L., Robertson, E. Y., Davison, K., Nelson, M. J., Karavirta, L., et al. (2017). The effect of functional overreaching on parameters of autonomic heart rate regulation. *Eur. J. Appl. Physiol.* 117, 541–550. doi: 10.1007/s00421-017-3549-5

Bellenger, C. R., Thomson, R. L., Robertson, E. Y., Davison, K., Nelson, M. J., Karavirta, L., et al. (2020). Heart rate acceleration at relative workloads during treadmill and overground running for tracking exercise performance during functional overreaching. *Sci. Rep.* 10, 1–9.

Bland, J. M., and Altman, D. G. (1995). Calculating correlation coefficients with repeated observations: part 1-correlation within subjects. *BMJ* 310:446. doi: 10.1136/bmj.310.6977.446

Bland, J. M., and Altman, D. G. (2010). Statistical methods for assessing agreement between two methods of clinical measurement. *Int. J. Nurs. Stud.* 47, 931–936. doi: 10.1016/j.ijnurstu.2009.10.001

Borresen, J., and Lambert, M. I. (2008). Autonomic control of heart rate during and after exercise: measurements and implications for monitoring training status. *Sports Med.* 38, 633–646. doi: 10.2165/00007256-200838080-00002

Borresen, J., and Lambert, M. I. (2009). The quantification of training load, the training response and the effect on performance. *Sports Med.* 39, 779–795. doi: 10.2165/11317780-000000000-00000

Bosquet, L., Merkari, S., Arvisais, D., and Aubert, A. E. (2008). Is heart rate a convenient tool to monitor overreaching? A systematic review of the literature. *Br. J. Sports Med.* 42, 709–714. doi: 10.1136/bjsm.2007.042200

Buchheit, M. (2014). Monitoring training status with heart rate measures: Do all roads lead to Rome? *Front. Physiol.* 5:73. doi: 10.3389/fphys.2014.00073

Buchheit, M., Chivot, A., Parouty, J., Mercier, D., Al Haddad, H., Laursen, P., et al. (2010). Monitoring endurance running performance using cardiac parasympathetic function. *Eur. J. Appl. Physiol.* 108, 1153–1167. doi: 10.1007/s00421-009-1317-x

Buchheit, M., Millet, G. P., Parisy, A., Pourchez, S., Laursen, P. B., and Ahmaidi, S. (2008). Supramaximal training and postexercise parasympathetic reactivation in adolescents. *Med. Sci. Sports Exerc.* 40, 362–371. doi: 10.1249/mss.0b013e31815aa2ee

Buchheit, M., Racinais, S., Bilsborough, J. C., Bourdon, P. C., Voss, S. C., Hocking, J., et al. (2012a). Monitoring fitness, fatigue and running performance during a pre-season training camp in elite football players. *J. Sci. Med. Sport.* 16, 550–555. doi: 10.1016/j.jsams.2012.12.003

Buchheit, M., Simpson, M., Al Haddad, H., Bourdon, P., and Mendez-Villanueva, A. (2012b). Monitoring changes in physical performance with heart rate measures in young soccer players. *Eur. J. Appl. Physiol.* 112, 711–723. doi: 10.1007/s00421-011-2014-0

Buchheit, M., Voss, S. C., Nybo, L., Mohr, M., and Racinais, S. (2011). Physiological and performance adaptations to an in-season soccer camp in the heat: associations with heart rate and heart rate variability. *Scand. J. Med. Sci. Sports.* 21, 477–485.

Cohen, J. (2010). *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ: Sage Publications.

Dupuy, O., Bherer, L., Audiffren, M., and Bosquet, L. (2013). Night and postexercise cardiac autonomic control in functional overreaching. *Appl. Physiol. Nutr. Metab.* 38:200. doi: 10.1139/apnm-2012-0203

Fuller, J. T., Bellenger, C. R., Thewlis, D., Arnold, J. B., Thomson, R. L., Tsiros, M. D., et al. (2017). Tracking performance changes with running-stride variability when athletes are functionally overreached. *Int. J. Sports Physiol. Perform.* 12, 357–363. doi: 10.1123/ijspp.2015-0618

Fuller, J. T., Thewlis, D., Tsiros, M. D., Brown, N. A. T., and Buckley, J. D. (2015). The long-term effect of minimalist shoes on running performance and injury: design of a randomised controlled trial. *BMJ Open* 5, 1–9. doi: 10.1007/978-3-319-30808-1_121-1

Greenham, G., Buckley, J. D., Garrett, J., Eston, R., and Norton, K. (2018). Biomarkers of physiological responses to periods of intensified, non-resistance based exercise training in well-trained male athletes: A systematic review and meta-analysis. *Sports Med.* 48, 2517–2548. doi: 10.1007/s40279-018-0969-2

Groslambert, A., and Mahon, A. D. (2006). Perceived exertion: Influence of age and cognitive development. *Sports Med.* 36, 911–928. doi: 10.2165/00007256-200636110-00001

Halson, S. L., Bridge, M. W., Romain, M., Bart, B., Michael, G., Jones, D. A., et al. (2002). Time course of performance changes and fatigue markers during intensified training in trained cyclists. *J. Appl. Physiol.* 93, 947–956. doi: 10.1152/japplphysiol.01164.2001

Hopkins, W. G. (2006). Spreadsheets for analysis of controlled trials, with adjustment for a subject characteristic. *Sportscience* 10, 46–50.

Hopkins, W. G., Marshall, S. W., Batterham, A. M., and Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Med. Sci. Sports Exerc.* 41:3. doi: 10.1249/mss.0b013e31818cb278

Jacobs, I. (1986). Blood lactate: Implications for training and sports performance. *Sports Med.* 3, 10–25. doi: 10.2165/00007256-198603010-00003

Lehmann, M., Gastmann, U., Petersen, G., Bachl, N., Seidel, A., Khalaf, A. N., et al. (1992). Training-overtraining: performance, and hormone levels, after a defined increase in training volume versus intensity in experienced middle and long-distance runners. *Br. J. Sports Med.* 26, 233–242. doi: 10.1136/bjsm.26.4.233

Le Meur, Y., Louis, J., Aubry, A., Guéneron, J., Pichon, A., Schaal, K., et al. (2014). Maximal exercise limitation in functionally overreached triathletes: Role of cardiac adrenergic stimulation. *J. Appl. Physiol.* 117, 214–222. doi: 10.1152/japplphysiol.00191.2014

Le Meur, Y., Pichon, A., Schaal, K., Schmitt, L., Louis, J., Gueneron, J., et al. (2013). Evidence of parasympathetic hyperactivity in functionally overreached athletes. *Med. Sci. Sports Exerc.* 45:2061. doi: 10.1249/mss.0b013e3182980125

McNair, D. M., Lorr, M., and Droppleman, L. F. (1971). *"Profile of Mood State Manual"*. San Diego, CA: Educational and Industrial Testing Service.

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., et al. (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: joint consensus statement of the european college of sport science and the american college of sports medicine. *Med. Sci. Sports Exerc.* 45, 186–205. doi: 10.1249/mss.0b013e318279a10a

Plews, D. J, Laursen, P., Stanley, J., Kilding, A., and Buchheit, M. (2013a). Training adaptation and heart rate variability in elite endurance athletes: Opening the door to effective monitoring. *Sports Med.* 43, 773–781. doi: 10.1007/s40279-013-0071-8

Plews, D. J., Laursen, P. B., Kilding, A. E., and Buchheit, M. (2013b). Evaluating training adaptation with heart-rate measures: a methodological comparison. *Int. J. Sports Physiol. Perform.* 8:688. doi: 10.1123/ijspp.8.6.688

Plews, D. J., Laursen, P. B., Le Meur, Y., Hausswirth, C., Kilding, A. E., and Buchheit, M. (2014). Monitoring training with heart rate-variability: How much compliance is needed for valid assessment? *Int. J. Sports Physiol. Perform.* 9:783. doi: 10.1123/ijspp.2013-0455

Rushall, B. S. (1990). A tool for measuring stress tolerance in elite athletes. *J. Appl. Sport Psychol.* 2, 51–66. doi: 10.1080/10413209008406420

Shephard, R. J. (2003). Limits to the measurement of habitual physical activity by questionnaires. *Br. J. Sports Med.* 37, 197–206. doi: 10.1136/bjsm.37.3.197

Urhausen, A., and Kindermann, W. (2002). Diagnosis of overtraining: What tools do we have? *Sports Med.* 32, 95–102. doi: 10.2165/00007256-200232020-00002

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 11 January 2021
doi: 10.3389/fnins.2020.612445

# Heart Rate Variability Modulates Interoceptive Accuracy

*Alexander Lischke[1]*, Rike Pahnke[2], Anett Mau-Moeller[2] and Matthias Weippert[2]**

*[1] Department of Psychology, University of Greifswald, Greifswald, Germany, [2] Department of Sport Science, University of Rostock, Rostock, Germany*

Our emotional experiences depend on our interoceptive ability to perceive and interpret changes in our autonomous nervous system. An inaccurate perception and interpretation of autonomic changes impairs our ability to understand and regulate our emotional reactions. Impairments in emotion understanding and emotion regulation increase our risk for mental disorders, indicating that interoceptive deficits play an important role in the etiology and pathogenesis of mental disorders. We, thus, need measures to identify those of us whose interoceptive deficits impair their emotion understanding and emotion regulation. Here, we used cardiac measures to investigate how our ability to engage prefrontal and (para-)limbic brain region regions affects our ability to perceive and interpret cardiac changes. We administered a heartbeat detection task to a sample of healthy individuals ($n$ = 113) whose prefrontal-(para-) limbic engagement had been determined on basis of a heart rate variability recording. We found a positive association between heartbeat detection and heart rate variability, implying that individuals with higher heart rate variability were more accurate in heartbeat detection than individuals with lower heart rate variability. These findings suggest that our interoceptive accuracy depends on our prefrontal-(para-)limbic engagement during the perception and interpretation of cardiac changes. Our findings also show that cardiac measures may be useful to investigate the association between interoceptive accuracy and prefrontal-(para-)limbic engagement in a time- and cost-efficient manner.

**Keywords: heartbeat detection, heart rate variability, interoception, attention, vagal tone, emotion**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Elena Makovac,*
*King's College London,*
*United Kingdom*
*Veronica Dusi,*
*University of Pavia, Italy*

****Correspondence:***
*Alexander Lischke*
*alexander.lischke@uni-greifswald.de*
*Matthias Weippert*
*matthias.weippert@uni-rostock.de*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *30 September 2020*
***Accepted:*** *02 December 2020*
***Published:*** *11 January 2021*

***Citation:***
*Lischke A, Pahnke R, Mau-Moeller A and Weippert M (2021) Heart Rate Variability Modulates Interoceptive Accuracy. Front. Neurosci. 14:612445. doi: 10.3389/fnins.2020.612445*

## INTRODUCTION

Interoception refers to our ability to perceive and interpret changes in our autonomous nervous system (Craig, 2002). Cardiac and respiratory activity are examples of autonomic processes that frequently change during emotional events (Kreibig, 2010). The perception and interpretation of these autonomic changes forms the basis of our emotional experiences and helps us to understand and to regulate our emotional reactions (Critchley and Garfinkel, 2017). For instance, those of us who are sensitive to cardiac changes have less difficulties in understanding and responding to emotional events than those of us who are insensitive to cardiac changes (Fustos et al., 2013; Shah et al., 2017; Lischke et al., 2020a,b). Given that emotion understanding and emotion regulation is central for our mental health (Gross and Jazaieri, 2014), it is not surprising that alterations in the perception and interpretation of autonomic changes increase our risk for mental disorders (Paulus and Stein, 2010). For instance, those of us whose cardiac sensitivity is in the abnormal range have

more mental health problems than those of us whose cardiac sensitivity is in the normal range (Pollatos et al., 2007b, 2009; Herbert et al., 2011; Shah et al., 2016). The way we perceive and interpret autonomic changes, thus, appears to be of utmost importance for our mental health.

To understand how we perceive and interpret autonomic changes, we need measures that differentiate between different aspects of interoception (Critchley and Garfinkel, 2017). Fortunately, these measures have already been developed by researchers. Depending on the research question, these measures assess interoceptive accuracy (i.e., objective accounts of interoception), interoceptive sensibility (i.e., subjective accounts of interoception) or interoceptive awareness (i.e., correspondence between objective and subjective accounts of interoception). The most popular measure is the heartbeat detection task (Schandry, 1981), an interceptive accuracy task that requires the tracking of heartbeats within different time intervals. By employing the heartbeat detection task, researchers were able to identify a network of brain regions that is relevant for the perception and interpretation of cardiac changes (Schulz, 2016). This network comprises several brain regions but prefrontal and (para-)limbic brain regions appear to be the most important ones. Prefrontal and (para-)limbic show the most pronounced activity changes during the perception and interpretation of cardiac changes (Critchley et al., 2004; Pollatos et al., 2005; Kuehn et al., 2016), implying a close association between prefrontal-(para-)limbic activity and interoceptive accuracy. It may, thus, be possible that our ability to perceive and interpret cardiac changes depends on our ability to engage prefrontal and (para-)limbic brain regions for this matter.

To test this possibility, we administered measures of interoceptive accuracy and prefrontal-(para-)limbic engagement to a sample of young adults. Interoceptive accuracy was measured with the heartbeat detection task and prefrontal-(para-)limbic engagement was measured with a heart rate recording. The heart rate recording was used for the determination of parasympathically induced heart rate changes, a measure of vagally mediated heart rate variability (Shaffer and Ginsberg, 2017). Parasympathetically induced heart rate changes are closely associated with activity changes in prefrontal and (para-)limbic brain regions (Thayer et al., 2012; Ruiz Vargas et al., 2016), indicating that vagally mediated heart rate variability reflects prefrontal-(para-)limibic engagement (Thayer and Lane, 2009; Smith et al., 2017). The heart rate recording, thus, allowed us to investigate the association between interoceptive accuracy and prefrontal-(para-)limbic engagement in an unobtrusive manner. In light of previous findings showing that activity changes in prefrontal and (para-)limbic brain regions are positively associated with the perception and interpretation of cardiac changes (Critchley et al., 2004; Pollatos et al., 2005; Kuehn et al., 2016), we expected to find a similar association between vagally mediated heart rate variability and heartbeat detection. Preliminary findings suggest that vagally mediated heart rate variability may be positively associated with heartbeat detection (Owens et al., 2018). We, thus, expected to find a positive rather than negative association between vagally mediated heartrate variability and heartbeat detection.

# MATERIALS AND METHODS

## Participants

We based our participant recruitment on an *a priori* power analysis with G*Power 3.1.9.2 (Faul et al., 2009). The power analysis suggested that 82 participants would provide sufficient data to detect meaningful associations between vagally mediated heartrate variability and heartbeat detection in our analyses [correlation analysis (two-tailed): $\alpha = 0.05$, $1\text{-}\beta = 0.80$, $r = 0.30$; regression analyses (total number of predictors: 8, number of tested predictors: 1): $\alpha = 0.05$, $1\text{-}\beta = 0.80$, $f^2 = 0.15$]. Following this suggestion, we recruited 113 participants for the study (see **Table 1**). In order to be included in the study, the participants had to be native speakers and to be aged between 18 and 35 years. Participants who were currently in psychotherapeutic treatment were excluded from the study. Inclusion and exclusion of participants was determined on basis of an in-house questionnaire assessing sociodemographic (age and sex), anthropometric (height and weight) and medical (physical activity in terms of aerobic fitness, smoking status, medication status, treatment status) information (Lischke et al., 2018a). All participants that were included in the study provided written-informed consent to the study protocol. The study protocol, which had been approved by the local ethics committee, was carried out in accordance with the Declaration of Helsinki.

## Procedure

We used a heart rate recording to determine participants' vagally mediated heart rate variability and heartbeat detection. Each recording session was scheduled during the daytime (at least 2 h after wakening time and 5 h before sleeping time) to control for circadian and diurnal variations in participants' heart rate (Yamasaki et al., 1996; Bonnemeier et al., 2003). Before we started with the recording session, we asked the participants to use the bathroom. This allowed us to rule out that bladder filling and gastric digestion had an effect on participants' heart rate (Fagius and Karhuvaara, 1989; Rossi et al., 1998). The participants completed the recording session in a comfortable chair that was located in a dimly lit room. The heart rate recording was performed with a mobile heart rate monitor (RS 800, Polar Electro Oy; Kempele, Finland) that has been shown to record heartbeats as accurate as mobile electrocardiogram systems (Weippert et al., 2010).

## Heartbeat Detection

Participants' heartbeat detection was determined during the first part of the recording session. Following an established procedure (Lischke et al., 2020a,b), we asked the participants to count their heartbeats during 25, 35, and 45 s lasting time intervals. They were not informed about the length of the time intervals and they were not allowed to use any measure that may have facilitated the heartbeat detection. We used the number of counted and recorded heartbeats to compute two

**TABLE 1 |** Participant characteristics.

| | M (SD) | 95% CI |
|---|---|---|
| Sex (f/m, n) | 40/69 | |
| Age | 26.30 (3.91) | [25.55, 27.04] |
| Tobacco use (n) | 28 | |
| Medication use (n) | 10 | |
| Anti-allergic medication | 3 | |
| Endocrine medication | 5 | |
| Psychotropic medication | 2 | |
| Contraceptive use (n) | 22 | |
| Unspecified contraceptives | 19 | |
| Androgenic contraceptives | 1 | |
| Anti-androgenic contraceptives | 2 | |
| Body mass index (kg/m$^2$) | 22.90 (2.83) | [22.40, 23.42] |
| Physical activity (h/week) | 6.19 (3.53) | [5.57, 6.86] |
| Respiratory activity (log pHF, Hz) | −0.71 (0.10) | [−0.72, −0.69] |
| Heart rate (bpm) | 74.70 (12.20) | [72.25, 76.88] |
| Heart rate variability | | |
| RMSSD (ms) | 43.54 (26.19) | [38.51, 48.79] |
| log RMSSD (ms) | 1.57 (0.25) | [1.52, 1.62] |
| pNN50 (%) | 20.72 (17.66) | [17.35, 24.20] |
| log pNN50 (%) | 1.14 (0.48) | [1.04, 1.23] |
| Heartbeat detection | | |
| $HBD_{SC}$ | 0.69 (0.18) | [0.66, 0.72] |
| $HBD_{GA}$ | 0.60 (0.26) | [0.56, 0.65] |

*pHF, peak of high frequency band (Shaffer and Ginsberg, 2017); RMSSD, root mean square of successive differences between consecutive heartbeats (Shaffer and Ginsberg, 2017); pNN50, number of successive heartbeat interval pairs that differ more than 50 ms divided by the total number of all heartbeat intervals (Shaffer and Ginsberg, 2017); $HBD_{SC}$, Heartbeat detection – traditional index (Schandry, 1981); $HBD_{GA}$, Heartbeat detection – alternative index (Garfinkel et al., 2015).*

different indices of participants' heartbeat detection, a traditional heartbeat detection index[1] (Schandry, 1981) and an alternative heartbeat detection index[2] (Hart et al., 2013) that has been shown to be to be less sensitive against outliers than the traditional heartbeat detection index (Garfinkel et al., 2015). We used both heartbeat detection indices in our analyses to test the robustness of our findings.

## Heart Rate Variability

Participants' vagally mediated heart rate variability was determined during the second part of the recording session. Following an established procedure (Lischke et al., 2018b, 2019), we asked the participants to sit still and to stay awake during a 300 s lasting time interval. The heartbeats that were recorded during this time interval were analyzed with Kubios HRV 2.2 (Tarvainen et al., 2014). The analysis followed the guidelines of the Task Force of the European Society of Cardiology (1996): The recordings were detrended (smoothn priors: $\lambda = 500$), visually inspected and artifact corrected (adaptive filtering: cubic spline interpolation) before they were subjected to a time-domain and spectral analysis. The time-domain analysis was used for the determination of a heart rate index (meanHR) and for the determination of two vagally mediated heartrate variability indices (the root mean square of successive differences between consecutive heartbeats, RMSSD; the number of successive heartbeat interval pairs that differ more than 50 ms divided by the total number of all heartbeat intervals, pNN50). The values of these indices were in the range of values that have been reported in comparable samples of participants (Dantas et al., 2018). The spectral analysis was used to determine a respiration index (Thayer et al., 2002), the peak frequency of the high frequency band (pHF). We used the respiration index to adjust the vagally mediated heartrate variability indices for respiration-induced alterations (Weippert et al., 2015). Using both vagally mediated heartrate variability indices in our analyses allowed us to test the robustness of our findings.

[1]
$$1\mathrm{HBD}_{\mathrm{SC}} = \frac{1}{3}\sum\left(1 - \frac{(|n\mathrm{heartbeats}_{\mathrm{real}} - n\mathrm{heartbeats}_{\mathrm{counted}}|)}{n\mathrm{heartbeats}_{\mathrm{real}}}\right)$$

[2]
$$2\mathrm{HBD}_{\mathrm{GF}} = \frac{1}{3}\sum\left(1 - \frac{(|n\mathrm{heartbeats}_{\mathrm{real}} - n\mathrm{heartbeats}_{\mathrm{counted}}|)}{(n\mathrm{heartbeats}_{\mathrm{real}} + n\mathrm{heartbeats}_{\mathrm{counted}})/2}\right)$$

## Statistical Analyses

We performed all analyses with the bootstrapping module of SPSS 27 (SPSS Inc., Chicago, IL, United States). Preliminary analyses revealed that the datasets of four participants were incomplete or invalid due to a recording error. We, thus, used the datasets of the remaining 109 participants for the main analyses. The main analyses comprised regression and correlation analyses. Multiple regressions were run to analyze the association between participants' vagally mediated heart rate variability and heartbeat detection. The vagally mediated heart rate variability indices constituted the predictor variables and the heartbeat detection indices constituted the criterion variables. Participant characteristics that may distort the association between vagally mediated heartrate variability and heartbeat detection were used as additional predictor variables (age, sex, body mass index, physical activity, respiratory activity, smoking status, and medication status). The regression analyses were complemented by correlation analyses. Partial correlations were run to quantify the association between participants' vagally mediated heart rate variability and heartbeat detection. The vagally mediated heart rate variability indices constituted the dependent variable, the heartbeat detection indices the independent variables and the aforementioned participant characteristics the control variables. We set the significance level for all analyses at $\alpha \leq 0.05$ and determined significance values ($p$), effect size measures ($r$, $R^2$, $\Delta R^2$, and $B$) and 95% confidence intervals (CIs).

# RESULTS

## Associations Between the Vagally Mediated Heart Rate Variability Indices (RMSSD, pNN50) and the Traditional Heartbeat Detection Index ($HBD_{SC}$)

In the first set of regression models, the traditional heartbeat detection index constituted the criterion variable. Entering the

**TABLE 2 |** Associations between the vagally mediated heart rate variability indices (RMSSD and pNN50) and the traditional heartbeat detection index ($HBD_{SC}$).

| | Heartbeat detection ($HBD_{SC}$) | | | | | | Heartbeat detection ($HBD_{SC}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Model One** | ***B*** | ***SE B*** | **95% CI** | ***t*** | ***p*** | **Model two** | ***B*** | ***SE B*** | **95% CI** | ***t*** | ***p*** |
| *Step one* | | | | | | *Step one* | | | | | |
| Sex | 0.13 | 0.05 | [0.03, 0.23] | 2.37 | 0.015* | Sex | 0.13 | 0.05 | [0.04, 0.23] | 2.37 | 0.018* |
| Age | 0.00 | 0.01 | [−0.01, 0.01] | −0.09 | 0.943 | Age | 0.00 | 0.01 | [−0.01, 0.01] | −0.09 | 0.915 |
| Tobacco use | −0.03 | 0.04 | [−0.11, 0.06] | −0.64 | 0.552 | Tobacco use | −0.03 | 0.04 | [−0.11, 0.06] | −0.64 | 0.545 |
| Medication use | 0.04 | 0.08 | [−0.10, 0.21] | 0.70 | 0.610 | Medication use | 0.04 | 0.08 | [−0.10, 0.20] | 0.70 | 0.574 |
| Contraceptive use | 0.03 | 0.05 | [−0.08, 0.14] | 0.54 | 0.545 | Contraceptive use | 0.03 | 0.05 | [−0.07, 0.14] | 0.54 | 0.559 |
| Body mass index | −0.01 | 0.01 | [−0.02, 0.01] | −0.97 | 0.342 | Body mass index | −0.01 | 0.01 | [−0.02, 0.01] | −0.97 | 0.312 |
| Physical activity | 0.01 | 0.01 | [0.00, 0.02] | 1.79 | 0.066 | Physical activity | 0.01 | 0.01 | [0.00, 0.02] | 1.79 | 0.068 |
| Respiratory activity (log pHF) | −0.03 | 0.16 | [−0.32, 0.30] | −0.15 | 0.891 | Respiratory activity (log pHF) | −0.03 | 0.16 | [−0.35, 0.30] | −0.15 | 0.870 |
| *Step two* | | | | | | *Step two* | | | | | |
| Sex | 0.13 | 0.05 | [0.03, 0.23] | 2.33 | 0.013** | Sex | 0.12 | 0.05 | [0.03, 0.22] | 2.27 | 0.020* |
| Age | 0.00 | 0.01 | [−0.01, 0.01] | 0.00 | 1.000 | Age | 0.00 | 0.00 | [−0.01, 0.01] | −0.07 | 0.946 |
| Tobacco use | −0.02 | 0.04 | [−0.10, 0.07] | −0.44 | 0.661 | Tobacco use | −0.02 | 0.04 | [−0.1, 0.07] | −0.41 | 0.694 |
| Medication use | 0.04 | 0.08 | [−0.1, 0.20] | 0.66 | 0.610 | Medication use | 0.04 | 0.07 | [−0.08, 0.19] | 0.72 | 0.551 |
| Contraceptive use | 0.05 | 0.05 | [−0.06, 0.15] | 0.81 | 0.374 | Contraceptive use | 0.04 | 0.05 | [−0.06, 0.15] | 0.69 | 0.452 |
| Body mass index | 0.00 | 0.01 | [−0.02, 0.01] | −0.50 | 0.669 | Body mass index | 0.00 | 0.01 | [−0.02, 0.01] | −0.41 | 0.687 |
| Physical activity | 0.01 | 0.00 | [0.00, 0.02] | 1.41 | 0.120 | Physical activity | 0.01 | 0.01 | [0.00, 0.02] | 1.24 | 0.185 |
| Respiratory activity (log pHF) | −0.11 | 0.16 | [−0.40, 0.22] | −0.59 | 0.486 | Respiratory activity (log pHF) | −0.10 | 0.16 | [−0.41, 0.25] | −0.53 | 0.558 |
| Heart rate variability (log RMSSD) | 0.15 | 0.06 | [0.02, 0.28] | 2.11 | 0.024* | Heart rate variability (log pNN50) | 0.09 | 0.04 | [0.02, 0.16] | 2.48 | 0.014** |

*Model one: step one: $R^2 = 0.15$, $F(8,100) = 2.13$, $p = 0.040$*, step two: $\Delta R^2 = 0.04$, $\Delta F(1,99) = 4.46$, $p = 0.037$*; model two: step one: $R^2 = 0.15$, $F(8,100) = 2.13$, $p = 0.040$*, step two: $\Delta R^2 = 0.05$, $\Delta F(1,99) = 6.13$, $p = 0.015$*. $HBD_{SC}$, Heartbeat detection – traditional index (Schandry, 1981); pHF, peak of high frequency band (Shaffer and Ginsberg, 2017); RMSSD, root mean square of successive differences between consecutive heartbeats (Shaffer and Ginsberg, 2017); pNN50, number of successive heartbeat interval pairs that differ more than 50 ms divided by the total number of all heartbeat intervals (Shaffer and Ginsberg, 2017). *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.*

participant characteristics as predictor variables in a first step into the regression models accounted for a significant proportion of the variance in the traditional heartbeat detection index [RMSSD: $R^2$ = 0.15, $F$(8,100) = 2.13, $p$ = 0.040; pNN50: $R^2$ = 0.15, $F$(8,100) = 2.13, $p$ = 0.040, see **Table 2**]. However, sex was the only predictor variable that turned out be significant in the regression models [RMSSD: $B$ = 0.14, $SE\ B$ = 0.05, 95% CI [0.03, 0.23], $t$(100) = 2.37, $p$ = 0.015; pNN50: $B$ = 0.13, $SE\ B$ = 0.05, 95% CI [0.04, 0.23], $t$(100) = 2.37, $p$ = 0.018; see **Table 2**]. Entering the vagally mediated heart rate variability indices as predictor variables in a second step into the regression models also accounted for a significant proportion of the variance in the traditional heartbeat detection index [RMSSD: $\Delta R^2$ = 0.04, $\Delta F$(1,99) = 4.46, $p$ = 0.037; pNN50: $\Delta R^2$ = 0.05, $F$(1,99) = 6.13, $p$ = 0.015, see **Table 2**]. The vagally mediated heart rate variability indices were, besides sex [RMSSD: $B$ = 0.13, $SE\ B$ = 0.05, 95% CI [0.03, 0.23], $t$(99) = 2.33, $p$ = 0.013; pNN50: $B$ = 0.12, $SE\ B$ = 0.05, 95% CI [0.02, 0.22], $t$(99) = 2.27, $p$ = 0.020; see **Table 2**], the only significant predictors in the regression models [RMSSD: $B$ = 0.15, $SE\ B$ = 0.06, 95% CI [0.02, 0.28], $t$(99) = 2.11, $p$ = 0.024; pNN50: $B$ = 0.09, $SE\ B$ = 0.04, 95% CI [0.02, 0.16], $t$(99) = 2.48, $p$ = 0.009; see **Table 2**]. Taken together, the regression models suggested that there was a small to medium sized association between the vagally mediated heart rate variability indices and the traditional heartbeat detection index [RMSSD: $r$(99) = 0.21, 95% CI [0.05, 0.37], $p$ = 0.037; pNN50: $r$(99) = 24, 95% CI [0.05, 0.41], $p$ = 0.015; see **Figure 1**].

## Associations Between the Vagally Mediated Heart Rate Variability Indices (RMSSD, pNN50) and the Alternative Heartbeat Detection Index ($HBD_{GA}$)

In the second set of regression models, the alternative heartbeat detection index constituted the criterion variable. Entering the participant characteristics as predictor variables in a first step into the regression models accounted for a significant proportion of the variance in the alternative heartbeat detection index [RMSSD: $R^2$ = 0.14, $F$(8,100) = 2.12, $p$ = 0.041; pNN50: $R^2$ = 0.14, $F$(8,100) = 2.12, $p$ = 0.041; see **Table 3**]. However, sex was the only predictor variable that turned out be significant in the regression models [RMSSD: $B$ = 0.18, $SE\ B$ = 0.08, 95% CI [0.03, 0.23], $t$(100) = 2.36, $p$ = 0.017; pNN50: $B$ = 0.18, $SE\ B$ = 0.07, 95% CI [0.04, 0.33], $t$(100) = 2.36, $p$ = 0.009; see **Table 3**]. Entering the vagally mediated heart rate variability indices as predictor variables in a second step into the regression models also accounted for a significant proportion of the variance in the alternative heartbeat detection index [RMSSD: $\Delta R^2$ = 0.04, $\Delta F$(1,99) = 4.79, $p$ = 0.031; pNN50: $\Delta R^2$ = 0.05, $F$(1,99) = 6.70, $p$ = 0.011; see **Table 3**]. The vagally mediated heart rate variability indices were, besides sex [RMSSD: $B$ = 0.18, $SE\ B$ = 0.08, 95% CI [0.03, 0.34], $t$(99) = 2.32, $p$ = 0.022; pNN50: $B$ = 0.17, $SE\ B$ = 0.07, 95% CI [0.04, 0.32], $t$(99) = 2.25, $p$ = 0.015; see **Table 3**], the only significant predictors in the regression models [RMSSD: $B$ = 0.23, $SE\ B$ = 0.09, 95% CI [0.04, 0.40], $t$(99) = 2.19, $p$ = 0.020; pNN50: $B$ = 0.13, $SE\ B$ = 0.05, 95% CI [0.03, 0.22], $t$(100) = 2.59, $p$ = 0.007; see **Table 3**]. Taken together, the regression models suggested that there was a small to medium sized association between the vagally mediated heart rate variability indices and the alternative heartbeat detection index [RMSSD: $r$(99) = 0.21, 95% CI [0.05, 0.37], $p$ = 0.031; pNN50: $r$(99) = 0.25, 95% CI [0.06, 0.42], $p$ = 0.011; see **Figure 2**].

# DISCUSSION

To explore the possibility that our interoceptive accuracy depends on our ability to engage prefrontal and (para-)limbic brain regions for this matter (Critchley et al., 2004; Pollatos et al., 2005, 2007a; Kuehn et al., 2016), we administered cardiac measures of interoceptive accuracy and prefontal-(para-)limbic engagement to a sample of young adults. Interoceptive accuracy was measured with a heartbeat detection task and prefrontal-(para-)limbic engagement with a heart rate recording. We used

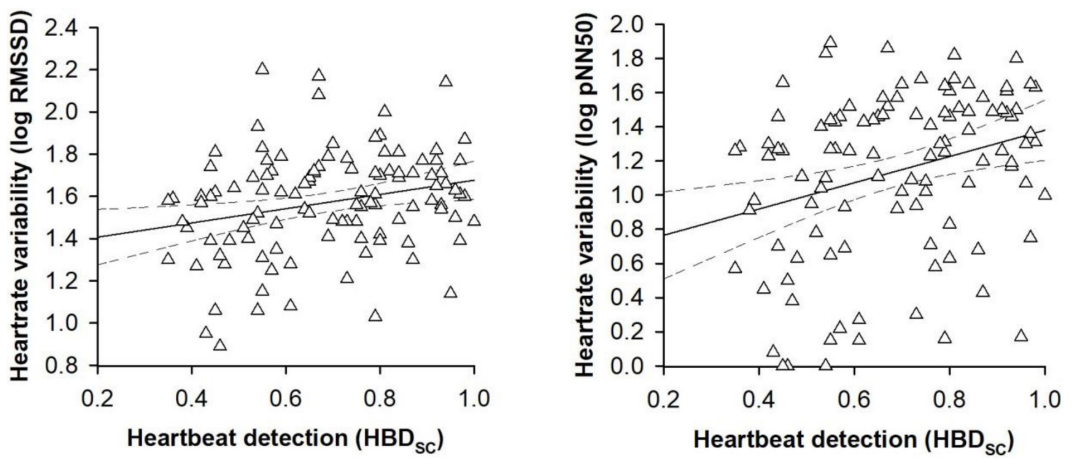



**FIGURE 1 |** Scatterplots with lines of best fit and 95% confidence intervals demonstrating raw associations between the vagally mediated heart rate variability indices (RMSSD and pNN50) and the traditional heartbeat detection index ($HBD_{SC}$).

**TABLE 3 |** Associations between the vagally mediated heart rate variability indices (RMSSD and pNN50) and the alternative heartbeat detection index ($HBD_{GA}$).

| | Heartbeat detection ($HBD_{GA}$) | | | | | | Heartbeat detection ($HBD_{GA}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Model One** | ***B*** | ***SE B*** | **95% CI** | ***t*** | ***p*** | **Model two** | ***B*** | ***SE B*** | **95% CI** | ***t*** | ***p*** |
| *Step one* | | | | | | *Step one* | | | | | |
| Sex | 0.18 | 0.08 | [0.03, 0.34] | 2.36 | 0.017* | Sex | 0.18 | 0.07 | [0.04, 0.33] | 2.36 | 0.009** |
| Age | 0.00 | 0.01 | [−0.02, 0.01] | −0.49 | 0.668 | Age | 0.00 | 0.01 | [−0.02, 0.01] | −0.49 | 0.656 |
| Tobacco use | −0.03 | 0.06 | [−0.14, 0.09] | −0.45 | 0.690 | Tobacco use | −0.03 | 0.06 | [−0.13, 0.09] | −0.45 | 0.696 |
| Medication use | 0.06 | 0.11 | [−0.15, 0.29] | 0.66 | 0.555 | Medication use | 0.06 | 0.11 | [−0.14, 0.28] | 0.66 | 0.583 |
| Contraceptive use | 0.04 | 0.08 | [−0.12, 0.18] | 0.53 | 0.579 | Contraceptive use | 0.04 | 0.08 | [−0.1, 0.20] | 0.53 | 0.565 |
| Body mass index | −0.01 | 0.01 | [−0.03, 0.01] | −1.05 | 0.290 | Body mass index | −0.01 | 0.01 | [−0.03, 0.01] | −1.05 | 0.318 |
| Physical activity | 0.01 | 0.01 | [0.00, 0.03] | 1.86 | 0.063 | Physical activity | 0.01 | 0.01 | [0.00, 0.03] | 1.86 | 0.064 |
| Respiratory activity (log pHF) | 0.02 | 0.23 | [−0.42, 0.49] | 0.07 | 0.930 | Respiratory activity (log pHF) | 0.02 | 0.23 | [−0.42, 0.45] | 0.07 | 0.933 |
| *Step two* | | | | | | *Step two* | | | | | |
| Sex | 0.18 | 0.08 | [0.03, 0.34] | 2.32 | 0.022* | Sex | 0.17 | 0.07 | [0.04, 0.32] | 2.25 | 0.015* |
| Age | 0.00 | 0.01 | [−0.02, 0.01] | −0.40 | 0.726 | Age | 0.00 | 0.01 | [−0.02, 0.01] | −0.47 | 0.688 |
| Tobacco use | −0.01 | 0.06 | [−0.13, 0.10] | −0.23 | 0.825 | Tobacco use | −0.01 | 0.06 | [−0.12, 0.1] | −0.20 | 0.842 |
| Medication use | 0.05 | 0.10 | [−0.15, 0.27] | 0.61 | 0.568 | Medication use | 0.06 | 0.10 | [−0.13, 0.27] | 0.68 | 0.552 |
| Contraceptive use | 0.07 | 0.08 | [−0.09, 0.21] | 0.82 | 0.384 | Contraceptive use | 0.06 | 0.08 | [−0.09, 0.2] | 0.70 | 0.468 |
| Body mass index | −0.01 | 0.01 | [−0.03, 0.01] | −0.56 | 0.553 | Body mass index | 0.00 | 0.01 | [−0.03, 0.02] | −0.47 | 0.665 |
| Physical activity | 0.01 | 0.01 | [0.00, 0.03] | 1.47 | 0.123 | Physical activity | 0.01 | 0.01 | [0.00, 0.02] | 1.29 | 0.162 |
| Respiratory activity (log pHF) | −0.10 | 0.24 | [−0.57, 0.4] | −0.40 | 0.647 | Respiratory activity (log pHF) | −0.08 | 0.23 | [−0.54, 0.35] | −0.32 | 0.734 |
| Heart rate variability (log RMSSD) | 0.23 | 0.09 | [0.04, 0.4] | 2.19 | 0.020* | Heart rate variability (log pNN50) | 0.13 | 0.05 | [0.03, 0.22] | 2.59 | 0.007** |

*Model one: step one: $R^2 = 0.14$, $F(8,100) = 2.12$, $p = 0.031$*, step two: $\Delta R^2 = 0.04$, $\Delta F(1,99) = 4.79$, $p = 0.031$*; model two: step one: $R^2 = 0.14$, $F(8,101) = 2.12$, $p = 0.041$*, step two: $\Delta R^2 = 0.05$, $\Delta F(1,99) = 6.70$, $p = 0.011$*. $HBD_{GA}$, Heartbeat detection – alternative index (Garfinkel et al., 2015); pHF, peak of high frequency band (Shaffer and Ginsberg, 2017); RMSSD, root mean square of successive differences between consecutive heartbeats (Shaffer and Ginsberg, 2017); pNN50, number of successive heartbeat interval pairs that differ more than 50 ms divided by the total number of all heartbeat intervals (Shaffer and Ginsberg, 2017). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

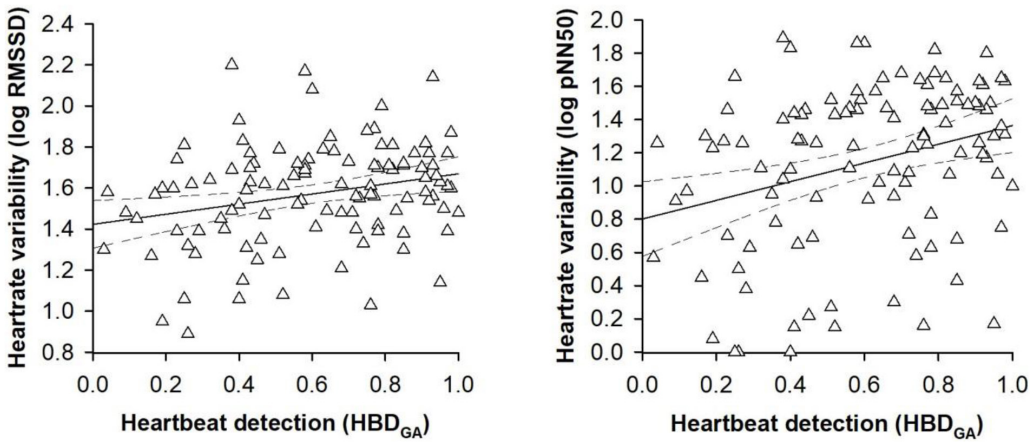



**FIGURE 2 |** Scatterplots with lines of best fit and 95% confidence intervals demonstrating raw associations between the vagally mediated heart rate variability indices (RMSSD and pNN50) and the alternative heartbeat detection index ($HBD_{GA}$).

the heartbeat detection task to determine two different heartbeat detection indices and the heart rate recording to determine two different vagally mediated heart rate variability indices (Shaffer and Ginsberg, 2017). The vagally mediated heart rate indices were both positively associated with the heartbeat detection indices, regardless whether the traditional or alternative heartbeat detection index were considered in the regression and correlation analyses. The regression and correlation analyses were well-powered and well-controlled, ruling out that the association between the vagally mediated heart rate variability index and the heartbeat detection indices was spurious. We, thus, found the expected association between vagally mediated heart rate variability and heartbeat detection. The association between vagally mediated heart rate variability and heartbeat detection supports the idea that our interoceptive accuracy depends on our ability to engage prefrontal and (para-)limbic brain regions for the perception and interpretation of cardiac changes.

To understand the association between vagally mediated heart rate variability and heartbeat detection, we have to delineate the processes that determine the performance on the heartbeat detection task. Heartbeat detection relies on executive control processes (Critchley and Garfinkel, 2017), in particular on those that are related to attention. For an accurate perception and interpretation of cardiac changes, attention has to be shifted from the outside to the inside of the body, to be shielded against sensations from the outside of the body and to be focused on sensations inside the body. Attention shifting, attention shielding and attention focusing are executive control processes that are driven by activity changes in prefrontal brain and (para-)limbic regions (Petersen and Posner, 2012). Activity changes in prefrontal and (para-)limbic brain regions are associated with vagally mediated heart rate variability (Thayer et al., 2012; Ruiz Vargas et al., 2016), indicating that vagally mediated heart rate variability may also be associated with these executive control processes. Vagally mediated heart rate variability is, in fact, associated with executive control processes (Zahn et al., 2016; Holzman and Bridgett, 2017), including attention shifting, attention shielding and attention focusing (Hansen et al., 2003; Williams et al., 2016; Siennicka et al., 2019; Sorensen et al., 2019). The association between vagally mediated heart rate variability and heartbeat detection may, thus, be mediated by executive control processes that are driven by activity changes in prefrontal and (para-)limbic brain regions.

There are several prefrontal and (para-)limbic brain regions that may mediate the association between vagally mediated heart rate variability and heartbeat detection through executive control processes. These brain regions are organized in networks that are implicated in the up- and downregulation of cardiac changes (Thayer and Lane, 2009), the perception and interpretation of cardiac changes (Schulz, 2016) and the execution of externally and internally oriented attention changes (Petersen and Posner, 2012). Some brain regions are part of more than one network. These brain regions provide functional and structural connections between the networks (Bullmore and Sporns, 2012). Most connections are provided by the anterior cingulate cortex and the insula (Medford and Critchley, 2010). These connections make the anterior cingulate cortex and the insula to central network hubs that coordinate the interplay between the networks (Dosenbach et al., 2007; Sridharan et al., 2008). The anterior cingulate cortex and the insula monitor and regulate the activity of all brain regions in the networks (Barrett and Simmons, 2015), which explains why activity changes in the anterior cingulate cortex and insula are closely associated with vagally mediated heart rate variability (Chang et al., 2013; Allen et al., 2015; Jennings et al., 2016), heartbeat detection (Critchley et al., 2004; Pollatos et al., 2005; Kuehn et al., 2016) and attention (Seeley et al., 2007; Eckert et al., 2009; Shulman et al., 2009). Activity changes in the anterior cingulate cortex and the insula may, thus, trigger executive control processes that mediate the association between vagally mediated heart rate variability and heartbeat detection.

To illustrate the importance of the anterior cingulate cortex and the insula for mediating the association between vagally mediated heart rate variability and heartbeat detection through

executive control processes, we only have to take a look at some of the most common mental disorders (Paulus and Stein, 2010). Mood and anxiety disorders are characterized by alterations in vagally mediated heart rate variability (Licht et al., 2008, 2009), heartbeat detection (Pollatos et al., 2009; Dunn et al., 2010) and executive control processes (Mogg et al., 1992, 1995; Lim and Kim, 2005). The alterations in vagally mediated heart rate variability and heartbeat detection are related to alterations in anterior cingulate cortex and insula activity during the perception and interpretation of cardiac changes (Caseras et al., 2013; Avery et al., 2014; Wiebking et al., 2015; Cui et al., 2020; DeVille et al., 2020), presumably via alterations in executive control processes (Mitterschiffthaler et al., 2008; Etkin et al., 2010). The alterations in anterior cingulate cortex and insula activity account for severe alterations in emotion, cognition and behavior (Caseras et al., 2013; Avery et al., 2014; Wiebking et al., 2015; Cui et al., 2020; DeVille et al., 2020), indicating that interoceptive deficits play an important role in the etiology and pathogenesis of mood and anxiety disorders (Paulus and Stein, 2010).

Considering the importance of interoceptive deficits for the etiology and pathogenesis of mood and anxiety disorders (Paulus and Stein, 2010), we need measures that allow us to identify those of us whose interoceptive deficits put them at risk for these disorders. As we have shown, cardiac measures may be useful for this purpose. We combined a cardiac measure of interoceptive accuracy, the heartbeat detection index, with a cardiac measure of prefrontal-(para-)limbic engagement, the vagally mediated heart rate variability index. Combining these measures allowed us to demonstrate that our interoceptive accuracy depends on our prefrontal and (para-)limbic engagement during the perception and interpretation of cardiac changes. It should be noted, however, that we only employed cardiac but not neural measures in our investigation. We, thus, can only assume that vagally mediated heart rate variability reflected prefrontal-(para-)limbic engagement during the perception and interpretation of cardiac changes. Future investigations that supplement cardiac measures with neural measures may help to test this assumption with more rigor (e.g., measuring vagally mediated heart rate variability and heartbeat detection during functional or structural imaging). Future investigations should also employ a more rigorous control of participant characteristics that affect cardiac measures than we did in our investigation (e.g., excluding participants with medication use or caffeine use). We hope that our investigation opens an avenue for these types of investigations because we believe that cardiac measures are a promising tool for researchers in the field of interoception. Cardiac measures can be obtained from unobtrusive heart rate recordings that do not require dedicated staff or equipment. These measures may, thus, be interesting for researchers who need to investigate the association between interoception and prefrontal-(para-)limbic engagement in a time- and cost-efficient manner.

## DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of ethical restrictions. Requests to access the datasets should be directed to AL, alexander.lischke@uni-greifswald.

## ETHICS STATEMENT

This study was reviewed and approved by the Ethics Committee of the University of Rostock. The participants provided their written informed consent to participate in the study.

## AUTHOR CONTRIBUTIONS

AL, RP, and MW designed the study. AM-M and MW collected the data. AL and MW analyzed the data. AL and RP wrote the manuscript. AM-M, MW, and RP contributed to writing, reviewing, and editing of the manuscript. All authors approved the final version of the manuscript.

## FUNDING

Funding for this study was supported by an Open Access Publishing grant that was provided by the German Research Foundation (DFG) and the University of Rostock. AL was supported by a grant provided by the DFG (LI 2517/2-1). The funding source had no further role in the design of the study, in the collection, analysis and interpretation of the data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

## REFERENCES

Allen, B., Jennings, J. R., Gianaros, P. J., Thayer, J. F., and Manuck, S. B. (2015). Resting high-frequency heart rate variability is related to resting brain perfusion. *Psychophysiology* 52, 277–287. doi: 10.1111/psyp.12321

Avery, J. A., Drevets, W. C., Moseman, S. E., Bodurka, J., Barcalow, J. C., and Simmons, W. K. (2014). Major depressive disorder is associated with abnormal interoceptive activity and functional connectivity in the insula. *Biol. Psychiatry* 76, 258–266. doi: 10.1016/j.biopsych.2013.11.027

Barrett, L. F., and Simmons, W. K. (2015). Interoceptive predictions in the brain. *Nat. Rev. Neurosci.* 16, 419–429. doi: 10.1038/nrn3950

Bonnemeier, H., Richardt, G., Potratz, J., Wiegand, U. K., Brandes, A., Kluge, N., et al. (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. *J. Cardiovasc. Electrophysiol.* 14, 791–799. doi: 10.1046/j.1540-8167.2003.03078.x

Bullmore, E., and Sporns, O. (2012). The economy of brain network organization. *Nat. Rev. Neurosci.* 13, 336–349. doi: 10.1038/nrn3214

Caseras, X., Murphy, K., Mataix-Cols, D., Lopez-Sola, M., Soriano-Mas, C., Ortriz, H., et al. (2013). Anatomical and functional overlap within the insula and anterior cingulate cortex during interoception and phobic symptom provocation. *Hum. Brain Mapp.* 34, 1220–1229. doi: 10.1002/hbm.21503

Chang, C., Metzger, C. D., Glover, G. H., Duyn, J. H., Heinze, H. J., and Walter, M. (2013). Association between heart rate variability and fluctuations in resting-state functional connectivity. *Neuroimage* 68, 93–104. doi: 10.1016/j.neuroimage.2012.11.038

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666. doi: 10.1038/nrn894

Critchley, H. D., and Garfinkel, S. N. (2017). Interoception and emotion. *Curr. Opin. Psychol.* 17, 7–14. doi: 10.1016/j.copsyc.2017.04.020

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., and Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7, 189–195. doi: 10.1038/nn1176

Cui, H., Zhang, B., Li, W., Li, H., Pang, J., Hu, Q., et al. (2020). Insula shows abnormal task-evoked and resting-state activity in first-episode drug-naive generalized anxiety disorder. *Depress Anxiety* 37, 632–644. doi: 10.1002/da.23009

Dantas, E. M., Kemp, A. H., Andreao, R. V., da Silva, V. J. D., Brunoni, A. R., Hoshi, R. A., et al. (2018). Reference values for short-term resting-state heart rate variability in healthy adults: Results from the Brazilian Longitudinal Study of Adult Health-ELSA-Brasil study. *Psychophysiology* 55:e13052. doi: 10.1111/psyp.13052

DeVille, D. C., Kuplicki, R., Stewart, J. L., Tulsa, I., Aupperle, R. L., Bodurka, J., et al. (2020). Diminished responses to bodily threat and blunted interoception in suicide attempters. *Elife* 9:51593.

Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., et al. (2007). Distinct brain networks for adaptive and stable task control in humans. *Proc. Natl. Acad. Sci. U S A.* 104, 11073–11078. doi: 10.1073/pnas.0704320104

Dunn, B. D., Stefanovitch, I., Evans, D., Oliver, C., Hawkins, A., and Dalgleish, T. (2010). Can you feel the beat? Interoceptive awareness is an interactive function of anxiety- and depression-specific symptom dimensions. *Behav. Res. Ther.* 48, 1133–1138. doi: 10.1016/j.brat.2010.07.006

Eckert, M. A., Menon, V., Walczak, A., Ahlstrom, J., Denslow, S., Horwitz, A., et al. (2009). At the heart of the ventral attention system: the right anterior insula. *Hum. Brain Mapp.* 30, 2530–2541. doi: 10.1002/hbm.20688

Etkin, A., Prater, K. E., Hoeft, F., Menon, V., and Schatzberg, A. F. (2010). Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *Am. J. Psychiatry* 167, 545–554. doi: 10.1176/appi.ajp.2009.09070931

Fagius, J., and Karhuvaara, S. (1989). Sympathetic activity and blood pressure increases with bladder distension in humans. *Hypertension* 14, 511–517. doi: 10.1161/01.hyp.14.5.511

Faul, F., Erdfelder, E., Buchner, A., and Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav. Res. Methods* 41, 1149–1160. doi: 10.3758/brm.41.4.1149

Fustos, J., Gramann, K., Herbert, B. M., and Pollatos, O. (2013). On the embodiment of emotion regulation: interoceptive awareness facilitates reappraisal. *Soc. Cogn. Affect. Neurosci.* 8, 911–917. doi: 10.1093/scan/nss089

Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., and Critchley, H. D. (2015). Knowing your own heart: distinguishing interoceptive accuracy from interoceptive awareness. *Biol. Psychol.* 104, 65–74. doi: 10.1016/j.biopsycho.2014.11.004

Gross, J. J., and Jazaieri, H. (2014). Emotion, emotion regulation, and psychopathology: An affective science perspective. *Clin. Psychol. Sci.* 2, 387–401. doi: 10.1177/2167702614536164

Hansen, A. L., Johnsen, B. H., and Thayer, J. F. (2003). Vagal influence on working memory and attention. *Int. J. Psychophysiol.* 48, 263–274. doi: 10.1016/s0167-8760(03)00073-4

Hart, N., McGowan, J., Minati, L., and Critchley, H. D. (2013). Emotional regulation and bodily sensation: interoceptive awareness is intact in borderline personality disorder. *J. Pers. Disord.* 27, 506–518. doi: 10.1521/pedi_2012_26_049

Herbert, B. M., Herbert, C., and Pollatos, O. (2011). On the relationship between interoceptive awareness and alexithymia: is interoceptive awareness related to emotional awareness? *J. Pers.* 79, 1149–1175. doi: 10.1111/j.1467-6494.2011.00717.x

Holzman, J. B., and Bridgett, D. J. (2017). Heart rate variability indices as bio-markers of top-down self-regulatory mechanisms: a meta-analytic review. *Neurosci. Biobehav. Rev.* 74(Pt A), 233–255. doi: 10.1016/j.neubiorev.2016.12.032

Jennings, J. R., Sheu, L. K., Kuan, D. C., Manuck, S. B., and Gianaros, P. J. (2016). Resting state connectivity of the medial prefrontal cortex covaries with individual differences in high-frequency heart rate variability. *Psychophysiology* 53, 444–454. doi: 10.1111/psyp.12586

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biol. Psychol.* 84, 394–421. doi: 10.1016/j.biopsycho.2010.03.010

Kuehn, E., Mueller, K., Lohmann, G., and Schuetz-Bosbach, S. (2016). Interoceptive awareness changes the posterior insula functional connectivity profile. *Brain Struct. Funct.* 221, 1555–1571. doi: 10.1007/s00429-015-0989-8

Licht, C. M., de Geus, E. J., van Dyck, R., and Penninx, B. W. (2009). Association between anxiety disorders and heart rate variability in The Netherlands Study of Depression and Anxiety (NESDA). *Psychosom. Med.* 71, 508–518. doi: 10.1097/psy.0b013e3181a292a6

Licht, C. M., de Geus, E. J., Zitman, F. G., Hoogendijk, W. J., van Dyck, R., and Penninx, B. W. (2008). Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). *Arch. Gen. Psychiatry* 65, 1358–1367. doi: 10.1001/archpsyc.65.12.1358

Lim, S. L., and Kim, J. H. (2005). Cognitive processing of emotional information in depression, panic, and somatoform disorder. *J. Abnorm. Psychol.* 114, 50–61. doi: 10.1037/0021-843x.114.1.50

Lischke, A., Jacksteit, R., Mau-Moeller, A., Pahnke, R., Hamm, A. O., and Weippert, M. (2018a). Heart rate variability is associated with psychosocial stress in distinct social domains. *J. Psychosom. Res.* 106, 56–61. doi: 10.1016/j.jpsychores.2018.01.005

Lischke, A., Mau-Moeller, A., Jacksteit, R., Pahnke, R., Hamm, A. O., and Weippert, M. (2018b). Heart rate variability is associated with social value orientation in males but not females. *Sci. Rep.* 8:7336.

Lischke, A., Pahnke, R., Mau-Moeller, A., Jacksteit, R., and Weippert, M. (2020a). Sex-specific relationships between interoceptive accuracy and emotion regulation. *Front. Behav. Neurosci.* 14:67. doi: 10.3389/fnbeh.2020.00067

Lischke, A., Weippert, M., Mau-Moeller, A., Jacksteit, R., and Pahnke, R. (2020b). Interoceptive accuracy is associated with emotional contagion in a valence- and sex-dependent manner. *Soc. Neurosci.* 15, 227–233. doi: 10.1080/17470919.2019.1690573

Lischke, A., Weippert, M., Mau-Moeller, A., Paschke, S., Jacksteit, R., Hamm, A. O., et al. (2019). Sex-specific associations between inter-individual differences in heart rate variability and inter-individual differences in emotion regulation. *Front. Neurosci.* 12:1040. doi: 10.3389/fnins.2018.01040

Medford, N., and Critchley, H. D. (2010). Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. *Brain Struct. Funct.* 214, 535–549. doi: 10.1007/s00429-010-0265-x

Mitterschiffthaler, M. T., Williams, S. C., Walsh, N. D., Cleare, A. J., Donaldson, C., Scott, J., et al. (2008). Neural basis of the emotional Stroop interference effect in major depression. *Psychol. Med.* 38, 247–256. doi: 10.1017/s0033291707001523

Mogg, K., Bradley, B. P., and Williams, R. (1995). Attentional bias in anxiety and depression: the role of awareness. *Br. J. Clin. Psychol.* 34, 17–36. doi: 10.1111/j.2044-8260.1995.tb01434.x

Mogg, K., Mathews, A., and Eysenck, M. (1992). Attentional bias to threat in clinical anxiety states. *Cogn. Emot.* 6, 149–159. doi: 10.1080/02699939208411064

Owens, A. P., Friston, K. J., Low, D. A., Mathias, C. J., and Critchley, H. D. (2018). Investigating the relationship between cardiac interoception and autonomic cardiac control using a predictive coding framework. *Auton. Neurosci.* 210, 65–71. doi: 10.1016/j.autneu.2018.01.001

Paulus, M. P., and Stein, M. B. (2010). Interoception in anxiety and depression. *Brain Struct. Funct.* 214, 451–463. doi: 10.1007/s00429-010-0258-9

Petersen, S. E., and Posner, M. I. (2012). The attention system of the human brain: 20 years after. *Annu. Rev. Neurosci.* 35, 73–89. doi: 10.1146/annurev-neuro-062111-150525

Pollatos, O., Kirsch, W., and Schandry, R. (2005). Brain structures involved in interoceptive awareness and cardioafferent signal processing: a dipole source localization study. *Hum. Brain Mapp.* 26, 54–64. doi: 10.1002/hbm.20121

Pollatos, O., Schandry, R., Auer, D. P., and Kaufmann, C. (2007a). Brain structures mediating cardiovascular arousal and interoceptive awareness. *Brain Res.* 1141, 178–187. doi: 10.1016/j.brainres.2007.01.026

Pollatos, O., Traut-Mattausch, E., and Schandry, R. (2009). Differential effects of anxiety and depression on interoceptive accuracy. *Depress Anxiety* 26, 167–173. doi: 10.1002/da.20504

Pollatos, O., Traut-Mattausch, E., Schroeder, H., and Schandry, R. (2007b). Interoceptive awareness mediates the relationship between anxiety and the

intensity of unpleasant feelings. *J. Anxiety Disord.* 21, 931–943. doi: 10.1016/j.janxdis.2006.12.004

Rossi, P., Andriesse, G. I., Oey, P. L., Wieneke, G. H., Roelofs, J. M., and Akkermans, L. M. (1998). Stomach distension increases efferent muscle sympathetic nerve activity and blood pressure in healthy humans. *J. Neurol. Sci.* 161, 148–155. doi: 10.1016/s0022-510x(98)00276-7

Ruiz Vargas, E., Soros, P., Shoemaker, J. K., and Hachinski, V. (2016). Human cerebral circuitry related to cardiac control: A neuroimaging meta-analysis. *Ann. Neurol.* 79, 709–716. doi: 10.1002/ana.24642

Schandry, R. (1981). Heart beat perception and emotional experience. *Psychophysiology* 18, 483–488. doi: 10.1111/j.1469-8986.1981.tb02486.x

Schulz, S. M. (2016). Neural correlates of heart-focused interoception: a functional magnetic resonance imaging meta-analysis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371:20160018. doi: 10.1098/rstb.2016.0018

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/jneurosci.5587-06.2007

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shah, P., Catmur, C., and Bird, G. (2017). From heart to mind: Linking interoception, emotion, and theory of mind. *Cortex* 93, 220–223. doi: 10.1016/j.cortex.2017.02.010

Shah, P., Hall, R., Catmur, C., and Bird, G. (2016). Alexithymia, not autism, is associated with impaired interoception. *Cortex* 81, 215–220. doi: 10.1016/j.cortex.2016.03.021

Shulman, G. L., Astafiev, S. V., Franke, D., Pope, D. L., Snyder, A. Z., McAvoy, M. P., et al. (2009). Interaction of stimulus-driven reorienting and expectation in ventral and dorsal frontoparietal and basal ganglia-cortical networks. *J. Neurosci.* 29, 4392–4407. doi: 10.1523/jneurosci.5609-08.2009

Siennicka, A., Quintana, D. S., Fedurek, P., Wijata, A., Paleczny, B., Ponikowska, B., et al. (2019). Resting heart rate variability, attention and attention maintenance in young adults. *Int. J. Psychophysiol.* 143, 126–131. doi: 10.1016/j.ijpsycho.2019.06.017

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Sorensen, L., Wass, S., Osnes, B., Schanche, E., Adolfsdottir, S., Svendsen, J. L., et al. (2019). A psychophysiological investigation of the interplay between orienting and executive control during stimulus conflict: A heart rate variability study. *Physiol. Behav.* 211:112657. doi: 10.1016/j.physbeh.2019.112657

Sridharan, D., Levitin, D. J., and Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U S A.* 105, 12569–12574. doi: 10.1073/pnas.0800005105

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220.

Task Force of the European Society of Cardiology (1996). Heart rate variability standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Sollers, J. J. III, Ruiz-Padial, E., and Vila, J. (2002). Estimating respiratory frequency from autoregressive spectral analysis of heart period. *IEEE Eng. Med. Biol. Mag.* 21, 41–45. doi: 10.1109/memb.2002.1032638

Weippert, M., Behrens, K., Rieger, A., Kumar, M., and Behrens, M. (2015). Effects of breathing patterns and light exercise on linear and nonlinear heart rate variability. *Appl. Physiol. Nutr. Metab.* 40, 762–768. doi: 10.1139/apnm-2014-0493

Weippert, M., Kumar, M., Kreuzfeld, S., Arndt, D., Rieger, A., and Stoll, R. (2010). Comparison of three mobile devices for measuring R-R intervals and heart rate variability: Polar S810i. Suunto t6 and an ambulatory ECG system. *Eur. J. Appl. Physiol.* 109, 779–786. doi: 10.1007/s00421-010-1415-9

Wiebking, C., de Greck, M., Duncan, N. W., Tempelmann, C., Bajbouj, M., and Northoff, G. (2015). Interoception in insula subregions as a possible state marker for depression-an exploratory fMRI study investigating healthy, depressed and remitted participants. *Front. Behav. Neurosci.* 9:82. doi: 10.3389/fnbeh.2015.00082

Williams, D. P., Thayer, J. F., and Koenig, J. (2016). Resting cardiac vagal tone predicts intraindividual reaction time variability during an attention task in a sample of young and healthy adults. *Psychophysiology* 53, 1843–1851. doi: 10.1111/psyp.12739

Yamasaki, Y., Kodama, M., Matsuhisa, M., Kishimoto, M., Ozaki, H., Tani, A., et al. (1996). Diurnal heart rate variability in healthy subjects: effects of aging and sex difference. *Am. J. Physiol.* 271(1 Pt 2), H303–H310.

Zahn, D., Adams, J., Krohn, J., Wenzel, M., Mann, C. G., Gomille, L. K., et al. (2016). Heart rate variability and self-control–A meta-analysis. *Biol. Psychol.* 115, 9–26. doi: 10.1016/j.biopsycho.2015.12.007

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 15 January 2021
doi: 10.3389/fphys.2020.596567

# A New Detection Method Defining the Aerobic Threshold for Endurance Exercise and Training Prescription Based on Fractal Correlation Properties of Heart Rate Variability

*Bruce Rogers[1]*, David Giles[2], Nick Draper[3], Olaf Hoos[4] and Thomas Gronwald[5]*

*[1] College of Medicine, University of Central Florida, Orlando, FL, United States, [2] Lattice Training Ltd., Chesterfield, United Kingdom, [3] School of Health Sciences, College of Education, Health and Human Development, University of Canterbury, Christchurch, New Zealand, [4] Center for Sports and Physical Education, Julius Maximilians University of Wuerzburg, Wuerzburg, Germany, [5] Department of Performance, Neuroscience, Therapy and Health, Faculty of Health Sciences, MSH Medical School Hamburg, University of Applied Sciences and Medical University, Hamburg, Germany*

OPEN ACCESS

**Edited by:**
*Clint Bellenger, University of South Australia, Australia*

**Reviewed by:**
*Daniel Boullosa, Federal University of Mato Grosso Do Sul, Brazil*
*Lars Brechtel, MSB Medical School Berlin, Germany*

***Correspondence:**
*Bruce Rogers*
*bjrmd@knights.ucf.edu;*
*brucerogersmd@gmail.com*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

**Received:** *19 August 2020*
**Accepted:** *18 December 2020*
**Published:** *15 January 2021*

**Citation:**
*Rogers B, Giles D, Draper N, Hoos O and Gronwald T (2021) A New Detection Method Defining the Aerobic Threshold for Endurance Exercise and Training Prescription Based on Fractal Correlation Properties of Heart Rate Variability. Front. Physiol. 11:596567. doi: 10.3389/fphys.2020.596567*

The short-term scaling exponent alpha1 of detrended fluctuation analysis (DFA a1), a nonlinear index of heart rate variability (HRV) based on fractal correlation properties, has been shown to steadily change with increasing exercise intensity. To date, no study has specifically examined using the behavior of this index as a method for defining a low intensity exercise zone. The aim of this report is to compare both oxygen intake ($VO_2$) and heart rate (HR) reached at the first ventilatory threshold (VT1), a well-established delimiter of low intensity exercise, to those derived from a predefined DFA a1 transitional value. Gas exchange and HRV data were obtained from 15 participants during an incremental treadmill run. Comparison of both $VO_2$ and HR reached at VT1 defined by gas exchange (VT1 GAS) was made to those parameters derived from analysis of DFA a1 reaching a value of 0.75 (HRVT). Based on Bland Altman analysis, linear regression, intraclass correlation (ICC) and *t* testing, there was strong agreement between VT1 GAS and HRVT as measured by both HR and $VO_2$. Mean VT1 GAS was reached at 39.8 ml/kg/min with a HR of 152 bpm compared to mean HRVT which was reached at 40.1 ml/kg/min with a HR of 154 bpm. Strong linear relationships were seen between test modalities, with Pearson's *r* values of 0.99 ($p < 0.001$) and.97 ($p < 0.001$) for $VO_2$ and HR comparisons, respectively. Intraclass correlation between VT1 GAS and HRVT was 0.99 for $VO_2$ and 0.96 for HR. In addition, comparison of VT1 GAS and HRVT showed no differences by *t* testing, also supporting the method validity. In conclusion, it appears that reaching a DFA a1 value of 0.75 on an incremental treadmill test is closely associated with crossing the first ventilatory threshold. As training intensity below the first ventilatory threshold is felt to have great importance for endurance sport, utilization of DFA a1 activity may provide guidance for a valid low training zone.

**Keywords: detrended fluctuation analysis, ventilatory threshold, aerobic threshold, intensity distribution, intensity zones, endurance exercise, endurance training, polarized training**

## INTRODUCTION

Training zone identification is part of the foundation for exercise intensity distribution study and implementation (Stöggl and Sperlich, 2019. Traditionally, the upper limit of the low intensity range (zone 1 in a 3 zone model) for intensity distribution for endurance exercise and training prescription has been represented by the first ventilatory (VT1) or lactate threshold (LT1) (Seiler and Kjerland, 2006; Esteve-Lanao et al., 2007; Mann et al., 2013; Pallarés et al., 2016). Although there may be different schools of thought on what type of distribution is "optimal" (polarized vs. pyramidal or threshold) both models are defined by having the major portion of training in zone 1. In addition, several training approaches for endurance athletes recommend spending large amounts of exercise time in a low intensity zone (Muñoz et al., 2014; Stöggl and Sperlich, 2014, 2019; Bourgois et al., 2019; Casado et al., 2019). Gold standard methods to obtain VT1 or LT1 revolve around either formal gas exchange testing or invasive blood lactate sampling. These procedures can be costly, require special test equipment, trained operators, ongoing calibration and verification. Even if these methods are utilized, there is disagreement on their accuracy as both visual (Yeh et al., 1983; Meyer et al., 1996) and automated (Ekkekakis et al., 2008) gas exchange analysis can be subject to substantial error. In addition, LT1 assessment can vary depending on the chosen concept of determination (Newell et al., 2007; Faude et al., 2009; Jamnick et al., 2018). Training guided by erroneous intensity targets could lead to potential adverse consequences such as prolonged cardiac parasympathetic recovery (Seiler and Kjerland, 2006; Stanley et al., 2013), central and muscular fatigue (Noakes et al., 2005; Venhorst et al., 2018), glycogen depletion (Beneke et al., 2011), and gastrointestinal barrier disruption (van Wijck et al., 2012). In view of the difficulties involved in gas exchange analysis, lactate test availability, invasiveness, and accuracy, a search for alternate methods of identifying the limits of low intensity exercise seem worthwhile.

Cardiac interbeat interval variation, commonly referred to as heart rate variability (HRV), has been extensively studied in both resting states (Shaffer and Ginsberg, 2017) as well as during dynamic exercise (Hottenrott and Hoos, 2017; Michael et al., 2017). Certain HRV indexes have been observed to change as exercise intensity rises, potentially providing information regarding an individual's physiologic status (Tulppo et al., 1996; Casties et al., 2006; Sandercock and Brodie, 2006; Karapetian et al., 2008; Michael et al., 2017; Gronwald et al., 2018, 2019a,b,c). It has also been shown that several of the examined HRV indexes also change during lower intensities (Tulppo et al., 1996; Sandercock and Brodie, 2006; Karapetian et al., 2008; Botek et al., 2010; Michael et al., 2017) making them potentially suitable for zone 1 delineation. However, despite some initial interest, widespread usage for the specific purpose of low intensity training limitation has not occurred. Frequency-domain parameters such as high frequency (HF) power have been noted to be unreliable in a sizable fraction of individuals with up to 20% of subjects not having identifiable breakpoints (Cottin et al., 2007). Time domain measures such as SDNN were found to closely relate with VT1 but little follow-up or verification has been done (Karapetian et al., 2008). The SD1 is another index that has been examined during exercise. It is based on a Poincare plot of each RR interval graphed against the preceding interval and is related to short term trends in RR patterns often assigned to nonlinear indexes (Shaffer and Ginsberg, 2017), although it is mathematically equivalent to another time domain index (Ciccone et al., 2017). While showing potential as a low intensity marker in some earlier studies (Tulppo et al., 1996) other evidence indicates that SD1 was already suppressed in young athletes at the first tested work rate of 60% $VO_{2MAX}$ making it less useful for zone 1 delineation (Blasco-Lafarga et al., 2017).

One nonlinear index, the short-term scaling exponent alpha1 based on Detrended Fluctuation Analysis (DFA a1), has generated interest as both an indicator of autonomic nervous system regulation as well as an overall marker of organismic demands Gronwald and Hoos, 2020). Originally, Peng et al. (1995) developed this method to measure scale-invariant behavior; this involved the evaluation of trends of all sizes in the presence or absence of fractal correlation properties in a heart rate (HR) time series (Yeh et al., 2010). Thus, the DFA method allows for the quantification of the degree of correlation and fractal scale of a HRV signal resulting in dimensionless measures. The short-term scaling exponent DFA a1 is based on the fractal dynamics (self-similarity) of the cardiac beat-to-beat pattern and provides insights into correlation properties of HR time series caused by physiological processes (Peng et al., 1995). DFA a1 values indicate time series correlation properties with approximately 1.5 indicating a strongly correlated pattern and $\leq$0.5 for anti-correlated pattern with random behavior; approximately 1.0 signifies a mix of uncorrelated and maximally correlated signal components (represents a balance between complete unpredictability (randomness) and predictability (strong correlations), also associated with fractal (self-similar) behavior (Platisa and Gal, 2008). Larger values of DFA a1 represent a smoother time series and smaller values of DFA a1 represent coarser ones (Peng et al., 1995; Goldberger et al., 2002). Within this framework, DFA a1 has been shown to decline as work rate rises, starting from strongly correlated patterns (value of 1.5) at rates well below the first ventilatory threshold (VT1), transitioning (values of 1.0–0.5) through values representing uncorrelated, less complex white noise behavior at moderate to high work rates, then finally showing anti-correlated behavior at the highest intensities (values of <0.5) (Gronwald et al., 2019c; Gronwald and Hoos, 2020). Given this relationship, there may be an opportunity to assist athletes in delineating intensity training zones by observing the change in DFA a1 with increasing exercise intensity (Gronwald et al., 2020; Rogers, 2020).

The purpose of this report is to validate a predefined DFA a1 value of 0.75 with the exercise intensity at VT1 obtained during an incremental treadmill run to exhaustion. This is to be done by a direct comparison of the VT1 intensity based on both absolute $VO_2$ and HR obtained during gas exchange with the same measures derived from analysis of DFA a1 behavior. If it can be shown that a predefined "boundary" value in the

DFA a1 index occurs near the VT1, this could establish a basis for further research exploiting a non-linear autonomic nervous system related marker in prospective exercise and training intensity distribution.

# MATERIALS AND METHODS

## Participants

Seventeen male volunteers aged 19–52, without previous medical history, current medications or physical issues were tested. A background questionnaire regarding medical history was reviewed along with information of the potential testing risks then institutionally approved consent was given. Approval for the study was granted by the University of Derby, United Kingdom (LSREC_1415_02) and conformed to the principles of the Declaration of Helsinki. Participants did not consume caffeine, alcohol or any stimulant for the 24 h before testing. Background data for each subject included, age, body weight, and training volume in hours per week (**Table 1**). All testing was done in the afternoon and at least 3 h post meal. No exercise was performed the day prior to the test. Two participants with a high degree of cardiac ectopy (frequent atrial premature beats and atrial trigeminy) during testing were excluded from analysis.

## Exercise Protocol

Participants performed an incremental $VO_{2MAX}$ test on a motorized treadmill (Woodway, Birmingham, United Kingdom). The treadmill was set for the Bruce protocol with increases in speed and inclination from 2.7 km/h at ten percent grade, increasing by 1.3 km/h and two percent grade every 3 min until volitional exhaustion. A fan was used for cooling.

**TABLE 1 |** Demographic data of all included participants ($n$ = 15) with training volume.

| Subject number | Age (years) | BW (Kg) | HT (cm) | TV (h/wk) |
|---|---|---|---|---|
| 1 | 19 | 82 | 182 | 3–6 |
| 2 | 19 | 82 | 176 | 3–6 |
| 3 | 20 | 82 | 190 | 3–6 |
| 4 | 23 | 77 | 180 | >6 |
| 5 | 24 | 69 | 171 | 3–6 |
| 6 | 24 | 65 | 165 | >6 |
| 7 | 24 | 76 | 186 | 3–6 |
| 8 | 25 | 78 | 171 | >6 |
| 9 | 26 | 69 | 169 | >6 |
| 10 | 30 | 92 | 189 | 1–3 |
| 11 | 30 | 73 | 175 | >6 |
| 12 | 32 | 65 | 161 | 1–3 |
| 13 | 36 | 75 | 182 | >6 |
| 14 | 50 | 94 | 178 | 3–6 |
| 15 | 52 | 71 | 171 | 1–3 |
| Mean (SD) | 29 (±10) | 77 (±9) | 176 (±9) | - |

*BW, Body weight; TV, Training volume. Mean (±standard deviation, SD) in last row.*

## Gas Exchange Testing and Calculation of the First Ventilatory Threshold

Gas exchange kinetics were recorded continuously using a breath-to-breath metabolic cart (Metalyzer 3B; Cortex Biophysik GmbH Germany). In addition, a Polar H7 (Polar Electro Oy, Kempele, Finland) was wirelessly paired to the Metalyzer cart for the purpose of HR recording concurrent with gas exchange data. $VO_2$, $VCO_2$, $PetO_2$, $PetCO_2$, $Ve/VO_2$, $Ve/VCO_2$, and HR were imported into Microsoft Excel 365 for analysis. The native gas exchange analysis feature of the Metalyzer was not used due to the unreliability of many automated VT1 calculations (Ekkekakis et al., 2008). Graphing of the above parameters were done to derive VT1, $VO_{2MAX}$, and $VO_2$ vs. time. No averaging was done for either gas exchange parameters or HR. Inspection of the $VO_2$ over time relationship was done to determine any significant plateau of the $VO_2$ curve for estimation of $VO_{2MAX}$ and $VO_2$ linearity. If a significant plateau was found, compensation for calculating both $VO_{2MAX}$ and the $VO_2$ over time equation was done. To reduce the chance of failure to identify the VT1 by gas exchange (VT1 GAS) based on a single method, evaluation was done according to the triple detection method consisting of V slope, $Ve/VO_2$, and excess $CO_2$ from Gaskill et al. (2001) as well as the $PetO_2$ nadir from Binder et al. (2008). Based on the quality and consistency of the plots, the excess $CO_2$ method was chosen to be used for all participants and reviewed independently by two investigators (**Figure 1A**). $VO_2$ was plotted over the elapsed time of the incremental test to produce a linear regression equation. $VO_2$ at the time of VT1 was based on linear regression from the $VO_2$ over time relationship.

## RR Measurements and Calculation of DFA a1 Derived Threshold

A 3-lead ECG (MP36; Biopac Systems Ltd.) with a sampling rate of 1,000 Hz was used to record the subject's ECG/RR times series. Biopac filter settings were set to 0.05 Hz high-pass filter and 150 Hz low-pass filter. Electrodes were placed in the CM5 distribution after appropriate skin cleansing and shaving if necessary. Sample data from the MP36 was saved as .acq files. ECG files for each subject were imported into Kubios 3.3.2 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). Kubios preprocessing settings were at the default values including the RR detrending method which was kept at "Smoothn priors" (Lambda = 500, Tarvainen et al., 2014). For DFA a1 estimation, the root mean square fluctuation of the integrated and detrended data is measured in observation windows of different sizes. The data are then plotted against the size of the window on a log-log scale. The scaling exponent represents the slope of the line, which relates (log) fluctuation to (log) window size (Mendonca et al., 2010). DFA a1 window width was set to $4 \leq n \leq 16$ beats.

For the detection of a HRV derived threshold, a DFA a1 value of 0.75 was chosen based on this being the midpoint between a fractal behavior of the HR time series of 1.0 (seen with very light exercise) and an uncorrelated value of 0.5 which represents white noise, random behavior (seen with high intensity exercise). A value of 0.75 has also been used as a cut-off value for survival

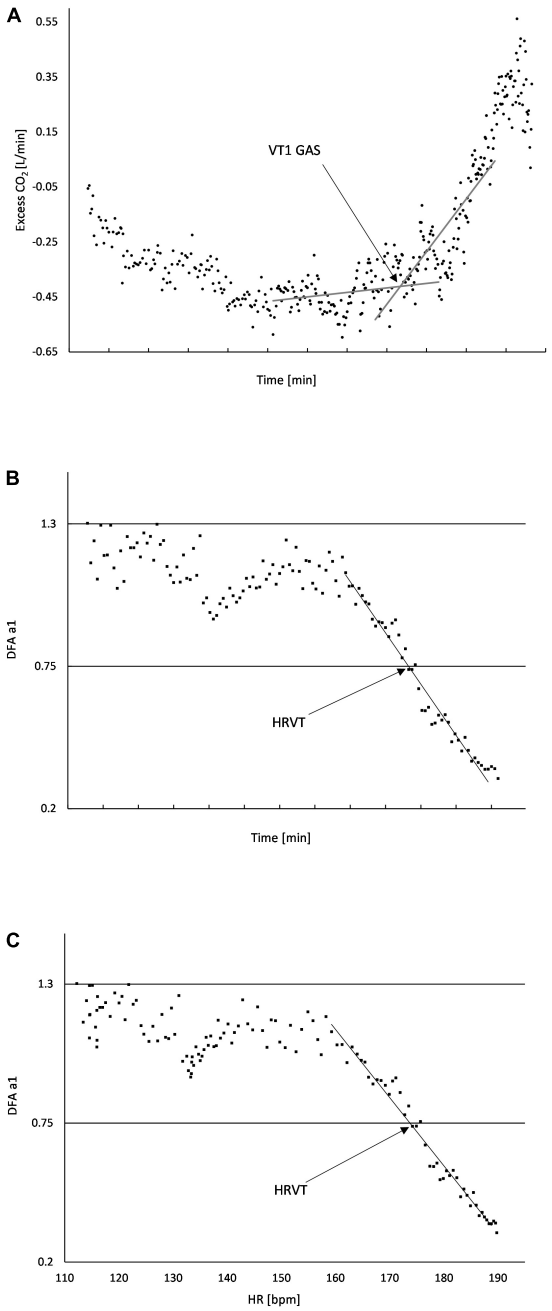



**FIGURE 1 | (A)** Usage of "Excess $CO_2$" technique to determine VT1 GAS. The intersection of the baseline and first rise in excess $CO_2$ corresponds to the time when VT1 occurs; **(B)** DFA a1 plotted over time, the area of linear drop of DFA a1 from about 1.0 to 0.5 is used to determine the $VO_2$ (from the $VO_2$ vs. time relation) at HRVT; **(C)** DFA a1 plotted against HR, the area of linear drop of DFA a1 from 1.0 to 0.5 is used to determine the HR at HRVT. All data taken from Subject #2.

curves and mortality rate assessment during resting conditions (Huikuri et al., 2000).

The following procedure was used to indicate at what level of running intensity (as $VO_2$ or HR) the DFA a1 would cross a value of 0.75: DFA a1 was calculated from the incremental exercise test RR series using 2 min time windows with a recalculation every 5 s throughout the test. Two minute time windowing was chosen based on the reasoning of Chen et al. (2002). The rolling time window measurement was used to better delineate rapid changes in the DFA a1 index over the course of the test. Each DFA a1 value is based on the RR series 1 min pre and 1 min post the designated time stamp. For example, at a time of 10 min into the testing, the DFA a1 is calculated from the 2 min window starting from minute 9 and ending at minute 11 and labeled as the DFA a1 at 10 min. Based on a rolling time recalculation every 5 s, the next data point would occur at 10:05 min (start 9:05 min and end 11:05 min).

Plotting of DFA a1 vs. time was then performed. Inspection of the DFA a1 relationship with time generally showed a reverse sigmoidal curve with a stable area above 1.0 at low work rates, a rapid, near linear drop reaching below 0.5 at higher intensity, then flattening without major change. A linear regression was done on the subset of data consisting of the rapid near linear decline from values near 1.0 (correlated) to approximately 0.5 (uncorrelated). The time of DFA a1 reaching 0.75 was calculated based on the linear regression equation from that straight section (**Figure 1B**). The time of DFA a1 reaching 0.75 was then converted to $VO_2$ using the $VO_2$ vs. time relation, resulting in the $VO_2$ at which DFA a1 equaled 0.75 (HRVT). A similar analysis was done for the HR reached at a DFA a1 of 0.75. First, ECG data from each 2 min rolling window was used to plot the average HR and DFA a1. The HR at which DFA a1 equaled 0.75 was found using the same technique as above, a linear regression through the rapid change section of DFA a1 values of 1.0 to below 0.5, with a subsequent equation for HR and DFA a1 (**Figure 1C**). Using a fixed variable of DFA a1 equals 0.75, the resulting HR was obtained. The HR at DFA a1 0.75 (based on ECG data) was then compared to the HR at VT1 GAS obtained from the metabolic cart data (based on the Polar H7).

Visual inspection of the entire test recording was done to determine sample quality, noise, arrhythmia, and missing beat artifact. As mentioned above, two participants with a high degree of atrial ectopy were excluded from analysis. The RR series of the included participants was then corrected by the Kubios "automatic method" and exported as text files for further analysis. Percent artifact reported refers those occurring during the linear regression segment (DFA a1 1.0 to near 0.5).

## Statistics

Statistical analysis was performed for the main variables, $VO_2$ at VT1 derived from gas exchange testing, $VO_2$ at DFA a1 0.75, HR at VT1 obtained from gas exchange testing and average HR at DFA a1 0.75. Standard statistical methods were used for the calculation of means and standard deviations (SD). Normal distribution of data was checked by Shapiro–Wilk's test. The agreement against the Gold Standard VT1 GAS was assessed using intraclass correlation coefficient (ICC), linear regression, Pearson's $r$ correlation coefficient, standard error of estimate (SEE), coefficient of determination ($R^2$) and Bland Altman plots with limits of agreement (Bland and Altman, 1999). The size of Pearson's $r$ correlations evaluated as follows; $0.3 \leq r < 0.5$ low; $0.6 \leq r < 0.8$ moderate and $r \geq 0.8$ high (Chan, 2003). The paired

t-test was used for comparison of VT1 GAS vs. HRVT for both $VO_2$ and HR parameters. For all tests, the statistical significance was accepted as $p \leq 0.05$. Cohen's $d$ was used to denote effect sizes (small effect = 0.2, medium effect = 0.5, large effect = 0.8; Cohen, 1988). Analysis was performed using Microsoft Excel 365 with Real Statistics Resource Pack software (Release 6.8).

# RESULTS

## Gas Exchange Testing

Individual gas exchange results are presented in **Table 2**. Both the $VO_{2MAX}$ as well as the percentage of $VO_{2MAX}$ and HR at VT1 GAS varied considerably among participants. $VO_{2MAX}$ ranged between 41 and 74 ml/kg/min. VT1 GAS was reached between 61 and 86% of the $VO_{2MAX}$ and at HRs between 108 and 183 bpm.

## RR Interval Quality

The percentage of artifacts was calculated based on the Kubios automatic correction method for each subject's test data. Since only a portion of the entire treadmill test was used for the linear interpolation of DFA a1, the artifact percentage listed refers to that section only. Artifact percentage for the linear plotted data series was between 0 and 3%, all consisting of atrial premature complexes (**Table 2**). There were no missed beats due to noise interference or loss of electrode contact. The two participants originally excluded from analysis had significant ectopy, leading to an uninterpretable DFA a1 pattern.

## Comparison of VT1 GAS vs. HRVT

The average VT1 GAS was 39.8 ml/kg/min (±8.9) compared to 40.1 ml/kg/min (±8.6) obtained by HRVT. The average HR at VT1 GAS was 152 bpm (±21) compared to 154 bpm (±20) obtained by HRVT. Strong linear relationships were seen between test modalities, with Pearson's $r$ values of 0.99 ($p < 0.001$) and.97 ($p < 0.001$) for $VO_2$ and HR comparisons respectively (**Figure 2**). Intraclass correlation between VT1 GAS and HRVT was 0.99 for $VO_2$ and 0.96 for HR. The comparison of VT1 GAS and HRVT showed no differences ($VO_2$: $p = 0.347$, $d = 0.030$; HR: $p = 0.191$, $d = 0.091$). Bland Altman analysis for VT1 GAS vs. HRVT for $VO_2$ (**Figure 3**) showed a mean difference of −0.33 ml/kg/min (±1.3) with upper and lower limits of 2.2 and −2.9 ml/kg/min. Bland Altman analysis for VT1 GAS vs. HRVT for HR (**Figure 3**) showed a mean difference of −1.9 bpm (±5) with upper and lower limits of 8 and −12 bpm.

# DISCUSSION

This study explored whether values of the nonlinear HRV index, DFA a1, pass through a defined transitional zone at workloads near VT1 during an incremental treadmill test. Since many prior reports have shown DFA a1 to decline during incremental exercise (Hautala et al., 2003; Casties et al., 2006; Platisa et al., 2008; Karavirta et al., 2009; Blasco-Lafarga et al., 2017; Gronwald et al., 2019c), our result showing a similar occurrence is not unanticipated. However, none have attempted to directly examine the possibility that the DFA a1 index has a distinct value at the VT1 work rate. Since many of the prior studies looking at DFA a1 response to incremental exercise intensity have used cycling as the exercise modality, it is also reassuring to see analogous results with treadmill running, adding validity to the behavior of this index during other endurance exercise types. The inclusion of a wide range of subject ages, body weights and fitness abilities, lends strength to the application of our results

**TABLE 2 |** Comparison of VT1 GAS and HRVT with measures of $VO_2$ and HR.

| Subject number | $VO_{2MAX}$ (ml/kg/min) | VT1 GAS $VO_2$ ($\%_{MAX}$) | VT1 GAS $VO_2$ (ml/kg/min) | HRVT $VO_2$ (ml/kg/min) | VT1 GAS HR (bpm) | HRVT HR (bpm) | Artifacts (%) |
|---|---|---|---|---|---|---|---|
| 1 | 58 | 77 | 45.2 | 46.1 | 167 | 170 | 0 |
| 2 | 57 | 75 | 42.8 | 45.1 | 169 | 175 | 0.5 |
| 3 | 47 | 70 | 32.7 | 31.6 | 178 | 175 | 0 |
| 4 | 71 | 86 | 61.2 | 61.2 | 155 | 156 | 0 |
| 5 | 64 | 68 | 43.6 | 43.0 | 143 | 137 | 0 |
| 6 | 54 | 74 | 40.1 | 38.1 | 165 | 163 | 0 |
| 7 | 47 | 76 | 35.8 | 37.2 | 164 | 171 | 2 |
| 8 | 54 | 70 | 37.8 | 37.9 | 137 | 135 | 0 |
| 9 | 72 | 69 | 49.3 | 49.2 | 183 | 184 | 0 |
| 10 | 46 | 61 | 27.7 | 29.2 | 108 | 122 | 0 |
| 11 | 74 | 65 | 48.1 | 46.4 | 151 | 148 | 3 |
| 12 | 49 | 66 | 32.0 | 33.3 | 154 | 160 | 1 |
| 13 | 57 | 66 | 37.6 | 39.4 | 154 | 159 | 1 |
| 14 | 41 | 70 | 28.6 | 28.8 | 139 | 136 | 0 |
| 15 | 54 | 64 | 34.5 | 35.5 | 118 | 122 | 1 |
| Mean (SD) | 56 (±10) | 70 (±6) | 39.8 (±8.9) | 40.1 (±8.6) | 152 (±21) | 154 (±20) | 0.6 (±0.9) |

*VT1 GAS, first ventilatory threshold; HRVT, DFA a1 derived threshold; HR, Heart rate; $VO_{2MAX}$, VT1 GAS percent of $VO_{2MAX}$ and Artifacts percentage. Mean (±standard deviation, SD) in last row.*

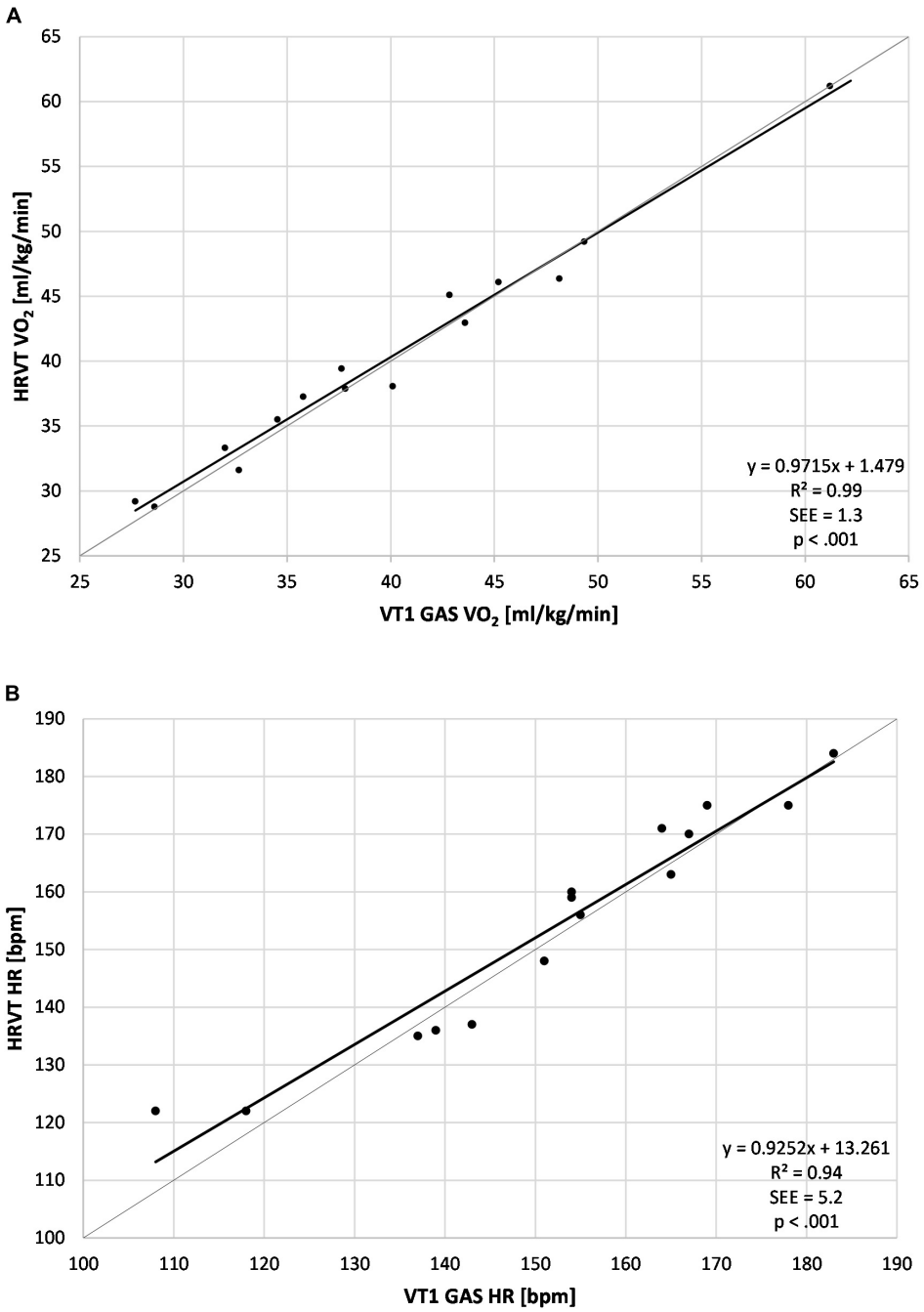



**FIGURE 2 |** Regression plots for all subject data. **(A)** Values of VT1 GAS vs. HRVT for $VO_2$; **(B)** Values of VT1 GAS vs. HRVT for HR. Bisection lines in light gray. SEE, standard error of estimate; $R^2$, coefficient of determination.

to the general population and its application in different fields of physical exercise and training.

In a recent perspective review (Gronwald et al., 2020), identification of a low intensity exercise zone based on DFA a1 for the purposes of endurance exercise and training prescription was discussed. The mechanism underlying DFA a1 decline with exercise is felt to be related to autonomic balance and a complex interaction of the two main branches, namely parasympathetic withdrawal, sympathetic intensification as well as other factors (Gronwald and Hoos, 2020). Since VT1 is usually seen at a point of significant parasympathetic withdrawal (Tulppo et al., 1996; Sales et al., 2019), leveraging HRV parameters that reliably reflect this occurrence can be of use during endurance exercise and training. Our methodology to determine HRVT utilized the rapid decline of DFA a1 from 1.0 to below 0.5, seen during progressive exercise intensity. The results presented here appear to indicate

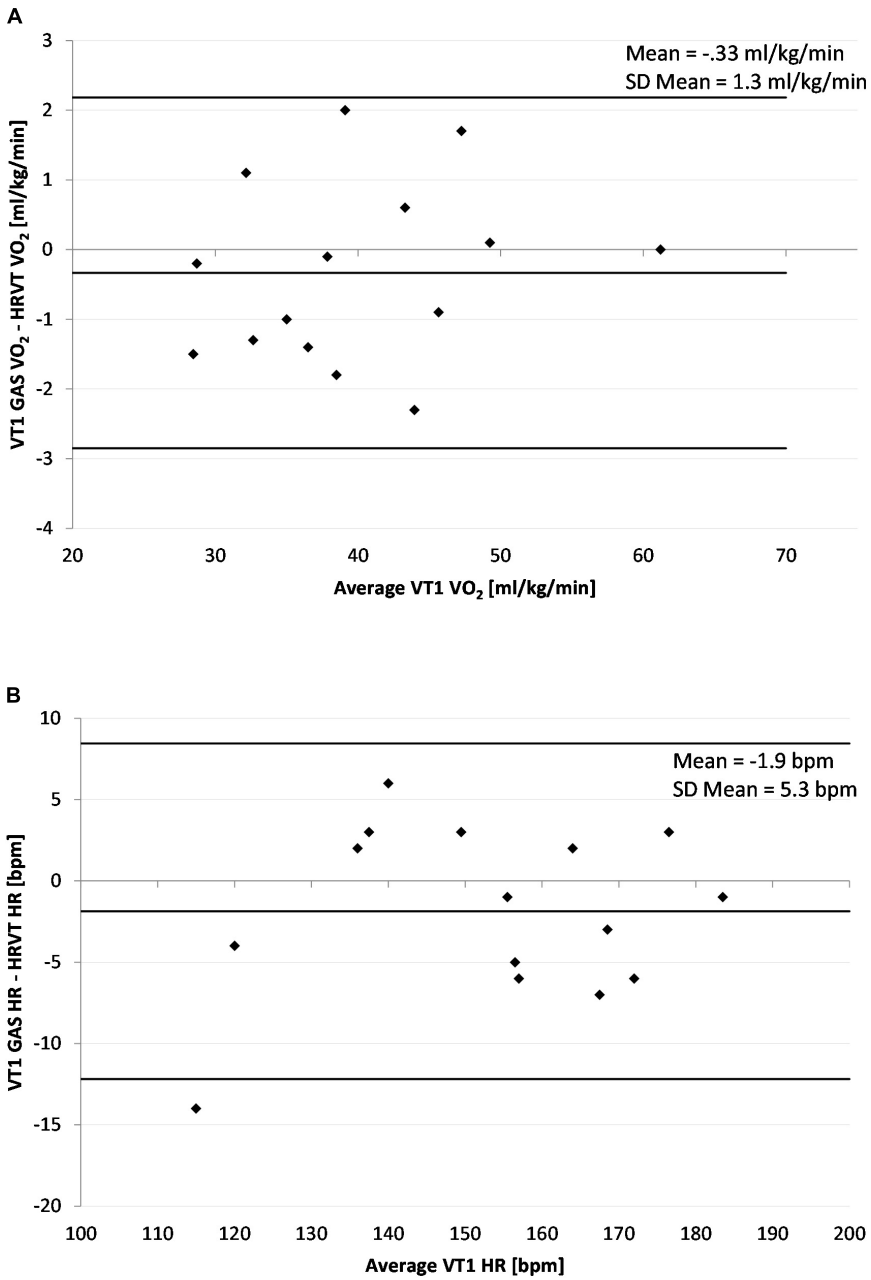



**FIGURE 3 |** Bland Altman Plot of VT1 GAS vs. HRVT for all participants. **(A)** Values of VT1 GAS vs. HRVT for $VO_2$; **(B)** Values of VT1 Gas vs. HRVT for HR. Center line in each plot represents the mean difference between each paired value, the top and bottom lines are 1.96 standard deviations from the mean difference.

that VT1 is reached at a midpoint between a fractal behavior of DFA a1 and a pattern of uncorrelated white noise with random behavior, corresponding to a DFA a1 of approximately 0.75. Bland Altman analysis with limits of agreement showed minimal difference between VT1 GAS and HRVT looking at either $VO_2$ or HR measurements. Correlation coefficients and ICC were high for both $VO_2$ and HR based comparisons.

Although the DFA a1 value of 0.75 was chosen theoretically, a brief review of prior investigation is supportive of this figure. In a study of young men performing a cycling ramp test, an average DFA a1 of 0.49 was associated with a lactate measurement of 2.49, indicating that LT1 had already been exceeded (Gronwald et al., 2019c). Other cycling ramp studies in men of different fitness levels seemed to indicate that DFA a1 crossed the value of 0.75 at about 73–78% of $VO_{2MAX}$ (Hautala et al., 2003; Hottenrott and Hoos, 2017), within the approximate realm of VT1 for many individuals (Gaskill et al., 2001; Pallarés et al., 2016). An examination of the DFA a1 response to incremental cycling exercise in teenage males (Blasco-Lafarga et al., 2017) showed an approximate crossing of the 0.75 value at an average

intensity near 65% of maximum, also near published ranges of VT1 (50–65% of $VO_{2MAX}$) in that age group (Runacres et al., 2019). In the current study, there appeared to be little bias in the $VO_2$ or HR associated with HRVT. Although there was some variability in VT1 GAS vs. HRVT parameters, for the most part, associations were similar to that of other comparisons of threshold approaches such as blood lactate or ventilatory parameters (Pallarés et al., 2016), or assessment of gas exchange techniques for VT1 determination (Gaskill et al., 2001). Several participants had relatively high heart rate at the VT1 (Azevedo et al., 2011) of which we do not have an explanation. A strength of this study is that of RR interval quality. Direct ECG visualization was done and a research grade device with a high sample rate was used. No missed or lost beats were seen, and the only artifact type present was APC aberrancy. In view of reports indicating substantial bias in nonlinear HRV indexes with artifact presence (Giles and Draper, 2018; Rincon Soler et al., 2018), a weakening of DFA a1 derived VT1 accuracy could occur with higher artifact occurrence.

A significant advantage of DFA a1 over other HRV indexes for the determination of a low intensity threshold revolves around the nature of testing. Other HRV metrics proposed to identify VT1 such as SDNN (Karapetian et al., 2008), HF power (Cottin et al., 2007) or SD1 (Tulppo et al., 1996) require testing into high intensity zones since they rely on curve interpretation that displays a demonstrable nadir. With DFA a1, once the VT1 boundary area is reached, little additional increase in exercise intensity should be required. The potential benefit of utilizing a fixed DFA a1 value as the VT1 delineation marker is especially attractive in populations unable or ill advised to enter high intensity regions. In addition, for athletes evaluating low intensity training limits, avoidance of exercise ramps to volitional failure may help avert undue stress in a polarized training model.

## Limitations and Future Direction

Given the issues with both availability and accuracy of gas exchange or blood lactate testing in determining VT1 for training zone purposes, an alternate modality that employs relatively simple wearable technology seems attractive. However, while DFA a1 monitoring may be a promising approach, several questions need to be addressed. Although this study was done with a wide range of subject age and fitness characteristics, no female participants were tested. If the DFA a1 index behavior is to be considered as a zone 1 delimiter for the general population, further investigation using female subjects is mandatory. Another area of concern is the transfer of the DFA a1 0.75 breakpoint obtained during incremental testing to that of one found during constant load exercise, including moderate length intervals (5 min). No data is available comparing DFA a1 behavior during an incremental ramp to constant load exercise (Gronwald and Hoos, 2020), making automatic transfer of zone boundaries unclear. Whether the index will remain stable for even longer exercise intervals (>60 min) performed below VT1 intensity is another open question as well as day to day repeatability. Another interesting subject to explore is the impact of athlete overtraining on DFA a1 behavior and VT1 prediction accuracy during exercise. Baumert et al. (2006) did show changes in DFA related scaling behavior after intense training, which may provide both a potential source of HRVT bias and an opportunity to screen for overtraining states. Although it seems that ramp protocol slope has minimal effect on the VT1 gas assessment (Weston et al., 2002; Boone and Bourgois, 2012), the analogous assumption needs to be shown in terms of HRVT thresholds. Another area for investigation is whether DFA a1 cut off values are equivalent between chest belt and research grade ECG recordings. Although in this study, the RR intervals were recorded with a research grade ECG device, it may be possible to reproduce similar results with chest belt ECG recordings. In that regard, two major questions need to be addressed. One is that of exercise associated missed beat artifact with possible faulty interpolation strategies by interpreting software, creating potential bias in the calculated DFA a1 values. As mentioned above, several reports have questioned the degree of bias of nonlinear HRV indexes if artifacts are present in the RR series (Giles and Draper, 2018; Rincon Soler et al., 2018; Stapelberg et al., 2018). Artifacts may be of different types such as missed beats or aberrancy. In the current report, no missed beats were seen, and only relatively rare atrial premature complexes were noted. However, two participants exhibited frequent APC aberrancy, had uninterpretable DFA a1 curves and were excluded from group analysis. Given the relative low numbers of participants, no definitive conclusion can be reached regarding artifact bias, but further investigation into effects of missed beats and aberrancy on the use of DFA a1 to delineate zone 1 transition is needed. Second is the question of DFA a1 value precision obtained by diverse monitoring devices possessing different sample rates and prepossessing strategies. Device sample rates have been shown to variably alter DFA a1 values at rest (Voss et al., 1996; Tapanainen et al., 1999; Singh et al., 2015) but may have more significant effects during exercise. Although no recent caffeine use was noted by history, we have no information on prior long term intake patterns which could affect autonomic balance on abrupt discontinuation (La Monica et al., 2018). Finally, it may be possible to answer many of these questions by "repurposing" prior work already done. For instance, a study by Boullosa et al. (2014) assessed the changes in DFA a1 before and after a typical incremental treadmill ramp to exhaustion. A look back at previously acquired RR recordings during the active ramp portion using the methods discussed here may be a way to rapidly acquire needed information about DFA a1 behavior during dynamic exercise.

## Conclusion

DFA a1, an index of fractal dynamics and correlations properties of the heart rate time series, was noted to decline during an incremental treadmill run test to exhaustion. The area of most rapid change of this index occurred near the first ventilatory threshold. The point of DFA a1 reaching a value of 0.75 during the incremental treadmill test was directly associated with the first ventilatory threshold as measured by heart rate and gas exchange $VO_2$. As training intensity below the first ventilatory threshold is felt to have great importance for exercise and training prescription in endurance sport, utilization of DFA a1

activity may provide guidance for a valid low training zone boundary without the need for gas exchange or blood lactate testing. Further study of DFA a1 behavior in female participants, during constant load intervals, index stability over long periods of time and across diverse recording devices is recommended. If investigation into these matters remain consistent with the results presented here, obtaining a low intensity zone boundary by automated analysis of a training session *via* an appropriate wearable device may be possible.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Derby, United Kingdom (LSREC_1415_02). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

BR and TG conceived the study. DG and ND performed the physiologic testing. BR wrote the first draft of the article. BR and TG performed the data analysis. All authors revised it critically for important intellectual content, final approval of the version to be published, and accountability for all aspects of the work.

## REFERENCES


Azevedo, L. F., Perlingeiro, P. S., Brum, P. C., Braga, A. M., Negrão, C. E., and de Matos, L. D. (2011). Exercise intensity optimization for men with high cardiorespiratory fitness. *J. Sports Sci.* 29, 555–561. doi: 10.1080/02640414.2010.549613

Baumert, M., Brechtel, L., Lock, J., and Voss, A. (2006). Changes in heart rate variability of athletes during a training camp. *Biomed. Tech.* 51, 201–204. doi: 10.1515/bmt.2006.037

Beneke, R., Leithäuser, R. M., and Ochentel, O. (2011). Blood lactate diagnostics in exercise testing and training. *Int. J. Sports Physiol. Perf.* 6, 8–24. doi: 10.1123/ijspp.6.1.8

Binder, R. K., Wonisch, M., Corra, U., Cohen-Solal, A., Vanhees, L., Saner, H., et al. (2008). Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. *Eur. J. Cardiovasc. Prev. Rehab.* 15, 726–734. doi: 10.1097/hjr.0b013e328304fed4

Bland, J. M., and Altman, D. G. (1999). Measuring agreement in method comparison studies. *Stat. Methods Med. Res.* 8, 135–160. doi: 10.1191/096228099673819272

Blasco-Lafarga, C., Camarena, B., and Mateo-March, M. (2017). Cardiovascular and Autonomic Responses to a Maximal Exercise Test in Elite Youngsters. *Int. J. Sports Med.* 38, 666–674. doi: 10.1055/s-0043-110680

Boone, J., and Bourgois, J. (2012). The oxygen uptake response to incremental ramp exercise: methodogical and physiological issues. *Sports Med. 1* 42, 511–526. doi: 10.2165/11599690-000000000-00000

Botek, M., Stejskal, P., Krejci, J., Jakubec, A., and Gaba, A. (2010). Vagal threshold determination. Effect of age and gender. *Int. J. Sports Med.* 31, 768–772. doi: 10.1055/s-0030-1263141

Boullosa, D. A., Barros, E. S., del Rosso, S., Nakamura, F. Y., and Leicht, A. S. (2014). Reliability of heart rate measures during walking before and after running maximal efforts. *Int. J. Sports Med.* 35, 999–1005. doi: 10.1055/s-0034-1372637

Bourgois, J. G., Bourgois, G., and Boone, J. (2019). Perspectives and Determinants for Training-Intensity Distribution in Elite Endurance Athletes. *Int. J. Sports Physiol. Perform.* 14, 1151–1156. doi: 10.1123/ijspp.2018-0722

Casado, A., Hanley, B., Santos-Concejero, J., and Ruiz-Pérez, L. M. (2019). World-Class Long-Distance Running Performances Are Best Predicted by Volume of Easy Runs and Deliberate Practice of Short-Interval and Tempo Runs. *J. Strength. Cond. Res.* doi: 10.1519/JSC.0000000000003176 [Online ahead of print].

Casties, J. F., Mottet, D., and Le Gallais, D. (2006). Non-linear analyses of heart rate variability during heavy exercise and recovery in cyclists. *Int. J. Sports Med.* 27, 780–785. doi: 10.1055/s-2005-872968

Chan, Y. H. (2003). Biostatistics 104: correlational analysis. *Singapore Med. J.* 44, 614–619.

Chen, Z., Ivanov, P. C., Hu, K., and Stanley, H. E. (2002). Effect of nonstationarities on detrended fluctuation analysis. *Phys. Rev. E* 65:041107.

Ciccone, A. B., Siedlik, J. A., Wecht, J. M., Deckert, J. A., Nguyen, N. D., and Weir, J. P. (2017). Reminder: RMSSD and SD1 are identical heart rate variability metrics. *Muscle Nerve.* 56, 674–678. doi: 10.1002/mus.25573

Cohen, J. (1988). *Statistical Power Analysis for the Behavioural Sciences*. Hillsdale: Erlbaum.

Cottin, F., Médigue, C., Lopes, P., Leprêtre, P. M., Heubert, R., and Billat, V. (2007). Ventilatory thresholds assessment from heart rate variability during an incremental exhaustive running test. *Int. J. Sports Med.* 28, 287–294. doi: 10.1055/s-2006-924355

Ekkekakis, P., Lind, E., Hall, E. E., and Petruzzello, S. J. (2008). Do regression-based computer algorithms for determining the ventilatory threshold agree? *J. Sports Sci.* 26, 967–976. doi: 10.1080/02640410801910269

Esteve-Lanao, J., Foster, C., Seiler, S., and Lucia, A. (2007). Impact of training intensity distribution on performance in endurance athletes. *J. Strength Cond. Res.* 21, 943–949. doi: 10.1519/00124278-200708000-00048

Faude, O., Kindermann, W., and Meyer, T. (2009). Lactate threshold concepts. *Sports Med.* 39, 469–490. doi: 10.2165/00007256-200939060-00003

Gaskill, S. E., Ruby, B. C., Walker, A. J., Sanchez, O. A., Serfass, R. C., and Leon, A. S. (2001). Validity and reliability of combining three methods to determine ventilatory threshold. *Med. Sci. Sports. Exerc.* 33, 1841–1848. doi: 10.1097/00005768-200111000-00007

Giles, D. A., and Draper, N. (2018). Heart Rate Variability During Exercise: A Comparison of Artefact Correction Methods. *J. Strength Cond. Res.* 32, 726–735. doi: 10.1519/jsc.0000000000001800

Goldberger, A. L., Amaral, L. A., Hausdorff, J. M., Ivanov, P. C., Peng, C. K., and Stanley, H. E. (2002). Fractal dynamics in physiology: Alterations with disease and aging. *PNAS* 99, 2466–2472. doi: 10.1073/pnas.012579499

Gronwald, T., and Hoos, O. (2020). Correlation properties of heart rate variability during endurance exercise: A systematic review. *Ann. Noninvas. Electrocardiol.* 25:e12697.

Gronwald, T., Hoos, O., and Hottenrott, K. (2019a). Effects of Acute Normobaric Hypoxia on Non-linear Dynamics of Cardiac Autonomic Activity During Constant Workload Cycling Exercise. *Front. Physiol.* 10:999. doi: 10.3389/fphys.2019.00999

Gronwald, T., Hoos, O., and Hottenrott, K. (2019b). Effects of a Short-Term Cycling Interval Session and Active Recovery on Non-Linear Dynamics of Cardiac Autonomic Activity in Endurance Trained Cyclists. *J. Clin. Med.* 6:8.

Gronwald, T., Hoos, O., Ludyga, S., and Hottenrott, K. (2019c). Non-linear dynamics of heart rate variability during incremental cycling exercise. *Res. Sports Med.* 27, 88–98. doi: 10.1080/15438627.2018.1502182

Gronwald, T., Ludyga, S., Hoos, O., and Hottenrott, K. (2018). Non-linear dynamics of cardiac autonomic activity during cycling exercise with varied cadence. *Hum. Mov. Sci.* 60, 225–233. doi: 10.1016/j.humov.2018.06.013

Gronwald, T., Rogers, B., and Hoos, O. (2020). Fractal correlation properties of heart rate variability: A new biomarker for intensity distribution in endurance exercise and training prescription? *Front. Physiol.* 10:3389. doi: 10.3389/fphys.2020.550572

Hautala, A. J., Mäkikallio, T. H., Seppänen, T., Huikuri, H. V., and Tulppo, M. P. (2003). Short-term correlation properties of R-R interval dynamics at different exercise intensity levels. *Clin. Physiol. Funct. Imag.* 23, 215–223. doi: 10.1046/j.1475-097x.2003.00499.x

Hottenrott, K., and Hoos, O. (2017). "Heart rate variability analysis in exercise physiology," in *ECG time series analysis: Engineering to medicine*, eds H. Jelinek, A. Khandoker, and D. Cornforth (London, UK: CRC Press), 245–257.

Huikuri, H. V., Mäkikallio, T. H., Peng, C. K., Goldberger, A. L., Hintze, U., and Møller, M. (2000). Fractal correlation properties of RR interval dynamics and mortality in patients with depressed left ventricular function after an acute myocardial infarction. *Circulation* 101, 47–53. doi: 10.1161/01.cir.101.1.47

Jamnick, N. A., Botella, J., Pyne, D. B., and Bishop, D. J. (2018). Manipulating graded exercise test variables affects the validity of the lactate threshold and VO2peak. *PLoS One* 13:e0199794. doi: 10.1371/journal.pone.0199794

Karapetian, G. K., Engels, H. J., and Gretebeck, R. J. (2008). Use of heart rate variability to estimate LT and VT. *Int. J. Sports Med.* 29, 652–657. doi: 10.1055/s-2007-989423

Karavirta, L., Tulppo, M. P., Laaksonen, D. E., Nyman, K., Laukkanen, R. T., Kinnunen, H., et al. (2009). Heart rate dynamics after combined endurance and strength training in older men. *Med. Sci. Sports Exerc.* 41, 1436–1443. doi: 10.1249/mss.0b013e3181994a91

La Monica, M. B., Fukuda, D. H., Wang, R., Gonzalez, A. M., Wells, A. J., Hoffman, J. R., et al. (2018). Maintenance of Vagal Tone with Time-Release Caffeine, But Vagal Withdrawal During Placebo in Caffeine-Habituated Men. *J. Caff. Adenos. Res.* 8, 59–64. doi: 10.1089/caff.2017.0039

Mann, T., Lamberts, R. P., and Lambert, M. I. (2013). Methods of prescribing relative exercise intensity: physiological and practical considerations. *Sports Med.* 43, 613–625. doi: 10.1007/s40279-013-0045-x

Mendonca, G. V., Heffernan, K. S., Rossow, L., Guerra, M., Pereira, F. D., and Fernhall, B. (2010). Sex differences in linear and nonlinear heart rate variability during early recovery from supramaximal exercise. *Appl. Physiol. Nutr. Metab.* 35, 439–446. doi: 10.1139/h10-028

Meyer, K., Hajric, R., Westbrook, S., Samek, L., Lehmann, M., Schwaibold, M., et al. (1996). Ventilatory and lactate threshold determinations in healthy normals and cardiac patients: methodological problems. *Eur. J. Appl. Physiol. Occupat. Physiol.* 72, 387–393. doi: 10.1007/bf00242266

Michael, S., Graham, K. S., and Oam Davis, G. M. (2017). Cardiac Autonomic Responses during Exercise and Post-exercise Recovery Using Heart Rate Variability and Systolic Time Intervals-A Review. *Front. Physiol.* 8:301. doi: 10.3389/fphys.2017.00301

Muñoz, I., Seiler, S., Bautista, J., España, J., Larumbe, E., and Esteve-Lanao, J. (2014). Does polarized training improve performance in recreational runners? *Int. J. Sports Physiol. Perform.* 9, 265–272. doi: 10.1123/ijspp.2012-0350

Newell, J., Higgins, D., Madden, N., Cruickshank, J., Einbeck, J., McMillan, K., et al. (2007). Software for calculating blood lactate endurance markers. *J. Sports Sci.* 25, 1403–1409. doi: 10.1080/02640410601128922

Noakes, T. D., Gibson, A. S. C., and Lambert, E. V. (2005). From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans: summary and conclusions. *Br. J. Sports Med.* 39, 120–124. doi: 10.1136/bjsm.2003.010330

Pallarés, J. G., Morán-Navarro, R., Ortega, J. F., Fernández-Elías, V. E., and Mora-Rodriguez, R. (2016). Validity and Reliability of Ventilatory and Blood Lactate Thresholds in Well-Trained Cyclists. *PLoS One.* 11:e0163389. doi: 10.1371/journal.pone.0163389

Peng, C. K., Havlin, S., Stanley, H. E., and Goldberger, A. L. (1995). Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos* 5, 82–87. doi: 10.1063/1.166141

Platisa, M. M., and Gal, V. (2008). Correlation properties of heartbeat dynamics. *Eur. Biophys. J.* 37, 1247–1252. doi: 10.1007/s00249-007-0254-z

Platisa, M. M., Mazic, S., Nestorovic, Z., and Gal, V. (2008). Complexity of heartbeat interval series in young healthy trained and untrained men. *Physiol. Meas.* 29, 439–450. doi: 10.1088/0967-3334/29/4/002

Rincon Soler, A. I., Silva, L. E. V., Fazan, R. Jr., and Murta, L. O. Jr. (2018). The impact of artefact correction methods of RR series on heart rate variability parameters. *J. Appl. Physiol.* 124, 646–652. doi: 10.1152/japplphysiol.00927.2016

Rogers, B. (2020). A personalized low intensity exercise prescription based on an index of non linear heart rate variability: a case report. *J. Athl. Enhanc.* 9:1.

Runacres, A., Mackintosh, K. A., and McNarry, M. A. (2019). The effect of constant-intensity endurance training and high-intensity interval training on aerobic and anaerobic parameters in youth. *J. Sports Sci.* 37, 2492–2498. doi: 10.1080/02640414.2019.1644890

Sales, M. M., Sousa, C. V., da Silva Aguiar, S., Knechtle, B., Nikolaidis, P. T., Alves, P. M., et al. (2019). An integrative perspective of the anaerobic threshold. *Physiol. Behav.* 205, 29–32. doi: 10.1016/j.physbeh.2017.12.015

Sandercock, G. R., and Brodie, D. A. (2006). The use of heart rate variability measures to assess autonomic control during exercise. *Scand. J. Med. Sci. Sports* 16, 302–313. doi: 10.1111/j.1600-0838.2006.00556.x

Seiler, K. S., and Kjerland, G. Ø (2006). Quantifying training intensity distribution in elite endurance athletes: is there evidence for an "optimal" distribution? *Scand. J. Med. Sci. Sports* 16, 49–56. doi: 10.1111/j.1600-0838.2004.00418.x

Shaffer, F., and Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Front. Publ. Health.* 5:258. doi: 10.3389/fpubh.2017.00258

Singh, M., Singh, M., and Banga, V. (2015). "Optimal ECG Sampling Rate for Non-Linear Heart Rate Variability," in *IJCA Proceedings on International Conference on Advancements in Engineering and Technology ICAET* (Korea: ICAET), 22–26.

Stanley, J., Peake, J. M., and Buchheit, M. (2013). Cardiac parasympathetic reactivation following exercise: implications for training prescription. *Sports Med.* 43, 1259–1277. doi: 10.1007/s40279-013-0083-4

Stapelberg, N. J. C., Neumann, D. L., Shum, D. H. K., McConnell, H., and Hamilton-Craig, I. (2018). The sensitivity of 38 heart rate variability measures to the addition of artefact in human and artificial 24-hr cardiac recordings. *Ann. Noninvas. Electrocardiol.* 23:e12483.

Stöggl, T., and Sperlich, B. (2014). Polarized training has greater impact on key endurance variables than threshold, high intensity, or high volume training. *Front. Physiol.* 5:33. doi: 10.3389/fphys.2014.00033

Stöggl, T. L., and Sperlich, B. (2019). Editorial: Training Intensity, Volume and Recovery Distribution Among Elite and Recreational Endurance Athletes. *Front. Physiol.* 10:592. doi: 10.3389/fphys.2019.00592

Tapanainen, J. M., Seppänen, T., Laukkanen, R., Loimaala, A., and Huikuri, H. V. (1999). Significance of the Accuracy of RR Interval Detection for the Analysis of New Dynamic Measures of Heart Rate Variability. *Anna. Noninvas. Electrocardiol.* 4, 10–17. doi: 10.1111/j.1542-474x.1999.tb00359.x

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Progr. Biomed.* 113, 210–220.

Tulppo, M. P., Mäkikallio, T. H., Takala, T. E., Seppänen, T., and Huikuri, H. V. (1996). Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *Am. J. Physiol.* 271, H244–H252.

van Wijck, K., Lenaerts, K., Grootjans, J., Wijnands, K. A., Poeze, M., Van Loon, L. J., et al. (2012). Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: strategies for evaluation and prevention. *Am. J. Physiol.* 303, G155–G168.

Venhorst, A., Micklewright, D., and Noakes, T. D. (2018). Towards a three-dimensional framework of centrally regulated and goal-directed exercise behaviour: a narrative review. *Br. J. Sports Med.* 52, 957–966. doi: 10.1136/bjsports-2016-096907

Voss, A., Wessel, N., Sander, A., Malberg, H., and Dietz, R. (1996). Requirements on sampling rate in Holter systems for analysis of heart rate variability. *Clin. Sci.* 91(Suppl.), 120–121. doi: 10.1042/cs0910120supp

Weston, S. B., Gray, A. B., Schneider, D. A., and Gass, G. C. (2002). Effect of ramp slope on ventilation thresholds and VO2peak in male cyclists. *Int. J. Sports Med.* 23, 22–27. doi: 10.1055/s-2002-19267

Yeh, R. G., Chen, G. Y., Shieh, J. S., and Kuo, C. D. (2010). Parameter investigation of detrended fluctuation analysis for short-term human heart rate variability. *J. Med. Biol. Eng.* 30, 277–282. doi: 10.5405/jmbe.30.5.02

Yeh, M. P., Gardner, R. M., Adams, T. D., Yanowitz, F. G., and Crapo, R. O. (1983). "Anaerobic threshold": problems of determination and validation. *J. Appl. Physiol.* 55, 1178–1186. doi: 10.1152/jappl.1983.55.4.1178

**Conflict of Interest:** DG was employed by company Lattice Training.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 28 January 2021
doi: 10.3389/fnins.2021.610955

# Survival Predictors of Heart Rate Variability After Myocardial Infarction With and Without Low Left Ventricular Ejection Fraction

*Junichiro Hayano[1]*, Norihiro Ueda[1], Masaya Kisohara[1], Emi Yuda[2], Robert M. Carney[3] and James A. Blumenthal[4]*

*[1] Department of Medical Education, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, [2] Tohoku University Graduate School of Engineering, Sendai, Japan, [3] Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, United States, [4] Department of Psychiatry, Duke University Medical Center, Durham, NC, United States*

**Background:** Heart rate variability (HRV) and heart rate (HR) dynamics are used to predict the survival probability of patients after acute myocardial infarction (AMI), but the association has been established in patients with mixed levels of left ventricular ejection fraction (LVEF).

**Objective:** We investigated whether the survival predictors of HRV and HR dynamics depend on LVEF after AMI.

**Methods:** We studied 687 post-AMI patients including 147 with LVEF ≤35% and 540 with LVEF >35%, of which 23 (16%) and 22 (4%) died during the 25 month follow-up period, respectively. None had an implanted cardioverter-defibrillator. From baseline 24 h ECG, the standard deviation (SDNN), root mean square of successive difference (rMSSD), percentage of successive difference >50 ms (pNN50) of normal-to-normal R-R interval, ultra-low (ULF), very-low (VLF), low (LF), and high (HF) frequency power, deceleration capacity (DC), short-term scaling exponent ($\alpha_1$), non-Gaussianity index ($\lambda_{25s}$), and the amplitude of cyclic variation of HR (Acv) were calculated.

**Results:** The predictors were categorized into three clusters; DC, SDNN, $\alpha_1$, ULF, VLF, LF, and Acv as Cluster 1, $\lambda_{25s}$ independently as Cluster 2, and rMSSD, pNN50, and HF as Cluster 3. In univariate analyses, mortality was best predicted by indices belonging to Cluster 1 regardless of LVEF. In multivariate analyses, however, mortality in patients with low LVEF was best predicted by the combinations of Cluster 1 predictors or Cluster 1 and 3 predictors, whereas in patients without low LVEF, it was best predicted by the combinations of Cluster 1 and 2 predictors.

**Conclusion:** The mortality risk in post-AMI patients with low LVEF is predicted by indices reflecting decreased HRV or HR responsiveness and cardiac parasympathetic dysfunction, whereas in patients without low LVEF, the risk is predicted by a combination of indices that reflect decreased HRV or HR responsiveness and indicator that reflects abrupt large HR changes suggesting sympathetic involvement.

**Keywords: heart rate dynamics, heart rate variability, myocardial Infarction, mortality, redundancy, risk stratification, survival, left ventricular ejection fraction**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Dorota Zyśko, Wrocław Medical University, Poland*
*Richard Sutton, Imperial College London, United Kingdom*

****Correspondence:***
*Junichiro Hayano*
*hayano@acm.org*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *28 September 2020*
***Accepted:*** *06 January 2021*
***Published:*** *28 January 2021*

***Citation:***
*Hayano J, Ueda N, Kisohara M, Yuda E, Carney RM and Blumenthal JA (2021) Survival Predictors of Heart Rate Variability After Myocardial Infarction With and Without Low Left Ventricular Ejection Fraction. Front. Neurosci. 15:610955. doi: 10.3389/fnins.2021.610955*

## INTRODUCTION

Despite significant achievements in its clinical management (Antman et al., 2004), acute myocardial infarction (AMI) remains a leading cause of death (Virani et al., 2020). AMIs occur in the United States at a rate of 1 person every 40 s, with an associated mortality of approximately 110,000 per year (Virani et al., 2020). Sudden cardiac death (SCD) is the most common cause of death after AMI (Zaman and Kovoor, 2014), and patients with a low left ventricular ejection fraction (LVEF) are at the highest risk of SCD during the early months to years after AMI (Solomon et al., 2005; Adabag et al., 2008). To prevent SCD, prophylactic implantation of cardioverter-defibrillators has been recommended for post-AMI patients with LVEF ≤35% (Moss et al., 2002; Goldberger and Lampert, 2006). However, the generalization of reperfusion therapy early after AMI onset (Aversano et al., 2002) has reduced the proportion of post-AMI patients with low LVEF, and consequently, the majority of SCDs occur in patients with LVEF >35%. It has become more important to find clinical markers to predict an increased risk of death in patients without low LVEF (Arevalo et al., 2016). In this study, we analyzed heart rate variability (HRV) and heart rate (HR) dynamics in post-AMI patients to determine the useful markers and combinations to predict mortality risk separately between patients with LVEF ≤35% and those with LVEF >35%.

The analysis of HRV and HR dynamics are widely used for survival risk stratification in cardiovascular diseases (Camm et al., 1996), particularly after AMI (Kleiger et al., 1987; Bigger et al., 1992; Peng et al., 1995; La Rovere et al., 1998; Huikuri et al., 2000; Bauer et al., 2006; Kantelhardt et al., 2007; Kiyono et al., 2007, 2008; Hayano et al., 2011a, 2017; Watanabe et al., 2016). The R-R interval time series data obtained from the 24 h Holter ECG are mainly used for these analyses and many indices have been proposed. The HRV indices are classified into time-domain and frequency-domain indices (Camm et al., 1996). The time-domain indices include the statistical measures of normal-to-normal (N-N) interval (R-R interval of consecutive sinus rhythms) variation, such as the standard deviation of 24 h N-N interval (SDNN) (Kleiger et al., 1987), root mean square of successive N-N interval difference (rMSSD), percentage of successive N-N intervals differing >50 ms (pNN50), deceleration capacity (DC) (Kantelhardt et al., 2007), and the amplitude of cyclic variation of HR (Acv) (Hayano et al., 2011b). Among these, rMSSD and pNN50 that quantify high-frequency N-N interval fluctuations reflect the tonic or sustained level of cardiac parasympathetic control (Berntson et al., 1997; Laborde et al., 2017). Due to a low-pass filter-like-transfer function, the sympathetic HR control cannot involve the modulation of these high-frequency fluctuations (Berger et al., 1989), and thus, these fluctuations are mediated purely by the vagus. In contrast, Acv reflects the HR responsiveness to apneic episodes during sleep. It quantifies the shortening in cardiac cycles caused by sleep-apnea-induced transient arousals. Because this HR response is abolished by atropine (Zwillich et al., 1982; Guilleminault et al., 1984), Acv is thought to reflect a reflex parasympathetic function. The tonic and reflex parasympathetic dysfunction is believed to be a risk for post-AMI mortality (Camm et al., 1996; Bauer et al., 2006; Hayano et al., 2017) because parasympathetic antagonism against sympathetic activation is important to maintain ventricular myocardial electric stability and to prevent the development of fatal ventricular arrhythmias (Hull et al., 1990, 1994; La Rovere et al., 1998).

The frequency-domain indices of HRV are calculated by the power spectral analysis of N-N interval time series and are quantified as the power of frequency components. Among such components, ultra-low frequency (<0.0033 Hz; ULF) and very-low-frequency (0.0033–0.04 Hz; VLF) components reflect fractal-like HR fluctuation that accounts for most of the power of 24 h HRV (Saul et al., 1988). A reduction in the VLF power is one of the most powerful predictors of post-AMI mortality (Bigger et al., 1992). In contrast, a reduction in the high-frequency component (HF, 0.15–0.40 Hz), which is thought to reflect cardiac parasympathetic dysfunction, paradoxically shows the lowest predictive power (Bigger et al., 1992). This paradox may be explained at least partly by the contamination of non-autonomic high-frequency R-R interval fluctuations caused by heart rate fragmentation (Costa et al., 2017; Hayano et al., 2020), which is a type of pacemaker dysfunction more likely to appear in high-risk patients (Costa et al., 2018).

The HR dynamics reflect the non-linear properties of HR fluctuation. Detrended fluctuation analysis (Peng et al., 1995) quantifies the scaling exponents of fractal-like HR dynamics and a reduction in the short-term (4–11 beats) scaling exponent ($\alpha_1$) is increased risk for post-AMI mortality (Huikuri et al., 2000). The non-Gaussianity index ($\lambda$) quantifies the probability density function for abrupt large HR changes suggesting sympathetic involvement (Kiyono et al., 2007). The $\lambda$ is increased in patients with heart failure, known as the state of increased sympathetic activity, while other HRV indices are decreased (Kiyono et al., 2007). Additionally, $\lambda$ is lower in these patients taking beta-blocker than in those without taking beta-blocker (Kiyono et al., 2007). An increase in $\lambda$ calculated at a time scale of 25 s ($\lambda_{25s}$) predicts increased risk for post-AMI cardiac mortality (Hayano et al., 2011a).

In the present study, we hypothesized that the HRV and HR dynamics indices and their combinations to predict post-AMI mortality risk differ between patients with and without low LVEF (≤35%). Most of earlier studies reporting predictive power of HRV and HR dynamics were conducted in post-AMI patients with mixed levels of LVEF (Kleiger et al., 1987; Bigger et al., 1992; Zuanetti et al., 1996; Lanza et al., 1998; Huikuri et al., 2000; Hayano et al., 2011a). The risk stratification models developed by the earlier studies may need to be reappraised separately depending on LVEF. The prophylactic ICD in post-AMI patients with low LVEF could also modify the risk structures. Considering these factors, we chose 24 h ECG data from the post-AMI cohort collected before ICD

**Abbreviations:** Acv, amplitude of cyclic variation of heart rate; ALLSTAR, Allostatic State Mapping by Ambulatory ECG Repository; AMI, acute myocardial infarction; DC, deceleration capacity; ENRICHD, Enhancing Recovery in Coronary Heart Disease; Fcv, frequency of cyclic variation of heart rate; HR, heart rate; HRF, heart rate fragmentation; HRV, heart rate variability; SDNN, standard deviation of normal-to-normal R-R interval; VLF, very low frequency.

became clinically widespread and we compared the HRV and HR dynamics indices associated with mortality risk between patients with and without low LVEF. Furthermore, considering the possible redundancy existing among the indices of HRV and HR dynamics (Yuda et al., 2020), we categorized the indices into classes by cluster analysis and analyzed the class-relationships with mortality risk.

# MATERIALS AND METHODS

## Study Cohort

We examined retrospective cohort data from a subset of the Enhancing Recovery in Coronary Heart Disease (ENRICHD) study (Berkman et al., 2003) consisting of 687 patients who had an AMI and were admitted to the coronary care units of 4 of the 8 ENRICHD clinical trial sites (Washington University, St. Louis, Missouri; Duke University, Durham, North Carolina; Harvard University, Boston, Massachusetts; Yale University, New Haven, Connecticut) between October 1997 and January 2000. The sample included 327 participants of the ENRICHD clinical trial who scored 10 or higher on the Beck Depression Inventory (Beck, 1972) and 360 AMI control participants who were not randomized in the ENRICHD trial because they were not depressed, but were otherwise medically eligible for the trial. Patients were included if they had analyzable Holter ECG data >20.4 h (85% of 24 h) including >3 h of sleep period (time in bed). Patients were excluded if they: (1) had other life-threatening illnesses; (2) were too ill or logistically unable to participate; (3) had ECG data in sinus rhythm <80% of total recorded beats, or (4) had atrial fibrillation, atrial flutter, or an implanted pacemaker or defibrillator. The collection and analysis of Holter ECG recordings were approved by the ethics committees of the corresponding clinical sites. All participants provided written informed consent to participate in the study.

The end-point of the present study was all-cause mortality. Patients underwent follow-up assessments 6 months after study enrollment and annually thereafter for up to 30 months. The end-points were identified from follow-up visits, telephone calls, routine hospital surveillance, and contacts with patients' physicians. The records of every identified hospitalization were obtained for review and confirmation by a panel of physicians. Death certificates were obtained for all reported deaths. The mortality endpoints used for the present study were either cardiac deaths (AMI, cardiac failure, and sudden cardiac death) or non-cardiac deaths.

## Measurements

Holter ECGs were recorded for 24 h within 28 [median (IQR), 13 (6–19)] days after the index AMI. The ECG recordings were scanned at the Heart Rate Variability Core laboratory at Washington University on a Marquette SXP Laser scanner with software version 5.8 (Marquette Electronics) using standard procedures. The annotated beat file was exported to a workstation for analysis of HRV and HR dynamics indices.

## Data Analysis

The time-domain and frequency-domain indices of HRV and the non-linear indices of HR dynamics that are known as major predictors of post-AMI mortality were calculated by the methods according to the recommended standard (Camm et al., 1996) and to previously published studies (Peng et al., 1995; Iyengar et al., 1996; Kantelhardt et al., 2007; Kiyono et al., 2007; Hayano et al., 2017).

Briefly, the time series of N-N intervals were derived from 24 h ECG data. For the time domain HRV indices, SDNN was computed as the 24 h standard deviation of N-N intervals, rMSSD was calculated as the square root of the mean square of 24 h successive N-N interval differences, pNN50 was obtained as the percentage of successive N-N intervals differing >50 ms, and DC was computed by the phase rectified signal averaging of the 24 h N-N interval time series (Kantelhardt et al., 2007). Acv was calculated by signal-averaging the amplitude of cyclic variation of HR detected by the method of auto-correlated wave detection with adaptive threshold algorithm (Hayano et al., 2011b).

For the frequency domain index, the N-N interval power spectrum was computed by a Fast Fourier transform with a Hanning window after interpolating with a horizontal step function and resampling at 2 Hz. The power spectral density was integrated for the power within the ULF (<0.0033 Hz), VLF (0.0033–0.04 Hz), LF (0.04–0.15 Hz), and HF (0.15–0.4 Hz) bands, respectively, and transformed into natural logarithmic values.

For the non-linear indices, the fractal correlation properties of HR dynamics were computed using the detrended fluctuation analysis and measured as the short-term (4–11 beat) scaling exponents ($\alpha_1$) (Peng et al., 1995; Iyengar et al., 1996). Also, the non-Gaussianity index of $\lambda$ was calculated at a time scale of 25 s ($\lambda_{25s}$) according to our previous work (Hayano et al., 2011a). This analysis detects the intermittency of HR increment. The intermittent behavior of HRV is related to non-Gaussian probability distribution with marked fat tails and a peak around the mean value, indicative of a higher probability of the interspersed appearance of large and small increments than the Gaussian fluctuations. The $\lambda$ quantifies the fatness of the tails of the non-Gaussian probability distribution. The mathematical description of the non-Gaussianity index has been reported elsewhere (Kiyono et al., 2004, 2007).

## Cluster Analysis of HRV and HR Dynamics Indices

To categorize HRV and HR dynamics indices, a cluster analysis was performed based on the correlation matrix between the indices. We used a divisive type cluster analysis. The analysis started with the assumption that all indices belong to a single cluster and continued to divide clusters until the eigenvalue of the second principal component of all clusters becomes less than 1. The cluster to which the index belongs was determined from the factor structure of the oblique principal component so that the index was classified into the clusters where the first principal component gives the highest correlation coefficient with the index.

## Evaluation of Predictive Performance

The predictive performance of the discriminant models, including those consisting of a single index and those of the combinations of multiple indices, was analyzed by logistic regression and evaluated by Somers' D and c-statistic. The logistic regression model provided prediction scores for individual participants and compared the scores between all possible pairs of survivors and non-survivors. Pairs with a survivor score higher than non-survivors were considered concordant, otherwise, they were considered discordant. Somers' D was calculated as the difference between the number of concordant and discordant pairs divided by the number of all possible pairs, taking a value from -1 (all pairs disagree) to 1 (all pairs agree). The c-statistic reflected the area under the receiver-operating characteristic curve for the predictive performance of the regression models.

## Statistical Analysis

We used SAS version 9.4 programs (SAS Institute, Cary, NC). Differences between survivors and non-survivors were evaluated by the Chi-square test for categorical data and by Wilcoxon two-sample test for continuous data. The SAS VARCLUS procedure with an oblique principal component cluster analysis was used to categorize the HRV and HR dynamics indices. The SAS LOGISTIC procedure was used for the logistic regression analysis for mortality risk stratification by HRV and HR dynamics indices and their combinations. All models included age as an independent predictor. For all statistical analyses, $P < 0.05$ was considered significant.

# RESULTS

## Patients' Characteristics

Patient characteristics are presented in **Table 1**. With baseline LVEF, the participants were divided into 147 patients with LVEF ≤35% (low LVEF) and 540 patients with LVEF >35%. During the follow-up period, 23 (16%) patients with low LVEF and 22 (4%) patients without low LVEF died from all-causes. Among patients with low LVEF, non-survivors were more often diabetic and mentally depressed, had lower LVEF, and had higher serum creatinine. Survivors were more likely to have had more frequent coronary angioplasty. Among patients without low LVEF, non-survivors were older and more often diabetic and smoker, had more frequent histories of coronary bypass surgery, had lower LVEF, had higher serum creatinine, and were more often Killip class III-IV after the index AMI. Survivors were more likely to have had an index AMI of the inferior wall and had more frequent acute reperfusion after the AMI.

## Cluster Analysis of HRV and HR Dynamics Indices

**Figure 1** shows the tree diagram of the hierarchical cluster based on the principal component of the correlation matrix. The cluster analysis was performed in all 687 patients without separating with LVEF. The predictors were found to be categorized into three clusters; DC, SDNN, $\alpha_1$, ULF, VLF, LF, and Acv as Cluster 1, $\lambda_{25s}$ independently as Cluster 2, and rMSSD, pNN50, and HF as Cluster 3.

## Univariate Associations of HRV and HR Dynamics With Post-AMI Mortality

**Table 2** shows the difference in HRV and HR dynamic indices between survivors and non-survivors. Regardless of LVEF, non-survivors had lower values for all indices in Cluster 1. Among patients with low LVEF, non-survivor has lower HF in Cluster 3, but $\lambda_{25s}$ (Cluster 2) did not differ significantly between survivors and non-survivors. Among patients without low LVEF, non-survivors had greater $\lambda_{25s}$(Cluster 2), and lower values for all indices in Cluster 3.

**Table 3** shows the results of the univariate logistic regression analysis. Regardless of LVEF, the top five predictors based on the c-statistic belonged to Cluster 1.

## Multivariate Associations of HRV and HR Dynamics With Post-AMI Mortality

**Table 4** shows the results of logistic regression analyses for all combinations between two predictors. Among patients with low LVEF, the top five performances were observed with the combinations between two predictors both in Cluster 1 and the combination between Cluster 1 and 3 predictors. In contrast, among patients without low LVEF, the top five performances were observed with the combinations between Cluster 1 and 2 predictors.

These features were also observed for the prediction models consisting of three predictors (**Table 5**). The mortality in patients with low LVEF was best predicted by the combinations of Cluster 1 and 3 predictors. In patients without low LVEF, the top four performances were observed with the combinations between Cluster 1 and 2, although the combinations of Cluster 1, 2, and 3 predictors also showed the 4th best performance.

# DISCUSSION

In this study, we sought to determine if HRV and HR dynamics indices that predict mortality risk after AMI differ between patients with and without low LVEF (≤35%). Considering the possible redundancy existing among HRV and HR dynamics indices (Yuda et al., 2020), we first categorized the predictors into classes. The cluster analysis revealed that the predictors can be classified into 3 clusters thought to reflect the magnitude of HRV or HR responsiveness (Cluster 1: DC, SDNN, $\alpha_1$, ULF, VLF, LF, and Acv), the frequency of abrupt large HR changes (Cluster 2: $\lambda_{25s}$), and cardiac parasympathetic function (Cluster 3: rMSSD, pNN50, and HF), respectively. Then, we examined the associations between clustered predictors and mortality risk in patients with and without low LVEF, separately. Univariate analyses showed that mortality was best predicted by indices belonging to Cluster 1 regardless of LVEF, but multivariate analyses showed that mortality in patients with low LVEF was best predicted by the combinations of two Cluster 1 predictors or Cluster 1 and 3 predictors, while in patients without low LVEF, it was best predicted by the combinations

**TABLE 1 |** Patients' characteristics.

| | LVEF ≤35% | | | LVEF >35% | | |
|---|---|---|---|---|---|---|
| | **Survivor** | **Non-survivor** | ***P**** | **Survivor** | **Non-survivor** | ***P**** |
| Number of patients, *n* | 124 (84%) | 23 (16%) | | 518 (96%) | 22 (4%) | |
| **Outcome** | | | | | | |
| Follow-up (days), median (IQR) | 778 (590–1,024) | 499 (175–657) | <0.0001 | 769 (574–974) | 373 (203–696) | <0.0001 |
| Cardiac death | 0 (0%) | 18 (78%) | | 0 (0%) | 14 (64%) | |
| **Demographic and clinical** | | | | | | |
| Age (years), median (IQR) | 62 (53–71) | 63 (53–75) | 0.3 | 58 (49–67) | 69 (59–71) | 0.001 |
| Women | 37 (30%) | 10 (43%) | 0.1 | 215 (42%) | 10 (45%) | 0.7 |
| Body mass index (kg/m$^2$), median (IQR) | 27.6 (24.5–31.2) | 28.3 (24.0–35.0) | 0.6 | 28.5 (25.4–32.1) | 28.4 (26.2–32.4) | 0.6 |
| Hypertension | 21 (17%) | 4 (17%) | 0.9 | 107 (21%) | 8 (36%) | 0.05 |
| Diabetes mellitus | 32 (26%) | 15 (65%) | 0.0002 | 128 (25%) | 17 (77%) | <0.0001 |
| Current smoker | 38 (31%) | 4 (17%) | 0.2 | 189 (36%) | 3 (14%) | 0.03 |
| BDI score ≥10 | 52 (42%) | 18 (78%) | 0.001 | 243 (47%) | 14 (64%) | 0.1 |
| History of myocardial infarction | 39 (31%) | 11 (48%) | 0.1 | 90 (17%) | 7 (32%) | 0.1 |
| History of coronary bypass surgery | 22 (18%) | 7 (30%) | 0.1 | 42 (8%) | 7 (32%) | <0.0001 |
| LVEF (%), median (IQR) | 30 (26–35) | 25 (20–30) | 0.0007 | 52 (45–55) | 45 (40–52) | 0.01 |
| Creatinine (mg/dL), median (IQR) | 1.0 (0.8–1.2) | 1.3 (1.1–2.3) | <0.0001 | 1.0(0.8–1.1) | 1.2 (0.9–2.1) | 0.01 |
| **Index AMI** | | | | | | |
| Killip class III-IV | 15 (12%) | 5 (22%) | 0.2 | 15 (3%) | 3 (14%) | 0.003 |
| Anterior wall AMI | 68 (55%) | 9 (39%) | 0.2 | 141 (27%) | 4 (18%) | 0.4 |
| Inferior wall AMI | 42 (34%) | 5 (22%) | 0.3 | 258 (50%) | 5 (23%) | 0.01 |
| **Treatment** | | | | | | |
| β-Blockers | 101 (81%) | 17 (74%) | 0.3 | 424 (82%) | 20 (91%) | 0.2 |
| Angiotensin converting enzyme inhibitors | 90 (73%) | 16 (70%) | 0.7 | 222 (43%) | 11 (50%) | 0.5 |
| Aspirin | 110 (89%) | 17 (74%) | 0.05 | 476 (92%) | 17 (77%) | 0.01 |
| Calcium channel blockers | 12 (10%) | 3 (13%) | 0.6 | 74 (14%) | 7 (32%) | 0.02 |
| Thrombolytic therapy after AMI | 38 (31%) | 6 (26%) | 0.7 | 173 (33%) | 2 (9%) | 0.02 |
| Coronary bypass after AMI | 27 (22%) | 1 (4%) | 0.05 | 73 (14%) | 2 (9%) | 0.5 |
| Coronary angioplasty ≤24 h after AMI | 66 (53%) | 4 (17%) | 0.004 | 334 (64%) | 12 (55%) | 0.2 |
| Acute reperfusion < = 12 h after AMI | 55 (44%) | 6 (26%) | 0.1 | 250 (48%) | 5 (23%) | 0.02 |

**Significance of difference by Wilcoxon two sample test for continuous variables and by chi-square test for categorical variables. AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction.*

of Cluster 1 and 2 predictors. Our findings indicate that the mortality risk in post-AMI patients with low LVEF is predicted by decreased HRV or HR responsiveness and cardiac parasympathetic dysfunction, whereas in patients without low LVEF, the risk is predicted by a combination of decreased HRV or HR responsiveness and increased abrupt large HR changes suggesting sympathetic involvement.

To our knowledge, this is the first study to compare HRV and HR dynamics indices that predict mortality between post-AMI patients with and without low LVEF. Most of earlier studies reporting predictive power of HRV and HR dynamics were conducted in post-AMI patients with mixed levels of LVEF, although they reported the independence of the predictive power of the indices from LVEF (Kleiger et al., 1987; Bigger et al., 1992; Zuanetti et al., 1996; Lanza et al., 1998; Hayano et al., 2011a). Also, Huikuri et al. (2000) examined the predictive value of HRV and HR dynamics in post-AMI patients with LVEF ≤35% and reported that a decrease in $\alpha_1$ had greater predictive power of post-AMI mortality than conventional HRV indices. Bauer et al. (2006) demonstrated that a decrease in DC had greater predictive power than SDNN and LVEF and reported that the risk stratification by DC was more useful in patients with LVEF >30% than in those with LVEF ≤30%. Liu et al. (2020) recently reported that decreased SDNN, VLF, and DC were independently associated with increased risk of sudden arrhythmic death in post-AMI patients with LVEF ≤35% and that combination of SDNN, VLF, and DC may help identify a high-risk patient group. Lombardi et al. (1996) compared HRV and HR dynamics indices between post-AMI patients with and without low LVEF and they observed reduced HRV power in the entire frequency range in patients with low LVEF, suggesting diminished responsiveness of sinus node to autonomic modulatory inputs in these patients. None of these studies, however, reported the difference in predictors between post-AMI patients with and without low LVEF.

In this study, we used retrospective cohort data of the ENRICHD study. The patients of this cohort had an AMI and admitted hospital between October 1977 and January 2000. Therefore, the fraction of patients who received a primary percutaneous coronary intervention was low and none of them

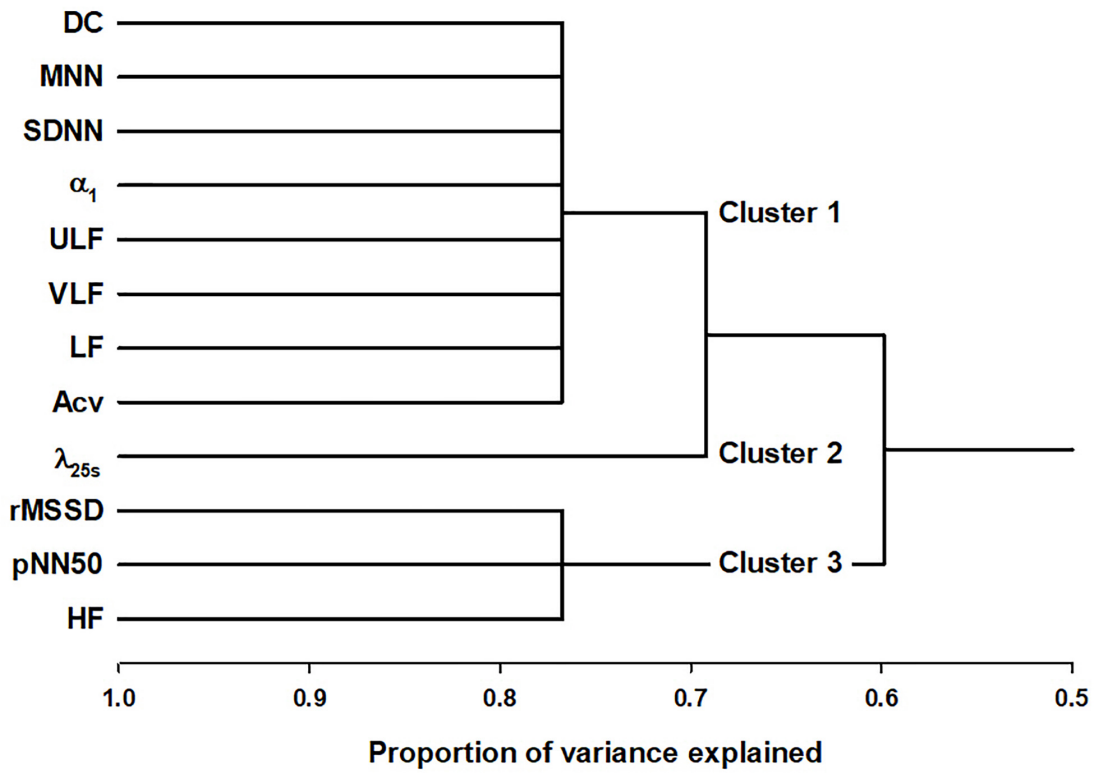



**FIGURE 1 |** Cluster analysis of HRV and HR dynamics indices in post-AMI patients. DC, deceleration capacity; SDNN, standard deviation of normal-to-normal R-R interval during 24 h; ULF, power of ultra-low-frequency (<0.0033 Hz) component; VLF, power of very-low-frequency (0.0033–0.04 Hz) component; LF, power of low-frequency (0.04–0.15 Hz) component; Acv, amplitude of the cyclic variation of heart rate; $\lambda_{25s}$, non-Gaussianity index for a segment length of 25 s; Fcv, frequency of the cyclic variation of heart rate; rMSSD, root mean square of successive R-R interval differences; pNN50, percentage of successive R-R intervals differing >50 ms, and HF, power of high-frequency (0.15–0.40 Hz) component.

had an ICD. We chose this cohort to allow comparison of post-AMI patients without low LVEF with a sufficiently sized group of patients with low LVEF whose survival risk is not affected by a prophylactic ICD. Additionally, the sample of this study included a subset of patients enrolled in the ENRICHD trial who had elevated symptoms of depression, which could affect the generalizability of our results. However, the proportion of the depressed patients with BDI scores ≥10 was 47.5%, which is comparable to the reported prevalence of depression (45–47%) in general post-AMI populations (Schleifer et al., 1989; Steeds et al., 2004).

We performed a cluster analysis of HRV and HR dynamics indices in the entire cohort of post-AMI patients. The indices were classified into three clusters and we observed that the associations between the HRV and HR dynamics indices and mortality risk were well explained as class-dependent relationships. These findings provide several insights into the underlying mechanisms.

First, the formation of clusters indicates that there are significant inter-correlations between these indices by the eigenvalue criteria of principal component analysis, supporting our previous finding of a big-data study reporting the substantial redundancy among HRV and HR dynamics indices (Yuda et al., 2020).

Second, the observation that all of the top five univariate predictors of post-AMI mortality belonged to Cluster 1 regardless of LVEF indicates the prognostic significance of the feature(s) common to the indices of this cluster. Although Cluster 1 includes a variety of indices, they commonly reflect the magnitude of HRV, such as SDNN, ULF, VLF, and LF, which are thought to be mediated by interactions between sympathetic and parasympathetic nerve activities, although parasympathetic dysfunction has been thought to be a primary cause of decreased HRV at rest and during sleep (Camm et al., 1996). Earlier studies reported that 92% of VLF power was suppressed by high dose atropine (0.04 mg/kg) (Taylor et al., 1998). DC has been developed to measure the rapid increase in R-R intervals caused only by parasympathetic control (Kantelhardt et al., 2007). The $\alpha_1$ increases with atropine and decreases with parasympathetic activation (Tulppo et al., 2001, 2005), although it decreases with increased levels of circulating noradrenaline in healthy men (Tulppo et al., 2001) and increases with β-blocker therapy in patients with heart failure (Lin et al., 2001; Ridha et al., 2002). Acv is thought to reflect a reflex parasympathetic function and its decrease indicates blunted parasympathetic responses to sleep apnea episodes (transient hypoxia, arousal, etc.) (Hayano et al., 2017). Acv is almost completely abolished by 2 mg of intravenous atropine but is unchanged by 5 mg of intravenous propranolol

**TABLE 2 |** Comparisons of baseline heart rate variability (HRV) and heart rate (HR) dynamics indices between survivors and non-survivors.

| Index, median (IQR) | LVEF ≤35% | | | LVEF >35% | | |
|---|---|---|---|---|---|---|
| | Survivor | Non-survivor | *P** | Survivor | Non-survivor | *P** |
| DC (ms) | 4.2 (3.1 – 6.2) | 2.9 (2.3 – 3.6) | 0.0002 | 5.3 (3.7 – 6.7) | 2.7 (2.2 – 3.8) | <0.0001 |
| SDNN (ms) | 87 (63 – 109) | 56 (47 – 73) | 0.0004 | 90 (69 – 118) | 65 (54 – 78) | 0.0008 |
| $\alpha_1$ | 1.2 (0.9 – 1.3) | 0.8 (0.6 – 1.1) | 0.002 | 1.2 (1 – 1.3) | 0.8 (0.7 – 1.2) | 0.001 |
| ULF [ln($ms^2$)] | 8.6 (7.9 – 9.1) | 7.7 (7.3 – 8.3) | 0.0005 | 8.7 (8 – 9.2) | 8 (7.6 – 8.4) | 0.001 |
| VLF [ln($ms^2$)] | 6.7 (6 – 7.3) | 5.7 (4.9 – 6.1) | 0.0002 | 6.8 (6 – 7.5) | 5.4 (4.6 – 6.3) | <0.0001 |
| LF[ln($ms^2$)] | 5.4 (4.3 – 6.4) | 4.1 (3.5 – 4.8) | 0.0005 | 5.6 (4.6 – 6.3) | 4.7 (2.8 – 5.3) | 0.0003 |
| Acv [ln(ms)] | 3.9 (3.5 – 4.3) | 3.3 (3 – 3.6) | <0.0001 | 4.2 (3.7 – 4.5) | 3.2 (2.9 – 3.7) | <0.0001 |
| $\lambda_{25s}$ | 0.5 (0.5 – 0.7) | 0.6 (0.4 – 0.7) | 0.7 | 0.5 (0.5 – 0.6) | 0.6 (0.5 – 0.6) | 0.002 |
| rMSSD (ms) | 20 (14 – 34) | 16 (12 – 24) | 0.1 | 23 (16 – 33) | 15 (11 – 29) | 0.01 |
| pNN50 (%) | 2.6 (0.4 – 10.7) | 0.8 (0.1 – 4.8) | 0.09 | 3.1 (0.8 – 10.2) | 0.5 (0.1 – 6.8) | 0.01 |
| HF [ln($ms^2$)] | 4.4 (3.5 – 5.5) | 3.6 (2.9 – 4.5) | 0.03 | 4.6 (3.9 – 5.5) | 3.5 (3 – 5.2) | 0.009 |

**Significance of difference by Wilcoxon two sample test. Abbreviations are explained in the legend of **Figure 1**.*

**TABLE 3 |** Predictive power of HRV and HR dynamics indices for post-AMI mortality (logistic regression analysis).

| Predictor | LVEF ≤35% | | | | LVEF >35% | | | |
|---|---|---|---|---|---|---|---|---|
| | Concordant,% | Discordant,% | Somers' D | c-Statistic | Concordant,% | Discordant,% | Somers' D | c-Statistic |
| DC | 74.4 | 25.6 | 0.489 | **0.744** | 82.7 | 17.3 | 0.655 | **0.827** |
| SDNN | 75.6 | 24.4 | 0.512 | **0.756** | 77.1 | 22.9 | 0.542 | 0.771 |
| $\alpha_1$ | 70.0 | 30.0 | 0.399 | 0.700 | 74.7 | 25.3 | 0.493 | 0.747 |
| ULF | 74.1 | 25.9 | 0.481 | 0.741 | 77.7 | 22.3 | 0.553 | **0.777** |
| VLF | 75.4 | 24.6 | 0.508 | **0.754** | 80.9 | 19.1 | 0.618 | **0.809** |
| LF | 74.5 | 25.5 | 0.490 | **0.745** | 79.1 | 20.9 | 0.582 | **0.791** |
| Acv | 80.7 | 19.3 | 0.614 | **0.807** | 82.5 | 17.5 | 0.649 | **0.825** |
| $\lambda_{25s}$ | 53.5 | 46.5 | 0.070 | 0.535 | 74.9 | 25.1 | 0.497 | 0.749 |
| rMSSD | 56.5 | 43.5 | 0.130 | 0.565 | 73.3 | 26.7 | 0.465 | 0.733 |
| pNN50 | 56.6 | 43.4 | 0.133 | 0.566 | 73.0 | 27.0 | 0.461 | 0.730 |
| HF | 65.4 | 34.6 | 0.309 | 0.654 | 76.8 | 23.2 | 0.536 | 0.768 |

*All classification models are adjusted for the effect of age. Boldface indicates the top five largest c-statistic values for LVEF ≥35% and LVEF >35%, respectively. Abbreviations are explained in the legend of **Figure 1**.*

**TABLE 4 |** Predictive performance (c-statistics) of combinations of two predictors among post-AMI patients grouped by LVEF.

| | DC | SDNN | $\alpha_1$ | ULF | VLF | LF | Acv | $\lambda_{25s}$ | rMSSD | pNN50 | HF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DC | – | 0.824 | 0.823 | 0.825 | 0.824 | 0.823 | **0.830** | **0.840** | 0.828 | 0.830 | 0.824 | **LVEF > 35%** |
| SDNN | 0.773 | – | 0.775 | 0.778 | 0.808 | 0.792 | 0.824 | 0.803 | 0.771 | 0.772 | 0.780 | |
| $\alpha_1$ | 0.768 | 0.773 | – | 0.782 | 0.802 | 0.789 | 0.816 | 0.769 | 0.791 | 0.792 | 0.791 | |
| ULF | 0.743 | 0.745 | 0.726 | – | 0.810 | 0.800 | 0.830 | 0.815 | 0.782 | 0.781 | 0.792 | |
| VLF | 0.758 | 0.762 | 0.751 | 0.754 | – | 0.809 | 0.826 | **0.832** | 0.808 | 0.808 | 0.808 | |
| LF | 0.765 | 0.767 | 0.766 | 0.742 | 0.733 | – | 0.825 | **0.832** | 0.790 | 0.791 | 0.790 | |
| Acv | 0.806 | **0.810** | **0.811** | 0.806 | 0.807 | **0.816** | – | **0.836** | 0.824 | 0.823 | 0.826 | |
| $\lambda_{25s}$ | 0.728 | 0.728 | 0.672 | 0.739 | 0.746 | 0.740 | 0.807 | – | 0.801 | 0.787 | 0.8327 | |
| rMSSD | 0.738 | 0.769 | 0.730 | 0.732 | 0.761 | 0.773 | **0.816** | 0.551 | – | 0.728 | 0.769 | |
| pNN50 | 0.740 | 0.759 | 0.737 | 0.741 | 0.760 | 0.765 | **0.817** | 0.581 | 0.556 | – | 0.769 | |
| HF | 0.738 | 0.744 | 0.768 | 0.732 | 0.759 | 0.751 | 0.808 | 0.684 | 0.717 | 0.699 | – | |
| | | | | | | **LVEF ≤ 35%** | | | | | | |

*Data are c-statistics calculated by logistic regression analysis. Values in lower left half and upper right half represent those for patients with LVEF ≤35% and those with LVEF > 35%, respectively. Boldface indicates the top five largest values for LVEF ≤35% and LVEF >35%, respectively. All classification models are adjusted for the effect of age. Abbreviations are explained in the legend of **Figure 1**.*

**TABLE 5 |** Combinations of three predictors with the top five predictive performance.

| Best combination | Concordant,% | Discordant,% | Somers' D | c-Statistic |
|---|---|---|---|---|
| **LVEF ≤35%** | | | | |
| $\alpha_1$ + Acv + rMSSD | 82.1 | 17.9 | 0.641 | 0.821 |
| Acv + rMSSD + HF | 81.8 | 18.2 | 0.636 | 0.818 |
| Acv + rMSSD + pNN50 | 81.5 | 18.5 | 0.629 | 0.815 |
| SDNN + Acv + rMSSD | 81.5 | 18.5 | 0.63 | 0.815 |
| ULF + Acv + rMSSD | 81.5 | 18.5 | 0.63 | 0.815 |
| **LVEF >35%** | | | | |
| ULF + Acv + $\lambda_{25s}$ | 84.4 | 15.6 | 0.689 | 0.844 |
| VLF + Acv + $\lambda_{25s}$ | 83.7 | 16.3 | 0.674 | 0.837 |
| ULF + VLF + $\lambda_{25s}$ | 83.2 | 16.8 | 0.665 | 0.832 |
| SDNN + VLF + $\lambda_{25s}$ | 83.1 | 16.9 | 0.661 | 0.831 |
| VLF + $\lambda_{25s}$ + pNN50 | 83.1 | 16.9 | 0.663 | 0.831 |

*All prediction models are adjusted for the effect of age. Abbreviations are explained in the footnote in **Table 2**.*

(Guilleminault et al., 1984). These indicate that decreased HRV or HR responsiveness mediated primarily by parasympathetic dysfunction is the most important single feature associated with mortality risk in post-AMI patients with and without low LVEF.

Third, the observation that mortality risk in patients with low LVEF was best predicted by the combinations of indices both in Cluster 1 or those in Cluster 1 and 3 indicates an increased risk of the coexistence of tonic/sustained and reflex parasympathetic disfunction. All of the top five combinations included Acv that reflects a reflex parasympathetic function. The other indices including those in Cluster 3 are thought to reflect the tonic or sustained level of parasympathetic function.

Fourth, the observation that mortality risk in patients without low LVEF was best predicted by the combinations of indices in Cluster 1 and 2 indicates an increased risk of the coexistence of decreased HRV or HR responsiveness and increased abrupt large HR changes. The $\lambda_{25s}$ reflects the fatness of tails of the probability density function of the magnitude of abrupt large HR changes. Its increase can occur when the relative frequency of large abrupt HR changes to smaller HR changes increases, suggesting the involvement of transient strong sympathetic activations (Kiyono et al., 2008, 2012). The $\lambda$ is increased in patients with heart failure and the level of increase is associated with mortality risk, while no other HRV or HR dynamics indices predict it (Kiyono et al., 2007). The $\lambda$ reflects the relative frequency of large abrupt HR changes but does not depend on the magnitude of HR change itself. Thus, this index could detect relative sympathetic overactivity even under the situation of reduces autonomic responsiveness.

Finally, the different predictive values of Cluster 2 predictor ($\lambda_{25s}$) between patients with and without low LVEF may be explained by the presence of overt or subclinical heart failure. In patients with low LVEF, the prognostic value of the indices of sympathetic overactivity could be less because sympathetic nerve activity is increased by heart failure, which most of these patients may have. In patients without low LVEF, the indices of sympathetic overactivity could have greater predictive value because it may reflect the presence or development of heart failure in a part of these patients.

## Limitations

Among Cluster 1 predictors, Acv was the best univariate predictor of post-AMI mortality, but this measure requires a cyclic variation of HR associated with sleep apnea episodes. Nevertheless, Acv was able to be calculated in all post-AMI patients. This is because the Holter ECG data having sleep period (time in bed) <3 h were not included in this study and because Acv can be calculated even in patients with subclinical sleep apnea if at least one episode of cyclic variation of HR is detected during sleep. Assuming cases in which Acv cannot be calculated, we examined logistic regression models excluding Acv, but the results for the relationships between clusters and mortality risk did not change (data not shown). Additionally, although study participants were recruited from four different clinical sites in diverse regions of the US, this study was performed using only one cohort of post-AMI patients. To confirm the present findings, future studies using different cohorts should be performed.

# CONCLUSION

We investigated whether the survival predictors of HRV and HR dynamics depend on LVEF after AMI. The mortality risk in post-AMI patients with low LVEF is predicted by indices that reflect decreased HRV or HR responsiveness and cardiac parasympathetic dysfunction, whereas in patients without low LVEF, the risk is predicted by a combination of predictors reflecting decreased HRV or HR responsiveness and increased abrupt large HR changes suggesting sympathetic involvement.

# DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data from this study are available upon request to the corresponding author. As the data contain potentially identifying or sensitive patient information, the use of the data is limited to the purpose and method of research approved by the ethics committees of the corresponding clinical

sites. Requests to access these datasets should be directed to Junichiro Hayano, hayano@acm.org.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the research ethics committees of Washington University, St. Louis, Missouri; Duke University, Durham, North Carolina; Harvard University, Boston, Massachusetts; and Yale University, New Haven, Connecticut. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JH and EY: conceptualization. JH: methodology, software, and writing—original draft preparation and visualization. EY, NU, and MK: validation. EY: formal analysis. MK and NU: investigation. RC and JB: resources, supervision, and funding acquisition. NU: data curation. EY and JB: writing—review and editing. JH and JB: project administration. All authors have read and agreed to the published version of the manuscript.

## FUNDING

This research was supported by the Grants HL 093374, HL080664, and HL58946 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland.

## ACKNOWLEDGMENTS

We thank Dr. Lisa Berkman at the Harvard T. H. Chan School of Public Health for providing data from the Yale University clinical site for our analysis.

## REFERENCES

Adabag, A. S., Therneau, T. M., Gersh, B. J., Weston, S. A., and Roger, V. L. (2008). Sudden death after myocardial infarction. *JAMA* 300, 2022–2029. doi: 10.1001/jama.2008.553

Antman, E. M., Anbe, D. T., Armstrong, P. W., Bates, E. R., Green, L. A., Hand, M., et al. (2004). ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction–executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to revise the 1999 guidelines for the management of patients with acute myocardial infarction). *J. Am. Coll. Cardiol.* 44, 671–719. doi: 10.1016/j.jacc.2004.07.002

Arevalo, H. J., Vadakkumpadan, F., Guallar, E., Jebb, A., Malamas, P., Wu, K. C., et al. (2016). Arrhythmia risk stratification of patients after myocardial infarction using personalized heart models. *Nat. Commun.* 7:11437. doi: 10.1038/ncomms11437

Aversano, T., Aversano, L. T., Passamani, E., Knatterud, G. L., Terrin, M. L., Williams, D. O., et al. (2002). Thrombolytic therapy vs primary percutaneous coronary intervention for myocardial infarction in patients presenting to hospitals without on-site cardiac surgery: a randomized controlled trial. *JAMA* 287, 1943–1951. doi: 10.1001/jama.287.15.1943

Bauer, A., Kantelhardt, J. W., Barthel, P., Schneider, R., Makikallio, T., Ulm, K., et al. (2006). Deceleration capacity of heart rate as a predictor of mortality after myocardial infarction: cohort study. *Lancet* 367, 1674–1681.

Beck, A. T. (1972). *Depression: Causes and Treatment*. Philadelphia, PA: University of Pennsylvania Press.

Berger, R. D., Saul, J. P., and Cohen, R. J. (1989). Transfer function analysis of autonomic regulation. I. Canine atrial rate response. *Am. J. Physiol.* 256, H142–H152.

Berkman, L. F., Blumenthal, J., Burg, M., Carney, R. M., Catellier, D., Cowan, M. J., et al. (2003). Effects of treating depression and low perceived social support on clinical events after myocardial infarction: the Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) Randomized Trial. *JAMA* 289, 3106–3116. doi: 10.1001/jama.289.23.3106

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648.

Bigger, J. T. Jr., Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., and Rottman, J. N. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 85, 164–171.

Camm, A. J., Malik, M., Bigger, J. T. Jr., Breithardt, G., Cerutti, S., Cohen, R. J., et al. (1996). Task Force of the european society of cardiology and the north american society of pacing and electrophysiology. heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93, 1043–1065.

Costa, M. D., Davis, R. B., and Goldberger, A. L. (2017). Heart rate fragmentation: a new approach to the analysis of cardiac interbeat interval dynamics. *Front. Physiol.* 8:255. doi: 10.3389/fphys.2017.00255

Costa, M. D., Redline, S., Davis, R. B., Heckbert, S. R., Soliman, E. Z., and Goldberger, A. L. (2018). Heart rate fragmentation as a novel biomarker of adverse cardiovascular events: the multi-ethnic study of atherosclerosis. *Front. Physiol.* 9:1117. doi: 10.3389/fphys.2018.01117

Goldberger, Z., and Lampert, R. (2006). Implantable cardioverter-defibrillators: expanding indications and technologies. *JAMA* 295, 809–818. doi: 10.1001/jama.295.7.809

Guilleminault, C., Connolly, S., Winkle, R., Melvin, K., and Tilkian, A. (1984). Cyclical variation of the heart rate in sleep apnoea syndrome. Mechanisms, and usefulness of 24 h electrocardiography as a screening technique. *Lancet* 1, 126–131.

Hayano, J., Kisohara, M., Ueda, N., and Yuda, E. (2020). Impact of Heart Rate Fragmentation on the Assessment of Heart Rate Variability. *Appl. Sci.* 10:3314.

Hayano, J., Kiyono, K., Struzik, Z. R., Yamamoto, Y., Watanabe, E., Stein, P. K., et al. (2011a). Increased non-gaussianity of heart rate variability predicts cardiac mortality after an acute myocardial infarction. *Front. Physiol.* 2:65. doi: 10.3389/fphys.2011.00065

Hayano, J., Watanabe, E., Saito, Y., Sasaki, F., Fujimoto, K., Nomiyama, T., et al. (2011b). Screening for obstructive sleep apnea by cyclic variation of heart rate. *Circ. Arrhythm. Electrophysiol.* 4, 64–72. doi: 10.1161/CIRCEP.110.958009

Hayano, J., Yasuma, F., Watanabe, E., Carney, R. M., Stein, P. K., Blumenthal, J. A., et al. (2017). Blunted cyclic variation of heart rate predicts mortality risk in post-myocardial infarction, end-stage renal disease, and chronic heart failure patients. *Europace* 19, 1392–1400. doi: 10.1093/europace/euw222

Huikuri, H. V., Makikallio, T. H., Peng, C. K., Goldberger, A. L., Hintze, U., and Moller, M. (2000). Fractal correlation properties of R-R interval dynamics and mortality in patients with depressed left ventricular function after an acute myocardial infarction. *Circulation* 101, 47–53.

Hull, S. S. Jr., Evans, A. R., Vanoli, E., Adamson, P. B., Stramba-Badiale, M., Albert, D. E., et al. (1990). Heart rate variability before and after myocardial infarction in conscious dogs at high and low risk of sudden death. *J. Am. Coll. Cardiol.* 16, 978–985.

Hull, S. S. Jr., Vanoli, E., Adamson, P. B., Verrier, R. L., Foreman, R. D., and Schwartz, P. J. (1994). Exercise training confers anticipatory protection from sudden death during acute myocardial ischemia. *Circulation* 89, 548–552.

Iyengar, N., Peng, C. K., Morin, R., Goldberger, A. L., and Lipsitz, L. A. (1996). Age-related alterations in the fractal scaling of cardiac interbeat interval dynamics. *Am. J. Physiol.* 271, R1078–R1084.

Kantelhardt, J. W., Bauer, A., Schumann, A. Y., Barthel, P., Schneider, R., Malik, M., et al. (2007). Phase-rectified signal averaging for the detection of quasi-periodicities and the prediction of cardiovascular risk. *CHAOS* 17:015112. doi: 10.1063/1.2430636

Kiyono, K., Hayano, J., Kwak, S., Watanabe, E., and Yamamoto, Y. (2012). Non-gaussianity of low frequency heart rate variability and sympathetic activation: lack of increases in multiple system atrophy and Parkinson disease. *Front. Physiol.* 3:34. doi: 10.3389/fphys.2012.00034

Kiyono, K., Hayano, J., Watanabe, E., Struzik, Z. R., and Yamamoto, Y. (2008). Non-Gaussian heart rate as an independent predictor of mortality in patients with chronic heart failure. *Heart Rhythm.* 5, 261–268.

Kiyono, K., Struzik, Z. R., Aoyagi, N., Sakata, S., Hayano, J., and Yamamoto, Y. (2004). Critical scale invariance in a healthy human heart rate. *Phys. Rev. Lett.* 93:178103.

Kiyono, K., Struzik, Z. R., and Yamamoto, Y. (2007). Estimator of a non-Gaussian parameter in multiplicative log-normal models. *Phys. Rev. E.* 76:041113.

Kleiger, R. E., Miller, J. P., Bigger, J. T. Jr., and Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 59, 256–262.

La Rovere, M. T., Bigger, J. T. Jr., Marcus, F. I., Mortara, A., and Schwartz, P. J. (1998). Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 351, 478–484.

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. *Frontiers in psychology.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lanza, G. A., Guido, V., Galeazzi, M. M., Mustilli, M., Natali, R., Ierardi, C., et al. (1998). Prognostic role of heart rate variability in patients with a recent acute myocardial infarction. *Am. J. Cardiol.* 82, 1323–1328. doi: 10.1016/s0002-9149(98)00635-3

Lin, L. Y., Lin, J. L., Du, C. C., Lai, L. P., Tseng, Y. Z., and Huang, S. K. (2001). Reversal of deteriorated fractal behavior of heart rate variability by beta-blocker therapy in patients with advanced congestive heart failure. *J. Cardiovasc. Electrophysiol.* 12, 26–32.

Liu, X., Xiang, L., and Tong, G. (2020). Predictive values of heart rate variability, deceleration and acceleration capacity of heart rate in post-infarction patients with LVEF >/=35. *Ann. Nonin. Electrocardiol.* 25:e12771. doi: 10.1111/anec.12771

Lombardi, F., Sandrone, G., Mortara, A., Torzillo, D., La Rovere, M. T., Signorini, M. G., et al. (1996). Linear and nonlinear dynamics of heart rate variability after acute myocardial infarction with normal and reduced left ventricular ejection fraction. *Am. J. Cardiol.* 77, 1283–1288. doi: 10.1016/s0002-9149(96)00193-2

Moss, A. J., Zareba, W., Hall, W. J., Klein, H., Wilber, D. J., Cannom, D. S., et al. (2002). Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. *N. Engl. J. Med.* 346, 877–883. doi: 10.1056/NEJMoa013474

Peng, C. K., Havlin, S., Stanley, H. E., and Goldberger, A. L. (1995). Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *CHAOS* 5, 82–87. doi: 10.1063/1.166141

Ridha, M., Makikallio, T. H., Lopera, G., Pastor, J., de Marchena, E., Chakko, S., et al. (2002). Effects of carvedilol on heart rate dynamics in patients with congestive heart failure. *Ann. Nonin. Electrocardiol.* 7, 133–138.

Saul, J. P., Albrecht, P., Berger, R. D., and Cohen, R. J. (1988). Analysis of long term heart rate variability: methods, 1/f scaling and implications. *Comput. Cardiol.* 14, 419–422.

Schleifer, S. J., Macari-Hinson, M. M., Coyle, D. A., Slater, W. R., Kahn, M., Gorlin, R., et al. (1989). The nature and course of depression following myocardial infarction. *Arch. Intern. Med.* 149, 1785–1789.

Solomon, S. D., Zelenkofske, S., McMurray, J. J., Finn, P. V., Velazquez, E., Ertl, G., et al. (2005). Sudden death in patients with myocardial infarction and left ventricular dysfunction, heart failure, or both. *N. Engl. J. Med.* 352, 2581–2588. doi: 10.1056/NEJMoa043938

Steeds, R. P., Bickerton, D., Smith, M. J., and Muthusamy, R. (2004). Assessment of depression following acute myocardial infarction using the Beck depression inventory. *Heart* 90, 217–218.

Taylor, J. A., Carr, D. L., Myers, C. W., and Eckberg, D. L. (1998). Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation* 98, 547–555. doi: 10.1161/01.cir.98.6.547

Tulppo, M. P., Kiviniemi, A. M., Hautala, A. J., Kallio, M., Seppanen, T., Makikallio, T. H., et al. (2005). Physiological background of the loss of fractal heart rate dynamics. *Circulation* 112, 314–319. doi: 10.1161/CIRCULATIONAHA.104.523712

Tulppo, M. P., Makikallio, T. H., Seppanen, T., Shoemaker, K., Tutungi, E., Hughson, R. L., et al. (2001). Effects of pharmacological adrenergic and vagal modulation on fractal heart rate dynamics. *Clin. Physiol.* 21, 515–523.

Virani, S. S., Alonso, A., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., Carson, A. P., et al. (2020). Heart Disease and stroke statistics-2020 update: a report from the american heart association. *Circulation* 141, e139–e596. doi: 10.1161/CIR.0000000000000757

Watanabe, E., Kiyono, K., Yamamoto, Y., and Hayano, J. (2016). "Heart rate variability and cardiac diseases," in *Clinical Assessment of the Autonomic Nervous System*, eds S. Iwase, J. Hayano, and S. Orimo (Berlin: Springer), 163–178.

Yuda, E., Ueda, N., Kisohara, M., and Hayano, J. (2020). Redundancy among risk predictors derived from heart rate variability and dynamics: ALLSTAR big data analysis. *Ann. Nonin. Electrocardiol.* 2:e12790. doi: 10.1111/anec.12790

Zaman, S., and Kovoor, P. (2014). Sudden cardiac death early after myocardial infarction: pathogenesis, risk stratification, and primary prevention. *Circulation* 129, 2426–2435. doi: 10.1161/CIRCULATIONAHA.113.007497

Zuanetti, G., Neilson, J. M., Latini, R., Santoro, E., Maggioni, A. P., and Ewing, D. J. (1996). Prognostic significance of heart rate variability in post-myocardial infarction patients in the fibrinolytic era. The GISSI-2 results. Gruppo Italiano per lo Studio della Sopravvivenza nell'. *Infarto Miocardico. Circulat.* 94, 432–436. doi: 10.1161/01.cir.94.3.432

Zwillich, C., Devlin, T., White, D., Douglas, N., Weil, J., and Martin, R. (1982). Bradycardia during sleep apnea. Characteristics and mechanism. *J. Clin. Invest.* 69, 1286–1292.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 11 February 2021
doi: 10.3389/fnins.2021.600865

# 24 h-Heart Rate Variability as a Communication Tool for a Personalized Psychosomatic Consultation in Occupational Health

*Marc N. Jarczok[1]*, Thomas Buckley[2], Harald O. Guendel[1,3], Irina Boeckelmann[4], Daniel Mauss[5], Julian F. Thayer[6] and Elisabeth M. Balint[1,3]*

*[1] Clinic for Psychosomatic Medicine and Psychotherapy, University Hospital Ulm, Ulm, Germany, [2] Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia, [3] Leadership Personal Center Ulm (LPCU), University of Ulm, Ulm, Germany, [4] Occupational Medicine, Faculty of Medicine, Otto-von-Guericke University, Magdeburg, Germany, [5] Mannheim Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, Heidelberg University, Heidelberg, Germany, [6] Department of Psychological Science, School of Social Ecology, University of California, Irvine, Irvine, CA, United States*

OPEN ACCESS

**Edited by:**
*Sylvain Laborde, German Sport University Cologne, Germany*

**Reviewed by:**
*Moacir Fernandes Godoy, Faculty of Medicine of São José do Rio Preto, Brazil*
*Michal Javorka, Comenius University, Slovakia*

***Correspondence:**
*Marc N. Jarczok*
*Marc.Jarczok@gmail.com; marc.jarczok@uniklinik-ulm.de*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

**Received:** *31 August 2020*
**Accepted:** *06 January 2021*
**Published:** *11 February 2021*

**Citation:**
*Jarczok MN, Buckley T, Guendel HO, Boeckelmann I, Mauss D, Thayer JF and Balint EM (2021) 24 h-Heart Rate Variability as a Communication Tool for a Personalized Psychosomatic Consultation in Occupational Health. Front. Neurosci. 15:600865. doi: 10.3389/fnins.2021.600865*

New tools for non-specific primary prevention strategies covering somatic and mental health in occupational medicine are urgently needed. Heart rate variability (HRV) reflects the capacity of the body to adapt to environmental challenges and of the mind to regulate emotions. Hence, a 24 h-measurement of HRV offers a unique possibility to quantify the interaction between situation-specific emotional regulation within a specific psychosocial environment and physiological state, thereby increasing self-perception and inducing motivation to change behavior. The focus of the present study represents such a 24 h-measurement of HRV and its presentation as a comprehensive graph including protocol situations of the client. A special training program for occupational health physicians and questionnaires for clients were developed and administered. The article reports the first data of the study "*healthy leadership and work – body signals for managers and employees*", an investigator-initiated, interventional, single-arm, open (non-blinded), multicenter, national trial with 168 participants. They reported a significantly improved perception of their bodily needs after the consultation (from Median = 7, interquartile range 5–8 to Median = 8, interquartile range 7–9; scale range from 1 to 10; $p < 0.001$, Wilcoxon rank test; effect size 0.49). The 16 occupational health physicians stated that the measurement of HRV was very well suited to enter into dialog with the managers and was feasible to show interactions between situations, thoughts, feelings, and bodily reactions. Taken together, we show that a 24 h-HRV-measurement can be a feasible and effective approach for holistic, psychosomatic primary prevention in occupational medicine. We discuss possible mechanisms for improving the individual health via the consultation, containing mindset and improved ANS activity.

**Keywords: 24 h-HRV, FFT, consultation, occupational health, workplace, screening**

## INTRODUCTION

The number of people with chronic diseases is increasing in society, for example, affecting approximately 40% of all Germans (Robert Koch-Institut (Hrsg.), 2014). The WHO attributes 60% of all deaths worldwide to chronic diseases, most of them to cardiovascular diseases (CVD) in Western countries (World Health Organisation, 2005). The costs of illness caused by CVD amounted to 46.4 billion Euros in Germany in 2015, whilst only 0.3% of this was spent on prevention in 2017 (DeStatis (Hrsg.), 2017; OECD (Hrsg.), 2020). Although CVD risk is partially determined by genetic factors, lifestyle plays an important preventative role; for example, physical activity and sleep can be associated with a reduced CVD risk of approximately 50% (Odegaard et al., 2011). Hence, opportunities for preventive activities are urgently needed. The present study investigates such an opportunity by implementing a 24 h-heart rate variability (HRV)-based consultation in a health check-up at the workplace, as this is a promising place for preventive activities.

Preventive activities at the workplace have the potential to address a large proportion of the population aged 20–64 as in Europe, the employment-to-population-ratio is 73%, meaning that 3 of 4 people interact with a workplace (Eurostat (Hrsg.), 2020). Thus, practical tools to A) easily assess an individuals current health state, B) increase knowledge about one's risks and resources, and improve understanding of bodily needs, and C) increase the motivation to change behavior are of particular interest in this area.

Nonetheless, existing preventive medical check-ups in occupational medicine often focus on secondary prevention only, as they merely detect an existing disease at an early stage or estimate the risk for a specific disease. For example, the *Findrisk* score assesses the risk of developing type 2 diabetes (Lindström and Tuomilehto, 2003) and the Framingham risk score estimates the cardiovascular (CV) risk within the next 10 years (Wilson et al., 1998). The risk of developing type 2 diabetes or CV diseases (CVD) is very low at younger ages, a time when primary prevention strategies should rather be implemented. On the other hand, addressing behavioral components of primary prevention such as engaging in physical activity, dietary habits, or stress prevention is not specific to a single disease. Trying to measure the effects of more general aspects like work stress raises several questions. Most commonly, the assessment of work stress is limited by missing objectivity as it is predominantly based on individuals' subjective perception and hence, self-report only (i.e., questionnaires). Although the association of perceived stress levels with various diseases has been reported (Backé et al., 2012; Lundberg, 2015; Theorell et al., 2015), the assessment of stress remains problematic at an individual level. The link between stress and disease risk is confounded by different variables such as individual genetic predisposition, sex, early life experience, individual resources, and actual coping strategies (Schneiderman et al., 2005; Rückholdt et al., 2019).

Unfortunately, most objective biological methods to measure stress levels are expensive, invasive, and often complicated to perform. For example, assessing a full cortisol circadian profile (a prominent stress hormone) comprising eight saliva samples is accompanied by low compliance because many participants perceive the strict protocol (exact timings, no food, no coffee, no cigarettes in the first hour post awakening) as impractical and protocol violation results in poor reliability (Stalder et al., 2016).

In addition to the hypothalamic-pituitary-adrenal (HPA) axis and associated cortisol response, there is another important stress pathway involving the central autonomic network (CAN) that is relaying its information primarily through the autonomic nervous system (ANS). This pathway can be indexed by measuring the variability between heartbeats, a valid measure of autonomic function (Wulsin et al., 2018). Two major components of the ANS can be differentiated. The parasympathetic component includes the cardiac branch of the 10th cranial nerve (i.e., the vagus nerve), which tonically inhibits intrinsic heart rate and modulates it on a beat to beat basis in milliseconds. In contrast, the sympathetic component modulates the heart rate at a slower frequency on a magnitude of seconds (below about 0.15 Hz). In detail, the vagus nerve represents a primary, bidirectional, and fast route transmitting physiological state to the brain (sensory part) as well as modulating somatic responses (motor part) to adapt to environmental challenges such as work demands (Bernard, 1867; Thayer and Lane, 2000; Wulsin et al., 2018). At the brain's end, the CAN controls the activity of preganglionic sympathetic and parasympathetic neurons. The CAN is involved in not only moment-to-moment modulation of visceral functions such as heart rate as described above but also in adaptation to internal or external challenges and the maintenance of homeostasis (Benarroch, 2014). Its network is organized on multiple levels, of which three are particularly important to this work: the forebrain – including the insular cortex, and anterior cingulate cortex, the limbic system including the amygdala, and the hypothalamus. Notably, these first three regions are involved in the integration of bodily sensations with emotional and goal-related autonomic responses, while the hypothalamus controls the integration of endocrine, autonomic, and sleep responses for homeostasis and adaptation. The second level includes, among others, the periaqueductal gray which integrates autonomic control of pain modulation and sleep, and the behavioral responses to environmental challenges (i.e., often termed stress) (Benarroch, 2014). Yet, it is the appraisal that turns an environmental or internal stimulus into a biological relevant stressor (Folkman et al., 1986). Thus, cardiac variability measures can provide a window to the working level of the CAN that reflects the capacity of the body to adapt to environmental challenges (Thayer et al., 2011) and to regulate emotions (Thayer and Lane, 2000), both pivotal in handling, e.g., work stress, like a problematic supervisor or deadline(s). The extent of the variability of heart rate is often used to predict intermediate or terminal endpoints (morbidity endpoints) such as the onset of depression or CVD, and mortality risk, such as from all causes, cardiovascular (e.g., hypertension), and cancer. For example, decreased values of HRV predict premature mortality and morbidity, e.g., higher inflammatory state (Jarczok et al., 2014; Aeschbacher et al., 2017), increased CVD risk (Kristal-Boneh et al., 1995; Mercedes et al., 2002; Jarczok et al., 2013b; Schuster et al., 2016) and myocardial infarction (MI) risk (Thayer and Lane, 2007). Research has also reported associations

with common mental disorders like depression (Kemp et al., 2014). Apart from diagnosed diseases, HRV parameters show associations to subjective measures like work stress (Jarczok et al., 2013a, 2020) and self-rated health (Jarczok et al., 2015). Results from five systematic reviews (partly overlapping) related to different aspects and years of occupational stress research suggest that various stress models such as the Job-Demand-Control Model (Karasek, 1979), the Effort-Reward-Imbalance Model (Siegrist, 1996), the concept of Organizational (In)justice (Elovainio et al., 2006; Herr et al., 2012), the perceived stress scale (Cohen et al., 1983) and burnout risk (MBI) (Maslach and Jackson, 1981) were found in the majority of studies to be associated with lower values of HRV measures. Specifically, higher questionnaire scores representing increased strain are associated with reduced parasympathetic activation (decreases in RMSSD and HF-power, but also LF-power and total power) (Togo and Takahashi, 2009; Jarczok et al., 2013a, 2020; Järvelin-Pasanen et al., 2019). In addition, lower SDNN values show a significant correlation with higher mortality in patients with prior MI in large cohort studies (Chattipakorn et al., 2007; Buccelletti et al., 2009; Huikuri and Stein, 2013), bypass surgeries (Lakusic et al., 2013) or heart failure (Sandercock and Brodie, 2006).

Beyond reported correlations between reduced short-time-measures of HRV and disease, measures of HRV capture a well-defined multi-phase course during progressively increasing physical exertion, under standardized working conditions, and during recovery after varying degrees of exertion (Kaikkonen et al., 2010). Thus, HRV can be used as a process-integrated measurement for an objective view of the response to the workload over the working day. Based on this measurement, working conditions can be analyzed for the identification of problematic patterns in the work environment (Bläsing, 2017).

Given these findings, the measurement of HRV is already used in various areas of occupational medicine and occupational science. This includes the analysis of the individual physical and mental workload and the identification of core areas of work-related stress, determination of recovery behavior, evaluation of the impact of new work equipment and new technologies, and application for the risk stratification of CVD (Sammito et al., 2015). Currently, HRV measurement is used for assessing stress and strain elicited by the introduction and usage of new work equipment and new technologies such as digital assistance systems, head-mounted displays, or exoskeleton (Schwerdtfeger et al., 2009) and in Space Medicine to describe the demands on flight personnel and to monitor individuals working under extreme conditions (Baevskiĭ, 2002).

Another example of a feasible application of HRV analysis to quantify 24-h stress/recovery balance in the workplace setting is the use of Firstbeat Bodyguard 2 (BG2) at StriveStronger, Sydney, an executive wellbeing and performance laboratory. The report is integrated into a physiology review consultation creating an opportunity to discuss the impact of lifestyle and health behaviors on stress and recovery (autonomic) balance and is used as an objective method to measure and quantify the effect of behavior change over time. This technology has been successfully used in over 1,000 clients and has been well-tolerated and acceptable as part of executive human performance and physiology assessments (May and Buckley, 2019). Taken together, ANS activity can index the overall functioning and adaptability of the body and mind (Holzman and Bridgett, 2017). Following from the writings of Darwin, in which the bi-directional communication between the heart and the brain was enunciated, the neurovisceral integration model has sought to further explicate this connection using modern methodologies and conceptions (Thayer et al., 2011).

Therefore, measuring ANS activity as a non-specific test would be a desirable instrument in primary prevention, as it allows on the one hand to assess the cumulative interaction of individuals with their environments and on the other hand capture the effect of prevention strategies. Taken together, measures of HRV fulfill many criteria of an early, non-specific screening tool in primary prevention in the area of occupational medicine.

Yet, using a short-term measurement of HRV (<1 h), it is only possible to give the client feedback on his current condition in the sense of a risk assessment such as blood pressure or cholesterol level. A 24 h measurement allows analysis of the course of the work load as described above. We state that far beyond the purely physical load, it represents a holistic assessment of the psychobiological interaction and functioning. Specifically, if the client writes a diary of memorable or important situations while using a wearable device that records HR, an offline analysis approach can be realized. This HR time series can be processed using spectral methods to show to the client a single graph, that contains the variability across the day paired with protocol situations of the client (Jarczok et al., 2019). The graph demonstrates the impact of situations relevant to the client on the visualized time series. This more holistic consultation has great potential to reveal the individual situation-specific psychophysiological reaction, thereby increasing self-perception and, even more importantly, induces a motivation to change behavior.

In particular, this novel approach offers the unique possibility to visualize the interaction between situation-specific emotion regulation (feelings and thoughts) within a specific psychosocial environment and the resulting situation and emotion-specific physiological states - actually the core definition of psychosomatics. The graph (spectrogram) serves as a "door opener" for the consulting clinician and allows to demonstrate individual somatic reactions in specific situations, preparing the ground for changes in attitudes and behavior. For example, a manager thought that he shows positive, healthy behavior by not reading any business emails before bedtime, only non-business ones. However, the HRV graph demonstrated to him that his physiological state was not different between private and business emails. As a result, he changed his bedtime routine and thus improved his sleep quality, which was confirmed in a follow-up HRV assessment. More generally spoken, the clients' benefit consists of mentalization and reflection of daily routine and corresponding physiological states, showing the individual "wear and tear" of the body, as Seeman would term it (Seeman et al., 1997). Furthermore, not only risky behaviors, but also resources are detected, so that not only negative behaviors are identified, but existing positive behaviors are encouraged. This personalized and salutogenic approach goes far beyond general

advice and can create more credibility, insight, and motivation for change. This approach could be seen as next-generation, psychosomatic biomonitoring that covers somatic and mental health in public health settings, empowering clients to take better care of themselves by improving their understanding of how their body and mind are working and where their risks and resources are.

The present article will describe the implementation of a 24 h-HRV-based consultation in a health check-up at the workplace and show the first results. We developed special training for occupational health physicians (OHPs) to enable them to record, analyze, interpret, and discuss these 24 h-measurements of HRV in a "psychosomatic way" (as described above) with the participants. We hypothesize that the HRV-based consultation improves the psychosomatic comprehension of the managers and employees as indicated by a significant increase in their self-reported notice of body signals and that the training is feasible, i.e., enables the OHPs to perform the HRV measurement, interpretation, and consultation.

# MATERIALS AND METHODS

## Methods

The study "*healthy leadership and work – body signals for managers and employees*" represents an investigator-initiated, interventional, single-arm, open (non-blinded), multicenter, national trial. It is registered in the German Clinical Trials Register (ID: *DRKS00014653*) and approved by the local ethics committee (ID *188/18*, IRB of Ulm University, Germany). Enrollment started on 04/07/2018 at four study sites: an automotive company (A), a multinational engineering and technology company (B), a printing group (C), and a pharmaceutical and consumer goods manufacturer (D). Consultation and data collection were completed at study sites A and B, and ongoing at sites C and D. Therefore, we report complete data from study sites A and B and partial data from the OHPs of study sites C and D.

## Psychometric Data

### Psychosomatic Interactions

Attitudes to and perceptions of psychosomatic interactions were assessed via eight questions using a Likert scale between 1 and 10 (see **Figure 3** for the exact wording).

Satisfaction with the consultation was assessed by 11 questions that were answered on a Likert Scale between 1 and 10 (see **Figure 2** for the exact wording).

### Ability to Work

Workability was assessed by the single question: "If you rate your best workability ever achieved with 10 points: How many points would you give for your current ability to work?" with potential responses between 0 (totally unfit for work) and 10 (currently the best working capacity) (Airaksinen et al., 2018).

### Irritation Scale

The irritation scale (Mohr et al., 2005) has a hierarchical structure with the two primary factors: cognitive irritation in the sense of not being able to switch off (indicated by items 1, 2, 4) and emotional irritation in the sense of an agitated irritation (items 3, 5, 6, 7, 8). Higher scores indicate higher irritation.

### Patient Health Questionnaire (PHQ-4)

The 4-item Patient Health Questionnaire (Löwe et al., 2010) consists of four items, two measuring anxiety, two depression, that are scored between 0 = never and 3 = almost every day and are summed, yielding a sum score between 0 and 12. Internal reliability Cronbach's Alpha previously reported for this questionnaire is 0.82 (Löwe et al., 2010). Higher values indicate more anxious and depressive symptoms. Sum scores of $\geq$3 in each subscale are categorized as probable cases of depression or anxiety, respectively (Löwe et al., 2005; Kroenke et al., 2009).

### Perceived Stress Scale (PSS-4)

Four questions ask participants how often they experienced stressful situations in the previous month on a Likert scale between 0 = never and 4 = very often (Cohen et al., 1983; Klein et al., 2016). Higher values indicate more stress. Cronbach's Alpha reported for this scale is 0.77 (Warttig et al., 2013).

## Details Regarding the HRV Measurement

A single-channel-ECG (Sampling rate 1000 Hz) was recorded using either three electrodes (Ambu BlueSensorR ECG Electrodes REF R-00-S/25) and a cable set (Bittium Corp., Oulu, Finland) or a textile chest belt with dry electrodes and the corresponding stingray adapter (Bittium Corp., Oulu, Finland) for at least 24 h using Bittium Faros$^{TM}$ 180 (Bittium Corp., Oulu, Finland). The ECG was imported into HRV-Scanner Software (BioSign GmbH, Ottenhofen, Germany) at study site A and into CardiscopeTM ANALYTICS professional edition (HASIBA medical GmbH, Graz, Austria) at study site B, screened and edited for artifacts and HRV values were calculated for consultation. The scientific HRV analysis was conducted by EB using the CardiscopeTM ANALYTICS professional edition (HASIBA medical GmbH, Graz, Austria). Only ECGs with a minimum recording length of 22 h (79,200 s) were entered into the analyses. The automated recognition of regular rhythm and artifacts of the software was checked manually and ECGs entered statistical analyses only if the rate of sinus rhythm was higher than 90%, regardless of the cause (aberrant rhythms or artifacts). The following parameters were extracted for analyses: HR (mean for 24 h), SDNN (standard deviation of all RR intervals), SDNN-i (mean value of the standard deviations of the average RR intervals of all 5-min segments of a measurement), SDANN (standard deviation of the average RR intervals of all 5-min segments of a measurement), RMSSD (square root of the squared mean of the sum of all differences of successive RR-intervals), Total Power-i (average power density in the total band, i.e., between 0.0 and 0.4 Hz of all 5-min-calculation windows), HFi (average energy density in the HF (high frequency) band, i.e., between 0.15 and 0.4 Hz of all 5-min-calculation windows), LFi (average energy density in the LF

(low frequency) band, i.e., between 0.04 and 0.15 Hz of all 5-min-calculation windows). For a description of the HRV parameters see Shaffer and Ginsberg (2017) and for further information of 24 h-analysis see Jarczok et al. (2019).

## Survey of the OHPs

The authors EB and MNJ met OHPs regularly (3, 6, and 12 months after the initial training) to review progress and address any questions. At each meeting, a standardized question catalog regarding difficulties in presenting the project, handling the devices, questionnaires and software, evaluating and interpreting the HRV measurement and conducting the consultations (see **Supplementary Table 1** for the exact wording) was discussed. The answers of the OHPs were recorded verbatim and summarized for this article. Further, the OHPs completed a questionnaire directly after the training with questions regarding the perceived quality of the training, scaled on a Likert Scale between 1 and 6, and a questionnaire at the end of the project (12 months after the initial trainings) asking for their opinion regarding the effort for explaining, analyzing, interpreting, discussing the measurement, how helpful it was for the consultation, whether and in which context they wanted to continue using the HRV measurement and questions about the training and meetings. All questions were answered on a Likert Scale between 1 and 10.

## Study Plan

We recruited OHPs through personal contact. The complete team of OHPs received training with a 1-day workshop to teach basic knowledge of HRV, details of measurement, analysis, and interpretation, including a hands-on part with clinical cases and ECG data (case vignettes) (see **Supplementary Table 1**). After practicing with some example cases with individual feedback from the supervisors EB and MNJ, the OHPs offered the HRV measurement and consultation at their company. Individual supervision for the OHPs was provided by study investigators (EMB) in case of need. Three, 6, and 12 months after the initial training, meetings were organized where the OHPs presented actual cases.

In study site A, the measurement was offered to every manager who consulted the OHP for his regular health check-up. In sites B–D, HRV-based consultation was offered not only to managers but also to employees by written information available at the company's medical service, not associated with a certain check-up.

Following written consent, participants received a 24 h recording of ECG and they were asked to complete a diary of that day and a questionnaire. The latter contained standard demographical questions, questions about hours actually worked weekly, smoking, hours of sports/physical activity per week, knowledge of relaxation methods like yoga, progressive muscle relaxation, autogenic training (yes/no), practicing a relaxation method, a question regarding subjective sleep quality ["*How do you rate your sleep overall?*" Scale from 1 (excellent) to 5 (poor)], perceived psychosomatic interactions [e.g. "*Feelings are expressed in me. – mentally (1) – physically (10)*"], workability, and the following standardized questionnaires: irritation scale (IS), perceived health questionnaire (PHQ-4) and perceived stress scale (PSS-4). Afterward, the OHP analyzed the ECG with regard to HRV and discussed the results with the study participant during their next meeting. Immediately after the consultation, the participant was asked to complete a questionnaire regarding his satisfaction with the consultation and questions about perceived psychosomatic interactions again. OHPs recorded medical diagnoses, medication, diastolic, and systolic blood pressure (only study site A), height, and weight (only study site A). CV risk factors comprised: smoking, arterial hypertension, hypercholesterinemia, adiposity (BMI $\geq$ 30 kg/m$^2$), diabetes (Type I or II), and hyperuricemia. At study site B, OHPs recorded the reason for the measurement (request of employee/manager, suggestion of OHP with explanation). Data were collected between July 2018 and June 2019 for 12 months at study site A, between December 2018 and August 2020 at site B, between May 2019 and ongoing at sites C and D.

Inclusion criteria for participation were age between 18 and 65 years and being a manager or employee at the cooperating company at the time point of study inclusion. Exclusion criteria were history of permanent cardiac arrhythmias (atrial fibrillation), pacemaker-dependent rhythm, or conditions under which calculation of HRV is not possible (e.g., frequent extrasystoles). The flowchart of the recruitment is shown in **Figure 1**.

The primary outcome was an increase in the self-reported notice of body signals in employees and managers pre/post via a question in the questionnaire: "*I can easily perceive what my body needs. . . Do not agree at all – strongly agree*" (scale from 1 to 10). This question was asked before the HRV measurement and immediately after the consultation.

## Details of HRV Analysis and Consultation

After importing the ECG data into the HRV software, the quality of data was reviewed by checking the parameters presented by the software (percentage of recognized regular rhythm) and by checking the ECG in time frames in which the software marked less recognized regular rhythm. For a valid interpretation of HRV, the artifact rates should be lower than 10%. However, over 24 h, time frames with sufficiently low artifact rates can be identified frequently, so that consultation can be based on these intervals. Time-domain parameters are checked for an approximate classification compared to a normal population to support the OHP's assessment. Then, the color spectrogram is reviewed thoroughly together with the participant's activity diary to identify resources and stressors which show distinct and different patterns in the spectrogram. Special interest is given to the existence of relaxation periods, i.e., if the night is a relaxation period (lower HR, higher HRV, distinct pattern in the HF band at night compared to day time) and if there are any other periods with markedly risen HRV, especially HF, during day time. The OHP concludes with some hypotheses of issues which the participant should improve, but importantly, the main conclusions can be drawn only at the end of the consultation when the OHP can merge these issues with the participant's thoughts, feelings, and motivation.

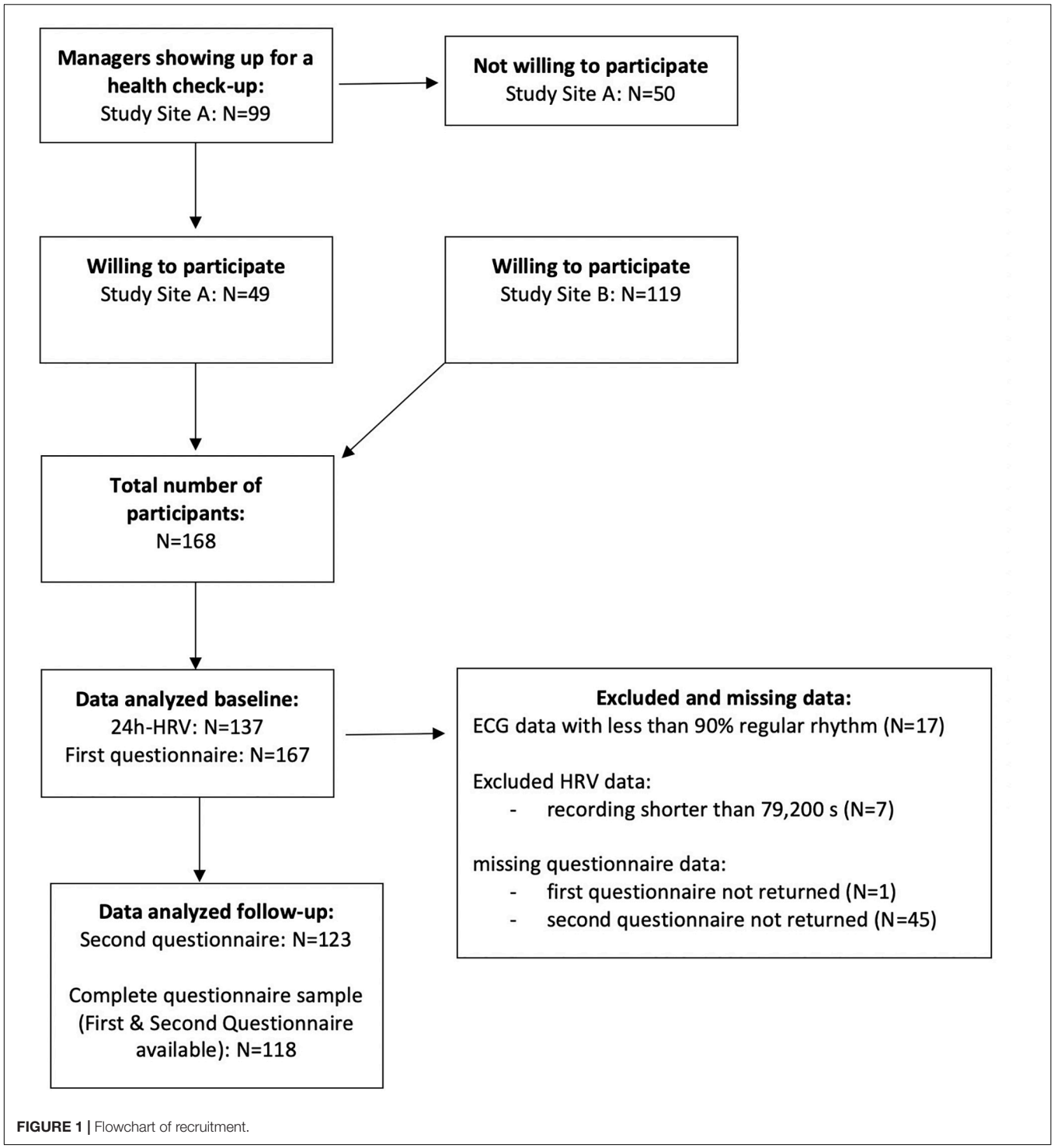



**FIGURE 1 |** Flowchart of recruitment.

The focus of the consultation is the linkage of HRV patterns to the diary to demonstrate both, strain and recovery periods in the HRV diagram and then to discuss these specific situations more deeply regarding the client's emotions, thoughts, and bodily perceptions during these periods. This intervention aims at strengthening the perception of signs of the body, thoughts, and feelings, with a focus on relaxation periods. Other situations of the measurement day, especially periods of reduced HRV despite a normal or low HR, are also discussed similarly. The focus of the consultation is not to promote avoidance of these situations, but recovery after these situations. Regarding the already discussed resources, possible actions that could be taken next time for better recovery are discussed at the end. The consultation closes with overall feedback about the level of variability and a summary of

conclusions resulting from the measurement and consultation, preferably developed together with the participant and not given as unidirectional advice. Details and examples of the consultation are given in (Jarczok et al., 2019).

## Data Sample

ECG data was not available for technical reasons for seven participants. A total of 17 ECGs showed more than 10% artifacts/non-sinus rhythm and were therefore excluded from HRV analyses. All other ECGs showed at least 90% sinus rhythm. Seven participants had a recording time shorter than 79,200 s and were therefore excluded from 24 h-analyses. Thus, the number of ECGs included in the analyses was 137.

One participant did not return the first questionnaire and 45 did not return the second questionnaire after the consultation, resulting in $N = 167$ for the first and $N = 123$ for the second questionnaire. As some participants did not submit a questionnaire for time point 1 but for time point 2, the complete analysis sample for hypothesis testing was $N = 118$.

Regarding the OHPs, $N = 16$ questionnaires are available for the first questionnaire and $N = 9$ for the final questionnaire, due to the ongoing training at study site C and D.

## Statistical Methods

The primary outcome is analyzed using Wilcoxon signed ranks tests. Effect size is calculated using Cohen's d. All data management and statistics were conducted using SPSS Statistics for Windows, version 25 (SPSS Inc., Chicago, IL, United States). A $p$-value smaller than 0.05 (one-sided) was considered statistically significant. Due to the exploratory nature, no adjustments were made for cumulative alpha error due to multiple testing.

# RESULTS

Between July 2018 and June 2019, $N = 99$ check-up consultations for managers were performed by the OHPs at study site A. All of them were offered the HRV-based consultation and $N = 49$ (49%) agreed to participate (see flow-chart **Figure 1**). Participants were not significantly different from non-participants regarding age and sex (all $p > 0.05$). At study site B, HRV-based consultation was offered not only to managers, but also to employees by written information available at the company's medical service and not associated to a certain check-up. A total of 89 (74%) of the $N = 118$ measurements of study site B were performed because the employee/manager requested it and 24 (20%) because the OHP offered the measurement. Reasons for a direct offer by the OHP were reported tiredness, tension, sleep problems, stress levels reported as high and burdening, and somatic symptoms like dizziness, pain.

The combined sample of 168 participants was between 23 and 63 years old (mean = 45.5, SD = 9.8) and 68% were male (**Table 1**). A third of them indicated that they work 50 h or more per week. Almost half reported engaging in regular physical activities more than 2 h/week and every second participant rated his/her sleep as satisfying. Two-thirds reported knowledge about relaxation techniques, while about the same amount reported never practicing any relaxation techniques. Elevated depression scores were present in 21 (13%) and elevated anxiety scores in 21 (13%) participants. The average workability index was high (mean = 7.3, SD = 1.7, scale range from 0 to 10). The participants were highly satisfied with the consultation (median: 10; interquartile range: 9-10, scale range from 1 to 10) (see **Figure 2**). The SDNN-i of each 24 h series was classified using percentiles from a large ($N > 7000$) working cohort (see appendix for the percentiles in

**TABLE 1** | Descriptive.

| | *N* | % | |
|---|---|---|---|
| CV risk factor count: | 163 | | |
| 0 | 131 | 80 | |
| 1 | 26 | 16 | |
| 2 | 5 | 3 | |
| 3 | 1 | 1 | |
| Diagnosis count: | 163 | | |
| 0 | 104 | 64 | |
| 1 | 30 | 18 | |
| 2 | 16 | 10 | |
| 3 and more | 13 | 8 | |
| Women | 57 | 35 | |
| Current Smokers | 14 | 8 | |
| Weekly working hours: >50 | 52 | 31 | |
| More than 2 h sport/week | 69 | 41 | |
| Relaxation method known | 114 | 68 | |
| Never using a relaxation method | 103 | 62 | |
| High quality sleep (subjective) | 88 | 54 | |
| | **N** | **Mean** | **SD** |
| Age (years) | 167 | 45.5 | 9.8 |
| BMI (kg/m$^2$) | 166 | 24.9 | 3.7 |
| Workability | 165 | 7.3 | 1.7 |
| Cognitive irritation | 166 | 11.3 | 4.8 |
| Emotional irritation | 166 | 14.2 | 5.9 |
| PSS sum score | 167 | 5.1 | 2.8 |
| PHQ sum score | 167 | 2.6 | 2.3 |
| | **N** | **Median** | **Interquartile range** |
| Overall satisfaction with consultation | 101 | 10 | 9–10 |
| Recommendation to a friend | 101 | 10 | 9–10 |
| New insights | 101 | 9 | 7–10 |
| Improved psychosomatic perspective | 101 | 8 | 6–9 |
| **24 h HRV parameters** | **N** | **Median** | **Interquartile range** |
| HR (beats per minute) | 137 | 74.81 | 69.78–79.87 |
| SDNN (msec) | 137 | 135.26 | 115.32–171.27 |
| SDNN-i (msec) | 137 | 60.12 | 50.64–73.64 |
| SDANN (msec) | 137 | 118.75 | 98.03–150.66 |
| rMSSD (msec) | 137 | 29.10 | 23.12–39.11 |
| LF (msec$^2$) | 137 | 1,106.56 | 763.46–1,727.52 |
| HF (msec$^2$) | 137 | 294.08 | 157.38–536.79 |
| Total power (msec$^2$) | 137 | 3,594.64 | 2,641.10–5,238.65 |

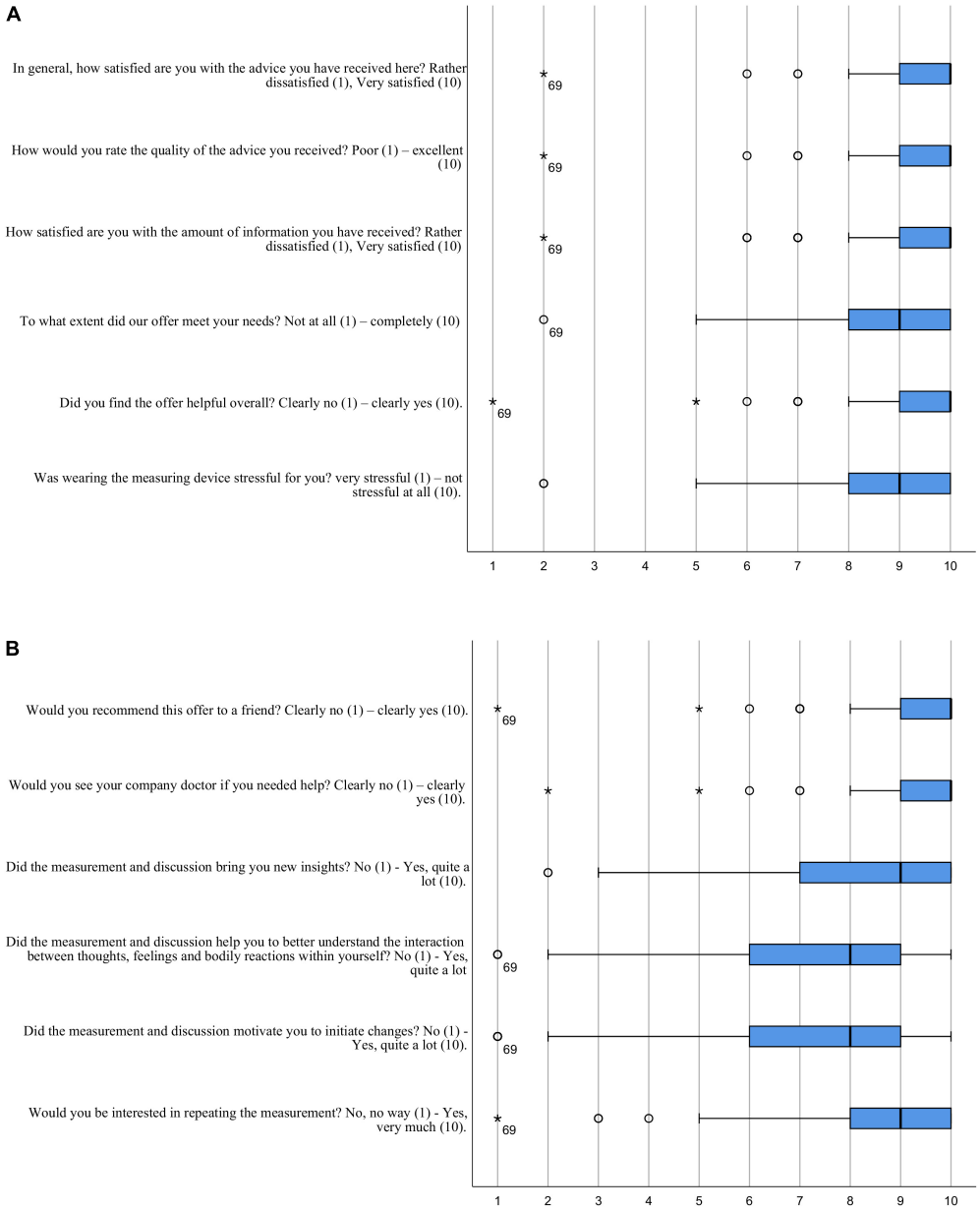



**FIGURE 2 | (A,B)** Satisfaction of the participants with the consultation. Boxplot from *N* = 123 participants.

**Supplementary Table 2**). Eleven participants (8%) had values lower than the $10^{th}$ percentile and 40 participants (29%) had values lower than the 25th percentile. Associations between HRV percentiles and psychometric/sociodemographic data are shown in **Supplementary Table 3**.

Participants reported a significantly improved perception of their bodily needs after the consultation (from Median = 7, interquartile range 5–8 to Median = 8, interquartile range 7–9; scale range from 1 to 10; $p < 0.001$, Wilcoxon rank test; Cohen's D = 0.49; *N* = 118) (see **Figure 3**).

A total of 16 OHPs were trained by our team. After the first training day, they felt sufficiently trained to conduct the HRV measurement (Median 5, interquartile range 4.25–5, scale between 1 = not at all and 6 = almost completely). During the regular meetings in the following months, the OHPs reported good acceptance of the HRV measurement. The effort to offer and explain the HRV measurement to potential participants was estimated as moderate (Median 5, interquartile range 3–8, scale between 1 = very large and 10 = very small). The procedure to equip participants, to return the HRV monitor, and transfer the recording to the local database was reported as time-consuming (about 30 min per participant). The procedure improved for the OHPs after they had instructed their medical staff to manage this. The reasons difficulties were encountered with the equipment

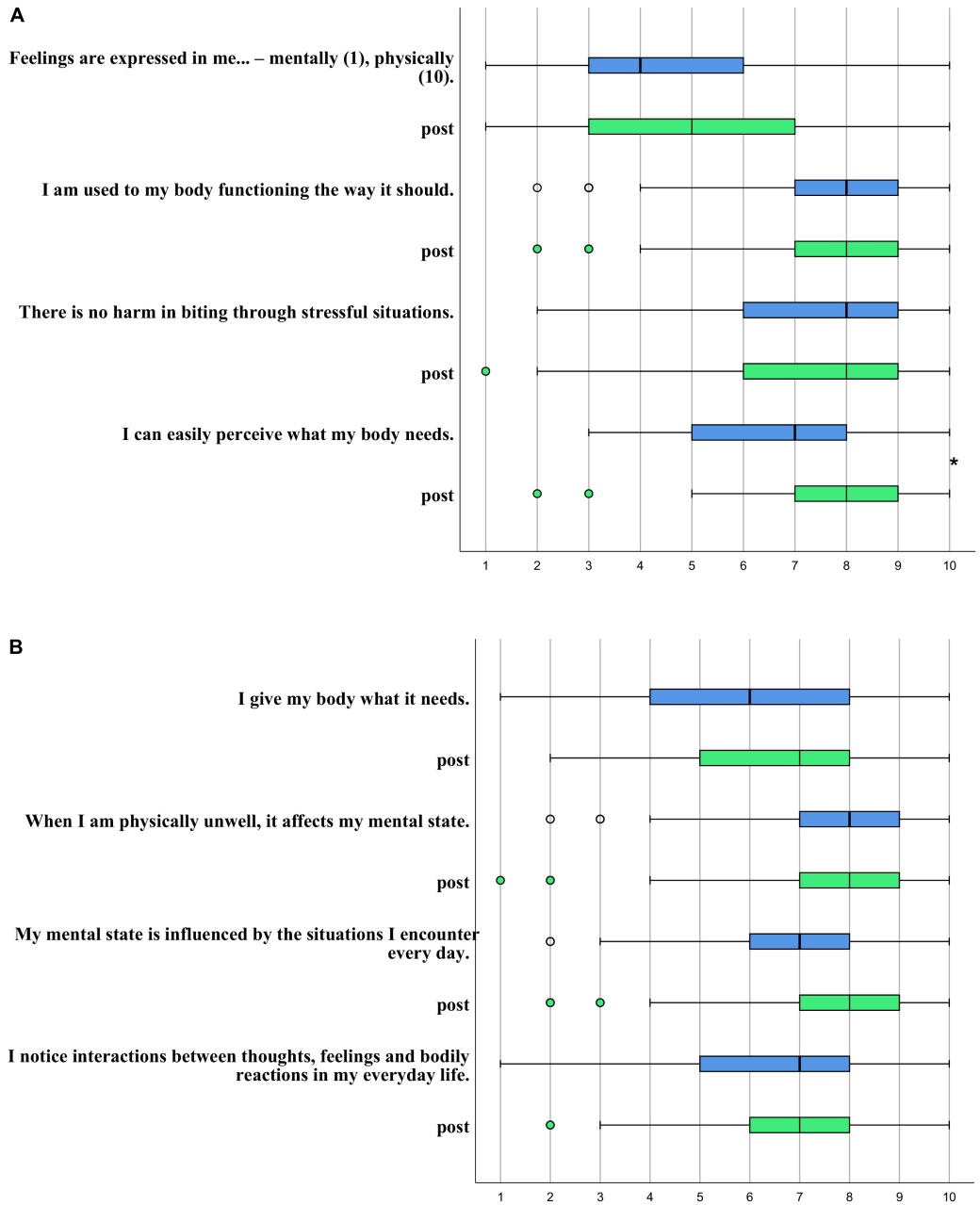



**FIGURE 3 | (A,B)** Psychosomatic perceptions before (blue) and after (green, marked as "post") the consultation. Boxplot from $N = 118$ participants. *$p < 0.05$. If not explicitly stated, the scale is rated between: Do not agree at all (1) – strongly agree (10).

was reported to be due to the exceptionally hot summer, and electrodes loosened quicker than normal and therefore started to move. This was improved by replacement with a textile chest belt with dry electrodes. Second, the electrodes were partially visible under light summer clothing that the participants found unpleasant. Logistic problems occurred on Fridays, as the participants did not want to conduct the measurement during their free weekend. Also, when participants worked at other sub-locations of study site A, they were unable to return the Holter monitor the next day.

The processing of ECG-data with the HRV software still took too much time for the OHPs (about 10 min per measurement; only technical processing, without interpretation) and we found no possibility to improve the process apart from changing the software to Cardiscope$^{TM}$, which we did at the study sites B–D. Still, the question of the effort to analyze and interpret the HRV was answered as high (Median 3, interquartile range 1.5 to 4, scale between 1 = very large and 10 = very small). Discussing this issue further, OHPs reported that it needed about 10 cases to get into a routine and be more time-efficient, both with handling the

software and interpreting the results. In the beginning, questions regarding interpretation of the results and communicating the results were frequent. Those with lesser cases still tended to feel insecure in analyzing, interpreting, and discussing the HRV graphs at the end of the year.

The extra time consumed by the HRV based consultation was reported as moderate (Median 4, interquartile range 2.5 to 4, scale between 1 = very high and 10 = very small). OHPs at study site A estimated an extra 15 min for HRV-based consultation adding this time to the pre-existing check-up consultation. The whole check-up examination and consultation took on average 2 1/2 h. At the other study sites, consultation times ranged between 30 min and one hour.

The OHPs reported the HRV measurement was, in general, helpful for consulting the participants (Median 4, interquartile range 2.5 to 4, scale between 1 = very helpful and 10 = not helpful at all), while they marked interesting cases where it was especially helpful, e.g., in the case of a young, hard-working female manager with a chronically ill child and tinnitus, which was rapidly assigned by her to her high strain. She felt quite bad about that because there was nothing she could change about her child and she did not want to reduce working hours as her job gave her high satisfaction. The results of the measurement were surprisingly positive, so she was encouraged to seek further medical help. A protracted otitis media and a perforated eardrum were detected and treated. Her tinnitus and her wellbeing improved. Another OHP reported that he was able to motivate a smoker with beginning arteriosclerosis and noticeably low HRV patterns at night to participate in a 1-week health training that is offered by the company.

Most OHPs wished to continue to use HRV-based consultation (Median 8; scale between 1 = not at all and 10 = yes, absolutely), but not for all check-ups, but selected cases like clients with sleeping problems, stress, or general fitness evaluation (free-text comments).

The OHPs reported that through the training, they felt empowered to continue applying HRV independently (Median 5, interquartile range 4.5 to 10, scale between 1 = not at all and 10 = yes, absolutely). The meetings with case studies were very helpful (median 10, interquartile range 9 to 10; scale between 1 = not at all and 10 = yes, absolutely) and the training met their expectations (median 8.5, interquartile range 8 to 10; scale between 1 = not at all and 10 = yes, absolutely). Most would recommend it to a colleague (median 8; interquartile range 6.25 to 10, scale between 1 = not at all and 10 = yes, absolutely). Still, insecurities in interpreting the results were reported mainly by those OHPs with less than 10 cases as reported above. Other OHPs stated that they found it difficult to conduct the consultation with the psychosomatic, resource-oriented focus. They requested more training in these special communication skills. Most of the OHPs reported that this kind of consultation cannot be shortened to less than 30 min, a time frame that is sometimes hard to offer if the workload is high.

Overall, the OHPs reported their impression that the participants were very satisfied with the consultations. Also, OHPs and participants (free-text comments) independently pointed out that the HRV measurement should be continued and offered to all employees; not necessarily during the check-up, but preferably for selected cases.

## DISCUSSION

This first data from our interventional trial shows that measuring 24 h-HRV and consulting based on the results can improve the perception of the bodily needs of employees and managers. An appropriate perception of needs is a basis for healthy behavior. Participants were highly satisfied with the consultation and OHPs reported that the measurement of HRV is very well suited to enter into dialog with the managers and employees and to show interactions between situations, thoughts, feelings, and bodily reactions. Therefore, we conclude that a 24 h-HRV-measurement can be a feasible and effective approach for holistic, psychosomatic primary prevention in occupational medicine.

We want to mark that our intervention is based on increasing awareness of bodily signals and psychosomatic interactions, while also enhancing resources and confirming situations in which managers and employees successfully recovered from stressful situations. Especially, we wanted to avoid marking "stressful situations" as "dangerous". We have achieved this goal, as stressful situations were not regarded as more harmful after compared with before the intervention (**Figure 3**, question 3). Most often, research results are classified under the headline "stress is bad for you". Studies have reported links between both work stress, especially deadline stress, and also high-level acute episodes of anger or anxiety with triggering of acute MI (McCormack et al., 2016; Buckley et al., 2019). However, that's a global finding and on an individual level, a comparable level of stress may be associated with heart disease in one person while another person may not be burdened at all. Beyond genetic predisposition, this probably depends on the frequency of exposure, intensity, and duration of the stressor. In other disciplines like cardiology, it turned out that the previously given common recommendation to do less physical exercise after being diagnosed with heart disease is counterproductive (Moraes-Silva et al., 2017). The circulatory system has to be used to function properly and to keep its functions – in other words, low to moderate, well-dosed stress appears essential for the system (when followed by recovery), while vigorous exertion is associated with increased CVD (Buckley et al., 2019). On a molecular level, recovery time played a pivotal role in a mouse model after traumatic stress exposure that triggered an acute heart injury. Interestingly, the repair process in the heart tissue was completed after 10 days. More importantly, group differences (long vs short exposure and long vs short recovery) were apparent in the recovery time but not in the stress exposure time, highlighting the important role of recovery time (Cho et al., 2014). Having this in mind, the presented consultation focuses on sufficient recovery (i.e., quality and quantity), not on avoiding stressful situations, i.e., avoiding chronic stress, not acute stressors – considering that this is primary prevention, the participants are healthy and the stressors are mild to moderate, far from

the type of stressors causing post-traumatic stress disorder. As we focus on recovery after stressful situations and many participants show sufficient recovery, they actually can see on the graph that the stressful situation did not do them any harm and that they can handle it. We think that this change of mindset even has a health-promoting effect, because the stress at work is then no longer considered harmful, but rather like a vaccination empowering the individual do deal better with stress. Meichenbaum transferred the model of vaccination to stress and developed a "stress inoculation training," claiming that regular exposure to mild stressors promotes resilience and the feeling of self-effectiveness (Meichenbaum and Deffenbacher, 1988). The importance of mindset on health is shown in another study by Crum who informed hotel room attendants that their work is a healthy exercise. Only this change in mindset without any behavioral changes led to a decrease in weight, blood pressure, and body fat compared to a control group who did not receive this information (Crum and Langer, 2007). We consider the changes found in psychosomatic attitudes and perception of the participants in our study as an indicator that the intervention positively changes their mindset, and we propose that this fact in itself may improve their health in addition to any behavioral changes we may have induced. We will further explore these hypotheses in our next study containing a repeated measurement after 3 months.

## Beyond This Study: What Are the Potential Mechanisms/Physiological Considerations

How can these mechanisms be linked to the ANS? Two very likely mechanisms appear to be pivotal. First, on the level of the brain, central autonomic processes, and processing shape the efferent projection activity via the lower brain regions such as to brain stem and further via the vagus nerve – that is the moment-to-moment evaluation of environmental threat and safety signals, its integration with memory, social functioning and emotion regulation, and also its projected trajectory into the near and distant future (Lane et al., 2009a,b; Thayer and Lane, 2009; Thayer et al., 2011; Holzman and Bridgett, 2017). If something is considered a threat or a safety signal is highly shaped by former experience, existing coping mechanisms, and actual beliefs. That's how the mindset of a person (that is his or her beliefs) can shape bodily response in a moment-to-moment fashion. The diseases that should be modified by this pathway should be those of the organs addressed by efferent ANS activity. The efferent vagal activity can inhibit (indirectly) cytokine release from immune cells, also known as the cholinergic anti-inflammatory pathway, acting through the α7 nicotinic acetylcholine receptor (α7 nAChR) on macrophages (Huston and Tracey, 2011). As excessive pro-inflammatory cytokine release is common in many chronic and non-communicable disease conditions (including MI, CVD, rheumatic diseases, and depression), the latter pathway appears to be highly relevant in disease etiology (Huston and Tracey, 2011). This is a possible mechanism of how changes in mindset can change bodily reaction patterns like blood pressure via the ANS.

## Limitations

We do not know if the study sample is representative of the managers and employees working in these companies. It may be that our sample consists of managers and employees that care more for their health. We do not have data from the companies regarding the sex and age of all their managers and employees. However, we can compare our study population with other data from Germany. The present sample showed a healthier lifestyle and fewer diagnoses than men of their age in Germany which we expected for a sample with high socioeconomic status (Stringhini et al., 2017): Mean BMI was 25 kg/m$^2$ and therefore lower than the mean BMI of 27 kg/m$^2$ reported for German men between 40 and 60 years old (Mikrozensus, 2017). The sample contained very few smokers: 2% compared to 34% of men smoking in the German general population and about 23% in a German sample of middle-aged men with higher education (Lampert, 2011). Regarding psychometric variables, the PSS scale score was lower than in other samples of healthy men (Lavoie and Douglas, 2012; Vallejo et al., 2018) and the PHQ sum scores were comparable (Löwe et al., 2010). It is interesting that though our study sample had a relatively healthy lifestyle and low burden of disease, the percentage of managers with low HRV values was not lower than in the sample from a large ($N > 7000$) working cohort (see **Supplementary Table 2**). A noticeable characteristic of this sample was the long working hours reported. As long working hours correlate with reduced HRV (Park et al., 2001), this might partially explain this unexpected finding.

Regarding the training of the OHPs, it is a limitation that the evaluation is based on subjective assessment only. A more sophisticated approach would contain systematic qualitative analyses as well as an objective examination of the skills. This was beyond the scope of the funding of the present project.

Further, the supposed primary prevention effects of the approach presented here need to be confirmed by a long-term follow-up study to evaluate the potential health benefits for the participants.

## Training of OHPs

Regarding the OHPs, the training to interpret the 24 h-spectrogram of HRV and learn the necessary techniques for this consultation, as well as the consultations themselves, take time. Future workshops will contain two instead of one training day at the start. Our concept of repeated meetings turned out to be helpful to improve the skills and knowledge that the company doctors still needed. As they required more training in resource-oriented and psychosomatically focused consultations, we added this to the meetings. We found it very important to focus on this. Otherwise, the HRV measurement is easily used like other risk scores in the sense that the risk and advice are told to the client, but the client has gained no insights into his body and the interactions between body, mind, and environment.

It turned out clearly that the preparation of the measurement and the technical details of analyzing should be as least time-consuming as possible for the OHPs so that they could concentrate on what is important: the consultation of the client. Thus, involving the medical staff from the outset is very important.

Taken together, we show that a 24 h-HRV-measurement can be a feasible and effective approach for holistic, psychosomatic primary prevention in occupational medicine.

## DATA AVAILABILITY STATEMENT

Due to data protection agreements, the research data cannot be uploaded to a public repository. However, the data can be reviewed upon request onsite at the Clinic for Psychosomatic Medicine and Psychotherapy (Ulm, Germany). Requests to access the datasets should be directed to EB, elisabeth.balint@uni-ulm.de.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Ulm University, Germany (ID 188/18). The participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MJ and EB: concept and design, acquisition, analysis, and interpretation of data, drafting of the manuscript, and statistical analysis. TB, HG, IB, DM, and JT: critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

## FUNDING

This project was funded by Karl-Schlecht-Stiftung, Gutenbergstr. 4, 72631 Aichtal, Germany and through core funds from the Clinic for Psychosomatic Medicine and Psychotherapy, University Hospital, Ulm (Germany).

## ACKNOWLEDGMENTS

We thank the participants and OHPs of the study sites for their collaboration and support. We are indebted thankful to Prof. Dr. med. Horst Kächele, who deceased while we were working on this article, for providing his support and encouragement as well as his support to establish contact with the KSG foundation. We will miss his support and clarity. We thank Dr. Franziska Kessemeier (LPCU Ulm) for supporting data management and Christian Hirning (LPCU Ulm) for supporting data management, analysis, and result section update.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.600865/full#supplementary-material

## REFERENCES

Aeschbacher, S., Schoen, T., Dörig, L., Kreuzmann, R., Neuhauser, C., Schmidt-Trucksäss, A., et al. (2017). Heart rate, heart rate variability and inflammatory biomarkers among young and healthy adults. *Ann. Med.* 49, 32–41. doi: 10.1080/07853890.2016.1226512

Airaksinen, J., Jokela, M., Virtanen, M., Oksanen, T., Koskenvuo, M., Pentti, J., et al. (2018). Prediction of long-term absence due to sickness in employees: development and validation of a multifactorial risk score in two cohort studies. *Scand. J. Work. Environ. Health* 44, 274–282. doi: 10.5271/sjweh.3713

Backé, E.-M., Seidler, A., Latza, U., Rossnagel, K., and Schumann, B. (2012). The role of psychosocial stress at work for the development of cardiovascular diseases: a systematic review. *Int. Arch. Occup. Environ. Health* 85, 67–79. doi: 10.1007/s00420-011-0643-6

Baevskiĭ, R. M. (2002). [Analysis of variability of cardiac rhythm in space medicine]. *Fiziol. Cheloveka* 28, 70–82.

Benarroch, E. E. (2014). "Central autonomic network," in *Autonomic Neurology*, ed. E. Benarroch (Oxford: Oxford University Press), 3–14. doi: 10.1093/med/9780199920198.003.0001

Bernard, C. (1867). *Lecture on the Physiology of the Heart and its Connections With the Brain, Delivered at the Sorbonne*, trans. J. S. Morel. 1 Edn. Savannah, GA: Purse & Son.

Bläsing, D. (2017). Erfassung von individuellem beanspruchungserleben am arbeitsplatz über herzratenvariabilität im pflegebereichheart rate variability as an individual parameter to describe and explain stress experience of nursing staff. *Z. Arbeitswiss* 71, 269–278. doi: 10.1007/s41449-017-0082-7

Buccelletti, E., Gilardi, E., Scaini, E., Galiuto, L., Persiani, R., Biondi, A., et al. (2009). Heart rate variability and myocardial infarction: systematic literature review and metanalysis. *Eur. Rev. Med. Pharmacol. Sci.* 13, 299–307.

Buckley, T., Soo Hoo, S. Y., Shaw, E., Hansen, P. S., Fethney, J., and Tofler, G. H. (2019). Triggering of acute coronary occlusion by episodes of vigorous physical exertion. *Hear. Lung Circ.* 28, 1773–1779. doi: 10.1016/j.hlc.2018.11.001

Chattipakorn, N., Incharoen, T., Kanlop, N., and Chattipakorn, S. (2007). Heart rate variability in myocardial infarction and heart failure. *Int. J. Cardiol.* 120, 289–296. doi: 10.1016/j.ijcard.2006.11.221

Cho, J. H., Lee, I., Hammamieh, R., Wang, K., Baxter, D., Scherler, K., et al. (2014). Molecular evidence of stress-induced acute heart injury in a mouse model simulating posttraumatic stress disorder. *Proc. Natl. Acad. Sci. U.S.A.* 111, 3188–3193. doi: 10.1073/pnas.1400113111

Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *J. Heal. Soc Behav.* 24, 385–396. doi: 10.2307/2136404

Crum, A. J., and Langer, E. J. (2007). Mind-set matters: exercise and the placebo effect. *Psychol. Sci.* 18, 165–171. doi: 10.1111/j.1467-9280.2007.01867.x

DeStatis (Hrsg.) (2017). Cardiovascular Diseases Cause Highest Costs. *Press Release No. 347 29.* September. 2017.

Elovainio, M., Kivimäki, M., Puttonen, S., Lindholm, H., Pohjonen, T., and Sinervo, T. (2006). Organisational injustice and impaired cardiovascular regulation among female employees. *Occup. Environ. Med.* 63, 141–144. doi: 10.1136/oem.2005.019737

Eurostat (Hrsg.) (2020). *Employment – Annual Statistics. Employ. (as % Population Aged 20 to 64)*. Luxembourg: Eurostat.

Folkman, S., Lazarus, R. S., Gruen, R. J., and DeLongis, A. (1986). Appraisal, coping, health status, and psychological symptoms. *J. Pers. Soc. Psychol.* 50, 571–579. doi: 10.1037/0022-3514.50.3.571

Herr, R. M., Li, J., Bosch, J. a., Schmidt, B., Dejoy, D. M., Fischer, J. E., et al. (2012). Psychometric properties of a German organizational justice questionnaire (G-OJQ) and its association with self-rated health: findings from the Mannheim Industrial Cohort Studies (MICS). *Int. Arch. Occup. Environ. Health* 87, 85–93. doi: 10.1007/s00420-012-0839-4

Holzman, J. B., and Bridgett, D. J. (2017). Heart rate variability indices as bio-markers of top-down self-regulatory mechanisms: a meta-analytic review. *Neurosci. Biobehav. Rev.* 74, 233–255. doi: 10.1016/j.neubiorev.2016.12.032

Huikuri, H. V., and Stein, P. K. (2013). Heart rate variability in risk stratification of cardiac patients. *Prog. Cardiovasc. Dis.* 56, 153–159. doi: 10.1016/j.pcad.2013.07.003

Huston, J. M., and Tracey, K. J. (2011). The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. *J. Intern. Med.* 269, 45–53. doi: 10.1111/j.1365-2796.2010.02321.x

Jarczok, M. N., Guendel, H., McGrath, J., and Balint, E. M. (2019). "Circadian rhythms of the autonomic nervous system – scientific implication and practical implementation," in *Chronobiology*, ed. P. Svorc (London: IntechOpen).

Jarczok, M. N., Jarczok, M., Mauss, D., Koenig, J., Li, J., Herr, R. M., et al. (2013a). Autonomic nervous system activity and workplace stressors-A systematic review. *Neurosci. Biobehav. Rev.* 37, 1810–1823. doi: 10.1016/j.neubiorev.2013.07.004

Jarczok, M. N., Jarczok, M., and Thayer, J. F. (2020). "Work stress and autonomic nervous system activity," in *Handbook of Socioeconomic Determinants of Occupational Health – From Macro-level to Micro-Level Evidence*, ed. T. Theorell (Basel: Springer International Publishing), 690. doi: 10.1007/978-3-030-05031-3_27-1

Jarczok, M. N., Kleber, M. E., Koenig, J., Loerbroks, A., Herr, R. M., Hoffmann, K., et al. (2015). Investigating the mechanisms of self-rated health: heart rate variability is more strongly associated than other frequently used biomarkers in a cross sectional occupational sample. *PLoS One* 10:e0117196. doi: 10.1371/journal.pone.0117196

Jarczok, M. N., Koenig, J., Mauss, D., Fischer, J. E., and Thayer, J. F. (2014). Lower heart rate variability predicts increased level of C-reactive protein 4 years later in healthy, nonsmoking adults. *J. Intern. Med.* 276, 667–671. doi: 10.1111/joim.12295

Jarczok, M. N., Li, J., Mauss, D., Fischer, J. E., and Thayer, J. F. (2013b). Heart rate variability is associated with glycemic status after controlling for components of the metabolic syndrome. *Int. J. Cardiol.* 167, 855–861. doi: 10.1016/j.ijcard.2012.02.002

Järvelin-Pasanen, S., Sinikallio, S., and Tarvainen, M. P. (2019). Heart rate variability and occupational stress— systematic review. *Ind. Health* 56, 500–511. doi: 10.2486/indhealth.2017-0190

Kaikkonen, P., Hynynen, E., Mann, T., Rusko, H., and Nummela, A. (2010). Can HRV be used to evaluate training load in constant load exercises? *Eur. J. Appl. Physiol.* 108, 435–442. doi: 10.1007/s00421-009-1240-1

Karasek, R. (1979). Job demands, job decision latitude and mental strain?: implications for job redesign. *Adm. Sci. Q.* 24, 285–308. doi: 10.2307/2392498

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., Carvalho, et al. (2014). Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. *Am. J. Psychiatry* 171, 1328–1334. doi: 10.1176/appi.ajp.2014.13121605

Klein, E. M., Brähler, E., Dreier, M., Reinecke, L., Müller, K. W., Schmutzer, G., et al. (2016). The german version of the perceived stress scale – psychometric characteristics in a representative German community sample. *BMC Psychiatry* 16:1–10. doi: 10.1186/s12888-016-0875-9

Kristal-Boneh, E., Raifel, M., Froom, P., and Ribak, J. (1995). Heart rate variability in health and disease. *Scand. J. Work. Environ. Health* 21, 85–95. doi: 10.5271/sjweh.15

Kroenke, K., Spitzer, R. L., Williams, J. B. W., and Löwe, B. (2009). An ultra-brief screening scale for anxiety and depression: the PHQ-4. *Psychosomatics* 50, 613–621. doi: 10.1176/appi.psy.50.6.613

Lakusic, N., Mahovic, D., Sonicki, Z., Slivnjak, V., and Baborski, F. (2013). Outcome of patients with normal and decreased heart rate variability after coronary artery bypass grafting surgery. *Int. J. Cardiol.* 166, 516–518. doi: 10.1016/j.ijcard.2012.04.040

Lampert, T. (2011). Rauchen – aktuelle entwicklungen bei erwachsenen. *GBE Kompakt Zahl Trends Gesundheitsbericht. Bundes* 2, 1–9.

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., and Thayer, J. F. (2009a). Neural correlates of heart rate variability during emotion. *Neuroimage* 44, 213–222. doi: 10.1016/j.neuroimage.2008.07.056

Lane, R. D., Waldstein, S. R., Critchley, H. D., Derbyshire, S. W. G., Drossman, D. A., Wager, T. D., et al. (2009b). The rebirth of neuroscience in psychosomatic medicine, Part II: clinical applications and implications for research. *Psychosom. Med.* 71, 135–151. doi: 10.1097/PSY.0b013e318198a11f

Lavoie, J. A. A., and Douglas, K. S. (2012). The perceived stress scale: evaluating configural, metric and scalar invariance across mental health status and gender. *J. Psychopathol. Behav. Assess.* 34, 48–57. doi: 10.1007/s10862-011-9266-1

Lindström, J., and Tuomilehto, J. (2003). The diabetes risk score: a practical tool to predict type 2 diabetes risk. *Diabetes Care* 26, 725–731. doi: 10.2337/diacare.26.3.725

Löwe, B., Kroenke, K., and Gräfe, K. (2005). Detecting and monitoring depression with a two-item questionnaire (PHQ-2). *J. Psychosom. Res.* 58, 163–171. doi: 10.1016/j.jpsychores.2004.09.006

Löwe, B., Wahl, I., Rose, M., Spitzer, C., Glaesmer, H., Wingenfeld, K., et al. (2010). A 4-item measure of depression and anxiety: Validation and standardization of the patient health questionnaire-4 (PHQ-4) in the general population. *J. Affect. Disord.* 122, 86–95. doi: 10.1016/j.jad.2009.06.019

Lundberg, U. (2015). Work conditions and back pain problems. *Stress Heal.* 31, 1–4. doi: 10.1002/smi.2633

Maslach, C., and Jackson, S. E. (1981). The measurement of experienced burnout. *J. Organ. Behav.* 2, 99–113. doi: 10.1002/job.4030020205

May, A., and Buckley, T. (2019). *Matchfit: The Complete Manual to get Your Body and Brain fit For Work and Fit for Life*, 1 Edn. Sydney, NSW: Brio Books.

McCormack, C., Tofler, G., Soo Hoo, S., Hansen, P., and Buckley, T. (2016). Onset of acute coronary syndrome following work-related events. *Heart. Lung Circ.* 25:S323. doi: 10.1016/j.hlc.2016.06.012

Meichenbaum, D. H., and Deffenbacher, J. L. (1988). Stress inoculation training. *Couns. Psychol.* 16, 69–90. doi: 10.1177/0011000088161005

Mercedes, R. C., Duanping, L., Evans, G. W., Cascio, W. E., Chambless, L. E., Rosamond, W. D., et al. (2002). Does the cardiac autonomic response to postural change predict incident coronary heart disease and mortality? The atherosclerosis risk in communities study – pubmed. *Am. J. Epidemiol.* 155, 48–56. doi: 10.1093/aje/155.1.48

Mikrozensus (2017). *Gesundheitszustand und -Relevantes Verhalten: Körpermaße nach Altersgruppen: Männer*. Available online at: https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Gesundheit/Gesundheitszustand-Relevantes-Verhalten/Tabellen/koerpermasse-maenner.html (accessed May 29, 2020).

Mohr, G., Rigotti, T., and Müller, A. (2005). Irritation – Ein instrument zur erfassung psychischer beanspruchung im arbeitskontext. Skalen- und itemparameter aus 15 studien. *Zeitschr. Arb. Organ* 49, 44–48. doi: 10.1026/0932-4089.49.1.44

Moraes-Silva, I. C., Rodrigues, B., Coelho-Junior, H. J., Feriani, D. J., and Irigoyen, M.-C. (2017). "Myocardial infarction and exercise training: evidence from basic science," in *Advances in Experimental Medicine and Biology*, ed. J. Xiao (New York, NY: Springer), 139–153. doi: 10.1007/978-981-10-4307-9_9

Odegaard, A. O., Koh, W. P., Gross, M. D., Yuan, J. M., and Pereira, M. A. (2011). Combined lifestyle factors and cardiovascular disease mortality in chinese men and women: the singapore chinese health study. *Circulation* 124, 2847–2854. doi: 10.1161/CIRCULATIONAHA.111.048843

OECD (Hrsg.) (2020). *Health Expenditures and Financing. Jt. OECD, EUROSTAT WHO Heal. Accounts SHA Quest*. Paris: OCED.

Park, J., Kim, Y., Cho, Y., Woo, K.-H., Chung, H. K., Iwasaki, K., et al. (2001). Regular overtime and cardiovascular functions. *Ind. Health* 39, 244–249. doi: 10.2486/indhealth.39.244

Robert Koch-Institut (Hrsg.) (2014). *Chronisches Kranksein. Faktenblatt zu GEDA 2012: Ergebnisse der Studie?»Gesundheit in Deutschland Aktuell 2012«*. Berlin: RKI.

Rückholdt, M., Tofler, G. H., Randall, S., and Buckley, T. (2019). Coping by family members of critically ill hospitalised patients: an integrative review. *Int. J. Nurs. Stud.* 97, 40–54. doi: 10.1016/j.ijnurstu.2019.04.016

Sammito, S., Thielmann, B., Seibt, R., Klussmann, A., Weippert, M., and Böckelmann, I. (2015). Guideline for the application of heart rate and heart rate

variability in occupational medicine and occupational science. *ASU Int.* 2015, 1–29. doi: 10.17147/asui.2015-06-09-03

Sandercock, G. R. H., and Brodie, D. A. (2006). The role of heart rate variability in prognosis for different modes of death in chronic heart failure. *Pacing Clin. Electrophysiol.* 29, 892–904. doi: 10.1111/j.1540-8159.2006.00457.x

Schneiderman, N., Ironson, G., and Siegel, S. D. (2005). Stress and health: psychological, behavioral, and biological determinants. *Annu. Rev. Clin. Psychol.* 1, 607–628. doi: 10.1146/annurev.clinpsy.1.102803.144141

Schuster, A. K., Fischer, J. E., Thayer, J. F., Mauss, D., and Jarczok, M. N. (2016). Decreased heart rate variability correlates to increased cardiovascular risk. *Int. J. Cardiol.* 203, 728–730. doi: 10.1016/j.ijcard.2015.11.027

Schwerdtfeger, B., Reif, R., Günthner, W. A., Klinker, G., Hamacher, D., Schega, L., et al. (2009). "Pick-by-vision: a first stress test," in *Proceedings of the 8th IEEE International Symposium on Mixed and Augmented Reality, ISMAR 2009*, Orlando, FL, 115–124. doi: 10.1109/ISMAR.2009.5336484

Seeman, T. E., Singer, B. H., Rowe, J. W., Horwitz, R. I., and McEwen, B. S. (1997). Price of adaptation–allostatic load and its health consequences. MacArthur studies of successful aging. *Arch. Intern. Med.* 157, 2259–2268. doi: 10.1001/archinte.157.19.2259

Shaffer, F., and Ginsberg, J. P. (2017). An Overview of heart rate variability metrics and norms. *Front. Public Heal.* 5:258. doi: 10.3389/fpubh.2017.00258

Siegrist, J. (1996). *Soziale Krisen und Gesundheit?: Eine Theorie der Gesundheitsförderung am Beispiel von Herz-Kreislauf-Risiken im Erwerbsleben*. Göttingen: Hogrefe Verl. für Psychologie.

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., et al. (2016). Psychoneuroendocrinology assessment of the cortisol awakening response: expert consensus guidelines. *Psychoneuroendocrinology* 63, 414–432. doi: 10.1016/j.psyneuen.2015.10.010

Stringhini, S., Carmeli, C., Jokela, M., Avendaño, M., Muennig, P., Guida, F., et al. (2017). Socioeconomic status and the 25×25 risk factors as determinants of premature mortality: a multicohort study and meta-analysis of 1°7 million men and women. *Lancet* 389, 1229–1237. doi: 10.1016/S0140-6736(16)32380-7

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers Iii, J. J., and Wager, T. D. (2011). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/s0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biol. Psychol.* 74, 224–242. doi: 10.1016/j.biopsycho.2005.11.013

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Theorell, T., Hammarström, A., Aronsson, G., Träskman, B. L., Grape, T., Hogstedt, C., et al. (2015). A systematic review including meta-analysis of work environment and depressive symptoms. *BMC Public Health* 15:738. doi: 10.1186/s12889-015-1954-4

Togo, F., and Takahashi, M. (2009). Heart rate variability in occupational health - a systematic review. *Ind. Health* 47, 589–602. doi: 10.2486/indhealth.47.589

Vallejo, M. A., Vallejo-Slocker, L., Fernández-Abascal, E. G., and Mañanes, G. (2018). Determining factors for stress perception assessed with the Perceived Stress Scale (PSS-4) in Spanish and other European samples. *Front. Psychol.* 9:37. doi: 10.3389/fpsyg.2018.00037

Warttig, S. L., Forshaw, M. J., South, J., and White, A. K. (2013). New, normative, English-sample data for the Short Form Perceived Stress Scale (PSS-4). *J. Health Psychol.* 18, 1617–1628. doi: 10.1177/1359105313508346

Wilson, P. W. F., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., and Kannel, W. B. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation* 97, 1837–1847. doi: 10.1161/01.CIR.97.18.1837

World Health Organisation (2005). *Preventing Chronic Diseases?: A Vital Investment?: WHO Global Report*. Geneva: WHO.

Wulsin, L., Herman, J., and Thayer, J. F. (2018). Stress, autonomic imbalance, and the prediction of metabolic risk: a model and a proposal for research. *Neurosci. Biobehav. Rev.* 86, 12–20. doi: 10.1016/j.neubiorev.2017.12.010

**Conflict of Interest:** IB received a fee for a HRV presentation (Training for doctors 09/2019 Dresden) from Firma Grünethal GmbH (Germany).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 17 February 2021
doi: 10.3389/fnins.2020.614141

# Beating Rate Variability of Isolated Mammal Sinoatrial Node Tissue: Insight Into Its Contribution to Heart Rate Variability

*Ori Shemla[1], Kenta Tsutsui[2,3], Joachim A. Behar[1]* and Yael Yaniv[1]**

[1] Biomedical Engineering Faculty, Technion-IIT, Haifa, Israel, [2] Department of Cardiovascular Medicine, Saitama Medical University International Medical Center, Saitama, Japan, [3] Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, Baltimore, MD, United States

OPEN ACCESS

**Edited by:**
Julian F. Thayer,
The Ohio State University,
United States

**Reviewed by:**
Richard Sutton,
Imperial College London,
United Kingdom
DeWayne P. Williams,
University of California, Irvine,
United States

***Correspondence:**
Joachim A. Behar
jbehar@technion.ac.il
Yael Yaniv
yaely@bm.technion.ac.il

**Specialty section:**
This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience

**Received:** 05 October 2020
**Accepted:** 28 December 2020
**Published:** 17 February 2021

**Citation:**
Shemla O, Tsutsui K, Behar JA and Yaniv Y (2021) Beating Rate Variability of Isolated Mammal Sinoatrial Node Tissue: Insight Into Its Contribution to Heart Rate Variability. Front. Neurosci. 14:614141. doi: 10.3389/fnins.2020.614141

**Background:** Because of the complexity of the interaction between the internal pacemaker mechanisms, cell interconnected signals, and interaction with other body systems, study of the role of individual systems must be performed under *in vivo* and *in situ* conditions. The *in situ* approach is valuable when exploring the mechanisms that govern the beating rate and rhythm of the sinoatrial node (SAN), the heart's primary pacemaker. SAN beating rate changes on a beat-to-beat basis. However, to date, there are no standard methods and tools for beating rate variability (BRV) analysis from electrograms (EGMs) collected from different mammals, and there is no centralized public database with such recordings.

**Methods:** We used EGM recordings obtained from control SAN tissues of rabbits ($n$ = 9) and mice ($n$ = 30) and from mouse SAN tissues ($n$ = 6) that were exposed to drug intervention. The data were harnessed to develop a beat detector to derive the beat-to-beat interval time series from EGM recordings. We adapted BRV measures from heart rate variability and reported their range for rabbit and mouse.

**Results:** The beat detector algorithm performed with 99% accuracy, sensitivity, and positive predictive value on the test (mouse) and validation (rabbit and mouse) sets. Differences in the frequency band cutoff were found between BRV of SAN tissue vs. heart rate variability (HRV) of *in vivo* recordings. A significant reduction in power spectrum density existed in the high frequency band, and a relative increase was seen in the low and very low frequency bands. In isolated SAN, the larger animal had a slower beating rate but with lower BRV, which contrasted the phenomena reported for *in vivo* analysis. Thus, the non-linear inverse relationship between the average HR and HRV is not maintained under *in situ* conditions. The beat detector, BRV measures, and databases were contributed to the open-source PhysioZoo software (available at: https://physiozoo.com/).

**Conclusion:** Our approach will enable standardization and reproducibility of BRV analysis in mammals. Different trends were found between beating rate and BRV or HRV in isolated SAN tissue vs. recordings collected under *in vivo* conditions, respectively, implying a complex interaction between the SAN and the autonomic nervous system in determining HRV *in vivo*.

**Keywords: heart rate variability, electrogram, animal models, pacemaker, sinoatrail node**

## INTRODUCTION

The normal heart beat dynamics involves orchestration of short- and long-scale periodic signals. These signals are generated by opening and closing of membranal channels (Adair, 2003) in heart pacemaker cells, interaction between pacemaker cells (Michaels et al., 1986), and the pacemaker cell interaction with other body systems (Yang and Xu-Friedman, 2013). To understand the role and relative contribution of each signal, experiments must be performed under *in vivo*, *in situ*, and *in vitro* conditions. When exploring the function of internal pacemaker mechanisms (see for example Yaniv et al., 2015; Behar et al., 2016), the *in vitro* conditions of isolated pacemaker cells is the optimal experimental model. However, when exploring the interconnected pacemaker cell mechanisms, the *in situ* environment of isolated sinoatrial node (SAN) tissue isolating it from all environmental effects (hormonal or nervous system) is the ideal model. While ECG recordings (i.e., *in vivo*) in a variety of mammals and electrical recordings of single pacemaker cells (*in vitro*) are routinely performed in many labs, electrical data from isolated pacemaker tissue are limited.

The heart rate variability (HRV, refers to variability measured under *in vivo* conditions) has been suggested as a powerful tool to explore system function (Burg et al., 1993; Bergfeldt and Haga, 2003; Rosenberg et al., 2020). HRV has been quantified *in vivo* (Goldberger et al., 2000; Behar et al., 2018b) and the beating rate variability (BRV, refers to variability measured under *in situ* or *in vitro* conditions) has been quantified in single pacemaker cells (Zaza and Lombardi, 2001; Yaniv et al., 2011). However, although the beating rate of the SAN changes on a beat-to-beat basis, BRV has not been extensively explored in isolated SAN tissue. A number of limitations hinder such research: (i) The electrogram (EGM) is used to measure electrical signals recorded on the isolated tissue surface and reflects the inner currents in this tissue. However, EGM signals differ in beat morphology and rate from *in vivo* ECG signals even if both are from the same mammal (see **Figure 1**). Therefore, different analysis tools are required to determine the beating rate from SAN-isolated tissue EGM than those used to determine beating rates from whole-body ECG recordings. To date, there is no database of mammalian EGM recordings available for the development of such a tool, and there are no standardized, state-of-the-art, partially or fully automated tools to analyze such recordings. (ii) Assuming that the first limitation is overcome, the beating rate of the tissue can be calculated from the EGM signals. However, there is no standard method to derive BRV from HRV, and there are no publicly available programs to analyze pacemaker tissue BRV. (iii) Isolating pacemaker tissue from healthy human patients is rare; consequently, other mammals are commonly used for cardiovascular research, with rabbits and mice being the most common mammal species used for such research. The rabbit is the smallest mammal with intracellular $Ca^{2+}$ dynamics similar to humans (Bers, 2002; Terentyev et al., 2014; Morrissey et al., 2017). On the other hand, mouse models are commonly used for overexpression or knockout of genes implicated in human cardiovascular diseases (Thireau et al., 2008; Tzimas et al., 2017; Hook et al., 2018). Furthermore, mice are practical as aging models due to their short lifespan (Liu et al., 2014; Yaniv et al., 2016). However, tissues from different mammals differ in their beating rate, and thus, BRV parameters must be adjusted for different mammals (Behar et al., 2018b).

We aim here to overcome these three limitations and design an open-source program to analyze mammal BRV derived from pacemaker tissue EGM recordings. The new tool will be applied to (i) test the effect of drugs on BRV indices, (ii) compare the BRV indices of the different mammals, and (iii) compare BRV indices to their corresponding *in vivo* indices. This analysis will enhance our understanding of the contribution of pacemaker mechanisms to HRV *in vivo*.

## MATERIALS AND METHODS

### Databases

EGM data from rabbits ($n$ = 9) (Yaniv et al., 2014) and mice ($n$ = 30) in basal state as well as data from mouse SAN tissues that were exposed to phosphodiesterase inhibition (using 3-isobutyl-1-methylxanthine; IBMX) ($n$ = 6) were used (Yaniv et al., 2016). All animal training and validation data used in the present paper were obtained from published studies for which the respective animal protocols and experimental procedures were approved by the local research committee. Rabbit and mouse SAN were fixed in a heated bath ($36 \pm 0.5°C$) and superfused with Tyrode's solution (see Materials in Yaniv et al., 2014) at a rate of

**Abbreviations**: ANS, autonomic nervous system; BI, beating interval; BR, beating rate; BRV, beating rate variability (analysis *in situ*); DFA, detrended fluctuations analysis; ECG, electrocardiogram; EGM, electrogram; FN, false negative; FNR, false negative rate; FP, false positive; FPR, false positive rate; GMM, Gaussian mixture model; HF, high frequency band in the PSD; HRV, heart rate variability (analysis *in vivo*); IBMX, 3-Isobutyl-1-methylxanthine, a phosphodiesterase inhibitor; IQR, interquartile range, the range between the 25th and 75th percentiles of a dataset; LF, low frequency band in the PSD; MSE, multiscale entropy; PSD, power spectrum density function; SAN, sinoatrial node; TP, true positive; TPR, true positive rate; VLF, very low frequency band in the PSD.

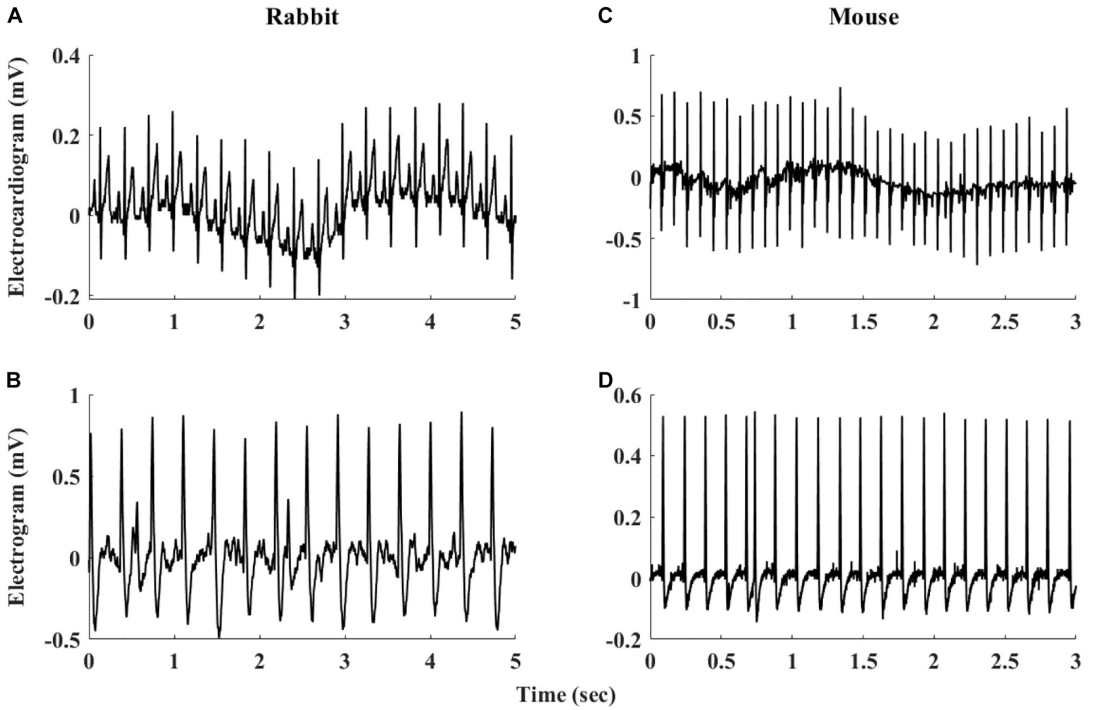



**FIGURE 1 |** Representative examples of rabbit **(A)** ECG and **(B)** sinoatrial node EGM and mouse **(C)** ECG and **(D)** sinoatrial node EGM.

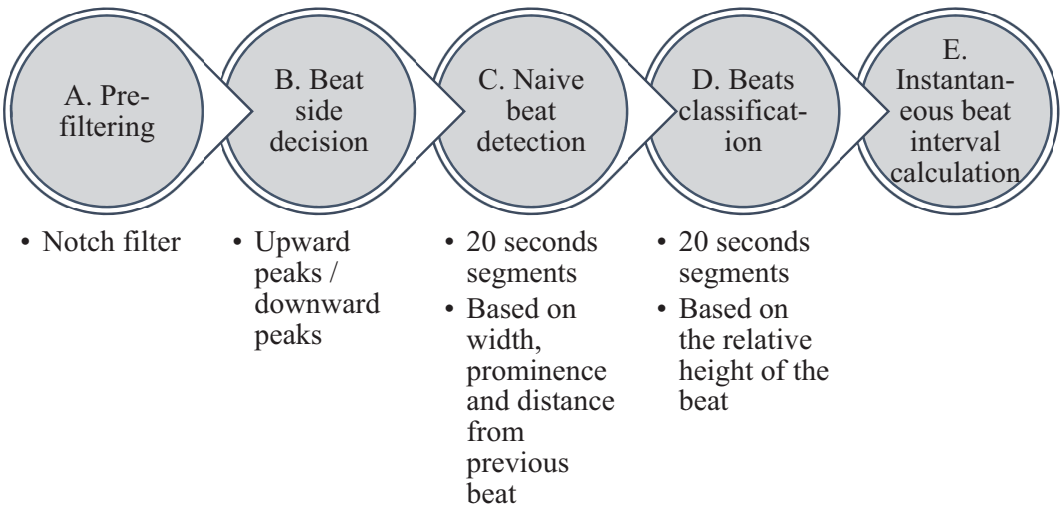



**FIGURE 2 |** Schematic description of the beat detection algorithm.

4 ml/min. An insulated Teflon-coated platinum electrode with a 0.25 (rabbit)- or 0.15 (mouse)-mm diameter tip was placed at the center of the SAN to record extracellular signals using a Neurolog system NL900D (Digitimer, Hertforsdire, United Kingdom), which were recorded at 10 kHz.

## Manual Beat Detection

Because no state-of-the-art beat detector is publicly available for mammal SAN EGM data to test our suggested algorithm, beats were manually annotated. The Matlab's "findpeaks.m" algorithm was used for initial peak detection. Then, a single trained annotator reviewed all the recordings and corrected the inaccurate annotations, i.e., false positive and false negative. These reference annotations were then used to evaluate our beat detector and to compute BRV measures. The manual annotations of the training sets were used to calculate the refractory period [minimal beating interval (BI)] and average BI of the SAN from each mammal.

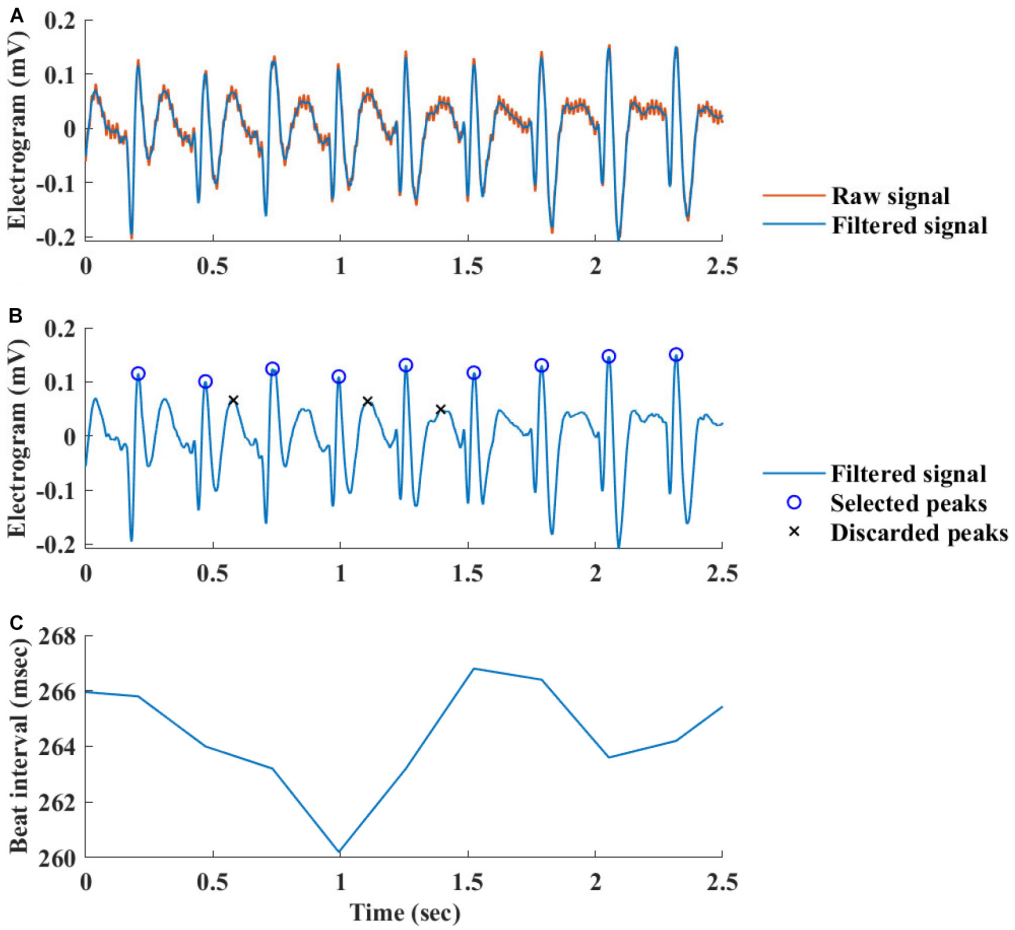



**FIGURE 3 |** Processing steps of the EGM beat detection algorithm. **(A)** Prefiltering, **(B)** naive peak detection and classification, and **(C)** instantaneous beat interval.

## Beat Detection Algorithms

**Figure 2** summarizes the steps used for beat detection in the EGM record, and **Figure 3** shows a representative example of the analysis step on one representative signal. In general, each EGM record went through (A) a prefiltering process to clear the data from environmental noise. A notch filter was used, which automatically identifies and reduces effects of the local electricity grid (e.g., 50/60 Hz noise) in the recording. (B) The signal upstream and downstream sign was determined based on the average frequency calculated from the power spectrum of the upward and downward parts of the filtered signal. In order to get more accurate BRV results, the side with the thinner peaks was chosen, reflected in higher average frequency. If the downward direction is preferred, the signal is reversed for the next steps. (C) Naive peak detection: Every 20 s of filtered signal was processed through Matlab's "findpeaks.m" algorithm. The peaks were defined as any point whose distance from a prior beat is longer than the refractory period and were higher than the neighboring points, however wider than 5 data points and not wider than twice the refractory period (width measured at half the height of the peak) and more prominent than a data-derived threshold. Peak threshold = (100 - Q)$^{th}$ percentile of the segment-Q$^{th}$ percentile of the segment (Q = 10 for rabbit and 5 for mouse). (D) After the prominence of all of the naively annotated beats was calculated, those with peaks that were less prominent than 0.7 times the median prominence of beats were eliminated. Finally, the instantaneous BI time series was calculated.

## Beating Rate Variability Measures

### Prefiltering

Before BRV can be calculated, two steps must be taken. First, to assure signal stationarity, a window length of 3 min for mice and 5 min for rabbits was used (Behar et al., 2018b). Second, range-based filtering was used. A certain constant range was defined, and every beat with a beating interval out of that range was excluded; for this purpose, every BI shorter than the refractory period or longer than three times the average BI of the mammal was discarded (Behar et al., 2018b). The resulting BI time series of the preprocessed signal was named NN.

### Time Domain Measures

The majority of time domain BRV measurements is not average BI dependent, thus do not need any adjustment (see **Table 1**). As was pointed out before (Behar et al., 2018b), only pNNxx

**TABLE 1 |** Beating rate variability parameters and their derivation from HRV.

| HRV measure | Units | Definition | Adjustment from *in vivo* to *in situ* |
|---|---|---|---|
| **Time domain** | | | |
| AVNN | (ms) | Average NN interval duration | No need |
| SDNN | (ms) | Standard deviation of NN interval duration | No need |
| RMSSD | (ms) | The square root of the mean of the sum of the squares of differences between adjacent NN intervals | No need |
| $pNN_{XX}$ | (%) | Percent of NN interval differenced greater than XX milliseconds | XX threshold, either the same or scaled according to the average BI to HR |
| SEM | (ms) | Standard error of the mean NN interval | No need |
| PIP | (%) | Percentage of inflection points in the NN interval time series | No need |
| IALS | (n.u.) | Inverse average length of the acceleration/deceleration segments | No need |
| PSS | (%) | Percentage of short segments | No need |
| PAS | (%) | The percentage of NN intervals in alternation segments | No need |
| **Frequency domain** | | | |
| Total power | ($ms^2$) | Total power of the PSD function in the frequency range | No need |
| VLF | ($ms^2$) | Power in the very low frequency band | Frequency band cutoffs |
| LF | ($ms^2$) | Power in the low frequency band | Frequency band cutoffs |
| HF | ($ms^2$) | Power in the high frequency band. Expected to be near 0 in BRV analysis of EGM from isolated tissue | Frequency band cutoffs |
| VLF norm | (%) | Power in the very low frequency band, normalized to the total power of the PSD | Frequency band cutoffs |
| LF norm | (%) | Power in the low frequency band, normalized to the total power in LF + HF bands | Frequency band cutoffs |
| HF norm | (%) | Power in the high frequency band, normalized to the total power in LF + HF bands | Frequency bands cutoffs |
| VLF-to-LF ratio | (n.u.) | The ratio between the power in the very low frequency and the power in the low frequency band | Frequency band cutoffs |
| LF-to-HF ratio | (n.u.) | The ratio between the power in the low frequency and the power in the high frequency band | Frequency band cutoffs |
| LF peak | (Hz) | Peak frequency in the low frequency band | Frequency band cutoffs |
| HF peak | (Hz) | Peak frequency in the low frequency band | Frequency bands cutoffs |
| **Non-linear domain** | | | |
| SD1 | (ms) | NN interval standard deviation along the perpendicular to the line-of-identity in the Poincare plot | No need |
| SD2 | (ms) | NN interval standard deviation along the line-of-identity in the Poincare plot | No need |
| Beta | (n.u.) | Slope of the linear interpolation of the spectrum in a log–log scale for frequencies in the VLF Beta range | VLF frequency band cutoffs |
| Alpha1 | (n.u.) | DFA low-scale slope | DFA cutoff |
| Alpha2 | (n.u.) | DFA high-scale slope | DFA cutoff |
| SampEn | (n.u.) | Sample entropy | No need |

measures that quantify the percent of NN interval differences greater than xx milliseconds must be adjusted for different mammals. We used two approaches to define the xx: one was related to the respiratory rate *in vivo*, thus the pNNxx *in situ* was similar to the value *in vivo*, and the other was to scale the xx parameter according to the scaling ratio of the BRV AVNN relative to the reported HRV AVNN.

## Frequency Domain Measures

The Welch's algorithm (Welch, 1967) was used for power spectrum density (PSD) estimation. We chose this spectral estimation method over auto-regressive model (Carvalho et al., 2003; Tarvainen et al., 2006), which is a less frequently used PSD estimate, and over the Lomb method (Lomb, 1976) because of the risk of aliasing (Behar et al., 2018a). Window lengths of 3 min for mice and 5 min for rabbits were used (Behar et al., 2018b). The PSD is traditionally divided into three main bands (Malik, 1996): the very low frequency (VLF) band, the low frequency (LF) band, and the high frequency (HF) band. To determine the cutoff frequencies between the bands, we used a Gaussian mixture model (GMM) of 2 Gaussians on the histogram of all prominent peaks (see **Figure 4**), following the approach described in Behar et al. (2018a). To calculate the prominent peaks in each band, we used a simple peak detection algorithm to look for the 16 most prominent peaks on each of the normalized PSDs, with a threshold of 0.01. The minimal frequency was determined as one over the window length in seconds and was used to defined the lower band of VLF. The HF band was set to be between the high cutoff frequency of the LF band and 2 Hz.

## Non-linear Domain Measures

Four measurements were used in this group: β coefficient, which corresponds to the slope of the linear interpolation of the spectrum in a log–log scale for frequencies below the upper bound of the VLF band, detrended fluctuations analysis (DFA) measures (Peng et al., 1994), Poincare analysis variation measure, and multiscale entropy (MSE) measures (Costa et al., 2005). Poincare analysis and MSE measures require no adaption. The

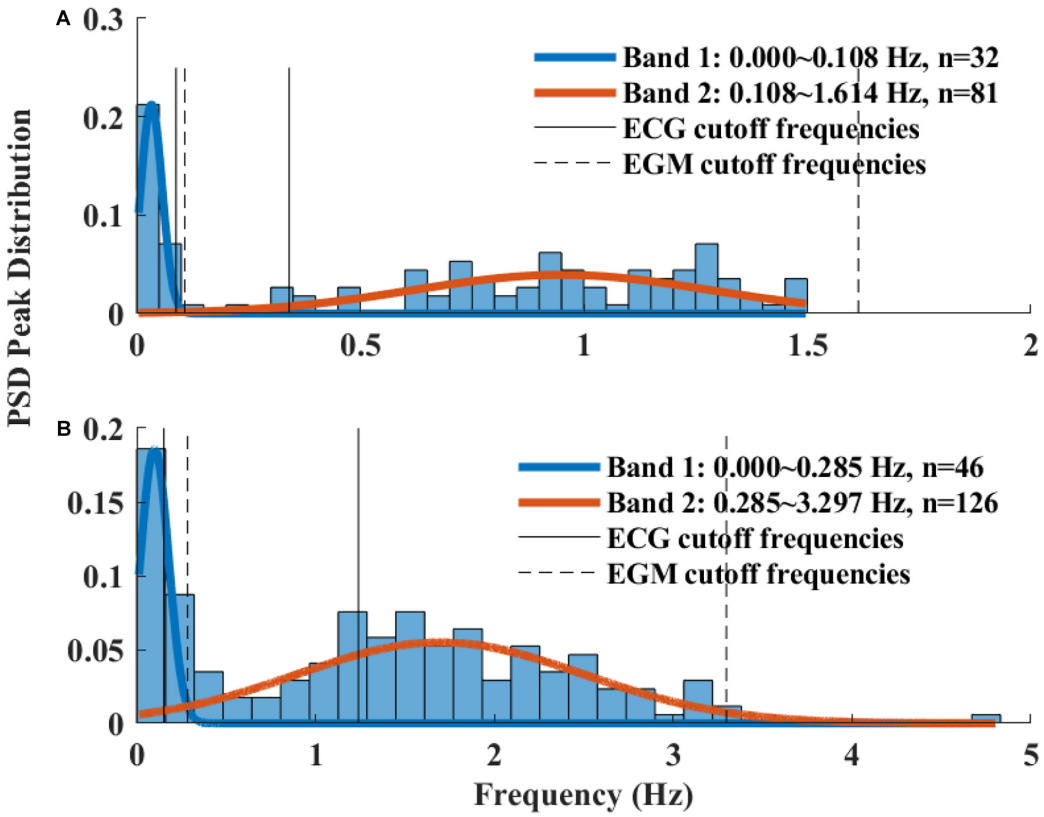



**FIGURE 4 |** A histogram of prominent power spectrum density (PSD) peaks and a fit model of two Gaussians of **(A)** rabbit (*n* = 9) and **(B)** mouse (*n* = 12). Two frequency bands were calculated for each of the mammals (dashed lines) and compared to the bands used in ECG HRV analysis (continuous line).

β coefficient was estimated in the adjusted VLF band. Originally, two DFA coefficients were reported for the slopes before and after 16 beats (cutoff). We evaluated this cutoff for BRV analysis.

## User Interface

The PhysioZoo open source software (Behar et al., 2018b) was used to calculate the different BRV measures. All of our analysis tools were implemented in PhysioZoo and are open to the public.

## Performance Statistics

The quality of the beat detector was assessed by comparing between the results of the algorithm and the manually annotated beats, by using a tolerance window of 10% of the average BI of the dataset and ignoring a constant difference between the beat locations. True positive (TP) annotations, false positive (FP) annotations, and the number of missed beats [false negative (FN)] were calculated. The following statistical measurements were used to report on the quality of our beat detector:

True positive rate (TPR)—the percentage of correctly annotated beats out of all the real beats of the dataset

$$TPR = 100^{*}\frac{TP}{TP+FN}\%. \quad (1)$$

False discovery rate (FDR)—the percentage of falsely annotated beats out of all the beat annotations

$$FDR = 100^{*}\frac{FP}{TP+FP}\%. \quad (2)$$

False negative rate (FNR)—the percentage of missed beats (real and not annotated by the algorithm) out of all the real beats of the dataset

$$FNR = 100^{*}\frac{FN}{TP+FN}\%. \quad (3)$$

## General Statistics

The rank-sum test was used to define the significance level of the differences between *in situ* vs. *in vivo* conditions of the same animal and between mouse vs. rabbit SAN tissue.

# RESULTS

This section presents the results of the performance of the beat detector on mouse and rabbit SAN tissues recordings collected under basal conditions, the range of BRV measures obtained for mouse and rabbit SAN tissues, the interpretation of BRV results in comparison to HRV, and an example of

insights on pacemaker function gained from drug response BRV analysis.

## Beat Detector

To validate the ability of the beat detector to perform on unknown EGM records, we divided the mouse data into training, validation, and test datasets. The dataset was randomly divided into a training set (40%), validation set (20%), and test set (40%). The algorithm was developed using the training set and then fine-tuned by evaluating its performance on the validation set. Finally, the generalization performance of the algorithm is reported for the test set. In the case of the rabbit data, because of the limited number of animals, the data were divided into training (67%) and validation (33%) sets. **Table 2** provides the performance statistics of the detector for mouse and rabbit. The beat detector algorithm very accurately detected the beat in the SAN EGM.

## BRV Measures

**Figure 5A** presents the BI histogram of representative examples of human-curated mouse and rabbit data. As can be seen, the BI scattering of mouse SAN tissue was higher than in the rabbit The Poincare plot (**Figure 5B**) was more scattered in mouse than rabbit, in accordance with the results of BRV time domain parameters.

**Figure 6** presents the log PSD vs. log frequency for all data; a tight linear relationship between the log (PSD) and log frequency in the VLF band was achieved. Thus, we can define β as the slope of this linear regression between the log (PSD) and log frequency in the VLF band, similar to that obtained by HRV analysis. **Figure 7** visualizes that the cutoff block size of DFA was similar to the cutoff under *in vivo* conditions [19.3 ± 7.6 ($n$ = 7) for rabbit and 17.5 ± 9 ($n$ = 11) for mouse]. **Table 3** summarizes the major changes in BRV analysis under *in situ* conditions vs. *in vivo*.

**Table 4** summarizes the analysis results of BRV measures of rabbit and mouse. The time domain analysis found a lower variability in rabbit vs. mouse SAN tissue. In both mammals, the HF band had the least information about PSD, as expected. The PSD information was divided between the VLF and LF bands (ratio of 4:1 in mouse and ratio of 1:2 in rabbit between VLF and LF bands). The non-linear parameters showed lower complexity in mouse than rabbit. To further explore the variability and complexity observed in SAN BRV, we plotted the Poincare plot and MSE vs. order, respectively. **Figure 8** shows that, in the lower scale, the MSE curve in rabbit was lower than that of mouse. However, at higher scales that represent the system complexity, the trends reverse.

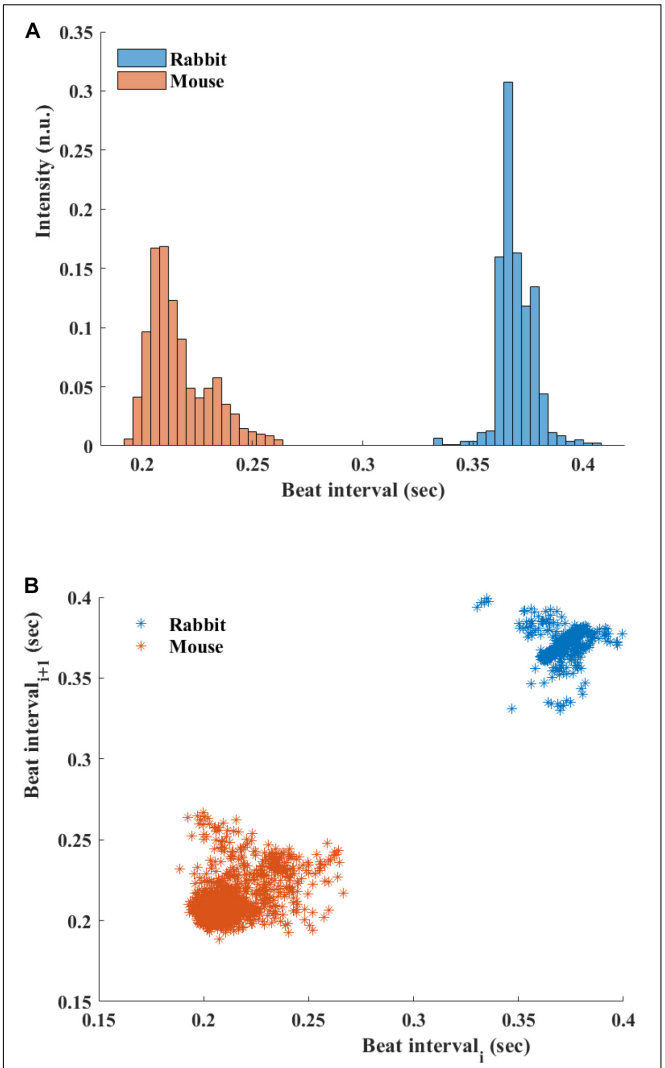



**FIGURE 5 | (A)** Beating interval (BI) histogram of a representative mouse and rabbit sinoatrial node (SAN) tissue. **(B)** Poincare plots of representative examples of SAN tissue from mice and rabbits.

**TABLE 2 |** Beat detector performance.

| | True positive rate | False discovery rate | False negative rate |
|---|---|---|---|
| **Mouse** | | | |
| Training set ($n$ = 11) | 99.6 (99.0–100)% | 0.5 (0.0–11.3)% | 0.4 (0.0–1.0)% |
| Validation set ($n$ = 5) | 99.8 (99.5–99.9)% | 0.2 (0.1–0.5)% | 0.2 (0.1–0.5)% |
| Test set ($n$ = 12) | 99.4 (99.2–99.5)% | 0.0 (0.0–0.0)% | 0.6 (0.5–0.8)% |
| **Rabbit** | | | |
| Training set ($n$ = 6) | 99.3 (99.2–99.8)% | 0.2 (0.1–0.5)% | 0.7 (0.2–0.8)% |
| Validation set ($n$ = 3) | 99.1 (99.0–99.3)% | 0.0 (0.0–0.2)% | 0.9 (0.7–1.0)% |

*All the results are presented as median and interquartile range: MED (Q1–Q3).*

## Studying BRV in Response to Conditions That Affect Pacemaker Function

To study how direct changes in pacemaker function affect the BRV, phosphodiesterase activity was inhibited by applying 100 μM 3-isobutyl-1-methylxanthine (IBMX),

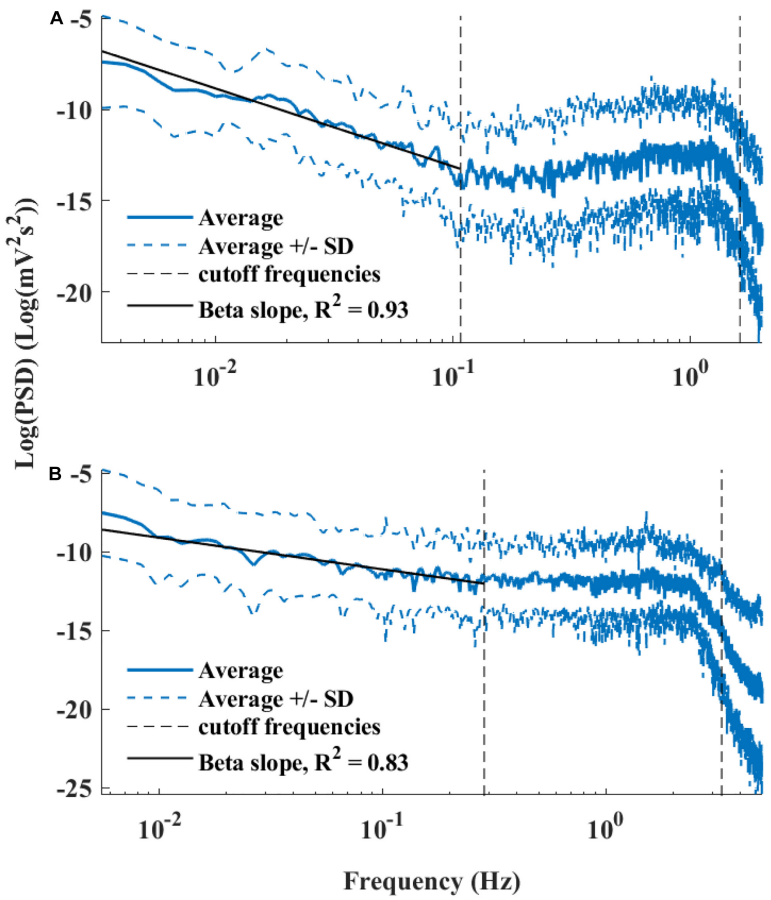



**FIGURE 6 |** Average power spectrum density (PSD) of **(A)** rabbits ($n$ = 9) and **(B)** mice ($n$ = 12), in a log–log scale. The dashed lines represent the standard deviation.

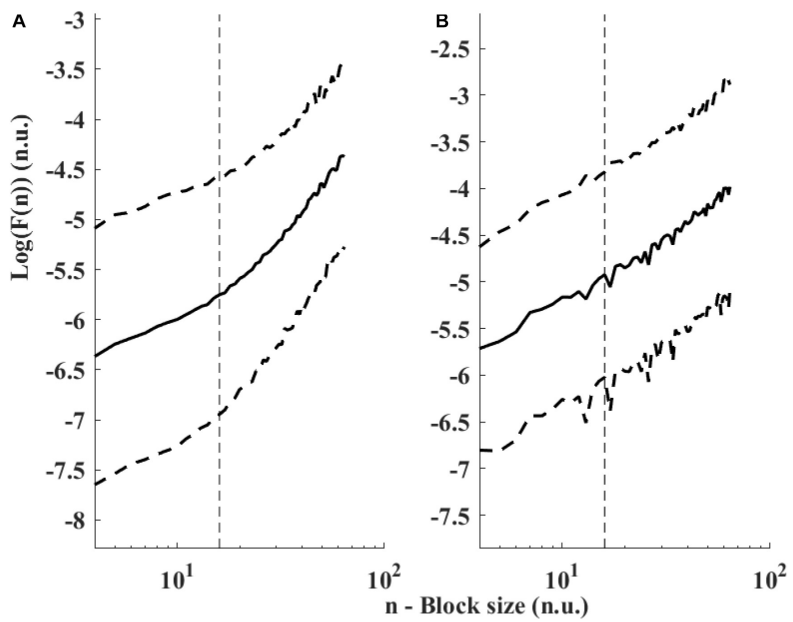



**FIGURE 7 |** Detrended fluctuations analysis visualized. Average and standard deviation (dashed lines) of F(n) for **(A)** rabbits ($n$ = 9) and **(B)** mice ($n$ = 12). A change in slope is visually noticeable around $15 < n < 20$. The traditional value used for ECG-derived HRV is $n$ = 16 (Peng et al., 1995) and shown with a dashed vertical line.

**TABLE 3 |** BRV analysis parameters and prefiltering parameters of *in vivo* vs. *in situ* analysis.

| Parameters | Rabbit—*in vivo* | Rabbit—*in situ* | Mouse—*in vivo* | Mouse—*in situ* |
|---|---|---|---|---|
| VLF–LF band cutoff (Hz) | 0.088 | 0.108 | 0.152 | 0.202 |
| LF–HF band cutoff (Hz) | 0.341 | 1.614 | 1.24 | 2.418 |
| XX for pNNXX (ms) | 17 | 24 | 5 | 12 |
| Minimum BI to filter (s) | 0.14 | 0.14 | 0.05 | 0.021 |
| Maximum BI to filter (s) | 0.58 | 0.982 | 0.24 | 0.724 |

*The band cutoff frequencies, the XX calculation for pNNXX, and minimum and maximum BI were calculated using the entire in situ dataset.*

which subsequently leads to an increase in phosphate activity and to an increase in BR. After 5 min of exposure to 100 μM IBMX, the BRV time domain measures are decreased (**Table 5** and **Figures 9A,B**). Additionally, a reduction in the relative PSD was documented in the LF, alongside an increase in the relative PSD in the HF band in response to 100 μM IBMX. There was also a shift in the LF peak (**Figure 9C**). The MSE curve showed an increase in the complexity in low-scale factors in response to 100 μM IBMX and similar behavior at high scale (**Figure 9D**).

# DISCUSSION

## Beat Detector

We present here a novel beat detector that is suitable for EGM data. Both the beat detector and the annotated data used to evaluate its performance are available at PhysioZoo platform (Behar et al., 2018b). To the best of our knowledge, this is the first time that a beat detector and annotated database are published. The beat detector was trained on mouse data and tested on a separate database with recordings from different mice. Because the amount of rabbit data was limited, the beat detector was trained and tested on data that came from the same rabbit. Future testing with recordings from additional rabbits and validation and training with other types of mammal data (see limitation) are expected to improve our beat detector.

**TABLE 4 |** BRV measures in rabbit and mouse SAN tissues and under *in vivo* conditions.

| Parameters | Rabbit *in vivo* ($p$ = 33, $n$ = 4) | Rabbit SAN tissue ($p$ = 9, $n$ = 9) | Mouse *in vivo* ($p$ = 64, $n$ = 8) | Mouse SAN tissue ($p$ = 12, $n$ = 12) |
|---|---|---|---|---|
| **Time domain** | | | | |
| AVNN (ms) | 264.95 (223.29–281.21) | 335.41* (305.78–348.82) | 108.50 (102.13–130.55) | 187.81*,# (165.47–203.14) |
| SDNN (ms) | 10.33 (6.23–15.00) | 8.69 (3.40–12.07) | 10.88 (5.60–14.32) | 10.43 (4.65–20.34) |
| RMSSD (ms) | 4.64 (2.72–9.33) | 4.36 (2.30–16.29) | 4.73 (2.89–7.61) | 9.30* (5.39–21.10) |
| pNN5 (%) | 0.38 (0.00–1.22) | 0.53 (0.00–5.89) | 16.54 (3.33–29.04) | 26.33# (3.92–51.86) |
| pNN12 (%) | 0.15 (0.00–0.48) | 0.09 (0.00–5.06) | 3.00 (0.36–8.00) | 5.47 (0.32–28.89) |
| SEM (ms) | 0.31 (0.17–0.42) | 0.29 (0.11–0.38) | 0.26 (0.14–0.35) | 0.31 (0.14–0.67) |
| PIP (%) | 41.68 (39.96–49.68) | 62.03* (60.69–67.10) | 43.09 (35.72–47.96) | 68.37*,# (66.47–75.05) |
| IALS () | 0.42 (0.40–0.50) | 0.62* (0.61–0.67) | 0.43 (0.36–0.48) | 0.69*,# (0.67–0.75) |
| PSS (%) | 36.95 (31.19–46.56) | 65.51* (62.77–75.45) | 38.95 (28.48–48.67) | 78.96*,# (74.06–82.55) |
| PAS (%) | 15.04 (10.30–19.59) | 33.99* (32.98–35.55) | 9.20 (7.07–13.24) | 39.70* (35.57–56.26) |
| **Frequency domain** | | | | |
| Total power ($ms^2$) | 63.39 (24.66–141.79) | 23.59 (8.30–88.71) | 86.62 (20.51–168.23) | 99.96 (16.51–159.82) |
| VLF ($ms^2$) | 25.84 (15.75–83.72) | 8.76* (2.68–20.43) | 48.33 (13.50–76.58) | 15.65 (2.48–77.8) |
| LF ($ms^2$) | 10.85 (5.13–25.40) | 6.46 (1.42–71.72) | 19.40 (5.18–56.22) | 33.71 (11.39–90.98) |
| HF ($ms^2$) | 5.36 (3.36–30.86) | 0.04* (0.03–1.90) | 7.69 (2.13–17.76) | 4.81*,# (1.98–22.55) |
| VLF norm () | 64.21 (49.27–70.41) | 65.35 (23.34–80.62) | 52.51 (40.82–76.73) | 14.66* (9.95–38) |
| LF norm () | 59.37 (49.10–74.22) | 97.94* (93.84–99.18) | 73.20 (62.57–80.84) | 89.70*,# (68.72–95.39) |
| HF norm () | 40.63 (25.78–50.90) | 2.06* (0.82–6.16) | 26.80 (19.16–37.43) | 10.30*,# (4.61–31.28) |
| VLF to LF ratio () | 2.95 (1.83–3.59) | 1.93 (0.33–4.43) | 1.81 (0.98–5.51) | 0.25* (0.12–0.79) |
| LF to HF ratio () | 1.46 (0.96–2.88) | 47.61* (16.51–124.13) | 2.73 (1.67–4.22) | 8.76*,# (2.44–20.97) |
| LF peak (Hz) | 0.12 (0.11–0.14) | 0.92* (0.63–1.21) | 0.25 (0.20–0.32) | 1.34* (0.72–1.77) |
| **Non-linear domain** | | | | |
| SD1 (ms) | 3.28 (1.93–6.60) | 3.08 (1.62–11.52) | 3.34 (2.05–5.38) | 6.58* (3.81–14.93) |
| SD2 (ms) | 13.47 (8.60–18.8) | 9.38 (4.41–13.15) | 14.59 (7.38–19.56) | 13.12 (5.36–20.49) |
| β () | −0.70 (−0.82 – −0.27) | −2.23* (−2.43 – −1.26) | −1.22 (−1.68 – −0.74) | −0.64*,# (−1.28 – −0.52) |
| Alpha1 () | 1.17 (1.04–1.30) | 0.45* (0.27–0.48) | 1.14 (0.97–1.25) | 0.54* (0.44–0.75) |
| Alpha2 () | 1.00 (0.89–1.15) | 1.17 (0.65–1.44) | 1.03 (0.93–1.18) | 0.70* (0.59–0.77) |
| SampEn () | 0.96 (0.60–1.18) | 0.74 (0.30–1.52) | 0.85 (0.54–1.29) | 0.89 (0.35–1.49) |

*All the results are presented as median and interquartile range: MED (Q1–Q3). *$p < 0.05$ the in situ vs. in vivo conditions of the same mammal. #$p < 0.05$ mouse vs. rabbit SAN tissue.*

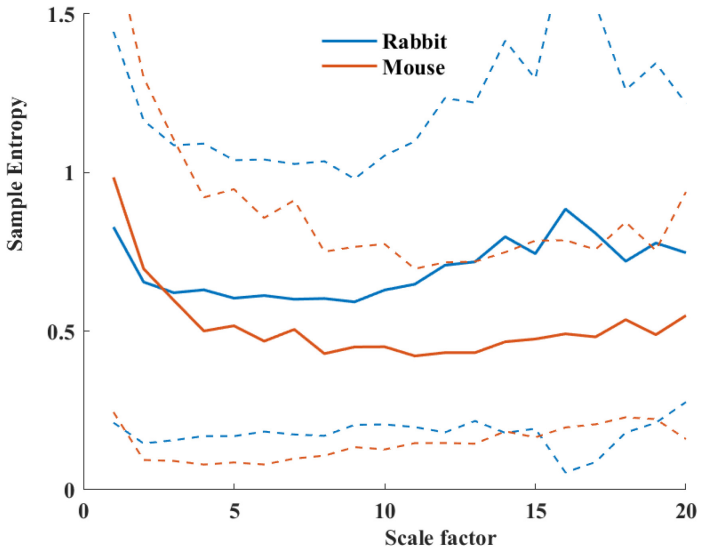



**FIGURE 8 |** Average sample entropy as function of scale factor for mouse ($n$ = 12) and rabbit ($n$ = 9) SAN tissues. The dashed lines represent the standard deviation.

**TABLE 5 |** BRV measures in mouse SAN tissues with and without phosphodiesterase inhibition by 100 μM IBMX.

| Parameters | Mouse SAN tissue ($n$ = 12) | Mouse SAN tissue + IBMX ($n$ = 6) |
|---|---|---|
| **Time domain** | | |
| AVNN (ms) | 187.81 (165.47–203.14) | 100.35 (91.42–106.69)* |
| SDNN (ms) | 10.43 (4.65–20.34) | 0.93 (0.55–4.29)* |
| RMSSD (ms) | 9.30 (5.39–21.10) | 1.48 (0.48–6.57)* |
| pNN5 (%) | 26.33 (3.92–51.86) | 0.33 (0.00–13.11)* |
| pNN12 (%) | 5.47 (0.32–28.89) | 0.00 (0.00–5.03)* |
| SEM (ms) | 0.31 (0.14–0.67) | 0.02 (0.01–0.10)* |
| PIP (%) | 68.37 (66.47–75.05) | 70.80 (64.45–72.13) |
| IALS () | 0.69 (0.67–0.75) | 0.71 (0.65–0.72) |
| PSS (%) | 78.96 (74.06–82.55) | 80.27 (67.85–82.34) |
| PAS (%) | 39.70 (35.57–56.26) | 45.31 (38.76–47.77) |
| **Frequency domain** | | |
| Total power ($ms^2$) | 99.96 (16.51–159.82) | 0.53 (0.17–7.28)* |
| VLF ($ms^2$) | 15.65 (2.48–77.80) | 0.10 (0.04–0.13)* |
| LF ($ms^2$) | 33.71 (11.39–90.98) | 0.15 (0.07–1.79)* |
| HF ($ms^2$) | 4.81 (1.98–22.55) | 0.31 (0.03–5.35) |
| VLF norm () | 14.66 (9.95–38.00) | 12.64 (2.47–42.77) |
| LF norm () | 89.70 (68.72–95.39) | 36.02 (31.85–41.91)* |
| HF norm () | 10.3 (4.61–31.28) | 63.98 (58.09–68.15)* |
| VLF to LF ratio () | 0.25 (0.12–0.79) | 0.46 (0.08–1.09) |
| LF to HF ratio () | 8.76 (2.44–20.97) | 0.57 (0.47–0.72)* |
| LF peak (Hz) | 1.34 (0.72–1.77) | 2.02 (1.74–2.20)* |
| **Non-linear domain** | | |
| SD1 | 6.58 (3.81–14.93) | 1.05 (0.34–4.65)* |
| SD2 | 13.12 (5.36–20.49) | 0.85 (0.58–3.88)* |
| Beta | −0.64 (−1.28 – −0.52) | −1.28 (−1.65 – −0.80) |
| Alpha1 | 0.54 (0.44–0.75) | 0.36 (0.27–0.44) |
| Alpha2 | 0.70 (0.59–0.77) | 0.45 (0.26–0.90) |
| SampEn | 0.89 (0.35–1.49) | 1.66 (1.05–2.05) |

*$p < 0.05$.

## BRV Measures

We present here, for the first time, a procedure for calculation of BRV measures in different mammals. The main adaptation from HRV to BRV is the changes in the band cutoff frequencies and the XX for pNNxx. Although the BR was lower under *in situ* conditions, the cutoff frequency between LF and HF was higher. These results are in contrast to the trend observed under *in vivo* conditions (Behar et al., 2018a). We also justified that the range of BI filtering should be different between *in vivo* and *in situ* conditions due to change in average BR, as expected. Under *in vivo* conditions, larger animals have a lower BR with increased HRV (Noujaim et al., 2007; Behar et al., 2018a). However, in isolated SAN tissue, a larger animal has lower BR but with lower BRV. Thus, the non-linear inverse relationship between the average HR and HRV is not maintained under *in situ* conditions. Because both the autonomic nervous system (ANS) and the SAN control the HR and HRV, lower HRV in small mammals is achieved via the balance between the sympathetic and parasympathetic systems. Thus, the ANS and the SAN have opposite effects on the HR vs. HRV relationship.

The division between the two ranges in DFA is assumed to be related to the relationship between the beating and breathing rates (Willson et al., 2002). However, we found that the cutoff between the two ranges under *in vivo* and under *in situ* conditions was similar. Thus, one may doubt that the breathing rate is the cause of the division, and it may be related to some internal pacemaker mechanism.

## BRV in Response to Conditions That Affect Pacemaker Function

We further explored the BRV in response to a drug that increases the beating rate (100 μM IBMX). As was shown before (Yaniv et al., 2016), an increase in mouse BR was associated with a decrease in the BRV time domain. Additional data regarding

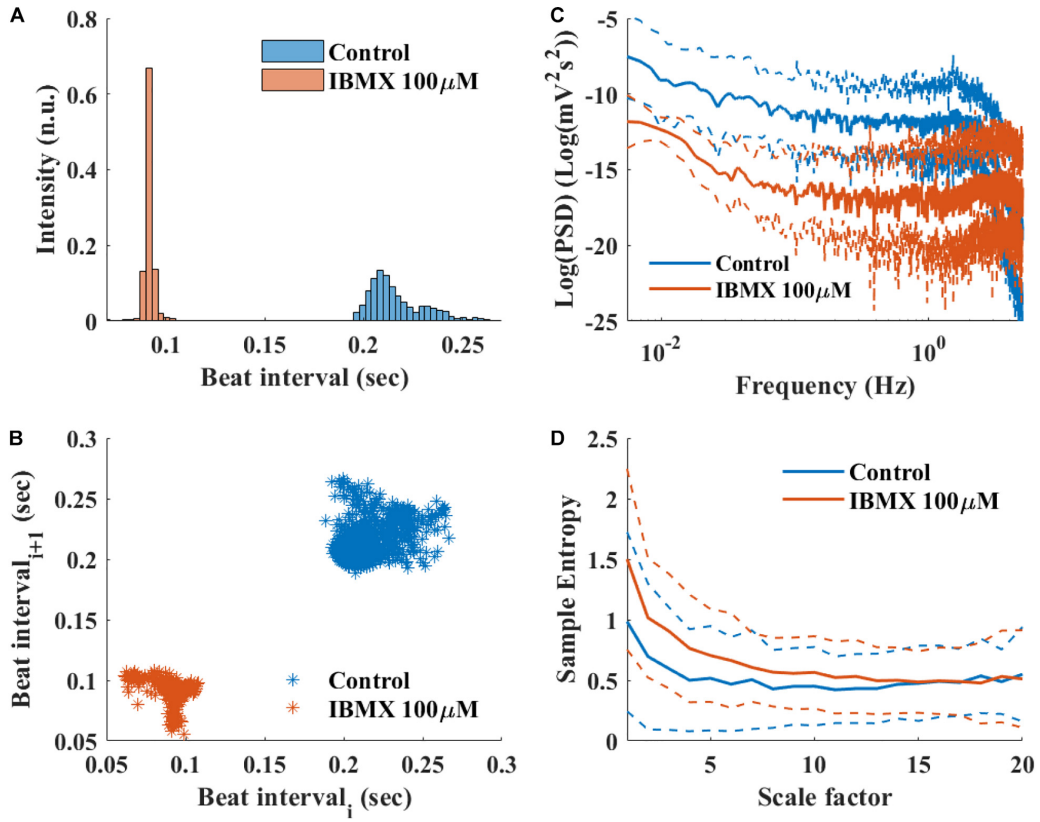



**FIGURE 9 |** Representative BRV measures if mice SAN tissues with and without phosphodiesterase inhibition by 100 μM IBMX. Representative examples **(A)** histograms, **(B)** Poincare plots and average (control *n* = 12, drug = 6), **(C)** PSD, and **(D)** sample entropy. The dashed lines represent the standard deviation.

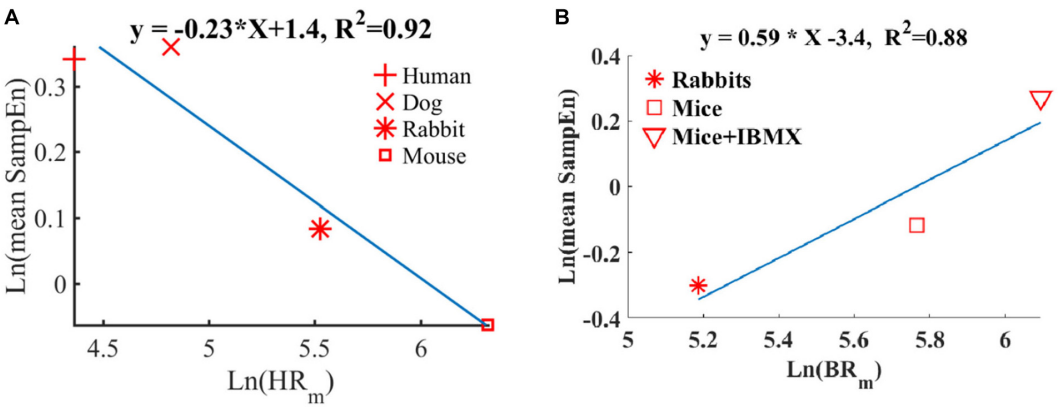



**FIGURE 10 |** Double-logarithmic plot of the mean SampEn vs. typical **(A)** heart rate (HRm) *in vivo* (adapted from Behar et al., 2018b) and **(B)** beating rate *in vitro*.

how drug intervention affects rabbit BRV, and BRV analysis from other mammals, will provide further insight into the BR vs. BRV relationship *in situ*.

## BRV vs. HRV Measures

**Table 4** summarizes the main differences between *in vivo* and *in situ* BRV. Based on the time domain analysis, there was an increase in fragmentation parameters in recordings of isolated tissue as compared to *in vivo* conditions (Behar et al., 2018b).

Exploring the frequency domain parameters revealed interesting phenomena. The majority of information was restored in the LF and VLF bands of isolated tissue compared to *in vivo* conditions. Thus, the SAN is the main contributor to the VLF and LF bands in rabbit and to the VLF band in mice. One of the consequences of such behavior in mice and rabbits can be

related to the balance between sympathetic and parasympathetic activity and to the relative magnitude of each of these activities in different mammals. The mice in our research were hosted in 20°C well below the thermoneutrality temperature and thus showed higher sympathetic activity, and sympathetic activity affects LF more than HF (Axsom et al., 2020). However, acclimation to a thermoneutral environment reversed the balance between sympathetic and parasympathetic activity (Swoap et al., 2008; Axsom et al., 2020), and thus, using SAN tissue from such mice in the future can reverse the information restored in different bands. As expected, there was almost no PSD in the HF band in both rabbit and mouse SAN tissue. Thus, the ANS is the main contributor to that band. Note that respiratory rate frequency is characteristic of the vagal activity. Because vagal activity is expressed in the HF band, it can explain the reduction in that band in the isolated tissue.

Based on the non-linear analysis parameters, the system complexity in the rabbit SAN tissue was higher as compared to *in vivo* conditions; the opposite trend was observed in mice. Thus, the system complexity is maintained mainly by the SAN in rabbit, and in mice, both SAN and ANS contribute to this complexity.

We and others have shown before that smaller mammals have a higher HR than larger mammals (Noujaim et al., 2007; Behar et al., 2018a). We also showed that an increase in HR was associated with a decrease in sample entropy (**Figure 10A**). In contrast to the *in vivo* conditions, in isolated SAN tissue, the opposite relationship between entropy index and BR was observed (**Figure 10B**). Thus, putting the *in vivo* and SAN tissue data together on one graph is expected to show biphasic behavior for the BR and BRV. One can therefore hypothesize that when the beating rate increases *in situ*, there is an increase in short-time entropy quantified by first-order entropy. Thus, the intrinsic properties of SAN lead to an increase in entropy in response to an increase in BR. However, the fact that this relationship reverses *in vivo* implies that (i) the ANS leads to an opposite relationship between entropy and BR, and (ii) under *in vivo* conditions, the ANS controls this first-order entropy scale as was shown before (Rosenberg et al., 2020).

## LIMITATIONS

The beat detector was trained and tested on SAN from healthy animals, without any drug interventions. Future training and examination of the beat detector on EGM of SAN tissue that was exposed to a drug will be necessary to validate the use of the beat detector. Similarly, we analyzed BRV using recordings of SAN of healthy mammals. Future analysis of BRV of SAN tissue from transgenic animals or animals with cardiac diseases and comparison to the respective *in vivo* conditions will allow us to learn how changes in SAN function affects HRV *in vivo*.

## CONCLUSION

The approach presented here will enable standardization and reproducibility of BRV analysis in mammalian. Different trends were found between BR and BRV of isolated SAN tissue vs. HRV *in vivo* conditions, implying a complex interaction between SAN and the ANS in determining HRV *in vivo*.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in PhysioZoo.com.

## ETHICS STATEMENT

The animal training and validation data used in the present paper were obtained from published studies for which the respective animal protocols and experimental procedures were approved by the local research committee.

## AUTHOR CONTRIBUTIONS

JB and YY conceived and designed the research. OS implemented the source code and interface and formatted the databases. YY drafted the manuscript. KT, JB, and YY edited and revised the manuscript. JB, YY, OS, and KT approved the final version. All authors contributed to the article and approved the submitted version.

## FUNDING

The work was supported by a Kamin Grant (YY) and ISF 330/19 (YY). This research was also supported by the Intramural Research Program of the NIH, National Institute on Aging (KT). KT was supported by the Japan Society for the Promotion of Science Research Fellowship for Japanese Biomedical and Behavioral Researchers at NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## REFERENCES

Adair, R. K. (2003). Noise and stochastic resonance in voltage-gated ion channels. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12099–12104. doi: 10.1073/pnas.2034447100

Axsom, J. E., Nanavati, A. P., Rutishauser, C. A., Bonin, J. E., Moen, J. M., and Lakatta, E. G. (2020). Acclimation to a thermoneutral environment abolishes age-associated alterations in heart rate and heart rate variability in conscious, unrestrained mice. *GeroScience* 42, 217–232. doi: 10.1007/s11357-019-00126-127

Behar, J., Ganesan, A., Zhang, J., and Yaniv, Y. (2016). The autonomic nervous system regulates the heart rate through cAMP-PKA dependent and independent coupled-clock pacemaker cell mechanisms. *Front. Physiol.* 7:419. doi: 10.3389/fphys.2016.00419

Behar, J. A., Rosenberg, A. A., Shemla, O., Murphy, K. R., Koren, G., Billman, G. E., et al. (2018a). A universal scaling relation for defining power spectral

bands in mammalian heart rate variability analysis. *Front. Physiol.* 9:1001. doi: 10.3389/fphys.2018.01001

Behar, J. A., Rosenberg, A. A., Weiser-Bitoun, I., Shemla, O., Alexandrovich, A., Konyukhov, E., et al. (2018b). PhysioZoo: a novel open access platform for heart rate variability analysis of mammalian electrocardiographic data. *Front. Physiol.* 9:1390. doi: 10.3389/fphys.2018.01390

Bergfeldt, L., and Haga, Y. (2003). Power spectral and Poincare plot characteristics in sinus node dysfunction. *J. Appl. Physiol.* 94, 2217–2224. doi: 10.1152/japplphysiol.01037.2002

Bers, D. M. (2002). Cardiac excitation–contraction coupling. *Nature* 415, 198–205. doi: 10.1038/415198a

Burg, M. M., Jain, D., Soufer, R., Kerns, R. D., and Zaret, B. L. (1993). Role of behavioral and psychological factors in mental stress-induced silent left ventricular dysfunction in coronary artery disease. *J. Am. Coll. Cardiol.* 22, 440–448. doi: 10.1016/0735-1097(93)90048-6

Carvalho, J. L. A., Rocha, A. F., Dos Santos, I., Itiki, C., Junqueira, L. F., and Nascimento, F. A. O. (2003). "Study on the optimal order for the auto-regressive time-frequency analysis of heart rate variability," in *Annual International Conference of the IEEE Engineering in Medicine and Biology - Proceedings*, (New York, NY: IEEE), 2621–2624. doi: 10.1109/iembs.2003.1280453

Costa, M., Goldberger, A. L., and Peng, C.-K. (2005). Multiscale entropy analysis of biological signals. *Phys. Rev. E* 71:021906. doi: 10.1103/PhysRevE.71.021906

Goldberger, A. L., Amaral, L. A. N., Glass, L., Hausdorff, J. M., Ivanov, P. C., Mark, R. G., et al. (2000). PhysioBank, physiotoolkit, and physionet. components of a new research resource for complex physiologic signals. *Circulation* 101, 215–220. doi: 10.1161/01.CIR.101.23.e215

Hook, M., Roy, S., Williams, E. G., Bou Sleiman, M., Mozhui, K., Nelson, J. F., et al. (2018). Genetic cartography of longevity in humans and mice: current landscape and horizons. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1864, 2718–2732. doi: 10.1016/J.BBADIS.2018.01.026

Liu, J., Sirenko, S., Juhaszova, M., Sollott, S. J., Shukla, S., Yaniv, Y., et al. (2014). Age-associated abnormalities of intrinsic automaticity of sinoatrial nodal cells are linked to deficient cAMP-PKA-Ca(2+) signaling. *Am. J. Physiol. Hear. Circ. Physiol.* 306, H1385–H1397. doi: 10.1152/ajpheart.00088.2014

Lomb, N. R. (1976). Least-squares frequency analysis of unequally spaced data. *Astrophys. Space Sci.* 39, 447–462. doi: 10.1007/BF00648343

Malik, M. (1996). Heart rate variability. standards of measurement, physiological interpretation, and clinical use. task force of the european society of cardiology and the north american society of pacing and electrophysiology. *Eur. Heart J.* 17, 354–381. doi: 10.1111/j.1542-474X.1996.tb00275.x

Michaels, D. C., Matyas, E. P., and Jalife, J. (1986). Dynamic interactions and mutual synchronization of sinoatrial node pacemaker cells. a mathematical model. *Circ. Res.* 58, 706–720. doi: 10.1161/01.res.58.5.706

Morrissey, P. J., Murphy, K. R., Daley, J. M., Schofield, L., Turan, N. N., Arunachalam, K., et al. (2017). A novel method of standardized myocardial infarction in aged rabbits. *Am. J. Physiol. Circ. Physiol.* 312, H959–H967. doi: 10.1152/ajpheart.00582.2016

Noujaim, S. F., Berenfeld, O., Kalifa, J., Cerrone, M., Nanthakumar, K., Atienza, F., et al. (2007). Universal scaling law of electrical turbulence in the mammalian heart. *Proc. Natl. Acad. Sci. U S A.* 104, 20985–20989. doi: 10.1073/pnas.0709758104

Peng, C.-K., Buldyrev, S. V., Havlin, S., Simons, M., Stanley, H. E., and Goldberger, A. L. (1994). Mosaic organization of DNA nucleotides. *Phys. Rev. E* 49, 1685–1689. doi: 10.1103/PhysRevE.49.1685

Peng, C.-K., Havlin, S., Stanley, H. E., and Goldberger, A. L. (1995). Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. *Chaos* 5, 82–87. doi: 10.1063/1.166141

Rosenberg, A. A., Weiser-Bitoun, I., Billman, G. E., and Yaniv, Y. (2020). Signatures of the autonomic nervous system and the heart's pacemaker cells in canine electrocardiograms and their applications to humans. *Sci. Rep.* 10:9971. doi: 10.1038/s41598-020-66709-z

Swoap, S. J., Li, C., Wess, J., Parsons, A. D., Williams, T. D., and Overton, J. M. (2008). Vagal tone dominates autonomic control of mouse heart rate at thermoneutrality. *Am. J. Physiol. - Hear. Circ. Physiol.* 294, 1581–1588. doi: 10.1152/ajpheart.01000.2007

Tarvainen, M. P., Georgiadis, S. D., Ranta-Aho, P. O., and Karjalainen, P. A. (2006). Time-varying analysis of heart rate variability signals with a Kalman smoother algorithm. *Physiol. Meas.* 27, 225–239. doi: 10.1088/0967-3334/27/3/002

Terentyev, D., Rees, C. M., Li, W., Cooper, L. L., Jindal, H. K., Peng, X., et al. (2014). Hyperphosphorylation of RyRs underlies triggered activity in transgenic rabbit model of LQT2 syndrome. *Circ. Res.* 115, 919–928. doi: 10.1161/CIRCRESAHA.115.305146

Thireau, J., Zhang, B. L., Poisson, D., and Babuty, D. (2008). Heart rate variability in mice: a theoretical and practical guide. *Exp. Physiol.* 93, 83–94. doi: 10.1113/expphysiol.2007.040733

Tzimas, C., Johnson, D. M., Santiago, D. J., Vafiadaki, E., Arvanitis, D. A., Davos, C. H., et al. (2017). Impaired calcium homeostasis is associated with sudden cardiac death and arrhythmias in a genetic equivalent mouse model of the human HRC-Ser96Ala variant. *Cardiovasc. Res.* 113, 1403–1417. doi: 10.1093/cvr/cvx113

Welch, P. D. (1967). The use of fast fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. *IEEE Trans. Audio Electroacoust.* 15, 70–73. doi: 10.1109/TAU.1967.1161901

Willson, K., Francis, D. P., Wensel, R., Coats, A. J. S., and Parker, K. H. (2002). Relationship between detrended fluctuation analysis and spectral analysis of heart-rate variability. *Physiol. Meas.* 23, 385–401. doi: 10.1088/0967-3334/23/2/314

Yang, H., and Xu-Friedman, M. A. (2013). Stochastic properties of neurotransmitter release expand the dynamic range of synapses. *J. Neurosci.* 33, 14406–14416. doi: 10.1523/JNEUROSCI.2487-13.2013

Yaniv, Y., Ahmet, I., Liu, J., Lyashkov, A. E., Guiriba, T.-R., Okamoto, Y., et al. (2014). Synchronization of sinoatrial node pacemaker cell clocks and its autonomic modulation impart complexity to heart beating intervals. *Hear. Rhythm* 11, 1210–1219. doi: 10.1016/j.hrthm.2014.03.049

Yaniv, Y., Ahmet, I., Tsutsui, K., Behar, J., Moen, J. M., Okamoto, Y., et al. (2016). Deterioration of autonomic neuronal receptor signaling and mechanisms intrinsic to heart pacemaker cells contribute to age-associated alterations in heart rate variability in vivo. *Aging Cell* 15, 716–724. doi: 10.1111/acel.12483

Yaniv, Y., Ganesan, A., Yang, D., Ziman, B. D., Lyashkov, A. E., Levchenko, A., et al. (2015). Real-time relationship between PKA biochemical signal network dynamics and increased action potential firing rate in heart pacemaker cells: kinetics of PKA activation in heart pacemaker cells. *J. Mol. Cell Cardiol.* 86, 168–178. doi: 10.1016/j.yjmcc.2015.07.024

Yaniv, Y., Maltsev, V. A., Escobar, A. L., Spurgeon, H. A., Ziman, B. D., Stern, M. D., et al. (2011). Beat-to-beat Ca2+-dependent regulation of sinoatrial nodal pacemaker cell rate and rhythm. *J. Mol. Cell. Cardiol.* 51, 902–905. doi: 10.1016/j.yjmcc.2011.08.029

Zaza, A., and Lombardi, F. (2001). Autonomic indexes based on the analysis of heart rate variability: a view from the sinus node. *Cardiovasc. Res.* 50, 434–442. doi: 10.1016/s0008-6363(01)00240-1

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

BRIEF RESEARCH REPORT
published: 12 March 2021
doi: 10.3389/fnins.2021.617443

# Compassion Is Not a Benzo: Distinctive Associations of Heart Rate Variability With Its Empathic and Action Components

*Maria Di Bello[1]*, Cristina Ottaviani[1,2] and Nicola Petrocchi[3]*

*[1] Department of Psychology, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy, [2] Functional Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy, [3] Department of Economics and Social Sciences, John Cabot University, Rome, Italy*

Recent studies have linked compassion with higher vagally mediated heart rate variability (vmHRV), a measure of parasympathetic activity, and meta-analytic evidence confirmed significant and positive associations. Compassion, however, is not to be confused with soothing positive emotions: in order to engage in actions aimed to alleviate (self or others) suffering, the pain should resonate, and empathic sensitivity should be experienced first. The present study examined the association between vmHRV and the empathic sensitivity and action components of trait and state compassion. To do so, several dispositional questionnaires were administered and two videos inducing empathic sensitivity (video 1) and compassionate actions (video 2) were shown, while the ECG was continuously recorded, and momentary affect was assessed. Results showed that (i) scores on subscales assessing the empathic component of trait compassion were inversely related to resting vmHRV; (ii) vmHRV decreased after video 1 but significantly increased after video 2. As to momentary affect, video 1 was accompanied with an increase in sadness and a decrease in positive affect, whereas video 2 was characterized by an increase in anger, a parallel decrease in sadness, and an increase (although non-significant) in positive affect. Overall, present findings support the notion that it is simplistic to link compassion with higher vmHRV. Compassion encompasses increased sensitivity to emotional pain, which is naturally associated with lower vmHRV, and action to alleviate others' suffering, which is ultimately associated with increased vmHRV. The importance of adopting a nuanced perspective on the complex physiological regulation that underlies compassionate responding to suffering is discussed.

**Keywords: compassion, action, heart rate variability, empathic engagement, empathic sensitivity**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Richard Sutton, Imperial College London, United Kingdom*
*James Kirby, The University of Queensland, Australia*

****Correspondence:***
*Maria Di Bello*
*maria.dibello@uniroma1.it*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *14 October 2020*
***Accepted:*** *17 February 2021*
***Published:*** *12 March 2021*

***Citation:***
*Di Bello M, Ottaviani C and Petrocchi N (2021) Compassion Is Not a Benzo: Distinctive Associations of Heart Rate Variability With Its Empathic and Action Components. Front. Neurosci. 15:617443. doi: 10.3389/fnins.2021.617443*

## INTRODUCTION

Over two decades, studies have shown the health promoting influences of compassion (Pace et al., 2009, 2013; Seppala et al., 2012; Slavich and Cole, 2013; Yarnell and Neff, 2013; Zessin et al., 2015), fostering the development of different compassion-focused interventions and scientific research on the nature of therapeutic process (Kirby, 2016; Ferrari et al., 2019; Gilbert, 2019). The aim of the

present study was to investigate the physiological signature (measured by vagally mediated heart rate variability; vmHRV) of the specific components of trait and state compassion.

Compassion has been defined as a sensitivity to suffering in self and others with a commitment to try to alleviate and prevent it (Gilbert, 2019); it does not refer to a positive emotional experience but to a suite of concrete prosocial behaviors, and may include positive emotional states such as kindness, empathy, generosity, and acceptance (Weidman and Tracy, 2020). Conversely, kindness is intended to create the conditions for happiness and prosperity; it does not require any sensitivity to and analysis of suffering (Gilbert et al., 2019).

As an evolved prosocial motivation, compassion requires complex social processing systems and evolved competencies which allow approach and engagement with distress signals to help alleviate the distress, such as, a sensitive awareness of suffering, empathic awareness, distress tolerance, a non-judgmental attitude, and the willingness to develop specific skills to enact the motive (knowing intentionality) (Gilbert, 2017a, 2019). This set of competencies does not translate compassion into an automatic response to suffering, but into a complex motivational process which guides the individual to be sensitive and receptive to signals of suffering, as opposed to trying to avoid or suppress them, and understand what is the most helpful thing to do in the specific circumstance.

Over the past decade, the psychophysiological perspective has contributed significantly to our understanding of compassion in an effort to provide unique insights into its nature and processes. A specific role of the 10th cranial nerve, namely the vagus, has been highlighted in compassion-related processes (Thayer et al., 2012; Porges, 2017; Petrocchi and Cheli, 2019). Efferent vagal fibers exert parasympathetic control of the heart. An indirect and non-invasive measure of vagal modulation of the heart is vmHRV, a measure of the variability of the time periods between adjacent heartbeats, resulting from the dynamic interplay between the parasympathetic and the sympathetic nervous systems (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In particular, high tonic vmHRV is a measure of robust parasympathetic control on the heart and appears to reflect the degree to which the prefrontal cortex provides context-appropriate control over the periphery. On the other hand, phasic vmHRV suppression represents the withdrawal of cardiac vagal control and the activation of the defensive systems (Thayer et al., 2009, 2012).

Recent studies (Svendsen et al., 2016, 2020; Luo et al., 2018) and meta-analytic evidence (Di Bello et al., 2020) showed that both state (i.e., induced) and trait (i.e., dispositional) compassion, toward oneself and others, are related to higher vmHRV (Di Bello et al., 2020). Additionally, compassion-focused practices can improve vmHRV (Rockliff et al., 2008; Arch et al., 2014; Matos et al., 2017; Petrocchi et al., 2017).

Previous studies, however, ignored a crucial ingredient of compassion, that is whether the ability to pay sensitive attention to suffering and effectively engage with it is accompanied by the intention and capacity to start the response process (an appropriate action) to alleviate the suffering (Gilbert, 2017b; Kirby et al., 2019; Gilbert, 2020). If both these components are taken into account, a non-linear association between compassion and vmHRV is expected (Miller, 2018). For example, attentional sensitivity and tolerance to other's distress may be associated with a decrease in vmHRV. Indeed, individuals with lower vmHRV show reduced ability to inhibit attention to affectively significant cues (Park et al., 2013) and the anticipation of social stress has shown to reduce vmHRV in individuals with high self-compassion (Luo et al., 2018). Additionally, in response to observation of others' emotional expressions, lower resting vmHRV was associated with higher activation in the mirror neuron system (Miller et al., 2019).

Indeed, a phasic decrease in vmHRV could be considered as a signature of the empathic engagement with others' suffering and it is the prerequisite to be touched by others' suffering and subsequently act to alleviate it (Miller, 2018; Gilbert, 2019; Steffen et al., 2020). Heart rate (and not vmHRV) has shown to increase more during compassion meditation than during neutral states, and HR increases are correlated with BOLD signal in the right middle insula (Lutz et al., 2008), suggesting that compassion enhances the emotional and somatosensory brain representations of others' emotions, amplifying the saliency of emotional stimuli.

Based on such evidence, compassion is expected to be first associated with reduced vmHRV to reflect empathic engagement with suffering, and then by increased vmHRV when the appropriate helpful action is performed. Indeed, in a pilot uncontrolled study, self-compassionate writing has been associated with a significant decrease in vmHRV during the task, and a significant increase in vmHRV during recovery (Steffen et al., 2020).

To date, the physiological signatures of each of the different components of compassion have not been thoroughly investigated, neither when dispositional (trait) nor when induced (state) compassion was examined. The study aimed to fill this gap, assessing resting vmHRV, the specific components of trait compassion, and vmHRV responses to and recovery from videos evoking the different components of compassion (i.e., empathic sensitivity and compassionate action). We hypothesized: (i) the association between tonic vmHRV and trait compassion to be negative for the empathic component and positive for the engagement component of compassion; (ii) to see a vmHRV decrease in response to a video eliciting empathetic sensitivity toward others' suffering, and a vmHRV increase in response to a video depicting intentional actions of giving help. We expected vmHRV reactivity to and recovery from such videos to be moderated by self and other-oriented trait compassion.

# MATERIALS AND METHODS

## Participants

The sample consisted of 45 students [88% female, mean age = 20.5 (1.05) years] of an American university based in Rome, Caucasian, and English speaking. Three subjects were not included in the analyses due to unreliable physiological measures ($N$ = 42, 88% female). Exclusionary criteria were self-reported major psychiatric or cognitive problems, organic illnesses,

substance abuse, cardiovascular disease, use of drugs/medications affecting cardiovascular function, body mass index $>30$ kg/m$^2$, menopause, use of oral contraceptives during the previous 6 months, pregnancy or childbirth within the last 12 months. The protocol was approved by the local Ethics Committee.

## Procedure and Design

The experiment was conducted as a repeated measures within-subjects experimental design (**Figure 1**). Written informed consent was provided. Participants were asked to refrain from drinking caffeinated or alcoholic beverages or exercising in the 2 h prior to the session. Data on socio-demographic information, medical background and currently prescribed medications were first collected. Then, several self-report trait measures of compassion were administered. Participants were asked to rest for a 5-min baseline ECG assessment, during which they rated their momentary affect by visual analog scales (VAS, baseline). Next, a 2.30-min video representing an "empathic sensitivity" condition was shown. This was followed by a post-video recovery assessment (2 min), during which the VAS were administered again (time 1). To collect a second baseline, participants were then instructed to relax for another 1.25 min. Subsequently, a

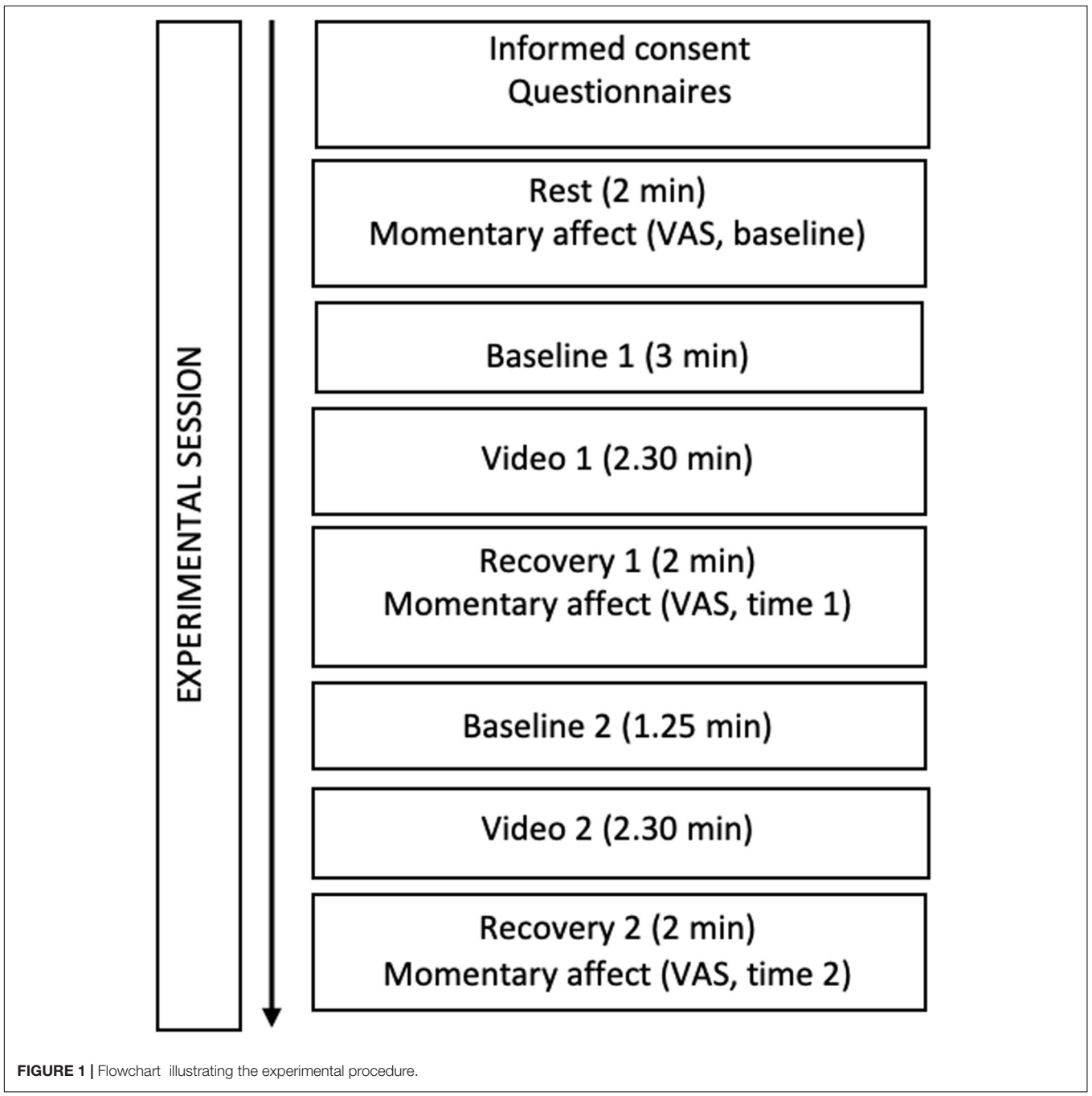



**FIGURE 1 |** Flowchart illustrating the experimental procedure.

second video representing a "compassionate action" condition was broadcast for 2.30 min, followed by a post-video recovery assessment (2 min), during which participants had to rate again their current affect by VAS (time 2). The videos are available from the last author on request.

## Stimulus Materials

Instructions were displayed on a computer monitor, while a guide-voice signaled participants to relax at baseline and gave indications on how to rate the experienced feelings on the VAS, and how to watch the videos. A pilot study was conducted to make sure that each video elicited one of the two specific components of compassion: empathic sensitivity by video 1 and intention to perform helpful actions by video 2. In video 1, others' suffering scenes were presented along with brief sentences describing the thoughts of each suffering individuals, aimed to facilitate the empathic attunement with them. Video 2 depicted scenes of a person actively engaged in giving help and support to suffering others. The participants had to pay attention, allowing themselves to experience thoughts and feelings, while taking the perspective of the person who gives help.

## Measures

### Socio-Demographic and Personal Information

Socio-demographic and personal information, included sex, age, height, and weight, and physical activity habits.

### Compassionate Engagement and Action Scales

Compassionate Engagement and Action Scales (CEAS; Gilbert et al., 2017) encompasses subscales assessing three "flows" of compassion (for others, from others, and self-compassion). Respondents are asked to think about distressing situations and rate how each sentence applies to them. For each scale, a total score and two subscales were calculated: Engagement and Actions. For the Compassion for Self scale, two subdimensions were analyzed in the Engagement subscale: Sensitivity to Suffering, and Engagement with Suffering.

### Compassionate Love for Humanity Scale

Compassionate Love for Humanity Scale (Sprecher and Fehr, 2005) is a 21-item scale designed to measure the compassionate attitude for strangers when they are most in need.

### Compassionate and Self-Image Goals Scale

Compassionate and Self-Image Goals Scale (Crocker and Canevello, 2008) consists of 13 items. Seven items assess compassionate goals, and six items assess self-image goals. The average for each subscale was calculated, with higher scores indicating higher interpersonal goals.

### Visual Analog Scales – VAS

At baseline and after each video (time 1 and time 2), participants rated their current levels of affective states (sad, angry, happy, anxious, calm, strong, weak, content, relieved, self-critical) on several 5-point VAS.

### Physiological Measures

Interbeat intervals were continuously recorded throughout the experimental session using the Firstbeat Bodyguard 2 with a standard electrode configuration. Time (root mean square successive difference; RMSSD) and frequency-domain (high-frequency HRV; HF-HRV) vmHRV measures were calculated. VmHRV analysis was performed using Kubios HRV software (Tarvainen et al., 2014). Artifacts and ectopic beats were corrected using a threshold-based correction.

## Data Analyses

The data were analyzed using IBM SPSS Statistics version 25 and Mplus 5.1. To evaluate the effects of dispositional variables (socio-demographic factors and trait measures) on the dependent variables, Pearson correlations were performed between BMI, physical activity, trait questionnaires, and vmHRV. Sex differences were evaluated by Student's *t*-test. The variables that resulted to be significantly associated with vmHRV measures were included in the subsequent analyses as covariate.

Following existing recommendations (Laborde et al., 2018), to determine whether the two videos induced a different physiological response, we computed reactivity and recovery scores by subtracting the baseline from each video-phase and the video-phase from each recovery-phase, respectively. A $2 \times 2$ mixed-model ANOVA was conducted on vmHRV with time (Reactivity; Recovery) as within-subjects variable, and video (video 1, video 2) as the between-subjects factor. Next, post hoc comparisons were used to identify significant differences between means (Reactivity to video 1 and 2, Recovery from video 1 and 2).

Moderation analyses were executed using the Hayes (2012) PROCESS macro version 3.5 to test conditional effects by trait variables on physiological responses. Specifically, Self-compassion and Compassion to Others scales of the CEAS were tested as moderators of the size of the effect of videos on physiological responses (Recovery and Reactivity). Both subscales (Engagement and Action) were tested for their moderating effects.

An exploratory factor analysis on VAS has been then conducted to identify associations within self-reported momentary affect variables. We used Principal Component Analysis (PCA) and Varimax orthogonal rotation as methods to extract the factors. Cattell's (1966) scree test was used as decision rule for identifying the number of factors to retain. Then, a structural equation model (SEM) was run using Mplus 5.1 (Muthén and Muthén, 2008) to conduct a set of confirmatory analyses to assess the relative fit of the three-factors model that emerged from the exploratory factor analysis (Positive Affect, low arousal Negative Affect, high arousal Negative Affect). A non-significant chi-square test was considered as evidence of good fit. Following the recommendations of Kline (1998), multiple indexes were used to evaluate the goodness of fit of the model. These included the comparative fit index (CFI), Tucker–Lewis index (TLI), and standardized root-mean-square residual (SRMR). Acceptable fit was defined as CFI and TLI values of 0.90 or greater and SRMR of 0.05 or less.

To determine if the two videos induced different emotional responses, a series of repeated-measure analysis of variance (ANOVA) was performed on factorial scores with time (baseline, video 1, video 2) as the within-subjects factor. Effect sizes were calculated using partial eta squares ($\eta^2_p$), with $\eta^2_p = 0.01$ referring to a small effect size, 0.06 to a medium effect size and 0.14 to a large effect size (Tabachnick and Fidell, 2013).

## RESULTS

Given the high correlation between the two vmHRV indices (rMSSD and HF-HRV; $r = 0.93$ for baseline 1; $r = 0.95$ for baseline 2; all $ps < 0.0001$), all the analyses have been performed on HF-HRV only. Correlation analysis showed negative and significant associations between HF-HRV and the following trait measures: Self Compassion total score ($r = -0.324$; $p = 0.036$), Sensitivity to Suffering-Self-Compassion component ($r = -0.307$; $p = 0.048$), Compassion to Others total score ($r = -0.407$; $p = 0.007$), and Compassion to Others-Engagement component ($r = -0.445$; $p = 0.003$).

In line with our main hypotheses, the mixed-model ANOVA revealed a main effect of Video for HF-HRV [$F_{(1,42)} = 7.465$; $p = 0.009$; $\eta^2_p = 0.151$] and significant Time × Video interaction [$F_{(1,42)} = 6.733$; $p = 0.013$; $\eta^2_p = 0.138$] (**Figure 2**). Reactivity did not differ between the two videos ($t = 0.148$; $p = 0.883$); however, recovery from video 2 showed a significant increase in HF-HRV compared to recovery from the video 1 ($t = 2.553$; $p = 0.003$). Given the lack of differences in reactivity to the two videos, moderation analyses were performed only on recovery values.

Multiple regression analysis showed no significant moderating effects on HF-HRV recovery from video 2 [$R^2 = 0.1447$; $F_{(3,38)} = 2.1435$; $p = 0.1108$], whereas a significant effect of Compassion to Others (β = 0.0111; $SE = 0.0044$, $p = 0.0153$) but not of Self Compassion (β = −0.0011; $SE = 0.0046$; $p = 0.8090$) emerged for Recovery from video 1, with a significant interaction effect (β = −0.0008; $SE = 0.0002$; $p = 0.0005$). Overall, the regression model explained the 50% of the observed variance of HF-HRV recovery from Video 1 [$F_{(3,38)} = 12.79$; $p = 0.0001$]. The analysis of simple slopes of the interaction effect highlighted that the relationship between Compassion to Others and Recovery 1 was significant for low (β = 0.0241; $SE = 0.0047$; $p = 0.0001$) and medium Self-Compassion scores ($-1 \times SD$) ($b = 0.0111$; $SE = 0.0044$; $p = 0.0153$), while for high scores the relationship was negative and non-significant (β = −0.0018; $SE = 0.0062$; $p = 0.7761$) (**Figure 3A**).

Results also revealed conditional effects of the Engagement with Suffering [$R^2 = 0.5247$; $F_{(3,38)} = 13.9851$; $p = 0.0001$; interaction effect: β = −0.0025; $SE = 0.0006$; $p = 0.0002$] (**Figure 3B**) and Action [$R^2 = 0.5069$; $F_{(3,38)} = 13.0196$; $p = 0.0001$; interaction effect: β = −0.0018; $SE = 0.0005$; $p = 0.0003$] (**Figure 3C**) subscales of Self Compassion on HF-HRV recovery from video 1. No significant moderating effect emerged by the Sensitivity to Suffering subscale.

The Scree plot based on exploratory factor analysis of VAS scores at baseline, revealed that the first 3 factors explained 69% of the variance in the data. The items exhibited loading values of ≥0.5, suggesting significant contribution. Thus, we selected the three-factor solution for further analyses. A three-factor model composed by (a) POSITIVE AFFECT (happy, calm, strong, content, relieved); (b) low arousal NEGATIVE AFFECT (sad, weak, anxious); and (c) high arousal NEGATIVE

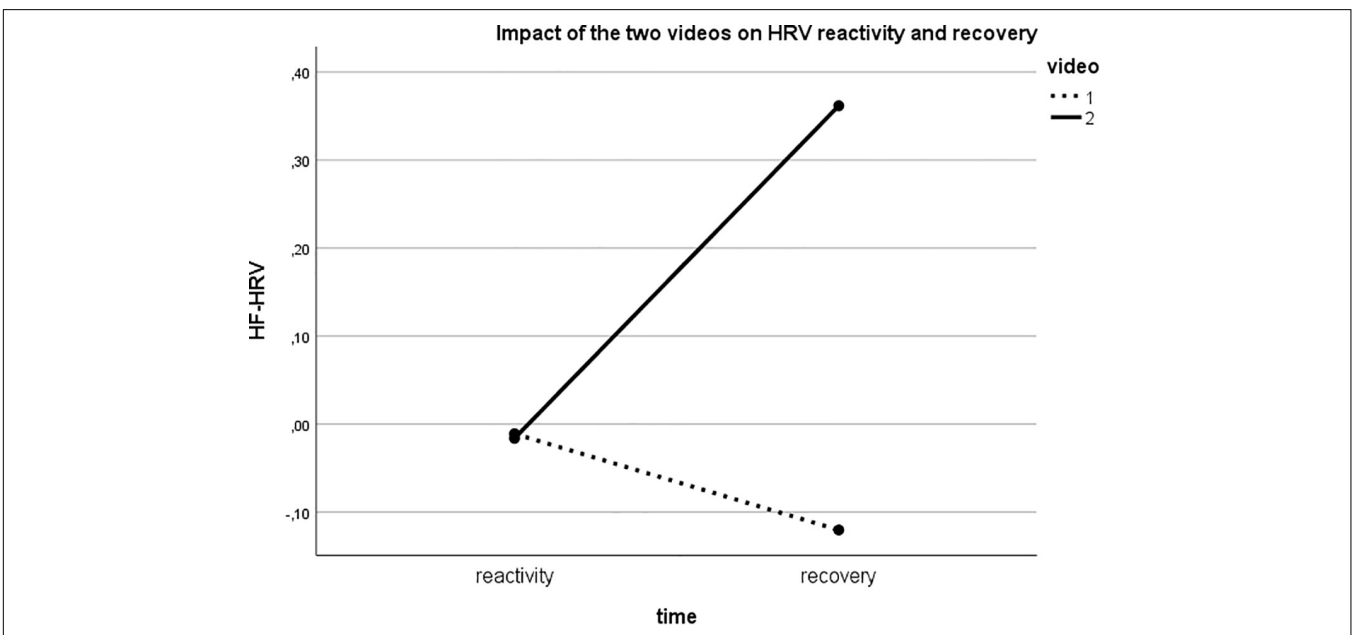



**FIGURE 2 |** Significant Time by Video interaction showing that while reactivity to the two videos did not differ, high-frequency heart rate variability (HF-HRV) increased during recovery from Video 2 (action component of compassion) but decreased during recovery from Video 1 (empathic sensitivity component of compassion).

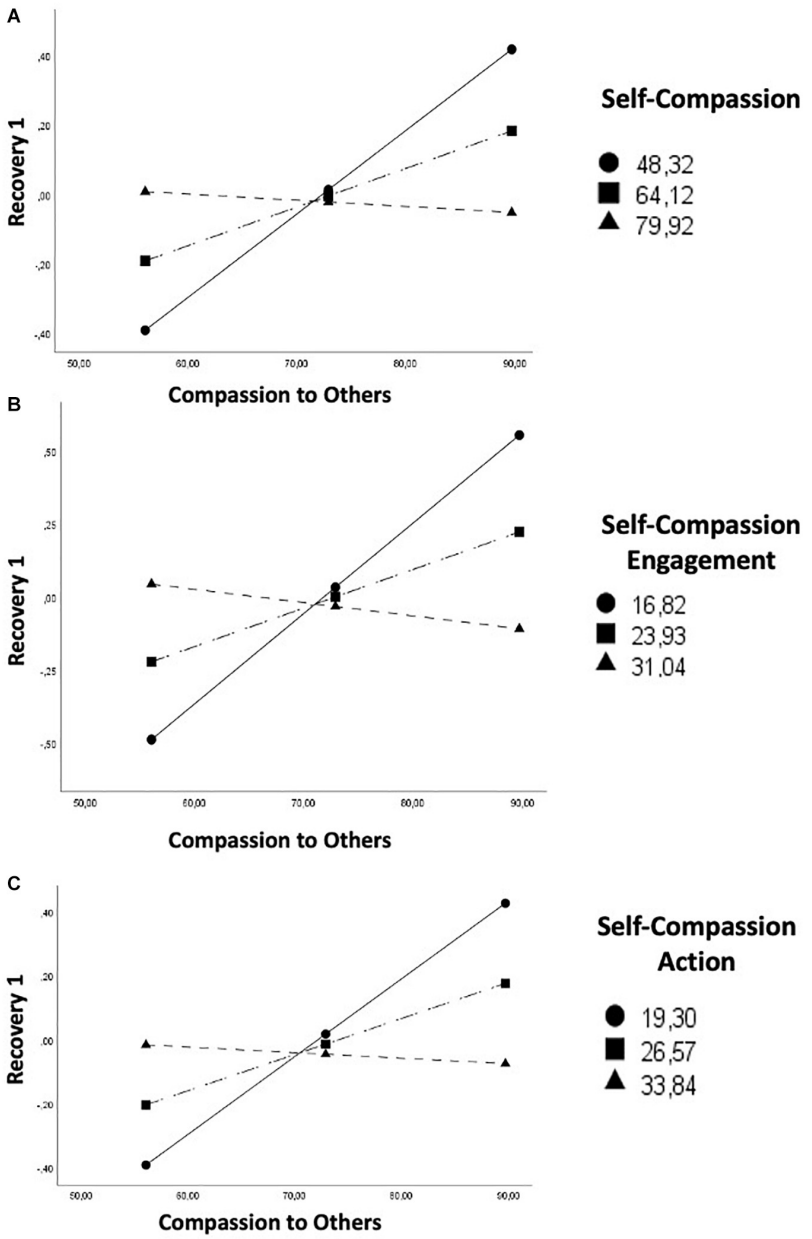



**FIGURE 3 |** Self-compassion [total score, **(A)**; Engagement subscale, **(B)**; and Action subscale, **(C)**] moderates the association between Compassion for Others and High-Frequency Heart Rate Variability (HF-HRV) recovery from Video 1.

AFFECT (angry, self-critical; **Table 1**) exhibited good fit. Coherently with modification indices, and according to the theoretical framework behind the present study (Gilbert, 2020), we have opted for a Model including the following three factors: (a) POSITIVE AFFECT (happy, calm, strong, content, relieved); (b) low arousal NEGATIVE AFFECT (sad, weak); (c) high arousal NEGATIVE AFFECT (angry, self-critical, anxious).

The repeated measures ANOVA revealed the following significant changes in response to the two videos: Positive Affect [$F_{(2,82)}$ = 12.106; $p$ = 0.0001; $\eta^2_p$ = 0.228; **Figure 4A**], high arousal Negative Affect [$F_{(2,82)}$ = 45.798; $p$ = 0.0001; $\eta^2_p$ = 0.528; **Figure 4B**], and low arousal Negative Affect [$F_{(2,82)}$ = 19.982; $p$ = 0.0001; $\eta^2_p$ = 0.328; **Figure 4C**]. Participants specifically reported to be sadder ($p$ = 0.0001, $M$ increase = 179%), and less happy and strong ($p$ = 0.0001, $M$ decrease = 309%) in response to video 1, whereas in response to video 2 they reported to be angrier, and more self-critical compared to baseline ($p$ = 0.0001, $M$ increase = 328%) and to video 1 ($p$ = 0.0001, $M$ increase = 273%) but less sad compared to video 1 ($p$ = 0.014, $M$ decrease = 83%). An increase in Positive Affect in

**TABLE 1 |** Correlations, means and standard deviations for visual analog scales (VAS) and goodness-fit indexes for the confirmatory factor analysis.

| Subtest | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| M | 1.62 | 1.24 | 3.10 | 2.29 | 3.02 | 2.98 | 1.93 | 3.36 | 2.10 | 2.67 |
| SD | 0.795 | 0.692 | 0.821 | 1.293 | 1.199 | 0.749 | 1.113 | 0.932 | 1.078 | 1.223 |
| 1. Sad | 1 | | | | | | | | | |
| 2. Angry | 0.346* | 1 | | | | | | | | |
| 3. Happy | −0.167 | −0.385* | 1 | | | | | | | |
| 4. Anxious | 0.298 | 0.195 | −0.417** | 1 | | | | | | |
| 5. Calm | −0.016 | −0.183 | 0.394** | −539** | 1 | | | | | |
| 6. Strong | −0.016 | 0.011 | 0.480** | −0.194 | 0.191 | 1 | | | | |
| 7. Weak | 0.575** | 0.213 | −0.393* | 0.625** | −0.200 | −0.324* | 1 | | | |
| 8. Content | −0.273 | −0.362* | 0.751** | −0.532** | 0.407** | 0.292 | −0.374* | 1 | | |
| 9. Relieved | −0.071 | −0.031 | 0.458** | −0.370* | 0.545** | 0.487** | −0.198 | 0.475** | 1 | |
| 10. Self-Critical | 0.468** | −0.163 | −0.138 | 0.401** | 0.022 | −0.009 | 0.466** | −0.192 | 0.025 | 1 |

| Model | $\chi 2$ | *df* | *p* | CFI | TLI | SRMR |
|---|---|---|---|---|---|---|
| **1** | *n.c.* | | | | | |
| **2** | 12.591 | 32 | 0.99 | 1.000 | 16.058 | 0.058 |
| **3** | 7.513 | 32 | 1.00 | 1.000 | 19.998 | 0.042 |

*$^*p < 0.05$; $^{**}p < 0.01$. n.c., no convergence.*

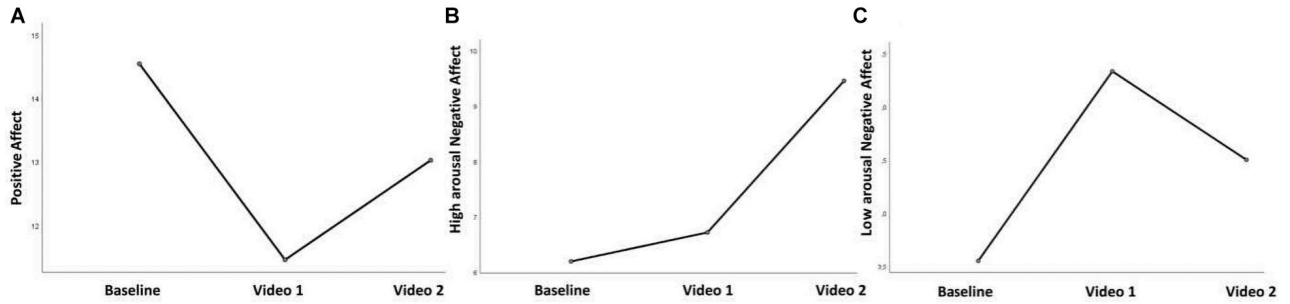



**FIGURE 4 |** Self-reported changes in Positive Affect **(A)**, high-arousal Negative Affect **(B)**, and low-arousal Negative Affect **(C)** in response to two videos.

response to video 2 also emerged, although not statistically significant (**Figure 4**).

## DISCUSSION

The present investigation aimed to examine the association between vmHRV and the specific components of trait and state (induced) compassion, namely empathic sensitivity and compassionate action. When the dispositional tendency to engage in compassion was examined, data evidenced that only the Compassionate Engagement Actions Scale (CEAS) showed associations with vmHRV. Notably, significant negative associations emerged between resting HF-HRV and dispositional compassion. This finding is not completely unexpected. Indeed, most of the studies that investigated the connection between compassion and vmHRV, used different measurement tools, which reflect different definitions of compassion and do not take into account the subdivision of engagement and action as fundamental components of compassion motivation (Gilbert, 2020). In line with our view that distress sensitivity and awareness involve specific physiological competencies for enabling emotional resonance to emotional pain, HF-HRV showed distinct negative associations with the element of empathic sensitivity (engagement) for both self- and other-oriented compassion. Notably, the sensitivity to suffering is an essential attribute of compassion that involves being responsive to one's own suffering or to other people's emotions (rather than activate defense mechanisms and avoidance), and perceiving when they need help (Gilbert, 2019).

Consistently, recent findings show that a more efficient shifting of attention from affective to non-affective aspects of negative information was related to lower resting vmHRV (Grol and De Raedt, 2020). In a compassionate approach, this may facilitate emotional pain awareness, and subsequent decisions for helpful actions. Thereby, the individual is able to learn that negative information does not always translate into an aversive outcome (Borkovec et al., 2004), when the compassionate motivation is active (Gilbert, 2020).

In line with our hypothesis, the data evidenced significant greater HF-HRV increase after watching the video depicting the intentional actions of giving help, compared to video eliciting empathic sensitivity toward others' suffering. Interestingly, most of the participants (88%) labeled the second video as more compassionate. This result replicates previous findings which identified increased vmHRV when individuals effectively engage in compassion interventions (Kim et al., 2020b), or improve their self-compassion competencies (Steffen et al., 2020). However, it also echoes recent evidence that both self-critical and self-compassionate writing were associated with a significant decrease in vmHRV during the task, but that only self-compassionate task produced a significant increase in vmHRV during recovery (Steffen et al., 2020).

Present data highlight that the engagement in compassion enables an appropriate autonomic response after seeing compassionate actions, indicative of how efficiently self-regulatory resources have been mobilized and used to overcome the emotional challenge and then return to resting level (Laborde et al., 2018). This quick return to parasympathetic response, called "vagal rebound" (Nederend et al., 2016), is crucial for therapy because, across time, it promotes an expansion of personal potential to self-regulate and react effectively, loosening resistances and blocks that in turn induce helplessness or shutdown states (Gilbert, 2020).

Our current findings suggest that compassion at first magnifies the saliency of emotional stimuli, consistent with the traditional function of this meditation (The Dalai Lama, 2001). Indeed, one aim of compassion focused training is to increase one's sensitivity to the painful emotional experience of oneself and others, along with the courage and commitment to try to alleviate it (Gilbert, 2020). In fact, personal distress is supposed to surface during compassion focused training; that is why a key part of the training is to provide individuals with a grounding and soothing body routine (breathing and posture) and a psychoeducation that help them develop a de-shaming, non-judgmental, and self-reassuring stance toward suffering and one's habitual patterns of emotional reactivity.

As to moderation analyses, a lower sensitivity to and motivation to engage with other's suffering moderated the association between compassion for others and vmHRV recovery from video 1 (empathic stress condition), whereas this moderating role did not emerge for recovery from video 2 (compassionate action condition). Specifically, in the first condition compassion toward others was positively associated with HF-HRV recovery via lower self-compassionate engagement and action.

We explored changes in different affective states distinguishing between positive affect, low arousal negative affect, and high arousal negative affect. Results revealed a significant increase in low arousal negative affect and a parallel decrease in positive affect in response to video 1, whereas an increase in high arousal negative affect and parallel decrease in low arousal negative affect emerged in response to video 2. Recently, Gilbert and collaborators highlighted that kindness and compassion are associated with different emotions. Whereas kindness is generally associated with positive feelings, engaging in compassionate actions can give rise to a different emotional experience and affective states, mostly associated with anxiety, sadness, disgust, and anger (Gilbert et al., 2019). Consistently, the automatic analysis of spontaneous facial expressions in response to a short video eliciting compassion, showed that anger, disgust, sadness, and surprise occurred more often than fear, happiness, and contempt. In line with our results, anger occurred more often during compassion compared to baseline (Kanovský et al., 2020).

Several limitations must be considered when interpreting the current results. First, being the first to explicitly investigate vmHRV in association with the two subcomponents of compassion, the present study was intended to be preliminary and therefore conducted on a relatively small sample. Replication with a larger and more diverse sample is warranted before we can draw any conclusion on this issue. Moreover, there was an unequal sex distribution in our sample comprising of mostly females, and this may have biased the results. Indeed, differences in resting vmHRV have been well-documented (Koenig and Thayer, 2016), with females showing greater vagal activity, despite lower RR intervals. Likewise, sex differences emerge in the stronger negative relationship between resting vmHRV and empathic concern toward another in pain in women than in men (Tracy and Giummarra, 2017). Indeed, this disparity may be the consequence of different evolutionary selective pressures on females, fostering the evolution of mutual physiological connection between oxytocin and vagal functioning consistently with models of parental investment (Carter, 2014). However, recent meta-analytic results argue against the role of sex as a moderator of the association between (self- and other-oriented) compassion and vmHRV (Di Bello et al., 2020).

Lastly, the two videos were not randomly presented. In order to exclude the role of carry-over effects, we statistically compared resting 1 with resting 2 and we found that full recovery occurred before the beginning of video 2. However, we are aware that this is a serious methodological limitation that future studies should avoid.

Limitations notwithstanding, current results have potential clinical importance, contributing to inform our comprehension of the processes that are active when one engages in compassion, and although preliminary, highlight the importance of adopting a nuanced perspective on the complex physiological regulation that underlies compassionate responding to suffering. Encouraging finding suggests that this issue could be fruitfully explored by a time series analysis of the vmHRV signal, to disentangle how it fluctuates over the course of the empathic sensitivity condition and across the course of recovery (Kim et al., 2020a).

To conclude, compassion should not be seen as an antidote for negative affect, as it requires a dosage of personal suffering and pain before reaching its emotional and health benefits.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the IRB of Sapienza University of Rome. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

NP conceptualized and conducted the study. MD analyzed the data and wrote the initial draft of the manuscript. All authors contributed to the interpretation of the results, provided critical feedback, helped shape the analysis and manuscript, and approved the submitted manuscript.

## REFERENCES


Arch, J. J., Brown, K. W., Dean, D. J., Landy, L. N., Brown, K. D., and Laudenslager, M. L. (2014). Self-compassion training modulates alpha-amylase, heart rate variability, and subjective responses to social evaluative threat in women. *Psychoneuroendocrinology* 42, 49–58. doi: 10.1016/j.psyneuen.2013.12.018

Borkovec, T., Alcaine, O., and Behar, E. (2004). "Avoidance theory of worry and generalized anxiety disorder," in *Generalized Anxiety Disorder: Advances in Research and Practice*, eds C. L. Turk and D. S. Mennin (New York, NY: Guilford Press), 77–108.

Carter, C. S. (2014). Oxytocin pathways and the evolution of human behavior. *Annu. Rev. Psychol.* 65, 17–39. doi: 10.1146/annurev-psych-010213-115110

Cattell, R. B. (1966). The scree test for the number of factors. *Multivariate Behav. Res.* 1, 245–276.

Crocker, J., and Canevello, A. (2008). Creating and undermining social support in communal relationships: the role of compassionate and self-image goals. *J. Pers. Soc. Psychol.* 95, 555–575. doi: 10.1037/0022-3514.95.3.555

Di Bello, M., Carnevali, L., Petrocchi, N., Thayer, J. F., Gilbert, P., and Ottaviani, C. (2020). The compassionate vagus: a meta-analysis on the connection between compassion and heart rate variability. *Neurosci. Biobehav. Rev.* 116, 21–30. doi: 10.1016/j.neubiorev.2020.06.016

Ferrari, M., Hunt, C., Harrysunker, A., Abbott, M. J., Beath, A. P., and Einstein, D. A. (2019). Self-compassion interventions and psychosocial outcomes: a meta-analysis of RCTs. *Mindfulness* 10, 1455–1473. doi: 10.1007/s12671-019-01134-6

Gilbert, P. (2017a). "Compassion as a social mentality: an evolutionary approach," in *Compassion, Concepts, Research and Applications*, ed. P. Gilbert (London: Routledge), 31–68.

Gilbert, P. (2017b). "Compassion. Definitions and controversies," in *Compassion: Concepts, Research and Applications*, ed. P. Gilbert (London: Routledge), 3–15.

Gilbert, P. (2019). Explorations into the nature and function of compassion. *Curr. Opin. Psychol.* 28, 108–114. doi: 10.1016/j.copsyc.2018.12.002

Gilbert, P. (2020). Compassion: from its evolution to a psychotherapy. *Front. Psychol.* 11:586161. doi: 10.3389/fpsyg.2020.586161

Gilbert, P., Basran, J., MacArthur, M., and Kirby, J. N. (2019). Differences in the semantics of prosocial words: an exploration of compassion and kindness. *Mindfulness* 10, 2259–2271. doi: 10.1007/s12671-019-01191-x

Gilbert, P., Catarino, F., Duarte, C., Matos, M., Kolts, R., Stubbs, J., et al. (2017). The development of compassionate engagement and action scales for self and others. *J. Compassionate Health Care* 4:4. doi: 10.1186/s40639-017-0033-3

Grol, M., and De Raedt, R. (2020). The link between resting heart rate variability and affective flexibility. *Cogn. Affect. Behav. Neurosci.* 20, 746–756. doi: 10.3758/s13415-020-00800-w

Hayes, A. F. (2012). PROCESS: A Versatile Computational Tool for Observed Variable Mediation, Moderation, and Conditional Process Modeling. [White paper]. Available online at: http://www.afhayes.com/public/process2012.pdf

Kanovský, M., Baránková, M., Halamová, J., Strnádelová, B., and Kóróniová, J. (2020). Analysis of facial expressions made while watching a video eliciting compassion. *Percept. Mot. Skills* 127, 317–346. doi: 10.1177/0031512519897512

Kim, J. J., Parker, S., Henderson, T., and Kirby, J. N. (2020a). Physiological fractals: visual and statistical evidence across timescales and experimental states. *J. R. Soc. Interface* 17:20200334. doi: 10.1098/rsif.2020.0334

Kim, J. J., Parker, S. L., Doty, J. R., Cunnington, R., Gilbert, P., and Kirby, J. N. (2020b). Neurophysiological and behavioural markers of compassion. *Sci. Rep.* 10:6789. doi: 10.1038/s41598-020-63846-3

Kirby, J. N. (2016). Compassion interventions: the programmes, the evidence, and implications for research and practice. *Psychol. Psychother.* 90, 432–455. doi: 10.1111/papt.12104

Kirby, J. N., Day, J., and Sagar, V. (2019). The 'Flow' of compassion: a meta-analysis of the fears of compassion scales and psychological functioning. *Clin. Psychol. Rev.* 70, 26–39. doi: 10.1016/j.cpr.2019.03.001

Kline, R. B. (1998). *Principles and Practice of Structural Equation Modeling*. New York, NY: Guilford Press.

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Laborde, S., Mosley, E., and Mertgen, A. (2018). Vagal tank theory: the three Rs resting, reactivity and recovery. *Front. Psychol.* 12:458. doi: 10.3389/fnins.2018.00458

Luo, X., Qiao, L., and Che, X. (2018). Self-compassion modulates heart rate variability and negative affect to experimentally induced stress. *Mindfulness* 9:1522. doi: 10.1007/s12671-018-0900-9

Lutz, A., Brefczynski-Lewis, J., Johnstone, T., and Davidson, R. J. (2008). Regulation of the neural circuitry of emotion by compassion meditation: effects of meditative expertise. *PLoS One* 3:e1897. doi: 10.1371/journal.pone.0001897

Matos, M., Duarte, C., Duarte, J., Pinto-Gouveia, J., Petrocchi, N., Basran, J., et al. (2017). Psychological and physiological effects of compassionate mind training: a pilot randomised controlled study. *Mindfulness* 8:1699. doi: 10.1007/s12671-017-0745-7

Miller, J. G. (2018). Physiological mechanisms of prosociality. *Curr. Opin. Psychol.* 20, 50–54. doi: 10.1016/j.copsyc.2017.08.018

Miller, J. G., Xia, G., and Hastings, P. D. (2019). Resting heart rate variability is negatively associated with mirror neuron and limbic response to emotional faces. *Biol. Psychol.* 146:107717. doi: 10.1016/j.biopsycho.2019.107717

Muthén, B., and Muthén, L. (2008). *Mplus Version 5.1* [computer software]. Los Angeles, CA: Muthén & Muthén. Available online at: http://www.statmodel.com/

Nederend, I., Schutte, N. M., Bartels, M., Ten Harkel, A. D., and de Geus, E. J. (2016). Heritability of heart rate recovery and vagal rebound after exercise. *Eur. J. Appl. Physiol.* 116, 2167–2176. doi: 10.1007/s00421-016-3459-y

Pace, T. W., Negi, L. T., Adame, D. D., Cole, S. P., Sivilli, T. I., Brown, T. D., et al. (2009). Effect of compassion meditation on neuroendocrine, innate immune and behavioral responses to psychosocial stress. *Psychoneuroendocrinology* 34, 87–98. doi: 10.1016/j.psyneuen.2008.08.011

Pace, T. W., Negi, L. T., Dodson-Lavelle, B., Ozawa-de Silva, B., Reddy, S. D., Cole, S. P., et al. (2013). Engagement with cognitively-based compassion training is associated with reduced salivary C-reactive protein from before to after training in foster care program adolescents. *Psychoneuroendocrinology* 38, 294–299. doi: 10.1016/j.psyneuen.2012.05.019

Park, G., Van Bavel, J. J., Vasey, M. W., and Thayer, J. F. (2013). Cardiac vagal tone predicts attentional engagement to and disengagement from fearful faces. *Emotion* 13, 645–656. doi: 10.1037/a0032971

Petrocchi, N., and Cheli, S. (2019). The social brain and heart rate variability: implications for psychotherapy. *Psychol. Psychother.* 92, 208–223. doi: 10.1111/papt.12224

Petrocchi, N., Ottaviani, C., and Couyoumdjian, A. (2017). Compassion at the mirror: exposure to a mirror increases the efficacy of a self-compassion manipulation in enhancing soothing positive affect and heart rate variability. *J. Posit. Psychol.* 12, 525–536. doi: 10.1080/17439760.2016.1209544

Porges, S. W. (2017). "Vagal pathways: portals to compassion," in *The Oxford Handbook of Compassion Science*, ed. E. M. Seppala (New York, NY: Oxford University Press), 189–202.

Rockliff, H., Gilbert, P., McEwan, K., Lightman, S., and Glover, D. (2008). A pilot exploration of heart rate variability and salivary cortisol responses to compassion-focused imagery. *Clin. Neuropsychiatry* 5, 132–139.

Seppala, E., Rossomando, T., and Doty, J. R. (2012). Social connection and compassion: important predictors of health and well-being. *Soc. Res.* 80, 411–430. doi: 10.1353/sor.2013.0027

Slavich, G. M., and Cole, S. W. (2013). The emerging field of human social genomics. *Clin. Psychol. Sci.* 1, 331–348. doi: 10.1177/2167702613478594

Sprecher, S., and Fehr, B. (2005). Compassionate love for close others and humanity. *J. Soc. Pers. Relat.* 22, 629–651. doi: 10.1177/0265407505056439

Steffen, P. R., Foxx, J., Cattani, K., Alldredge, C., Austin, T., and Burlingame, G. M. (2020). Impact of a 12-week group-based compassion focused therapy intervention on heart rate variability. *Appl. Psychophysiol. Biofeedback* 46, 61–68. doi: 10.1007/s10484-020-09487-8

Svendsen, J. L., Osnes, B., Binder, P. E., Dundas, I., Visted, E., Nordby, H., et al. (2016). Trait self-compassion reflects emotional flexibility through an association with high vagally mediated heart rate variability. *Mindfulness* 7, 1103–1113. doi: 10.1007/s12671-016-0549-1

Svendsen, J. L., Schanche, E., Osnes, B., Vøllestad, J., Visted, E., Dundas, I., et al. (2020). Is dispositional self-compassion associated with psychophysiological flexibility beyond mindfulness? An exploratory pilot study. *Front. Psychol.* 11:614. doi: 10.3389/fpsyg.2020.00614

Tabachnick, B., and Fidell, L. (2013). *Using Multivariate Statistics*, 6th Edn. Boston, MA: Pearson.

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–Heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

The Dalai Lama (2001). *An Open Heart: Practicing Compassion in Everyday Life*. Boston, MA: Little Brown and Company.

Tracy, L. M., and Giummarra, M. J. (2017). Sex differences in empathy for pain: what is the role of autonomic regulation? *Psychophysiology* 54, 1549–1558. doi: 10.1111/psyp.12895

Weidman, A. C., and Tracy, J. L. (2020). Picking up good vibrations: uncovering the content of distinct positive emotion subjective experience. *Emotion* 20, 1311–1331. doi: 10.1037/emo0000677

Yarnell, L. M., and Neff, K. D. (2013). Self-compassion, interpersonal conflict resolutions, and well-being. *Self Identity* 12, 146–159. doi: 10.1080/15298868.2011.649545

Zessin, U., Dickhäuser, O., and Garbade, S. (2015). The relationship between self-compassion and well-being: a meta-analysis. *Appl. Psychol.* 7, 340–364. doi: 10.1111/aphw.12051

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 30 March 2021
doi: 10.3389/fphys.2021.596060

# Is Ultra-Short-Term Heart Rate Variability Valid in Non-static Conditions?

*Jin Woong Kim†, Hyeon Seok Seok† and Hangsik Shin**

*Department of Biomedical Engineering, Chonnam National University, Yeosu-si, South Korea*

In mobile healthcare, heart rate variability (HRV) is increasingly being used in dynamic patient states. In this situation, shortening of the measurement time is required. This study aimed to validate ultra-short-term HRV in non-static conditions. We conducted electrocardiogram (ECG) measurements at rest, during exercise, and in the post-exercise recovery period in 30 subjects and analyzed ultra-short-term HRV in time and frequency domains by ECG in 10, 30, 60, 120, 180, and 240-s intervals, and compared the values to the 5-min HRV. For statistical analysis, null hypothesis testing, Cohen's *d* statistics, Pearson's correlation coefficient, and Bland-Altman analysis were used, with a statistical significance level of $P < 0.05$. The feasibility of ultra-short-term HRV and the minimum time required for analysis showed differences in each condition and for each analysis method. If the strict criteria satisfying all the statistical methods were followed, the ultra-short-term HRV could be derived from a from 30 to 240-s length of ECG. However, at least 120 s was required in the post-exercise recovery or exercise conditions, and even ultra-short-term HRV was not measurable in some variables. In contrast, according to the lenient criteria needed to satisfy only one of the statistical criteria, the minimum time required for ultra-short-term HRV analysis was 10–60 s in the resting condition, 10–180 s in the exercise condition, and 10–120 s in the post-exercise recovery condition. In conclusion, the results of this study showed that a longer measurement time was required for ultra-short-term HRV analysis in dynamic conditions. This suggests that the existing ultra-short-term HRV research results derived from the static condition cannot applied to the non-static conditions of daily life and that a criterion specific to the non-static conditions are necessary.

**Keywords: autonomic nervous system, heart rate variability, mobile healthcare, ultra-short-term heart rate variability, electrocadiogram**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Moacir Fernandes Godoy, Faculty of Medicine of São José do Rio Preto, Brazil*
*Paolo Melillo, University of Campania Luigi Vanvitelli, Italy*

***Correspondence:**
*Hangsik Shin*
*hangsik.shin@jnu.ac.kr*

*† These authors have contributed equally to this work*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

***Received:*** *18 August 2020*
***Accepted:*** *10 March 2021*
***Published:*** *30 March 2021*

***Citation:***
*Kim JW, Seok HS and Shin H (2021) Is Ultra-Short-Term Heart Rate Variability Valid in Non-static Conditions? Front. Physiol. 12:596060. doi: 10.3389/fphys.2021.596060*

## INTRODUCTION

The measurement of heart rate variability (HRV) is used to assess autonomic nervous system (ANS) activity and is known to be meaningful in assessing cardiac vagal activity (Malik et al., 1996; Berntson et al., 1997; Laborde et al., 2017). HRV measurement analyzes variations in the inter-beat interval seen in the electrocardiogram (ECG). In general, long-term HRV is derived from a 24 h ECG, and short-term HRV is derived from a 5 min ECG (Camm et al., 1996). While long-term recording has the potential to analyze physiological statuses, such as congestive heart failure,

mitral regurgitation, and mortality (Camm et al., 1996), short-term HRV is becoming more popular because long-term HRV is difficult to measure in everyday life. Recently, for monitoring the health situations of an individual in daily life, the use of HRV has greatly increased in connection with mobile and wearable technologies. However, when used for routine healthcare purposes, the subject may feel that even the 5 min steady-state measurement required for short-term HRV analysis is long, which may result in measurement discomfort. Moreover, real-time measurements are difficult while the human body is in motion, and are likely to yield inaccurate results. To overcome these practical limitations and increase the usefulness of HRV in mobile and wearable situations, ultra-short-term HRV with an analysis interval of less than 5 min has been studied (Hamilton et al., 2004; Flatt and Esco, 2013; Castaldo et al., 2017). Previous studies have calculated HRV at intervals between 10 s and 10 min, the minimum time required to identify statistically significant differences for each ultra-short-term HRV variable compared to short-term HRV, as summarized in **Table 1**. While the previous results may seem to suggest the validity of ultra-short-term HRV, they cannot be generalized because each variable had a very large variation, which depended upon the analysis method or condition.

The biggest pitfall of the previous studies was that ultra-short-term HRV was only evaluated in non-dynamic conditions, such as resting, despite its suggested use in situation including mobile healthcare. Dynamic activities occurring in daily life can activate and inhibit the autonomic nervous system, which can greatly affect HRV. Previous studies demonstrated that HRV was a reliable tool for assessing autonomic control of the HR during dynamic conditions such as walking before and after maximal effort (Hunt and Saengsuwan, 2018). However due to the exercise performed, average of normal-to-normal intervals (AVNN), standard deviation of normal-to-normal intervals (SDNN), root-mean-square of successive difference (RMSSD), percent of successive difference of normal-to-normal interval exceeds 50 ms (pNN50), total power (TP), low frequency (LF), high frequency (HF), and very low frequency (VLF) changed significantly from the resting state (Javorka et al., 2002; Leicht et al., 2008). In addition, significant changes in the SDNN, RMSSD, LF, and HF were observed according to exercise intensity (Buchheit et al., 2007; Martinmäki et al., 2008). Changes in HRV in post-exercise recovery conditions were also reported (Barak et al., 2010; Stanley et al., 2013). The above results demonstrate that the dynamic state causes a change in HRV, which may have a big impact on the analysis interval needed for ultra-short-term HRV. Thus, a separate analysis and dedicated criteria for ultra-short-term HRV in dynamic conditions are essential.

In the mobile healthcare environment, HRV is measured not only in the resting state, but also in various dynamic states such as standing, moving, and stopping. In addition, due to the nature of dynamic state measurements, a measurement time as short as possible is required. This study aimed to examine the feasibility of ultra-short-term HRV measurements in steady-state conditions, as well as dynamic conditions, using various statistical techniques. To this end, ultra-short-term HRV analysis was performed using ECGs obtained during rest, while exercising, and during post-exercise recovery, and the minimum required time for ultra-short-term HRV analysis was investigated in each condition and compared to the short-term HRV results.

**TABLE 1 |** Minimum time requirements for ultra-short-term HRV analysis.

| HRV variable | Minimum time requirements for ultra-short-term HRV analysis | References |
|---|---|---|
| **Time domain** | | |
| Average of normal-to-normal interval (AVNN) | 10 s | McNames and Aboy, 2006; Salahuddin et al., 2007; Nussinovitch et al., 2011; Baek et al., 2015 |
| Standard deviation of normal-to-normal interval (SDNN) | 30–240 s | McNames and Aboy, 2006; Nussinovitch et al., 2011; Baek et al., 2015; Munoz et al., 2015 |
| Root-mean-square of successive difference (RMSSD) | 10–30 s | McNames and Aboy, 2006; Salahuddin et al., 2007; Nussinovitch et al., 2011; Baek et al., 2015; Munoz et al., 2015 |
| The percentage of adjacent NN intervals that differ from each other by more than 50 ms (pNN50) | 30–60 s | Salahuddin et al., 2007; Baek et al., 2015 |
| **Frequency domain** | | |
| Total power (TP) | 240 s | Baek et al., 2015 |
| Very-low-frequency power (VLF) | 270 s | Baek et al., 2015 |
| Low-frequency power (LF) | 40–250 s | Malik et al., 1996; Salahuddin et al., 2007; Baek et al., 2015 |
| High-frequency power (HF) | 20–180 s | Malik et al., 1996; Salahuddin et al., 2007; Baek et al., 2015 |
| The ratio of low-frequency power to high-frequency power (LF/HF) | 50–90 s | McNames and Aboy, 2006; Salahuddin et al., 2007; Baek et al., 2015 |
| Normalized low-frequency power (nLF) | 50–90 s | Salahuddin et al., 2007; Baek et al., 2015 |
| Normalized high-frequency power (nHF) | 50–90 s | Salahuddin et al., 2007; Baek et al., 2015 |

# MATERIALS AND METHODS

## Exercise Protocol

The experiments in this study were performed in the order of resting, while performing progressive resistance exercises, and during post-exercise recovery to induce autonomic activation in each condition (**Figure 1**). To minimize motion artifacts due to upper body movement during exercise, a stationary bicycle,

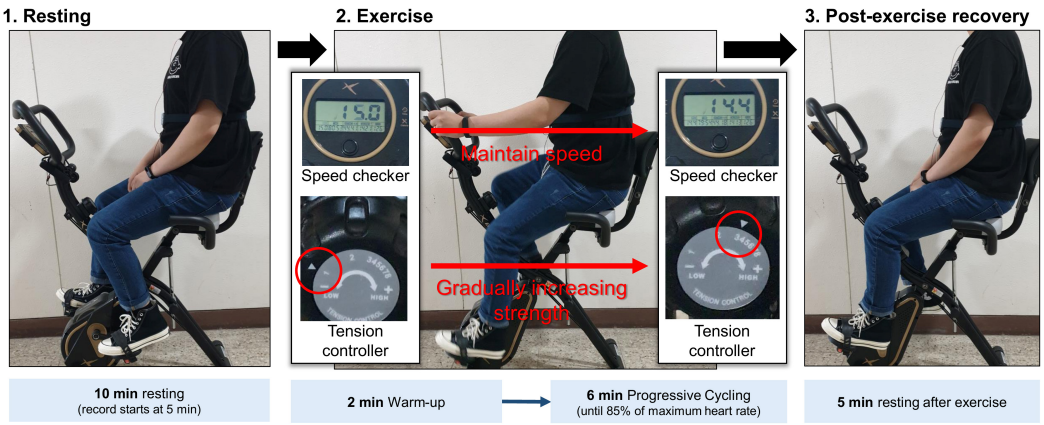



**FIGURE 1 |** Experimental protocol.

DP-652-G-1 (Iwhasmp Inc., Seoul, South Korea) was used. The stationary bicycle used in the study had a total of eight levels of exercise intensity. Steps 1 and 2 were set to warm-up, steps 3, 4, 5, and 6 were set to moderate-intensity training, and steps 7 and 8 were set to high-intensity training. In the resting stage, the subject took a 10-min rest in the sitting position, and the latter 5 min of the ECG was used for HRV analysis. The exercise was performed for a total of 8 min, including 2 min of warm-up, taking into account the recommended exercise time for cardiopulmonary exercise testing (CPET) (Datta et al., 2015). During the exercise, the speed was maintained at 14–16 m/s. In the warm-up phase, the tension controller of the stationary bicycle was set to step one. After that, the step of the tension controller was increased by one step every two min to gradually increase the exercise intensity. In general, a heart rate greater than 85% of the age-predicted maximal heart rate is defined as high-intensity exercise, so when the subject's heart rate reached 85% of the maximum age-related heart rate, the subject immediately stopped exercising and rested (Fletcher et al., 2013). Measurements in the post-exercise recovery condition were performed with the subject sitting comfortably on the stationary bicycle for 5 min. To exclude factors affecting HRV, subjects who had been drinking alcohol, consuming caffeine, smoking, had a lack of sleep, or were taking medication within 24 h that could affect the autonomic nerves were excluded. Since autonomic nervous system activity can change according to temperature and humidity (Zhu et al., 2018), the humidity of the measurement space was maintained at 65% and the temperature was maintained at room temperature at (20–28°C). The proposed protocol was approved by the Institutional Review Board (IRB) of Chonnam National University (IRB No. 1040198-190821-HR-090-02, Gwangju, South Korea). **Figure 1** shows the experimental protocol and environment. All subjects provided written informed consent.

## Data Recording and Signal Preprocessing

The experimental protocol lasted a total of 23 min and ECG recordings were made for 18 min, except for the initial 5 min of the resting stage. ECG was measured with lead I at 1 kHz sampling frequency. An MP150 (BIOPAC Systems, Inc., CA, United States) and wireless module RSPEC-R (BIOPAC Systems, Inc., CA, United States) were used for the ECG measurements (**Figure 2**). The measured ECGs were stored on a laptop using BIOPAC's AcqKnowledge software. As exercise can change the R–R interval (RRI) rapidly during warm-up, only the progressive cycling data after warm-up were used. ECG was bandpass filtered with a 0.05–30 Hz passband, and the QRS-complex was detected by the Pan and Tompkins (1985) QRS detection algorithm (threshold = 0.2). Experienced researchers checked whether the QRS complex was appropriately detected and manually corrected falsely detected QRS complex. Prior to data analysis, pre-screening was conducted and cases where the QRS waveform could not be intuitively observed due to severe motion artifacts were excluded from the analysis. During measurement, instrument errors and severe subject movements may cause errors in QRS detection such as a mis-detected QRS or missing QRS, which may result in abnormal RRIs. For example, a falsely detected QRS complex decreases or increases the RRI, and a missed QRS complex prolongs the RRI. Thus, the abnormal range of RRIs were corrected by removing and interpolating when the current RRI increased by more than 32.5% or decreased by more than 24.5% from the previous RRI (Marked, 1995; Choi and Shin, 2018). Linear interpolation was used for interpolating the RRIs. To analyze HRV according to the analysis time, the analysis interval was set to 10, 30, 60, 120, 180, 240, and 300-s lengths (**Figure 3**). After calculating the RRI for each analysis interval, time domain and frequency domain HRV variables were derived. As a result of the HRV analysis, seven resting condition datasets, seven exercise condition datasets, and seven post-exercise recovery condition datasets were derived per subject, including six ultra-short-term HRVs per measurement condition.

## HRV Parameters

The HRV variables used in this study were AVNN, SDNN, standard deviation of successive difference (SDSD), RMSSD, and pNN50 in the time domain, and TP, VLF, LF, HF, LF/HF,

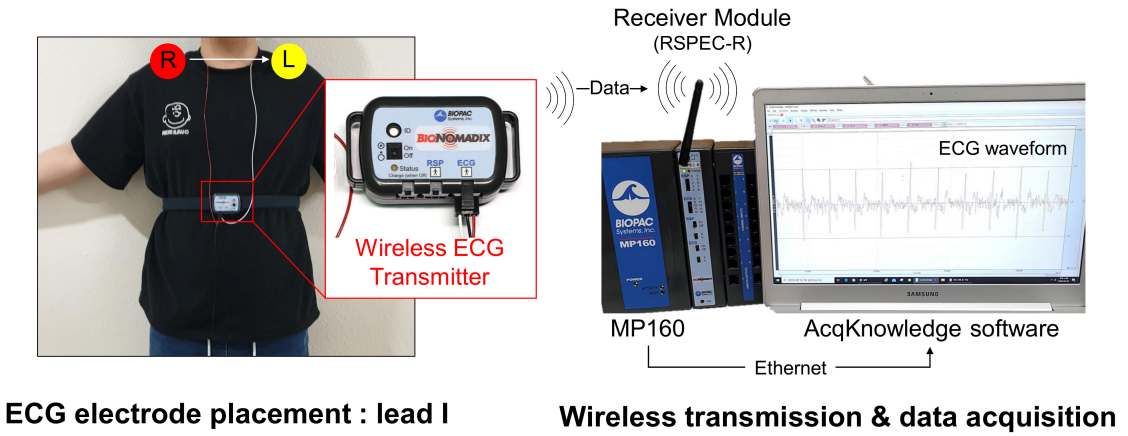



**FIGURE 2 |** System configuration for obtaining electrocardiograms.

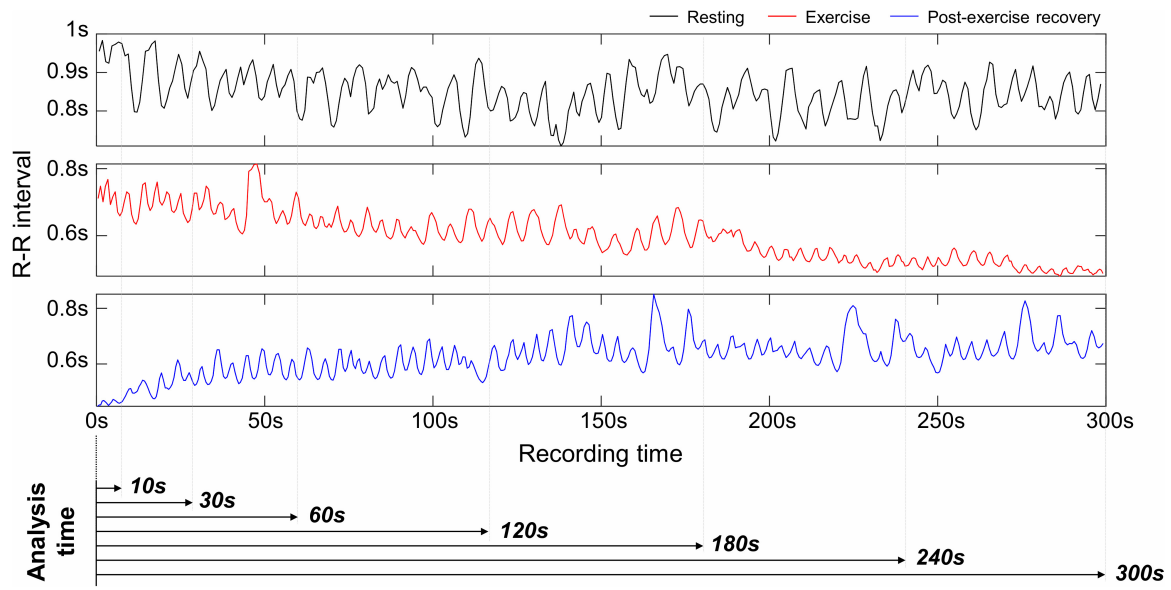



**FIGURE 3 |** Examples of R–R interval in resting, exercising, and post-exercise recovery conditions, and analysis intervals for ultra-short-term heart rate variability analysis.

normalized LF (nLF), and normalized HF (nHF) in the frequency domain (Acharya et al., 2006). **Table 2** shows the definition of each HRV variable. For the HRV frequency domain analysis, the RRIs were transformed to an evenly sampled time series by resampling with 4 Hz after 1 kHz linear interpolation, and both the mean and linear trends were removed. The power spectral density was estimated by fast Fourier transform. The frequencies corresponding to VLF, LF, and HF were set to 0.0033–0.04, 0.04–0.15, and 0.15–0.4 Hz, respectively. HRV variables reflect various physiological activities related to the autonomic nervous system. The HF reflects parasympathetic or vagal activity frequently called the respiratory band. The LF is known to primarily reflect baroreceptor activity while at rest (Malliani, 1995).

Since this study aimed to verify the significance of short-term and ultra-short-term HRVs, the SDNN index (the mean of the 5 min standard deviation of the average NN intervals), the standard deviation of the average NN intervals (SDANN), and ultra-low-frequency power (ULF) were excluded from the analysis.

## Statistical Analysis

Statistical significance was assessed for each variable of the ultra-short-term HRV and short-term HRV measured at rest, while exercising, and during post-exercise recovery. Prior to the analyses, all HRV variables were log-transformed to obtain approximately normal distributions. For statistical analysis, to test agreement with the null hypothesis, the ANOVA test was performed when the equivariance and normality conditions were satisfied, and the Kruskal-Wallis test was performed when the equivariance was satisfied without normality. Otherwise,

**TABLE 2 |** Definition of HRV variables.

| HRV variables | Unit | Description |
|---|---|---|
| **Time domain** | | |
| AVNN | ms | Average of all NN intervals |
| SDNN | ms | Standard deviation of all NN intervals |
| SDSD | ms | Standard deviation of differences between adjacent NN intervals |
| RMSSD | ms | The square root of the mean of the sum of the squares of differences between adjacent NN intervals |
| pNN50 | % | NN50 count divided by the total number of all NN intervals |
| **Frequency domain** | | |
| TP | $ms^2$ | The variance of NN intervals over the temporal segment |
| VLF | $ms^2$ | Power in the very-low-frequency range |
| LF | $ms^2$ | Power in the low-frequency range |
| HF | $ms^2$ | Power in the high-frequency range |
| LF/HF | n.u. | LF power in normalized units |
| nLF | n.u. | HF power in normalized units |
| nHF | n.u. | LF/HF ratio |

*n.u., null unit.*

Friedman's test was performed. Bonferroni's post hoc test was carried out for inter-group comparisons. Prior to the analysis of variance, Levene's test was used to assess the homogeneity of variance, and the Kolmogorov-Smirnov test was used to test normality. *P*-values of less than 0.05 were considered to indicate statistical significance.

We calculated Cohen's *d* statistics to quantify the bias of the HRV measurements of different analysis intervals relative to their within-group variations (Cohen, 1988). Cohen's *d* is an appropriate effect size indicating the standardized measure of the size of the mean difference or the relationship among the study groups and is used to indicate the standardized difference between two means. Cohen's *d* is determined by calculating the mean difference between the two groups and then dividing the result by the pooled standard deviation that is a weighted average of the standard deviations of two or more groups. The individual standard deviations are averaged, with more weight given to larger sample sizes (Equation 1).

$$\text{Cohen}'\text{s } d = \frac{(M_2 - M_1)}{\text{SD}_{\text{pooled}}} \quad (1)$$

where, $\text{SD}_{\text{pooled}}$ is $\sqrt{\left(\text{SD}_1^2 + \text{SD}_2^2\right)/2}$, and $M_1$ and $M_2$, and $\text{SD}_1$ and $\text{SD}_2$ are the mean and standard deviation of the two groups, respectively.

The *d* value is interpreted in a range from 0.01 to 2.0, where 0.01 is very small, 0.2 is small, 0.5 is medium, 0.8 is large, 1.2 is very large, and 2 is huge (Cohen, 1988; Rosenthal, 1990). A *d* of 1 indicates that the two groups differ by 1 standard deviation, while a *d* of 2 indicates that they differ by 2 standard deviations.

In this study, a *d* value of less than 0.5 was set as the criterion indicating two groups had similar values, and the minimum required interval was evaluated using shortest ultra-short-term HRV interval measured as the minimum time interval for which this criterion was maintained. Pearson's correlation coefficients (*R*) were calculated between the short-term HRV and the ultra-short-term HRV values. In this study, $R > 0.8$, which is generally used to represent a strong correlation, was set as the ultra-short-term HRV measurability criterion. However, a strong correlation does not guarantee a close agreement between two groups. Therefore, the Bland–Altman (BA) analysis was carried out to analyze the agreement between short-term HRV and ultra-short-term HRV with 95% limits of agreement (LoA) (Altman and Bland, 1983; Bland and Altman, 2003). We performed a BA analysis with the *x*-axis as the ground truth (short-term HRV) (Krouwer, 2008). The bias was calculated as the mean difference between the short-term HRV and ultra-short-term HRV measurements. In this study, we examined whether the LoA of the ultra-short-term HRV variable included the zero-difference line of the BA plot, indicating the short-term HRV equaled the ultra-short-term HRV. This is not the method used in the general BA analysis but was used to check the measurability based on whether the LoA of the estimated value of the ultra-short-term HRV included the ground truth. We used 50% LoA as a decision threshold, which means that the interquartile range of the derived ultra-short-term HRV variable included the ground truth. Matlab 2016a (Mathworks, Inc., MA, United States) was used for signal processing in the HRV analysis and statistical analyses.

# RESULTS

## Experimental Data

Experiments and data acquisition were performed on 30 healthy adults without cardiovascular disease, eight of whom were excluded due to severe motion artifacts during the experiment, preventing the QRS from being distinguished intuitively. Finally, the data from 22 participants were used for the analysis. **Table 3** shows the demographics of the subjects who participated in the experiment.

## Null Hypothesis Testing

**Figure 4** and **Table 4** show the ultra- and short-term HRV results in resting, exercising, and post-exercise recovery conditions. The results of the null hypothesis test showed that all HRV variables were significantly ($P < 0.05$) different, except pNN50 in the resting and exercising conditions, and HF in the resting condition. The Bonferroni post hoc test showed significant differences ($P < 0.05$) in the resting state between ultra-short-term HRV and short-term HRV at analysis intervals of ≤10 s in

**TABLE 3 |** Participant demographics.

| Sex (N) | Age (years) | Height (cm) | Weight (kg) | Body mass index ($kg/m^2$) |
|---|---|---|---|---|
| Male (15) | 25.5 ± 4.5 | 175.1 ± 3.9 | 71.2 ± 11.5 | 23.1 ± 3.3 |
| Female (7) | 21.5 ± 1.9 | 159.4 ± 1.9 | 54.8 ± 4.2 | 21.2 ± 2.0 |
| Total (22) | 24.2 ± 4.3 | 170.1 ± 8.0 | 66.0 ± 12.4 | 22.5 ± 3.1 |

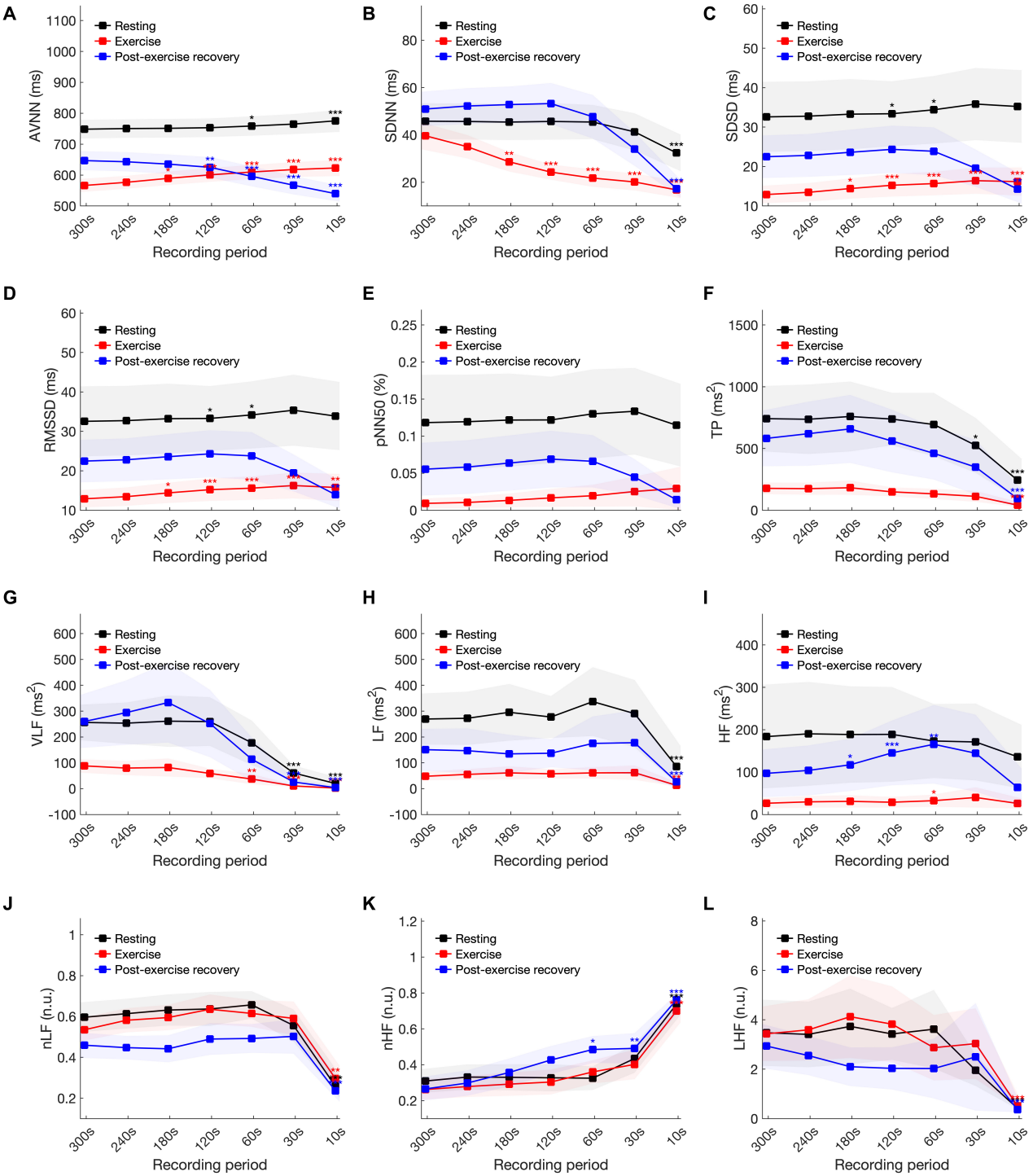



**FIGURE 4 |** Statistical analysis of heart rate variability variables in resting, exercising, and post-exercise recovery conditions according to shortening of the analysis interval. **(A)** AVNN, **(B)** SDNN, **(C)** SDSD, **(D)** RMSSD, **(E)** pNN50, **(F)** TP, **(G)** VLF, **(H)** LF, **(I)** HF, **(J)** LH/HF, **(K)** nLF, and **(L)** nHF. Statistical significance was evaluated compared to the 300-s analysis results at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

the SDNN, LF, LF/HF, and HF; ≤30 s intervals in the AVNN; and ≤60 s intervals in the SDSD and RMSSD. In the exercising state, significant differences were found at ≤10-s intervals in the TP, LF, LF/HF, nLF, and nHF; at ≤60-s intervals in the VLF and HF; and at ≤180-s intervals in the SDNN, SDSD, and RMSSD. In the recovery stage, significant differences ($P < 0.05$)

**TABLE 4 |** Ultra-short-term and short-term HRV group statistical results in resting, exercising, and post-exercise recovery conditions.

| HRV Variable | Condition | Analysis time | | | | | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | | 300 s | 240 s | 180 s | 120 s | 60 s | 30 s | 10 s | |
| AVNN (ms) | Resting | 758.0 (85.3) | 759.0 (85.2) | 760.0 (86.6) | 762.7 (85.8) | **766.5 (90.5)*** | 771.1 (93.5) | **782.4 (100.8)***** | <0.001[f] |
| | Exercising | 571.1 (55.7) | 580.9 (54.8) | **593.3 (56.1)*** | **603.9 (58.0)***** | **612.3 (59.1)***** | **620.7 (64.3)***** | **624.8 (67.4)***** | <0.001[f] |
| | Post-exercise recovery | 655.3 (85.1) | 651.3 (85.8) | 642.4 (88.8) | **631.3 (86.5)**** | **605.4 (78.6)***** | **577.7 (69.3)***** | **548.9 (69.3)***** | <0.001[f] |
| SDNN (ms) | Resting | 45.0 (20.7) | 45.6 (20.9) | 45.1 (21.1) | 45.0 (21.0) | 43.5 (18.8) | 37.7 (20.0) | **27.4 (14.6)***** | <0.001[f] |
| | Exercising | 38.9 (15.6) | 34.5 (13.2) | **28.2 (10.7)** | **24.3 (8.8)***** | **22.1 (10.2)***** | **20.5 (9.4)***** | **16.6 (8.3)***** | <0.001[f] |
| | Post-exercise recovery | 50.4 (20.4) | 51.5 (20.8) | 51.2 (19.8) | 50.7 (21.0) | 47.4 (23.5) | 35.7 (9.8) | **17.3 (9.8)***** | <0.001[k] |
| SDSD (ms) | Resting | 32.3 (24.1) | 32.4 (23.8) | 32.8 (23.9) | **32.7 (21.4)*** | **33.0 (21.5)*** | 33.6 (23.3) | 30.2 (17.9) | <0.01[f] |
| | Exercising | 12.8 (5.8) | 13.3 (6.0) | **14.2 (6.4)*** | **14.8 (7.1)***** | **15.3 (7.8)***** | **16.4 (9.2)***** | **15.7 (9.3)***** | <0.001[f] |
| | Post-exercise recovery | 23.7 (15.9) | 24.1 (16.0) | 24.7 (16.9) | 25.3 (17.6) | 25.3 (17.4) | 21.4 (13.3) | **15.4 (10.7)**** | <0.001[f] |
| RMSSD (ms) | Resting | 32.2 (24.1) | 32.4 (23.8) | 32.8 (23.9) | **32.6 (21.3)*** | **32.8 (21.3)*** | 33.1 (22.9) | 29.2 (16.9) | <0.01[f] |
| | Exercising | 12.8 (5.8) | 13.2 (6.0) | **14.2 (6.4)*** | **14.8 (7.0)***** | **15.2 (7.7)***** | **16.2 (9.0)***** | **15.4 (9.0)**** | <0.001[f] |
| | Post-exercise recovery | 23.7 (15.9) | 24.0 (16.0) | 24.7 (16.9) | 25.2 (17.6) | 25.2 (17.3) | 21.3 (13.2) | **15.0 (10.3)**** | <0.001[f] |
| pNN50 (%) | Resting | 32.2 (16.9) | 32.4 (16.7) | 32.8 (16.6) | 32.6 (15.6) | 32.8 (15.4) | 33.1 (14.7) | 29.2 (11.9) | 0.984[k] |
| | Exercise | 0.9 (1.7) | 0.9 (2.0) | 1.2 (2.0) | 1.4 (2.6) | 1.8 (3.8) | 2.5 (5.3) | 2.6 (7.4) | 0.147[k] |
| | Post-exercise recovery | 6.5 (10.6) | 6.8 (10.8) | 7.3 (11.1) | 7.7 (11.3) | 7.3 (10.3) | 5.5 (7.0) | 1.8 (3.8) | <0.01[k] |
| TP ($ms^2$) | Resting | 757.6 (756.2) | 762.8 (813.9) | 781.2 (815.6) | 729.7 (553.2) | 650.9 (649.0) | **474.4 (619.0)*** | **111.2 (98.9)***** | <0.001[f] |
| | Exercising | 181.2 (123.6) | 179.0 (125.8) | 184.3 (137.2) | 153.7 (92.5) | 137.8 (113.0) | 121.3 (128.9) | **41.1 (48.2)***** | <0.001[k] |
| | Post-exercise recovery | 575.8 (643.4) | 580.7 (706.6) | 586.4 (671.2) | 565.1 (738.8) | 471.5 (547.9) | 405.8 (547.9) | **110.7 (227.6)***** | <0.001[k] |
| VLF ($ms^2$) | Resting | 255.0 (188.3) | 254.5 (229.5) | 259.9 (292.5) | 254.4 (268.1) | 171.7 (237.6) | **39.5 (60.3)***** | **5.7 (6.7)***** | <0.001[k] |
| | Exercising | 90.3 (73.5) | 82.0 (80.1) | 84.9 (101.3) | 63.5 (53.3) | **35.7 (54.3)**** | **9.8 (10.6)***** | **2.1 (2.8)***** | <0.001[k] |
| | Post-exercise recovery | 249.7 (278.0) | 258.3 (301.0) | 267.4 (310.8) | 235.6 (368.7) | 82.5 (104.4) | **29.1 (104.4)***** | **4.6 (14.0)***** | <0.001[k] |
| LF ($ms^2$) | Resting | 285.0 (292.7) | 294.6 (305.3) | 324.6 (323.9) | 295.2 (232.2) | 296.4 (240.5) | 275.5 (380.6) | **29.0 (31.4)***** | <0.001[k] |
| | Exercise | 47.9 (38.9) | 53.4 (41.5) | 55.8 (40.8) | 53.5 (36.8) | 62.5 (60.8) | 65.0 (81.8) | **11.5 (14.4)**** | <0.001[k] |
| | Post-exercise recovery | 157.8 (241.3) | 153.2 (260.1) | 140.4 (223.3) | 140.0 (154.7) | 186.3 (303.4) | 200.8 (303.4) | **30.5 (83.4)***** | <0.001[k] |
| HF ($ms^2$) | Resting | 185.1 (335.9) | 192.2 (334.9) | 183.8 (290.4) | 168.7 (212.2) | 177.4 (240.7) | 157.5 (247.7) | 76.2 (67.3) | 0.583[f] |
| | Exercising | 30.9 (35.5) | 34.7 (40.2) | 35.7 (42.1) | 33.0 (36.7) | **37.7 (41.9)*** | 46.2 (71.2) | 27.3 (34.1) | <0.05[f] |
| | Post-exercise recovery | 113.5 (167.9) | 119.7 (176.8) | **132.6 (185.1)*** | **166.5 (228.0)***** | **198.4 (273.9)**** | 174.7 (269.0) | 75.4 (132.9) | <0.001[f] |
| LF/HF (n.u.) | Resting | 2.9 (2.5) | 2.9 (2.7) | 3.6 (4.1) | 3.1 (2.5) | 2.8 (2.4) | 1.7 (1.4) | **0.4 (0.3)***** | <0.001[k] |
| | Exercising | 3.0 (2.6) | 3.2 (3.1) | 3.8 (4.5) | 3.5 (4.4) | 2.6 (3.7) | 2.5 (2.8) | **0.5 (0.4)**** | <0.001[k] |
| | Post-exercise recovery | 2.7 (2.2) | 2.4 (2.2) | 2.0 (2.3) | 1.8 (2.3) | 1.8 (3.5) | 1.3 (1.2) | **0.3 (0.2)***** | <0.001[k] |
| nLF ($ms^2$) | Resting | 0.6 (0.2) | 0.6 (0.2) | 0.6 (0.2) | 0.6 (0.2) | 0.6 (0.2) | 0.5 (0.2) | **0.2 (0.1)***** | <0.001[k] |
| | Exercising | 0.5 (0.2) | 0.6 (0.2) | 0.6 (0.2) | 0.6 (0.2) | 0.6 (0.2) | 0.6 (0.2) | **0.3 (0.2)***** | <0.001[k] |
| | Post-exercise recovery | 0.5 (0.2) | 0.5 (0.2) | 0.4 (0.2) | 0.5 (0.2) | **0.5 (0.2)*** | **0.5 (0.2)**** | **0.2 (0.2)***** | <0.001[k] |
| nHF ($ms^2$) | Resting | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) | 0.5 (0.2) | **0.7 (0.1)***** | <0.001[f] |
| | Exercising | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) | 0.3 (0.2) | 0.4 (0.2) | 0.4 (0.2) | **0.7 (0.2)***** | <0.001[f] |
| | Post-exercise recovery | 0.3 (0.2) | 0.3 (0.2) | 0.4 (0.2) | 0.5 (0.2) | 0.5 (0.2) | 0.5 (0.2) | **0.8 (0.1)***** | 0.001[f] |

*All values are mean (SD), except for P-value. NN, normal-to-normal interval; AVNN, average of NN; SDNN, standard deviation of NN; SDSD, standard deviation of successive difference of NN; RMSSD, root-mean-square of successive difference of NN; pNN50, percentage of adjacent NNs that differ from each other by more than 50 ms; TP, total power; VLF, very-low-frequency power of 0.0033–0.04 Hz; LF, low-frequency power of 0.04–0.15 Hz; HF, high-frequency power of 0.15–0.4 Hz; LF/HF, ratio of low-frequency power to high-frequency power; nLF, normalized low-frequency power [LF/(TP–VLF)]; nHF, normalized high-frequency power [HF/(TP–VLF)]. Bold means statistically different cases. Statistical significance was evaluated compared to the 300-s analysis results at *P < 0.05, **P < 0.01, ***P < 0.001. [k]Kruskal-Wallis test. [f]Friedman's test. Bonferroni's post hoc test was performed with the Kruskal-Wallis test or the Friedman test.*

were found at ≤10-s intervals in the SDNN, SDSD, RMSSD, TP, LF, LF/HF, and nHF; at ≤30-s intervals in the VLF; at ≤60-s intervals in the nLF; ≤120-s intervals in the AVNN; and ≤180-s intervals in the HF.

## Cohen's *d* Statistics

**Figure 5** and **Supplementary Table 1** of the **Supplementary Information** (SI) show Cohen's *d* statistics for the ultra-short-term HRV values. The minimum recording time required for each variable except for the ultra-short-term HRV values indicated as not available (n/a) was as follows for resting, exercising, and post-exercise recovery, respectively: AVNN: 120 s, n/a, and n/a; SDNN: 60 s, n/a, and n/a; SDSD: 240 s, n/a, and n/a; RMSSD: 240 s, n/a, and n/a; pNN50: 30, 120 s, and n/a; TP: 60, 120, and 120 s; VLF: 120, 180, and 120 s; LF: 30 s, n/a, and 30 s; HF: 10 s, n/a, and n/a; LF/HF: 30, 30, and 30 s; nLF: 60 s, n/a, n/a; and nHF: 30, 30 s, and n/a. The 95% confidence interval of *d* was >0.5 in all cases, and the interval was wider in the post-exercise recovery and exercising states than in the resting condition. Cohen's *d* tended to increase with decreasing analysis interval, regardless of the experimental conditions. In addition, except for some cases, *d* was increased in the exercising and recovery conditions compared to the resting state. Based on the 240 s results, in some cases, *d* was smaller compared to the resting condition results. For example, TP was lower in the resting condition than in the exercising and post-exercise recovery conditions. In LF/HF, *d* decreased in the post-exercise recovery condition, whereas pNN50 and nHF decreased in the exercising condition.

## Pearson's Correlation

**Figure 6** and **Supplementary Table 2** of the SI show the results of Pearson's correlation analysis. In all cases, the correlation coefficient decreased according to the shortening of the analysis interval. The trend of the decrease was different for each condition, but variables such as SDNN, pNN50, TP, VLF, and LF/HF showed clear differences in each condition. In most cases, the correlation coefficient was largely decreased in the dynamic condition compared to the resting condition as the analysis interval decreased. The minimum analysis interval required for each HRV variable for resting, exercising, and post-exercise recovery, respectively, was as follows: AVNN: 10, 10, and 10 s; SDNN: 30, 180, and 60 s; SDSD: 10, 10, and 30 s; RMSSD: 10, 10, and 30 s; pNN50: 60, 180, and 120 s; TP: 30, 120, and 120 s; VLF: 120, 240, and 180 s; LF: 120, 120, and 180 s; HF: 30, 30, and 120 s; LF/HF: 180, 240, and 240 s; nLF: 120, 120, and 120 s; and nHF: 120, 120, and 180 s. The correlation coefficient also showed a drastic decrease in specific analysis intervals. For example, the correlation coefficient in the resting pNN50 decreased from 0.700 to 0.497 at <30-s intervals, the VLF from 0.806 to 0.482 at <120-s intervals, and 0.916–0.488 at <120-s intervals.

## Bland–Altman Limits of Agreement

**Figure 7** and **Supplementary Table 3** in the SI, show increases in bias, and the width of the 50% LoA interval was observed to increase as the analysis interval decreased for all HRV variables in every condition. This means that the shorter the analysis interval, the greater the likelihood of error. The minimum time for ultra-short-term HRV analysis based on 50% LoA for resting, exercising, and post-exercise recovery states, respectively, was as follows: AVNN: 30 s, n/a, and n/a; SDNN: 30 s, n/a, and n/a; SDSD: 240, 240, and 30 s; RMSSD: 10, 240, and 30 s; pNN50: 10, 30 s, and n/a; TP: 60, 60, and 60 s; VLF: 60, 120, and 120 s; LF: 30, 30, and 30 s; HF:10 s, n/a, and 240 s; LF/HF: 30, 30, and 30 s; nLF: 30, 120 s, and n/a; and nHF: 30, 30 s, and n/a.

# DISCUSSION

Unlike the existing ultra-short-term HRV studies conducted in resting conditions, we focused on analyzing the minimum time required for ultra-short-term HRV analysis in resting, exercising, and post-exercise recovery conditions. In this study, we used statistical methods such as null hypothesis testing, Cohen's *d* statistics, Pearson's correlation, or Bland-Altman LoA in combination to derive the required time for ultra-short-term HRV analysis.

## The Minimum Time Requirement for Ultra-Short-Term HRV Analysis

**Table 5** shows the summary of the minimum time required for the analysis of ultra-short-term HRV variables in each condition investigated using various statistical metrics. Although the minimum requirement times derived from the various analysis methods differed from each other, in general, the minimum analysis time requirement in the dynamic condition increased in all results. In addition, while Cohen's *d*, the LoA, and the null hypothesis tests showed a relatively longer minimum time requirement in the time domain analysis and a shorter minimum time requirement in the frequency domain analysis, Pearson's *R* tests showed a short minimum time requirement for time-domain analysis and a longer time requirement for frequency domain analysis, revealing mutually opposite results. Although it is very difficult to synthesize these results, conclusions can be drawn from the most strict or lenient conditions that satisfy all conditions. Strict conditions are mainly derived from the results of Cohen's *d*, the LoA, or the null hypothesis test, indicating that many HRV variables cannot be used for dynamic analysis or require at least 120 s of analysis time for some variables. Lenient conditions showed that the time domain variables were mainly derived from Cohen's *d*, the LoA, and the hypothesis tests, while the frequency domain variables were mainly derived from Pearson's *R*, which showed the ability of ultra-short-term HRV analysis in 60-s recording intervals. While the two suggestions show a difference in the minimum required time, they suggest that in common, longer recording is required in almost all cases for the ultra-short-term HRV analysis of dynamic conditions compared to the static condition.

## Ultra-Short-Term HRV in Dynamic Conditions

### HRV in Exercise Conditions

Before discussing ultra-short-term HRV in dynamic conditions, consideration should be given to cardiac autonomic regulation

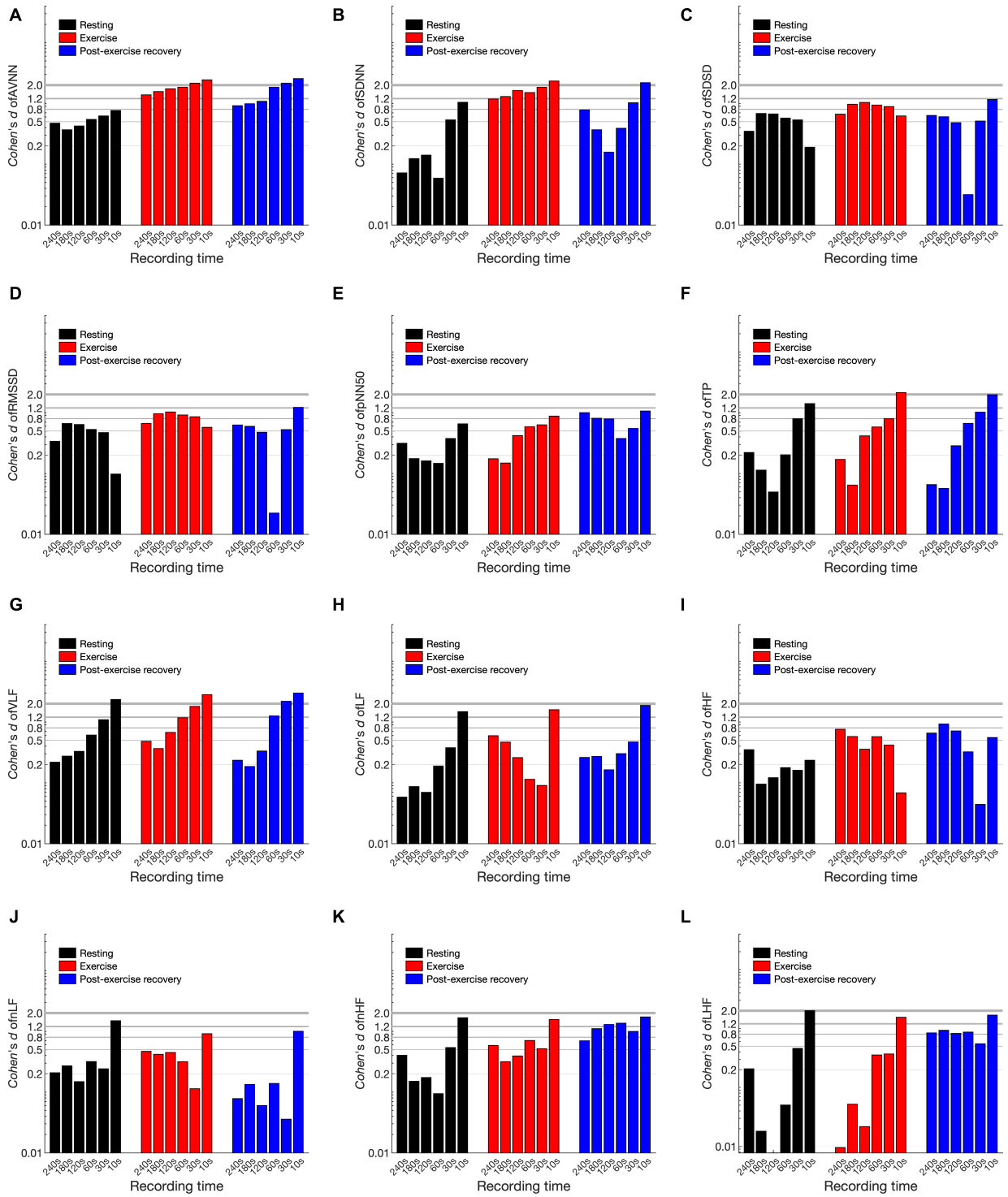



**FIGURE 5 |** Cohen's *d* statistics of heart rate variability variables in resting, exercising, and post-exercise recovery conditions according to shortening of the analysis interval. **(A)** AVNN, **(B)** SDNN, **(C)** SDSD, **(D)** RMSSD, **(E)** pNN50, **(F)** TP, **(G)** VLF, **(H)** LF, **(I)** HF, **(J)** LH/HF, **(K)** nLF, and **(L)** nHF.

during exercising or post-exercise recovery. In the emerged cardiac autonomic regulation model (Raven et al., 2006; Nobrega et al., 2014; White and Raven, 2014; Michelini et al., 2015), the HR rapidly increases, primarily mediated by reduced cardiac parasympathetic neural activity and reductions in cardiac sympathetic neural activity. In cardiac rhythm

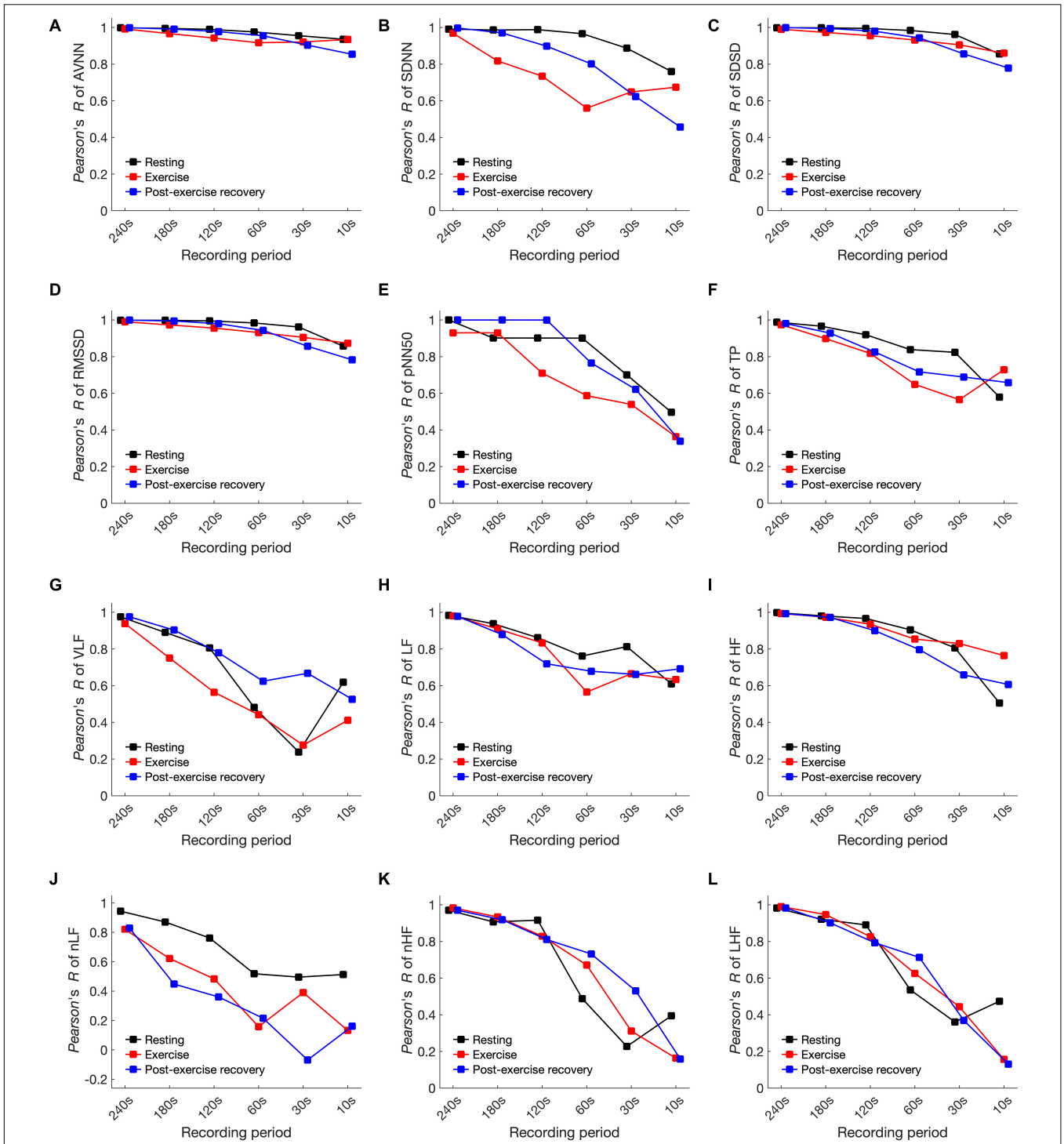



**FIGURE 6 |** Pearson's correlation coefficient of heart rate variability variables in resting, exercising, and post-exercise recovery conditions according to shortening of the analysis interval. **(A)** AVNN, **(B)** SDNN, **(C)** SDSD, **(D)** RMSSD, **(E)** pNN50, **(F)** TP, **(G)** VLF, **(H)** LF, **(I)** HF, **(J)** LH/HF, **(K)** nLF, and **(L)** nHF.

regulation, both the SNS (Sympathetic Nervous System) and PNS (Parasympathetic Nervous System) regulate HR throughout the entire exercise duration, where the SNS works as a tone-setter and the PNS operates as a rapid responder/modulator (Michael et al., 2017). In resting or low-level activity conditions, parasympathetic control is dominant. However, sympathetic control becomes dominant according to increases in exercise intensity (White and Raven, 2014). As a result, higher exercise intensity was reportedly

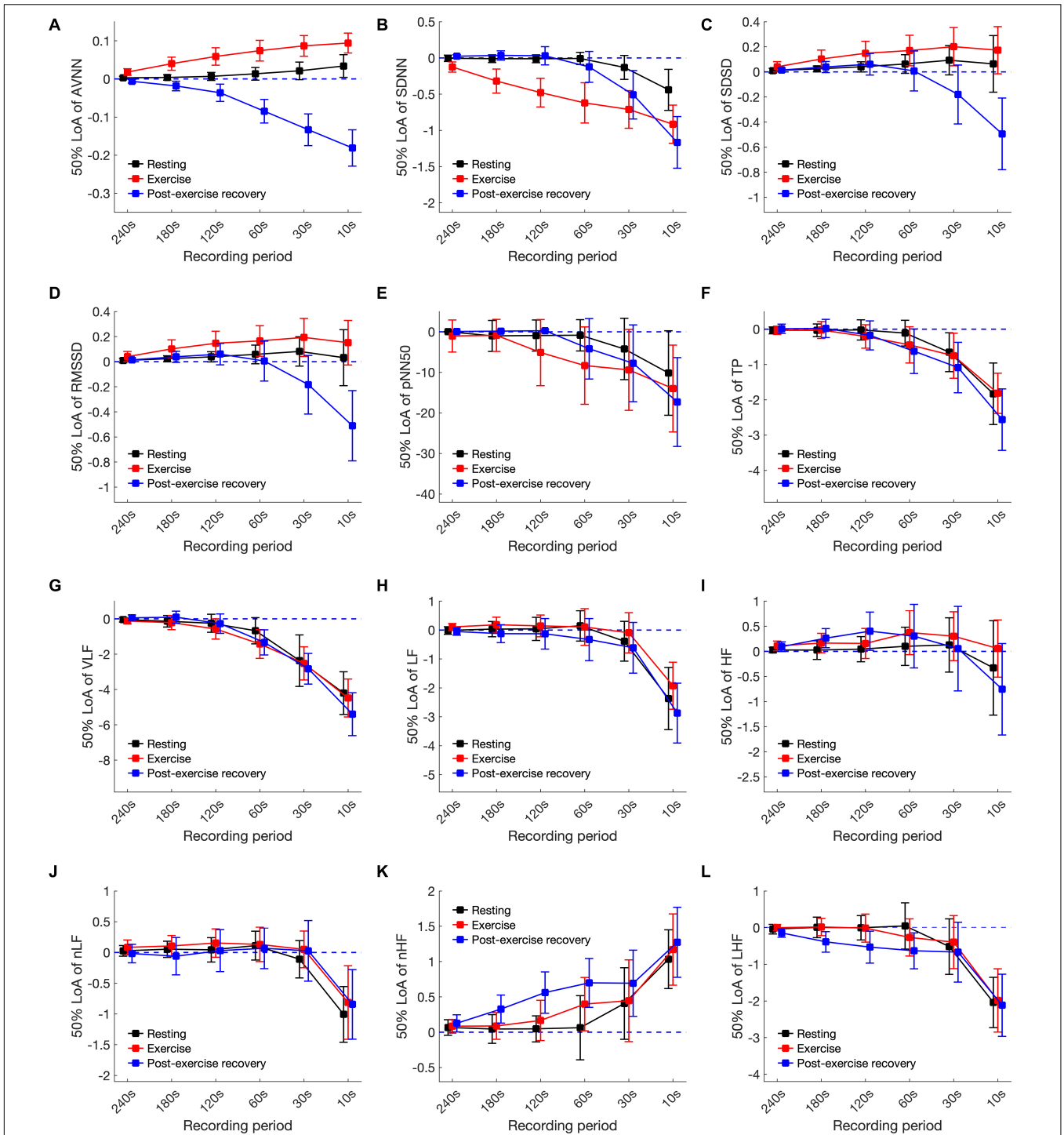



**FIGURE 7 |** The 50% limits of agreement of heart rate variability variables in resting, exercising, and post-exercise recovery conditions according to shortening of the analysis interval. **(A)** AVNN, **(B)** SDNN, **(C)** SDSD, **(D)** RMSSD, **(E)** pNN50, **(F)** TP, **(G)** VLF, **(H)** LF, **(I)** HF, **(J)** LH/HF, **(K)** nLF, and **(L)** nHF.

associated with decreases in the SDNN (Tulppo et al., 1996; Hautala et al., 2003); RMSSD (Tulppo et al., 1996; Leicht et al., 2008; Boettger et al., 2010; Karapetian et al., 2012); LF, HF, and TP (Perini et al., 1989; Tulppo et al., 1996; Avery et al., 2001; Hautala et al., 2003; Povea et al., 2005; Casties et al., 2006; Spadacini et al., 2006; Fisher et al., 2009; Boettger et al., 2010; Karapetian et al., 2012); and HF (Cottin et al., 2006, 2007). Typically, the nLF increases during low-moderate intensity exercise and decreases during higher intensity exercise, whereas the nHF shows the opposite response (Perini et al., 1990, 1993;

**TABLE 5 |** Suggested minimum analysis intervals for ultra-short-term heart rate variability analysis according to the statistical metrics.

| Metric | Condition | HRV Variables (s) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVNN | SDNN | SDSD | RMSSD | pNN50 | TP | VLF | LF | HF | LF/HF | nLF | nHF |
| Cohen's *d* | Resting | 120 | 60 | 240 | 240 | 30 | 60 | 120 | 30 | 10 | 30 | 60 | 30 |
| | Exercising | n/a | n/a | n/a | n/a | 120 | 120 | 180 | n/a | n/a | 30 | n/a | 30 |
| | Post-exercise recovery | n/a | n/a | n/a | n/a | n/a | 120 | 120 | 30 | n/a | 30 | n/a | n/a |
| Pearson's *R* | Resting | 10 | 30 | 10 | 10 | 60 | 30 | 120 | 120 | 30 | 180 | 120 | 120 |
| | Exercising | 10 | 180 | 10 | 10 | 180 | 120 | 240 | 120 | 30 | 240 | 120 | 120 |
| | Post-exercise recovery | 10 | 60 | 30 | 30 | 120 | 120 | 180 | 180 | 120 | 240 | 120 | 180 |
| Limits of agreements | Resting | 30 | 30 | 240 | 10 | 10 | 60 | 60 | 30 | 10 | 30 | 30 | 30 |
| | Exercising | n/a | n/a | 240 | 240 | 30 | 60 | 120 | 30 | n/a | 30 | 120 | 30 |
| | Post-exercise recovery | n/a | n/a | 30 | 30 | n/a | 60 | 120 | 30 | 240 | 30 | n/a | n/a |
| Hypothesis test | Resting | 120 | 30 | 180 | 180 | 10 | 60 | 60 | 30 | 10 | 30 | 30 | 30 |
| | Exercising | 240 | 240 | 240 | 240 | 10 | 30 | 120 | 30 | 120 | 30 | 30 | 30 |
| | Post-exercise recovery | 180 | 30 | 30 | 30 | 10 | 30 | 60 | 30 | 240 | 30 | 120 | 30 |
| Recommendation (strict) | Resting | 120 | 60 | 240 | 240 | 60 | 60 | 120 | 120 | 30 | 180 | 120 | 120 |
| | Exercising | 240 | n/a | n/a | n/a | 180 | 120 | 240 | n/a | n/a | 240 | n/a | 120 |
| | Post-exercise recovery | 180 | n/a | n/a | n/a | n/a | 120 | 180 | 180 | n/a | 240 | n/a | n/a |
| Recommendation (lenient) | Resting | 10 | 30 | 10 | 10 | 10 | 60 | 60 | 30 | 10 | 30 | 30 | 30 |
| | Exercising | 10 | 180 | 10 | 10 | 10 | 30 | 120 | 30 | 30 | 30 | 30 | 30 |
| | Post-exercise recovery | 10 | 60 | 30 | 30 | 10 | 30 | 60 | 30 | 120 | 30 | 120 | 30 |

*Decision criteria for availability: d < 0.5 (small) for Cohen's d, R > 0.8 for Pearson's R, 50% limits of agreement, P < 0.05 for the null hypothesis test. n/a, not available. The strict condition was a combination of the values with the longest analysis intervals in the same conditions and the lenient condition was a combination of the shortest analysis intervals in the same conditions.*

Hautala et al., 2003; Pichon et al., 2004; Povea et al., 2005; Martinmäki and Rusko, 2008), although conflicting responses have also been reported (Avery et al., 2001; Casties et al., 2006). The LF/HF demonstrates inconsistent responses to exercise. Some studies reported an increase in low-moderate intensity exercise and a decrease during higher intensity exercises (Radaelli et al., 1996; Tulppo et al., 1996; Hautala et al., 2003). However, other studies reported a progressive decrease from rest with increasing exercise intensity (Casties et al., 2006) or a progressive increase from rest (Saito and Nakamura, 1995; Avery et al., 2001).

### HRV in Post-exercise Recovery Conditions

In post-exercise recovery conditions, the aforementioned processes mediating cardio-acceleration during exercise occur in reverse, and finally, the HR and HRV demonstrate a time-dependent recovery and eventual return to pre-exercise levels (Stanley et al., 2013). Heart rate changes in the post-exercise recovery period are not theoretically well-established, and there is a view that rapid HR reduction immediately after exercise is influenced by parasympathetic reactions (Cole et al., 1999; Coote, 2010; Peçanha et al., 2014) or is affected by sympathetic activity as well (Kannankeril et al., 2004; Pichon et al., 2004). All of the above demonstrate that during exercise or recovery, either the SNS or the PNS may be dominant and that this effect may vary from person to person. In addition, the balance of the SNS and PNS may change according to exercise intensity and the post-exercise recovery status. For example, which nervous system will be dominant depends upon the exercise intensity, with the PNS dominant in low-moderate exercise and the SNS in strenuous exercise (White and Raven, 2014). Similarly, during recovery, autonomic activity may be different depending upon early and late recovery periods. In the early phase of recovery, although some evidence has suggested sympathetic involvement as well (Nandi and Spodick, 1977; Kannankeril et al., 2004; Pichon et al., 2004), the "fast phase" of HR recovery has often been attributed to parasympathetic reactivation (Perini et al., 1989; Imai et al., 1994; Cole et al., 1999; Peçanha et al., 2014), and in the late phase of recovery, a more gradual "slow phase" of cardio-deceleration is observed, mediated by both progressive parasympathetic reactivation and sympathetic withdrawal (Michael et al., 2017).

Thus, in a dynamic state, such as movement or recovery, the response of the autonomic nervous system is not steady-state, but transient. Transient indicates a status in which the autonomic nervous system is not stabilized and continuously changes. Therefore, longer analysis intervals than transient intervals can be used to ensure stable analysis without significant differences compared to the steady-state. Therefore, finding the minimum required interval for ultra-short-term HRV means finding the transient interval under each condition? Thus, it is generally expected that the time required for HRV analysis in the dynamic condition will be longer than in the resting condition, assuming steady-state. In addition, since the length of the transient interval for exercise and recovery differs for each individual, it is necessary to consider the time required for ultra-short-term HRV analysis under the various conditions.

## Limitations

This study was the first study to investigate the application of ultra-short-term HRV in dynamic conditions. However, the following limitations impact the generalizability of the results and emphasize the necessity of further research. First, the

conventional HF band of 0.15–0.40 Hz was used in this research. However, this band may not be suitable during exercise where higher respiratory frequencies are observed. This was already suggested by the results of previous studies where the standard spectral HRV analysis was more susceptible to anomalies compared to the time domain analysis (Ng et al., 2009), and thus, should not be used in exercise conditions (Michael et al., 2017).

Second, because the subjects of this study were limited to young and healthy adults, the results of this study cannot be generalized to all age groups, including diseased populations, and the number of subjects was also insufficient for parameter validation. In addition, this study did not include the analysis of various activities, such as walking, running, sitting, standing, and climbing stairs that can be encountered in daily life nor did it include an analysis by emotional state. Therefore, for the application to general mobile healthcare research, expansion of the research to various dynamic states is required.

## CONCLUSION

Dynamic behavior causes non-stationary transient changes in the autonomic nervous system, which can greatly affect the minimum interval required for HRV analysis. The results of this study suggest that the application of ultra-short-term HRV in dynamic conditions, such as exercising and post-exercise recovery periods that may occur in daily life, requires dedicated criteria for the analysis interval that is different from the existing resting ultra-short-term HRV analysis criteria. In conclusion, ultra-short-term HRV analysis in dynamic conditions required longer analysis intervals compared to resting conditions, and in dynamic conditions, when strict criteria are applied to satisfy various statistical analysis techniques, ultra-short-term HRV analysis is not recommended.

## DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The datasets analyzed during the current study are not publicly available due to privacy issue but are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to HS, hangsik.shin@jnu.ac.kr.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Asan medical center. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

HS and JK designed the experimental protocol. JK carried out experiment and collected data. HS and HSS analyzed data statistically. HS supervised the project. All authors contributed to the article and approved the submitted version.

## FUNDING

This research was supported by two grants from the Basic Science Research Program through the National Research Foundation of Korea (NRF), one funded by the Ministry of Science and ICT (NRF-2018R1A4A1025704), and one funded by the Ministry of Education (NRF-2018R1D1A3B07046442), South Korea.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.596060/full#supplementary-material

## REFERENCES

Acharya, U. R., Joseph, K. P., Kannathal, N., Lim, C. M., and Suri, J. S. (2006). Heart rate variability: a review. *Med. Biol. Eng. Comput.* 44, 1031–1051.

Altman, D. G., and Bland, J. M. (1983). Measurement in medicine: the analysis of method comparison studies. *J. R. Statist. Soc. Ser. D (Statist.)* 32, 307–317. doi: 10.2307/2987937

Avery, N., Wolfe, L., Amara, C., Davies, G., and Mcgrath, M. (2001). Effects of human pregnancy on cardiac autonomic function above and below the ventilatory threshold. *J. Appl. Physiol.* 90, 321–328. doi: 10.1152/jappl.2001.90.1.321

Baek, H. J., Cho, C. H., Cho, J., and Woo, J. M. (2015). Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability. *Telemed. J. E Health*, 21, 404–414.

Barak, O. F., Jakovljevic, D. G., Gacesa, J. Z. P., Ovcin, Z. B., Brodie, D. A., and Grujic, N. G. (2010). Heart rate variability before and after cycle exercise in relation to different body positions. *J. Sports Sci. Med.* 9:176.

Berntson, G. G., Thomas Bigger, J. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Bland, J. M., and Altman, D. G. (2003). Applying the right statistics: analyses of measurement studies. *Ultrasound Obstetr. Gynecol. Off. J. Int. Soc. Ultrasound Obstetr. Gynecol.* 22, 85–93. doi: 10.1002/uog.122

Boettger, S., Puta, C., Yeragani, V. K., Donath, L., Mueller, H.-J., Gabriel, H. H., et al. (2010). Heart rate variability, QT variability, and electrodermal activity during exercise. *Med. Sci. Sports Exerc.* 42, 443–448. doi: 10.1249/mss.0b013e3181b64db1

Buchheit, M., Solano, R., and Millet, G. P. (2007). Heart-rate deflection point and the second heart-rate variability threshold during running exercise in trained boys. *Pediatr. Exerc. Sci.* 19, 192–204. doi: 10.1123/pes.19.2.192

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R., et al. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Castaldo, R., Montesinos, L., Melillo, P., Massaro, S., and Pecchia, L. (2017). "To what extent can we shorten HRV analysis in wearable sensing? A case study on mental stress detection," in *EMBEC & NBC 2017*, eds H. Eskola, O. Väisänen, J. Viik, and J. Hyttinen (Singapore: Springer), 643–646. doi: 10.1007/978-981-10-5122-7_161

Casties, J.-F., Mottet, D., and Le Gallais, D. (2006). Non-linear analyses of heart rate variability during heavy exercise and recovery in cyclists. *Int. J. Sports Med.* 27, 780–785. doi: 10.1055/s-2005-872968

Choi, A., and Shin, H. (2018). Quantitative analysis of the effect of an ectopic beat on the heart rate variability in the resting condition. *Front. Physiol.* 9:922.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale. NJ: Erihaum.

Cole, C. R., Blackstone, E. H., Pashkow, F. J., Snader, C. E., and Lauer, M. S. (1999). Heart-rate recovery immediately after exercise as a predictor of mortality. *N. Engl. J. Med.* 341, 1351–1357. doi: 10.1056/nejm199910283411804

Coote, J. H. (2010). Recovery of heart rate following intense dynamic exercise. *Exp. Physiol.* 95, 431–440. doi: 10.1113/expphysiol.2009.047548

Cottin, F., Leprêtre, P.-M., Lopes, P., Papelier, Y., Médigue, C., and Billat, V. (2006). Assessment of ventilatory thresholds from heart rate variability in well-trained subjects during cycling. *Int. J. Sports Med.* 27, 959–967. doi: 10.1055/s-2006-923849

Cottin, F., Medigue, C., Lopes, P., Leprêtre, P.-M., Heubert, R., and Billat, V. (2007). Ventilatory thresholds assessment from heart rate variability during an incremental exhaustive running test. *Int. J. Sports Med.* 28, 287–294. doi: 10.1055/s-2006-924355

Datta, D., Normandin, E., and Zuwallack, R. (2015). Cardiopulmonary exercise testing in the assessment of exertional dyspnea. *Annal. Thorac. Med.* 10:77. doi: 10.4103/1817-1737.151438

Fisher, J. P., Ogoh, S., Junor, C., Khaja, A., Northrup, M., and Fadel, P. J. (2009). Spontaneous baroreflex measures are unable to detect age-related impairments in cardiac baroreflex function during dynamic exercise in humans. *Exp. Physiol.* 94, 447–458. doi: 10.1113/expphysiol.2008.044867

Flatt, A. A., and Esco, M. R. (2013). Validity of the ithleteTM smart phone application for determining ultra-short-term heart rate variability. *J. Hum. Kinet.* 39, 85–92. doi: 10.2478/hukin-2013-0071

Fletcher, G. F., Ades, P. A., Kligfield, P., Arena, R., Balady, G. J., Bittner, V. A., et al. (2013). Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation* 128, 873–934. doi: 10.1161/cir.0b013e31829b5b44

Hamilton, R. M., Mckechnie, P. S., and Macfarlane, P. W. (2004). Can cardiac vagal tone be estimated from the 10-second ECG? *Int. J. Cardiol.* 95, 109–115. doi: 10.1016/j.ijcard.2003.07.005

Hautala, A. J., Mäkikallio, T. H., Seppänen, T., Huikuri, H. V., and Tulppo, M. P. (2003). Short-term correlation properties of R–R interval dynamics at different exercise intensity levels. *Clin. Physiol. Funct. Imag.* 23, 215–223. doi: 10.1046/j.1475-097x.2003.00499.x

Hunt, K. J., and Saengsuwan, J. (2018). Changes in heart rate variability with respect to exercise intensity and time during treadmill running. *Biomed. Eng. Online* 17, 1–12.

Imai, K., Sato, H., Hori, M., Kusuoka, H., Ozaki, H., Yokoyama, H., et al. (1994). Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J. Am. College Cardiol.* 24, 1529–1535. doi: 10.1016/0735-1097(94)90150-3

Javorka, M., Zila, I., Balharek, T., and Javorka, K. (2002). Heart rate recovery after exercise: relations to heart rate variability and complexity. *Brazil. J. Med. Biol. Res.* 35, 991–1000. doi: 10.1590/s0100-879x2002000800018

Kannankeril, P. J., Le, F. K., Kadish, A. H., and Goldberger, J. J. (2004). Parasympathetic effects on heart rate recovery after exercise. *J. Investigat. Med.* 52, 394–401. doi: 10.2310/6650.2004.00611

Karapetian, G., Engels, H., Gretebeck, K., and Gretebeck, R. (2012). Effect of caffeine on LT, VT and HRVT. *Int. J. Sports Med.* 33, 507–513. doi: 10.1055/s-0032-1301904

Krouwer, J. S. (2008). Why Bland–Altman plots should use X, not (Y+X)/2 when X is a reference method. *Statist. Med.* 27, 778–780. doi: 10.1002/sim.3086

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research–recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213.

Leicht, A. S., Sinclair, W. H., and Spinks, W. L. (2008). Effect of exercise mode on heart rate variability during steady state exercise. *Eur. J. Appl. Physiol.* 102, 195–204. doi: 10.1007/s00421-007-0574-9

Malik, M., Bigger, J. T., Camm, A. J., Kleiger, R. E., Malliani, A., Moss, A. J., et al. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Malliani, A. (1995). Association of heart rate variability components with physiological regulatory mechanisms. *Heart Rate Variabil.* 8, 202–242.

Marked, V. (1995). "Correction of the heart rate variability signal for ectopics and missing beats," in *Heart Rate variability*, eds M. Malik and A. J. Camm (Armonk, NY: Futura Publishing Co. Inc.).

Martinmäki, K., Häkkinen, K., Mikkola, J., and Rusko, H. (2008). Effect of low-dose endurance training on heart rate variability at rest and during an incremental maximal exercise test. *Eur. J. Appl. Physiol.* 104:541. doi: 10.1007/s00421-008-0804-9

Martinmäki, K., and Rusko, H. (2008). Time-frequency analysis of heart rate variability during immediate recovery from low and high intensity exercise. *Eur. J. Appl. Physiol.* 102, 353–360. doi: 10.1007/s00421-007-0594-5

McNames, J., and Aboy, M. (2006). Reliability and accuracy of heart rate variability metrics versus ECG segment duration. *Med. Biol. Eng. Comput*. 44, 747–756.

Michael, S., Graham, K. S., and Davis, G. M. (2017). Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals—a review. *Front. Physiol.* 8:301.

Michelini, L. C., O'leary, D. S., Raven, P. B., and Nóbrega, A. C. (2015). Neural control of circulation and exercise: a translational approach disclosing interactions between central command, arterial baroreflex, and muscle metaboreflex. *Am. J. Physiol. Heart Circulat. Physiol.* 309, H381–H392.

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., and Snieder, H. (2015). Validity of (ultra-) short recordings for heart rate variability measurements. *PLoS One* 10:e0138921. doi: 10.1371/journal.pone.0138921

Nandi, P. S., and Spodick, D. H. (1977). Recovery from exercise at varying work loads. Time course of responses of heart rate and systolic intervals. *Br. Heart J.* 39:958. doi: 10.1136/hrt.39.9.958

Ng, J., Sundaram, S., Kadish, A. H., and Goldberger, J. J. (2009). Autonomic effects on the spectral analysis of heart rate variability after exercise. *Am. J. Physiol. Heart Circulat. Physiol.* 297, H1421–H1428.

Nobrega, A. C., O'leary, D., Silva, B. M., Marongiu, E., Piepoli, M. F., and Crisafulli, A. (2014). Neural regulation of cardiovascular response to exercise: role of central command and peripheral afferents. *BioMed Res. Int.* 2014:478965.

Nussinovitch, U., Elishkevitz, K. P., Katz, K., Nussinovitch, M., Segev, S., Volovitz, B., and Nussinovitch, N. (2011). Reliability of ultra-short ECG indices for heart rate variability. *Ann. Noninvasive Electrocardiol.* 16, 117–122.

Pan, J., and Tompkins, W. J. (1985). A real-time QRS detection algorithm. *IEEE Transact. Biomed. Eng.* 32, 230–236. doi: 10.1109/tbme.1985.325532

Peçanha, T., Silva-Júnior, N. D., and Forjaz, C. L. D. M. (2014). Heart rate recovery: autonomic determinants, methods of assessment and association with mortality and cardiovascular diseases. *Clin. Physiol. Funct. Imag.* 34, 327–339. doi: 10.1111/cpf.12102

Perini, R., Orizio, C., Baselli, G., Cerutti, S., and Veicsteinas, A. (1990). The influence of exercise intensity on the power spectrum of heart rate variability. *Eur. J. Appl. Physiol. Occupat. Physiol.* 61, 143–148. doi: 10.1007/bf00236709

Perini, R., Orizio, C., Comandè, A., Castellano, M., Beschi, M., and Veicsteinas, A. (1989). Plasma norepinephrine and heart rate dynamics during recovery from submaximal exercise in man. *Eur. J. Appl. Physiol. Occupat. Physiol.* 58, 879–883. doi: 10.1007/bf02332222

Perini, R., Orizio, C., Milesi, S., Biancardi, L., Baselli, G., and Veicsteinas, A. (1993). Body position affects the power spectrum of heart rate variability during

dynamic exercise. *Eur. J. Appl. Physiol. Occupat. Physiol.* 66, 207–213. doi: 10.1007/bf00235095

Pichon, A. P., De Bisschop, C., Roulaud, M., Denjean, A., and Papelier, Y. (2004). Spectral analysis of heart rate variability during exercise in trained subjects. *Med. Sci. Sports Exerc.* 36, 1702–1708. doi: 10.1249/01.mss.0000142403.93205.35

Povea, C., Schmitt, L., Brugniaux, J., Nicolet, G., Richalet, J.-P., and Fouillot, J.-P. (2005). Effects of intermittent hypoxia on heart rate variability during rest and exercise. *High Altitude Med. Biol.* 6, 215–225. doi: 10.1089/ham.2005.6.215

Radaelli, A., Valle, F., Falcone, C., Calciati, A., Leuzzi, S., Martinelli, L., et al. (1996). Determinants of heart rate variability in heart transplanted subjects during physical exercise. *Eur. Heart J.* 17, 462–471. doi: 10.1093/oxfordjournals.eurheartj.a014881

Raven, P. B., Fadel, P. J., and Ogoh, S. (2006). Arterial baroreflex resetting during exercise: a current perspective. *Exp. Physiol.* 91, 37–49. doi: 10.1113/expphysiol.2005.032250

Rosenthal, R. (1990). How are we doing in soft psychology? *Am. Psychol.* 45:775. doi: 10.1037/0003-066x.45.6.775

Saito, M., and Nakamura, Y. (1995). Cardiac autonomic control and muscle sympathetic nerve activity during dynamic exercise. *Jpn. J. Physiol.* 45, 961–977. doi: 10.2170/jjphysiol.45.961

Salahuddin, L., Cho, J., Jeong, M. G., and Kim, D. (2007). "Ultra short term analysis of heart rate variability for monitoring mental stress in mobile settings," in *Proceedings of the 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society* (Piscataway, NJ: IEEE), 4656–4659.

Spadacini, G., Passino, C., Leuzzi, S., Valle, F., Piepoli, M., Calciati, A., et al. (2006). Frequency-dependent baroreflex control of blood pressure and heart rate during physical exercise. *Int. J. Cardiol.* 107, 171–179. doi: 10.1016/j.ijcard.2005.03.011

Stanley, J., Peake, J. M., and Buchheit, M. (2013). Cardiac parasympathetic reactivation following exercise: implications for training prescription. *Sports Med.* 43, 1259–1277. doi: 10.1007/s40279-013-0083-4

Tulppo, M. P., Makikallio, T., Takala, T., Seppanen, T., and Huikuri, H. V. (1996). Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *Am. J. Physiol. Heart Circul. Physiol.* 271, H244–H252.

White, D. W., and Raven, P. B. (2014). Autonomic neural control of heart rate during dynamic exercise: revisited. *J. Physiol.* 592, 2491–2500. doi: 10.1113/jphysiol.2014.271858

Zhu, H., Wang, H., Liu, Z., Li, D., Kou, G., and Li, C. (2018). Experimental study on the human thermal comfort based on the heart rate variability (HRV) analysis under different environments. *Sci. Total Environ.* 616, 1124–1133. doi: 10.1016/j.scitotenv.2017.10.208

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 30 April 2021
doi: 10.3389/fnins.2021.625276

# A Close Examination of the Use of Systolic Time Intervals in the Calculation of Impedance Derived Cardiac Autonomic Balance and Regulation

*Cameron R. Wiley[1], Vida Pourmand[2], Julian F. Thayer[1] and DeWayne P. Williams[1]**

[1] Department of Psychological Science, University of California, Irvine, Irvine, CA, United States, [2] Department of Psychology, Western Washington University, Bellingham, WA, United States

Traditionally, impedance derived measures of cardiac autonomic balance (CAB) and regulation (CAR) are calculated using indices of heart rate variability (HRV) that primarily reflect parasympathetic nervous system activity (e.g., high-frequency HRV | HF-HRV) and pre-ejection period (PEP; a systolic time interval and measure of sympathetic activity). However, HF-HRV and PEP are considered measures of chronotropic and inotropic cardiac influence, respectively. Left ventricular ejection time (LVET) is a systolic time interval that reflects sympathetic chronotropic influence, and therefore may be a more appropriate measure for calculating CAB and CAR compared to PEP. Thus, the current study evaluates both PEP and LVET in the calculation of CAB and CAR. Data from 158 healthy participants (mean age = 19.09 years old, SD = 1.84 years) were available for analyses. CAB and CAR values were calculated using both HF-HRV and the root mean square of successive differences, in addition to both PEP and LVET, in accordance with previously established guidelines. Analyses showed that correlations were significantly weaker between CAB and CAR calculated using LVET for both HF ($z = 5.12$, $p < 0.001$) and RMSSD ($z = 5.26$, $p < 0.001$) than with PEP. These data suggest that LVET, compared to PEP, provides better "autonomic space" as evidenced by a lack of correlation between CAB and CAR computed using LVET. We stress that future research consider calculating CAB and CAR using chronotropic measures for both parasympathetic and sympathetic activity, as doing so may yield more accurate and independent measures of cardiac autonomic activity compared to a mixture of inotropic (i.e., PEP) and chronotropic (i.e., HF-HRV) measures.

**Keywords: cardiac autonomic balance, cardiac autonomic regulation, heart rate variability, pre-ejection period, left ventricular ejection time**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Antonio Roberto Zamunér, Catholic University of Maule, Chile*
*Vitor Engracia Valenti, São Paulo State University, Brazil*

****Correspondence:***
*DeWayne P. Williams*
*dewaynpw@uci.edu*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *02 November 2020*
***Accepted:*** *29 March 2021*
***Published:*** *30 April 2021*

***Citation:***
*Wiley CR, Pourmand V, Thayer JF and Williams DP (2021) A Close Examination of the Use of Systolic Time Intervals in the Calculation of Impedance Derived Cardiac Autonomic Balance and Regulation. Front. Neurosci. 15:625276. doi: 10.3389/fnins.2021.625276*

## INTRODUCTION

The dynamic between the parasympathetic and sympathetic branches of the autonomic nervous system is a multifaceted one that is implicated in psychological and physiological processes and health (Sleight, 1997; Thayer et al., 2009). Good health is generally marked by a relative equilibrium between the parasympathetic and sympathetic branches, referred to as autonomic balance

(Thayer and Friedman, 1997; Malliani, 2005). Conversely, poor health is linked to autonomic imbalance, which is characterized by hyperactive sympathetic activity and hypoactive parasympathetic activity (Malliani et al., 1994; Thayer et al., 2010b). Therefore, examining the association between cardiac autonomic activity, health outcomes, and psychological factors is of interest to many psychologists and physicians alike. In this effort, impedance derived measures of cardiac autonomic balance (CAB) and regulation (CAR) have been developed (Berntson et al., 2008). Traditionally, both CAB and CAR are calculated using respiratory sinus arrythmia or high frequency heart rate variability (HF-HRV; an index of heart rate variability and measure of parasympathetic activity) and pre-ejection period (PEP; a systolic time interval and measure of sympathetic activity) (Berntson et al., 2008; Singh et al., 2009; Kreibig et al., 2012; Bylsma et al., 2015). However, HF-HRV is considered a measure of chronotropic influence, defined as control of the heart via the sinoatrial node (Thayer et al., 2010a). In contrast, PEP is considered a measure of inotropic influence, defined as myocardial contractility (Levy, 1997). Thus, it is important to consider the calculation of CAB and CAR using indices of chronotropic influence for both parasympathetic and sympathetic measures. The left ventricular ejection time (LVET) is a systolic time interval that reflects sympathetic chronotropic influence, and therefore may be a superior measure (compared to PEP) for calculating CAB and CAR (Stemmler, 1993; Uijtdehaage and Thayer, 2000; Thayer and Uijtdehaage, 2001). Thus, the current study investigates both PEP and LVET in the calculation of CAB and CAR, and we highlight implications for how these differential calculations may impact psychophysiological research.

## Autonomic Balance and Health

Autonomic nervous system imbalance, or an increase in sympathetic activity coupled with a decrease in parasympathetic activity, has been associated with poorer physiological health outcomes including metabolic abnormalities (Licht et al., 2013) and cardiovascular disease risk factors (i.e., hypertension, diabetes) (Thayer et al., 2010b), as well as worse psychological outcomes, including anxiety (Friedman and Thayer, 1998), depression (Stone et al., 2020), and increased levels of daily stress (Mitchell et al., 2017). Due to its importance in health research, there have been several attempts to accurately measure cardiac autonomic balance and regulation over the years using various physiological measures. Berntson et al. (2008) proposed two indices of cardiac autonomic activity using impedance cardiography known as Cardiac Autonomic Balance (CAB) and Cardiac Autonomic Regulation (CAR). CAB is defined as the reciprocal balance between parasympathetic and sympathetic nervous system activity, while CAR is defined as the total activity of both branches. CAB and CAR can be calculated using indices of parasympathetically mediated HRV (e.g., the root mean square of successive differences [RMSSD], HF-HRV) and impedance derived systolic time intervals (i.e., pre-ejection period [PEP]) as an index of sympathetic activity (Berntson et al., 2008; Williams et al., 2017). Both CAB and CAR have been used as indices of autonomic balance and activity in a myriad of studies, showing associations with affective responses (Kreibig et al., 2012), psychopathologies (Bylsma et al., 2015; Stone et al., 2020), stress (Gump et al., 2011; Mitchell et al., 2017), inflammatory markers (Singh et al., 2009; Alen et al., 2020), and physiological health (Berntson et al., 2008; Vrijkotte et al., 2015). For example, a history of myocardial infarctions and type 2 diabetes diagnoses are more likely to be linked to low levels of CAR and CAB, respectively (Berntson et al., 2008), while lower CAB has also been shown to be associated with increased levels of inflammatory cytokines such as interleukin-6 and tumor necrosis factor alpha (Alen et al., 2020).

## Chronotropic vs. Inotropic Cardiac Influence

Autonomic influences on the heart can differ based on whether activation occurs at the sinoatrial (SA) node or the atrioventricular (AV) node. Autonomic nervous system activation at the SA node results in control of heart rate, known as chronotropy, which is associated with several cardiac measures including RMSSD (Thayer et al., 2010a). Among these measures is the left ventricular ejection time (LVET), a systolic time interval reflective of sympathetic activity (Stemmler, 1993; Thayer and Uijtdehaage, 2001). LVET is defined as the duration of the left ventricle to eject blood corresponding to the opening and closing of the aortic valve. More specifically, LVET refers to the interval between the B- and X-point on the dZ/dt waveform (Sherwood et al., 1990; Lozano et al., 2007). On the other hand, autonomic stimulation at the AV node results in changes in myocardial contractility, known as inotropy (Levy, 1997). A common inotropic measure is PEP, also a systolic time interval reflective of sympathetic activity, defined as the duration between initial ventricular depolarization and opening of the aortic valve. More specifically, PEP reflects the interval from the onset of the ECG Q-wave to the onset of left ventricular ejection (the interval preceding LVET) (Sherwood et al., 1990; Berntson et al., 2004).

Whereas both LVET and PEP are systolic time intervals that reflect sympathetic activity, the physiological foundations of these measures differ significantly. Therefore, a closer examination of the calculation of both CAB and CAR using PEP and LVET is warranted. Berntson et al. (1991) even acknowledged this potential issue in an earlier article, stating: "Moreover, in view of the highly specific patterns of autonomic activity that can be seen across organ systems, measures of the two autonomic divisions should be derived from the same organ. Finally, even chronotropic and inotropic influences on the heart, for example, are mediated by separate efferent pathways that may be subject to differential central control. Consequently, indices should be optimally derived from the same functional dimension of the target organ" (pp. 482-483).

## The Autonomic Space Model

Further exploration of the differential autonomic contributions of various cardiovascular measures led to the development of the Autonomic Space Model (Berntson et al., 1993), which proposed that chronotropic control of the heart via parasympathetic and sympathetic influence can vary

reciprocally, independently, or coactively; laying the foundation for the future development of CAB and CAR. The autonomic "space" in question refers to the transformations that take place between psychophysiological antecedents and autonomic outflows (e.g., reciprocal, independent, or coactive), and between autonomic outflows and functional effects on target organs (i.e., chronotropic and inotropic influences on the heart) (Berntson et al., 1994b). The varying modes of autonomic control that the Autonomic Space Model describes can be illustrated using a bivariate model where the x-axis represents independent sympathetic control using a normalized sympathetic measure of cardiac activity (i.e., *z*-scores of PEP values) and the *y*-axis represents independent parasympathetic control using a normalized parasympathetic measure of cardiac activity (i.e., *z*-scores of HF-HRV). The graphical space within these axes can be divided into four quadrants that represent the modes of autonomic activity (reciprocal sympathetic, reciprocal parasympathetic, coactivation, and co-inhibition; see **Figure 1** for example). Overall, this model provided a more comprehensive conceptualization of the flexibility of the autonomic nervous system and also serves as an additional way to examine the influence of different parasympathetic = and sympathetic measures on CAB and CAR. Importantly, one piece of such conceptualization, however, is that CAB and CAR are not significantly related. In other words, these various autonomic states as defined by CAB and CAR values can be *independent* from one another. For example, individuals could conceivably be higher in CAB, but not necessarily CAR. This is important, as CAB and CAR are thought to differentially predict cardiac disease states (e.g., myocardial infraction, diabetes; Berntson et al., 2008) and thus, CAB and CAR values should not be related or dependent on one another.

## Present Study

Given the importance of the autonomic nervous system in linking psychological and physiological health, it is crucial that the dynamic between its two branches be conceptualized in a way that optimally and accurately captures pure parasympathetic and sympathetic nervous system activity. Taking this into account, the purpose of this paper is to evaluate both PEP and LVET in the calculation of CAB and CAR. Specifically, we aim to determine the differential contributions of PEP and LVET in autonomic space by comparing measures of CAB and CAR that are calculated using each systolic time interval independently (i.e., comparing CAB_PEP to CAR_PEP and comparing CAB_LVET to CAR_LVET). If LVET and HRV measures represent chronotropic cardiac influence, and PEP represents inotropic cardiac influence, then CAB and CAR calculated using LVET should more accurately depict autonomic "space" compared to CAB and CAR calculated using PEP. Therefore, the following investigation examines the impact different systolic time intervals (PEP and LVET) can have on the association between CAB and CAR. We hypothesize that CAB and CAR calculated using PEP will be more closely associated compared to CAB and CAR calculated using LVET. Support for these hypotheses would suggest that PEP provides less of a distinction (or less autonomic space) between CAB and CAR compared to LVET. Thus, the current investigation evaluates the impact of chronotropic (LVET) verses inotropic (PEP) measures in both the calculation and validity of impedance derived measures of CAB and CAR.

# METHODS

## Participants and Procedures

Participants were recruited via two methods: (a) an introductory level psychology course research pool, where students earned class credit for participating; and (b) outside of the research pool, with these individuals being compensated with cash. Data were pooled over three studies ($N = 158$, 107 females, 57 minorities, $M$ age = 19.09, SD = 1.84, age range: 18–30). All participants were apparently healthy and did not readily present any mental or physical disorders.

We asked all participants not to smoke, undergo vigorous physical activity, or drink caffeine or alcohol in the six hours prior to the experiment. The methods of each study were approved by the institutional review board at The Ohio State University (IRB Protocol Number: 2012B0580) and followed the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) checklist (Knottnerus and Tugwell, 2008),All participants gave written informed consent. All experimental sessions were conducted between 9am and 5pm. Prior to each session, participants were asked if they wanted to use the restroom and were given the opportunity to do so if necessary. In all studies, participants were placed in a soundproof experimental room that was held at room temperature (70 to 73 degrees Fahrenheit, or 21 to 23 degrees Celsius) and equipped with a camera and microphone for safety and instructional purposes as well as a high-definition TV (for stimuli presentation which did not occur in the present study). Participants were given a detailed explanation of the procedures that would take place without indicating the specific hypothesis under study or the manipulations applied. Electrocardiogram (ECG) leads were attached to the subjects and while in a separate control room, the experimenter led the subjects to the initial phases of the experiment. All participants first completed a 5-min baseline resting period, where participants, while spontaneously breathing, sat and viewed a blank, gray screen, and were instructed not to move or fall asleep while their cardiac activity was recorded via ECG. The "blank gray screen" contained no additional stimuli; the TV was turned on with a blank and "gray" screen so that participants were not able to view themselves via the reflection when powered off. The data for the present study was derived from this baseline period.

## Cardiovascular Measures

Cardiac data was recorded continuously throughout each experiment via a three-lead ECG with three additional leads for the ICG signal at a 1000 Hz sampling rate using a Mindware$^{TM}$ 2000D (MW2000D) Impedance Cardiograph package. Electrodes were placed on the clavicle (1), ribs (2), lower back (1), lower sternum (1), notch of the throat (1), and back of the neck (1). Successive R-spikes were obtained from ECG recordings

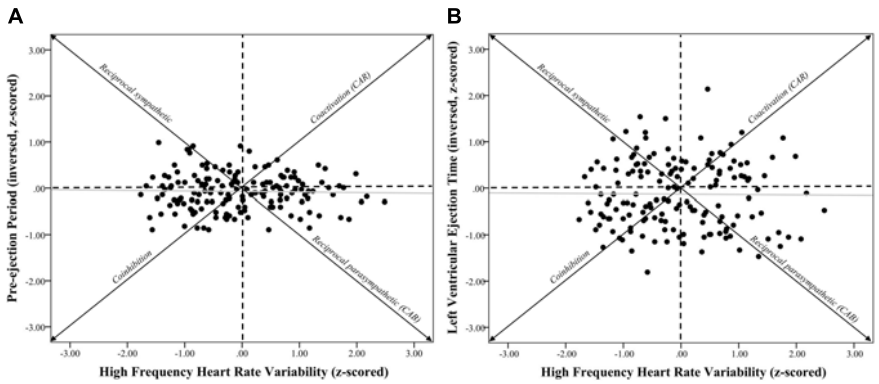



**FIGURE 1 |** Systolic time intervals and high frequency heart rate variability scatterplots. *Note*. **(A)** shows a scatterplot between pre-ejection periods (PEP *z*-scored and inversed, see section "METHODS" for details) and high frequency heart rate variability (HF-HRV z-scored) ($r = –0.03$, $p = 0.73$). **(B)** shows a scatterplot between left ventricular ejection time (LVET inversed and z-scored) and HF-HRV ($r = –0.01$, $p = 0.90$). Individuals in the coinhibition quadrant would show lower CAR scores, while individuals in the coactivation quadrant would show higher CAR scores. Individuals in the reciprocal sympathetic quadrant would show lower CAB scores, while individuals in the reciprocal parasympathetic quadrant would show higher CAB scores.

to calculate baseline HR and variability in these R-spikes was employed to calculate baseline HRV. Participants' successive IBIs (in milliseconds) were extracted using Mindware$^{TM}$ HRV Analysis software. IBIs were written in a text file and analyzed using Kubios HRV analysis package 2.0 (Tarvainen et al., 2014), allowing for the calculation of time- and frequency-domain indices of resting HRV (Task Force of the European Society of Cardiology, 1996). Artifacts within the R-to-R series were visually detected, and we applied an artifact correction level that would differentiate and remove artifacts (differing abnormal IBIs from the mean IBI). The detrending of time- and frequency-domain HRV measures was accomplished via the smoothness priors approach (see Tarvainen et al., 2014, for review). The root mean square of successive differences (RMSSD), measured in milliseconds, was calculated and is considered to be a stable (Li et al., 2009) and valid (Thayer et al., 2010a), time-domain measure of HRV. Autoregressive estimates were also calculated, yielding high-frequency power HRV (HF, 0.15–0.4 Hz; Thayer et al., 2010a). High-frequency peak values (HF hz) were obtained from a spectral-domain analysis as a measure of respiration frequency to control for potential bias (Thayer et al., 2002). Using Mindware$^{TM}$ Impedance Cardiography Analysis software, mean PEPs and LVETs were also calculated (in milliseconds) in accordance with previously published guidelines (Sherwood et al., 1990). Specifically, Mindware Impedance Cardiography applies an algorithm that accurately identifies the Q peak (R onset) and the B point (start of the dz/dt peak) in the dz/dt wave form allowing for the calculation of both PEP and LVET for each individual (for more details, please see Berntson et al., 2004 and Lozano et al., 2007). As previously stated, CAB can be defined as a relative balance between parasympathetic and sympathetic nervous system activity. Therefore, it is calculated by subtracting the HRV measure for parasympathetic activity from the impedance measure for sympathetic activity, resulting in the relative difference in control between the two branches (Berntson et al., 2008). Conversely, CAR is defined as the total activity of both branches of the autonomic nervous system. Therefore, it is calculated by adding the HRV measure for sympathetic activity to the HRV measure for parasympathetic activity, resulting in a measure of total autonomic control (Berntson et al., 2008). Berntson et al. (2008) original formulas expressed the dynamics between the parasympathetic and sympathetic as $CAB = HFz - (-PEPz)$ and $CAR = HFz + (-PEPz)$, which employ a chronotropic frequency-domain measure of parasympathetic activity (HF) and an inotropic impedance-derived measure of sympathetic activity (PEP). However, other research has identified RMSSD as an equally reliable time-domain measure of parasympathetic activity compared to HF (Penttilä et al., 2001; Balocchi et al., 2006; Sollers et al., 2007; Hill et al., 2009; Williams et al., 2017), while LVET has long been established as an impedance-derived index of sympathetic activity (Stemmler, 1993; Thayer and Uijtdehaage, 2001). Based on this information, the current conceptualizations of both CAB and CAR use either HF-HRV or RMSSD to reflect parasympathetic activity, and either PEP or LVET to reflect sympathetic activity. CAB and CAR were first calculated using the original parasympathetic measure from the Berntson et al. (2008) study, yielding four formulas for CAB and CAR that can be expressed as $CAB\_H\_PEP = HFz - (-PEPz)$, $CAR\_H\_PEP = (HFz) + (-PEPz)$, $CAB\_H\_LVET = HFz - (-LVETz)$, and $CAR\_H\_LVET = (HFz) + (-LVETz)$. CAB and CAR were then calculated using a time-domain measure of HRV to reflect parasympathetic activity, yielding four additional formulas that can be expressed as $CAB\_R\_PEP = RMSSDz - (-PEPz)$, $CAR\_R\_PEP = (RMSSDz) + (-PEPz)$, $CAB\_R\_LVET = RMSSDz - (-LVETz)$, and $CAR\_R\_LVET = (RMSSDz) + (-LVETz)$. In all calculations, z-scores are computed for the parasympathetic and sympathetic measures to account for disparities in their means and units of measurement, while the sympathetic measure is multiplied by −1 to reflect the fact that smaller values are indicative of greater sympathetic activity.

## Statistical Analyses

All statistical tests were conducted using SPSS (ver. 27, IBM Chicago, IL, United States). Zero-order correlations were performed between variables of interest including *z*-scored variables used to calculate CAB and CAR, as well as log-transformed variables used to calculate CAB and CAR. Confidence intervals (95%) were obtained for all correlation coefficients and are reported in brackets. Fisher's *z*-to-*r* transformation was used to test differences between correlation coefficients. Statistics reported include Pearson's r correlation values, 95% confidence intervals (in square brackets), and *p*-values.

Hierarchical regression analyses were also conducted to see whether CAR predicted CAB differentially based on calculations of the measures. Step one included covariates that were sex, age, body mass index, and race. An individual's ethnicity can determine relative levels of resting HRV (Choi et al., 2006; Hill et al., 2015) and thus, was included as a covariate in applicable analyses (ethnicity coded as 0 = European American, 1 = Other). It is well-known that resting HRV decreases with age (e.g., Choi et al., 2006; Voss et al., 2015), therefore age was also included as a covariate. Body mass index was also included as previous research has shown that higher body mass index is associated with decreased resting HRV (e.g., Koenig et al., 2014; Molfino et al., 2009). Step two included respiration rate (HF Hz; Thayer et al., 2002). CAR calculated from either PEP (Model 1) or LVET (Model 2) were variables in their respective third step. Statistics reported include, change in $R^2$ ($\Delta R^2$), unstandardized beta ($b$) coefficients, standard errors (SE), 95% confidence intervals (in square brackets), partial correlation coefficients, and $p$ values.

# RESULTS

## Descriptive Statistics

Extreme outliers (+ 2SD) were removed, leaving a total sample of 158 participants (107 females, 57 minorities, $M$age = 19.09, $SDage$ = 1.84, $M$BMI = 22.96, $SDBMI$ = 3.77). Averages of raw scores for PEP ($M$ = 118.20, $SD$ = 10.71), LVET ($M$ = 241.92, $SD$ = 36.58), log-transformed HF ($M$ = 6.65, $SD$ = 0.93), and log-transformed RMSSD ($M$ = 3.73, $SD$ = 0.45), were obtained. We also reported averages for variables calculated using HF, which included CAB_H_PEP ($M$ = 0.02, $SD$ = 1.04), CAR_H_PEP ($M$ = −0.13, $SD$ = 1.02), CAB_H_LVET ($M$ = 0.08, $SD$ = 1.20), and CAR_H_LVET ($M$ = −0.19, $SD$ = 1.19), as well as variables calculated using RMSSD, which included CAB_R_PEP ($M$ = −0.06, $SD$ = 0.92), CAR_R_PEP ($M$ = −0.20, $SD$ = 0.86), CAB_R_LVET ($M$ = 0.00, $SD$ = 1.07), and CAR_R_LVET ($M$ = −0.26, $SD$ = 1.08). Please see **Table 1** for descriptive statistics.

**TABLE 1 |** Descriptive statistics of variables of interest.

| | *M* | *SD* | *Range (min, max)* |
|---|---|---|---|
| Age | 19.09 | 1.84 | 18.00, 30.00 |
| BMI | 22.96 | 3.77 | 14.98, 35.29 |
| Respiration Rate | 0.25 | 0.06 | 0.15, 0.38 |
| RMSSD | 45.90 | 19.12 | 9.90, 102.47 |
| HF | 36.61 | 19.08 | 2.13, 87.74 |
| PEP | 118.20 | 10.71 | 90.00, 140.00 |
| LVET | 241.92 | 36.58 | 128.00, 326.00 |
| *ln*RMSSD | 3.73 | 0.45 | 2.29, 4.63 |
| *ln*HF | 6.65 | 0.93 | 3.96, 8.87 |
| *ln*PEP | 4.77 | 0.09 | 4.50, 4.94 |
| *ln*LVET | 5.48 | 0.16 | 4.85, 5.79 |
| *z*RMSSD | −0.13 | 0.79 | −1.63, 2.22 |
| *z*HF | −0.05 | 0.95 | −1.77, 2.49 |
| -*z*PEP | −0.07 | 0.40 | −0.89, 0.99 |
| -*z*LVET | −0.13 | 0.73 | −1.81, 2.14 |
| CAB_HF_PEP | 0.02 | 1.04 | −2.44, 2.78 |
| CAR_HF_PEP | −0.13 | 1.02 | −2.46, 2.30 |
| CAB_HF_LVET | 0.08 | 1.20 | −2.25, 3.17 |
| CAR_HF_LVET | −0.19 | 1.19 | −2.62, 2.85 |
| CAB_RMSSD_PEP | −0.06 | 0.92 | −2.25, 2.51 |
| CAR_RMSSD_PEP | −0.20 | 0.86 | −2.14, 2.04 |
| CAB_RMSSD_LVET | 0.00 | 1.07 | −2.83, 2.90 |
| CAR_RMSSD_LVET | −0.26 | 1.08 | −2.10, 2.80 |

*The table above includes means (M), standard deviations (SD), and the range (minimum, maximum) for raw scores of root mean square of successive differences (RMSSD), high frequency heart rate variability (HF), pre-ejection period (PEP), left ventricular ejection time (LVET), and log-transformed scores of RMSSD, HF, PEP, LVET, z-scored RMSSD, HF, PEP, LVET. It also includes M, SD and ranges for cardiac autonomic balance (CAB) calculated using HF, RMSSD, PEP, and LVET as well as cardiac autonomic regulation (CAR) using those same variables. ln = natural log-transformed; z = z-scored variable; −z = inverse of z-scored variable.*

**TABLE 2 |** Zero-order correlations among variables used to calculate cardiac autonomic balance and cardiac autonomic regulation.

| | | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1. | RMSSD*z* | – | | | |
| 2. | HF*z* | **0.44**** | – | | |
| 3. | −PEP*z* | −0.09 | −0.03 | – | |
| 4. | −LVET*z* | 0.01 | −0.01 | **0.36**** | – |

*Zero-order correlations between root mean square of successive differences (RMSSD), high frequency heart rate variability (HF), pre-ejection period (PEP), and left ventricular ejection time (LVET). These variables were used to calculate cardiac autonomic balance and cardiac autonomic regulation variables. Significant correlations are bolded; z = z-scored variable, −z = inverse of z-scored variable, **p < 0.01.*

## Zero-Order Correlations

Zero-order correlational analyses were conducted (see **Tables 2**, **3**) and plotted (see **Figures 1**–**4**) for various measures of HRV, impedance, CAB, and CAR.

Zero-order correlational analyses were conducted (see **Tables 2**, **3**). Results showed that there was a moderate, significant correlation between HF*z* and RMSSD*z* ($r$ = 0.44, CI [0.31, 0.56], $p$ < 0.001). Importantly, there was a significant strong correlation between *ln*HF and *ln*RMSSD ($r$ = 0.90, CI [0.87, 0.93], $p$ < 0.001). Results also showed a significant positive association between −PEP*z* and −LVET*z* ($r$ = 0.36, CI [0.22, 0.49], $p$ < 0.001).

Results showed that correlations between HF*z* and −PEP*z* ($r$ = −0.03, CI [−0.19, 0.13], $p$ = 0.73) as well as −LVET*z* ($r$ = −0.01, CI [−0.17, 0.15], $p$ = 0.90) were not statistically

**TABLE 3 |** Zero-order correlations among CAB and CAR variables.

| **A** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| 1. CAB_H_PEP | – | | | |
| 2. CAR_H_PEP | **0.69**** | – | | |
| 3. CAB_H_LVET | **0.82**** | **0.64**** | – | |
| 4. CAR_H_LVET. | **64**0.81**** | **0.26**** | – | |
| **B** | **1** | **2** | **3** | **4** |
| 1. CAB_R_PEP | – | | | |
| 2. CAR_R_PEP | **0.59**** | – | | |
| 3. CAB_R_LVET | **0.77**** | **0.53**** | – | |
| 4. CAR_R_LVET | **0.56**** | **0.77**** | 0.09 | – |

***Table 3A*** *shows zero-order correlations between cardiac autonomic balance (CAB) and cardiac autonomic regulation (CAR) variables that were calculated using high frequency heart rate variability (denoted as "H") and pre-ejection period (PEP) or left ventricular ejection time (LVET), respectively.* ***Table 3B*** *shows CAB and CAR calculated using the root mean square of successive differences (denoted as "R") and pre-ejection period (PEP) or left ventricular ejection time (LVET), respectively. Statistically significant correlations are bolded, **p < 0.01.*

significant. Additionally, correlations between RMSD$z$ and −PEP$z$ ($r = -0.09$, CI [−0.24, 0.07], $p = 0.28$) as well as −LVET$z$ ($r = 0.01$, CI [−0.15, 0.17], $p = 0.91$) were also not significant.

Results revealed that there was a significant relationship between CAR_PEP and CAB_PEP calculated using both HF ($r = 0.69$, CI [0.60, 0.76], $p < 0.001$) and RMSSD ($r = 0.59$, CI [0.48, 0.68], $p < 0.001$). There was a significant correlation between CAB_LVET and CAR_LVET calculated using HF ($r = 0.26$, CI [0.11, 0.40], $p < 0.001$) but not RMSSD ($r = 0.08$, CI [−0.08, 0.23], $p = 0.28$). The correlation coefficient between CAB_PEP and CAR_PEP was significantly stronger compared to the correlation found between CAB_LVET and CAR_LVET for both HF ($z = 5.12, p < 0.001$) and RMSSD ($z = 5.26, p < 0.001$).

## Regression Analyses

### Cardiac Autonomic Balance and Regulation Calculated Using HF

Regression analyses (see **Table 4**) revealed that CAR_PEP significantly predicted 49.9% of the variance in CAB_PEP ($\Delta R^2 = 0.40$, $b = 0.69$, $SE = 0.06$, $rpartial = 0.67$, CI [0.56, 0.81], $p < 0.001$). In contrast, CAR_LVET significantly predicted 13.0% of the variance in CAB_LVET ($\Delta R^2 = 0.04$, $b = 0.22$, $SE = 0.08$, $rpartial = 0.22$, CI [0.06, 0.38], $p = 0.01$). The association between CAR_PEP and CAB_PEP ($rpartial = 0.67$) was significantly stronger ($z = 5.17$, $p < 0.001$) compared to the association between CAR_LVET and CAB_LVET ($rpartial = 0.22$).

### Cardiac Autonomic Balance and Regulation Calculated Using RMSSD

For CAR and CAB computed using RMSSD (see **Table 5**), results showed that CAR_PEP significantly predicted 37.4% of the variance in CAB_PEP ($\Delta R^2 = 0.34$, $b = 0.63$, $SE = 0.07$, $rpartial = 0.59$, CI [0.49, 0.77], $p < 0.001$). In contrast, CAR_LVET did not significantly predict CAB_LVET ($\Delta R^2 = 0.01$, $b = 0.09$, $SE = 0.08$, $rpartial = 0.10$, CI [−0.06, 0.25], $p = 0.24$) and only explained 5.5% of the variance in CAB_LVET. The association between CAR_PEP and CAB_PEP ($rpartial = 0.59$) was significantly stronger ($z = 5.08$, $p < 0.001$) compared to the association between CAR_LVET and CAB_LVET ($rpartial = 0.10$).

# DISCUSSION

The purpose of the current investigation was to evaluate PEP and LVET in the calculation of CAB and CAR in order to determine which systolic time interval provided CAB and CAR with optimal autonomic space. Our results showed the association between z-transformed HRV (HF and RMSSD) and both PEP and LVET to be near zero, however, HRV and LVET appear to show better space given the spread of data points. Importantly, there was a stronger association between CAB and CAR when calculated using PEP compared to LVET, which show little (calculated using HF) to no (calculated using RMSSD) association between CAB and CAR. In other words, when calculated using PEP, individuals higher in CAB are more likely to be higher in CAR. In contrast when calculated using LVET, the association between CAB and CAR is significantly lower, and when calculated using RMSSD, is negligible. Taken together, these data suggest that LVET provides better autonomic space compared to PEP when paired with HRV in the calculation of CAB and CAR. Furthermore, we highlight that the association between CAB and CAR computed using RMSSD and LVET was not significant. This may further suggest RMSSD as a better measure for the calculation of CAB and CAR.

Cardiac autonomic balance (CAB) and CAR are designed to capture opposing modes of autonomic activity, with CAB reflecting a propensity toward the dominance of either the sympathetic or parasympathetic branch, and CAR reflecting the co-activation or co-inhibition of both branches. With this in mind, our findings may suggest that CAR and CAB calculated using PEP may not sufficiently reflect these functional differences, as indicated by their strong agreement. Given that the associations seen between PEP-derived CAR and CAB remain strong regardless of which chronotropic HRV measure is used in their calculations, it is likely these associations are the result of PEP failing to provide adequate coverage of autonomic space. One potential reason behind this is that, as previously mentioned, PEP represents an inotropic measure of sympathetic activity, influencing myocardial contractility at the atrioventricular (AV) node of the heart. In contrast, LVET shares a functional foundation with chronotropic measures HF and RMSSD (Stemmler, 1993). When CAB and CAR are calculated using LVET we see that the two measures are in little-to-no association; especially when they are calculated using RMSSD. This suggests that while there may be circumstances under which CAB and CAR may be significantly associated when calculated using LVET (i.e., using HF), it is significantly weaker compared to using PEP, and not significant when calculated using RMSSD. Overall, these results suggest that LVET-derived CAB and CAR represent more distinct patterns of autonomic activity due to LVET providing better autonomic space compared to PEP. Furthermore, these results also suggest that chronotropic time-domain measures of HRV (i.e., RMSSD)

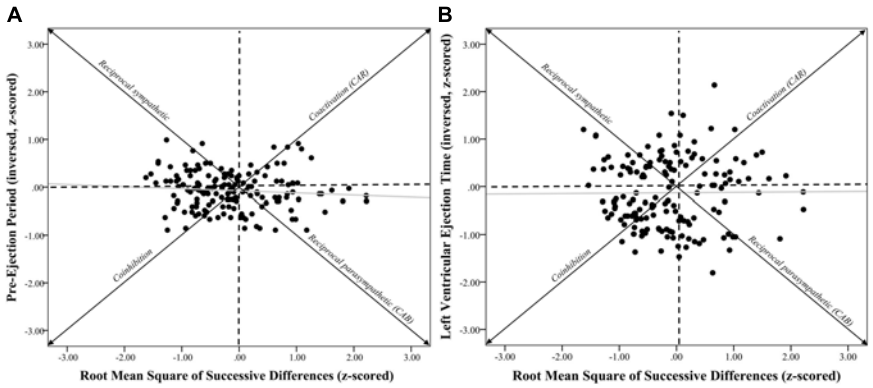



**FIGURE 2 |** Systolic time intervals and root mean square of successive differences scatterplots. *Note*. (**A**) shows a scatterplot between pre-ejection periods (PEP *z*-scored and inversed, see section "METHODS" for details) and root mean square of successive differences (RMSSD-HRV, *z*-scored) ($r = –0.09$, $p = 0.28$). (**B**) shows a scatterplot between left ventricular ejection time (LVET inversed and z-scored) and RMSSD-HRV ($r = 0.01$, $p = 0.91$). Individuals in the coinhibition quadrant would show lower CAR scores, while individuals in the coactivation quadrant would show higher CAR scores. Individuals in the reciprocal sympathetic quadrant would show lower CAB scores, while individuals in the reciprocal parasympathetic quadrant would show higher CAB scores.

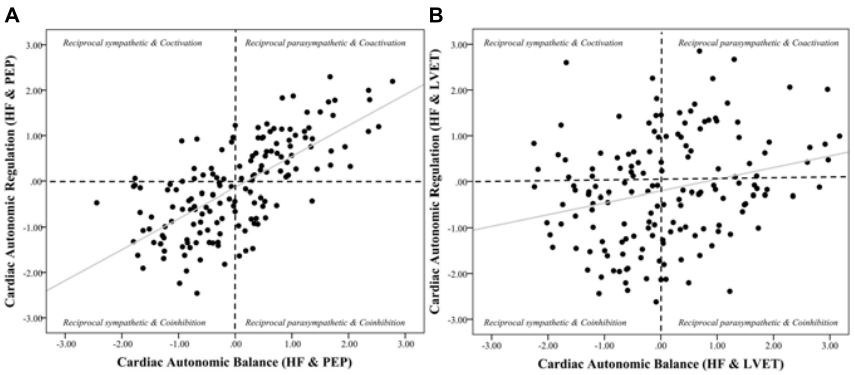



**FIGURE 3 |** Scatterplots of cardiac autonomic balance and regulation computed using HF-HRV and both PEP and LVET. *Note.* (**A**) depicts the strong significant association between cardiac autonomic balance (CAB) and regulation (CAR) calculated using high frequency heart rate variability (HF) and pre-ejection periods (PEP) ($r = 0.69$, $p < 0.001$). (**B**) depicts a significantly weaker association between CAB and CAR calculated using left ventricular ejection time (LVET) ($r = 0.26$, $p < 0.001$). The correlation coefficient between CAR and CAB computed using PEP was significantly stronger than when computed using LVET ($z = 5.12$, $p < 0.001$).

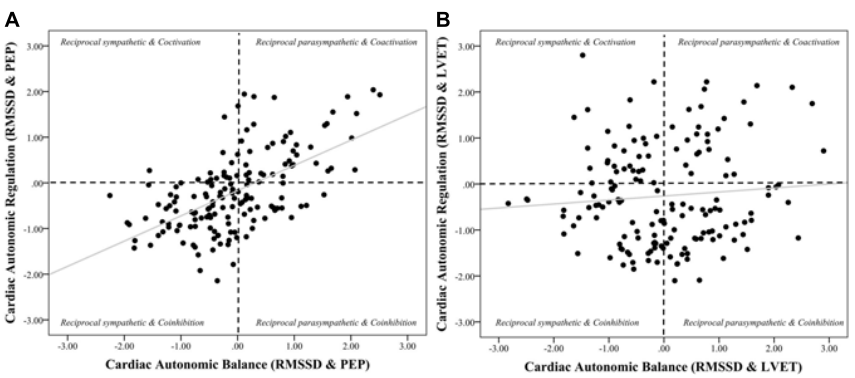



**FIGURE 4 |** Scatterplots of cardiac autonomic balance and regulation computed using RMSSD-HRV and both PEP and LVET. *Note.* (**A**) depicts the strong significant association between cardiac autonomic balance (CAB) and regulation (CAR) calculated using high frequency heart rate variability (HF) and pre-ejection periods (PEP) ($r = 0.59$, $p < 0.001$). (**B**) depicts the lack of an association between CAB and CAR calculated using left ventricular ejection time (LVET) ($r = 0.08$, $p = 0.28$). The correlation coefficient between CAR and CAB computed using PEP was significantly stronger than when computed using LVET ($z = 5.26$, $p < 0.001$).

**TABLE 4 |** Hierarchical regression analyses calculated from HF, PEP, LVET.

| | Cardiac Autonomic Balance (PEP) | | | | | | Cardiac Autonomic Balance (LVET) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor Step** | **$\Delta R^2$** | ***b*** | ***SE*** | ***p*** | **95%CI** | ***rpartial*** | **$\Delta R^2$** | ***b*** | ***SE*** | ***p*** | **95%CI** | ***rpartial*** |
| $R^2$ | | 0.50** | | | | | | 0.13** | | | | |
| Sex | | −0.02 | 0.13 | 0.89 | [−0.28, 0.25] | −0.01 | | 0.21 | 0.20 | 0.30 | [−0.19, 0.61] | 0.09 |
| Age | | 0.00 | 0.03 | 0.93 | [−0.06, 0.07] | 0.01 | | 0.01 | 0.05 | 0.91 | [−0.09, 0.11] | 0.01 |
| BMI | | −0.02 | 0.02 | 0.35 | [−0.05, 0.02] | −0.08 | | −0.01 | 0.03 | 0.57 | [−0.07, 0.04] | −0.05 |
| Race | | 0.06 | 0.13 | 0.62 | [−0.19, 0.32] | 0.04 | | 0.09 | 0.20 | 0.63 | [−0.29, 0.48] | 0.04 |
| Respiration | | | | | | | | | | | | |
| Rate | | 1.90 | 0.98 | 0.06 | [−0.04, 3.85] | 0.16 | | 4.23** | 1.48 | 0.01 | [1.30, 7.16] | 0.23 |
| *CAR_HF_PEP* | 0.40** | 0.69** | 0.06 | < 0.001 | [0.56, 0.81] | 0.67 | | – | – | – | – | – |
| *CAR_HF_LVET* | | – | – | – | – | – | 0.04** | 0.22** | 0.08 | 0.01 | [0.06, 0.38] | 0.22 |

*The table above shows the unstandardized beta coefficients (b) with associated significant levels at each step in the regression model. Regression analyses (left) of cardiac autonomic regulation (CAR) calculated from pre-ejection period (PEP) and high frequency heart rate variability (HF) predicting cardiac autonomic balance (CAB) calculated from PEP and HF. Regression analyses (right) of CAR calculated from left ventricular ejection time (LVET) and HF predicting CAB calculated from LVET and HF, **p < 0.01.*

**TABLE 5 |** Hierarchical regression analyses calculated from RMSSD, PEP, LVET.

| | Cardiac Autonomic Balance (PEP) | | | | | | Cardiac Autonomic Balance (LVET) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor Step** | **$\Delta R^2$** | ***b*** | ***SE*** | ***p*** | **95%CI** | ***rpartial*** | **$\Delta R^2$** | ***b*** | ***SE*** | ***p*** | **95%CI** | ***rpartial*** |
| $R^2$ | | 0.37** | | | | | | 0.06 | | | | |
| Sex | | −0.16 | 0.13 | 0.22 | [−0.42, 0.10] | −0.10 | | −0.15 | 0.19 | 0.43 | [−0.52, 0.22] | −0.06 |
| Age | | −0.01 | 0.03 | 0.75 | [−0.08, 0.06] | −0.03 | | −0.03 | 0.05 | 0.57 | [−0.12, 0.07] | −0.05 |
| BMI | | −0.03 | 0.02 | 0.10 | [−0.06, 0.01] | −0.14 | | −0.04 | 0.02 | 0.08 | [−0.09, 0.00] | −0.14 |
| Race | | 0.01 | 0.13 | 0.91 | [−0.23, 0.26] | 0.01 | | −0.02 | 0.18 | 0.91 | [−0.38, 0.34] | −0.01 |
| Respiration | | | | | | | | | | | | |
| Rate | | 1.31 | 0.96 | 0.17 | [−0.59, 3.21] | 0.11 | | 2.63 | 1.37 | 0.06 | [−0.08, 5.34] | 0.15 |
| *CAR_R_PEP* | 0.34** | 0.63** | 0.07 | < 0.001 | [0.49, 0.77] | 0.59 | | – | – | – | – | – |
| *CAR_R_LVET* | | – | – | – | – | – | 0.01 | 0.09 | 0.08 | 0.24 | [−0.06, 0.25] | 0.10 |

*The table above shows the unstandardized beta coefficients (b) with associated significant levels at each step in the regression model. Regression analyses (left) of cardiac autonomic regulation (CAR) calculated from pre-ejection period (PEP) and root mean square of successive differences (RMSSD; denoted as "R") predicting cardiac autonomic balance (CAB) calculated from PEP and RMSSD. Regression analyses (right) of CAR calculated from left ventricular ejection time (LVET) and RMSSD predicting CAB calculated from LVET and RMSSD, **p < 0.01.*

and impedance cardiography may be superior indices of parasympathetic and sympathetic activity when calculating CAB and CAR. A potential reason for this pattern may be due to time-domain HRV measures (especially RMSSD) being more resistant to violations of stationarity compared to frequency domain measures (Tarvainen et al., 2002).

## Implications

The psychophysiological connection between the autonomic nervous and cardiovascular systems continues to be at the forefront of health research. An imbalance or dysregulation of this relationship is of particular interest, given its association with stress (Wulsin et al., 2018), psychopathologies (Thayer and Brosschot, 2005), difficulties in emotion regulation (Williams et al., 2015), cardiovascular disease risk factors (Thayer et al., 2010b), and all-cause mortality (Thayer and Sternberg, 2006). As such, special attention should be given to the methods and formulas designed to quantify this relationship, especially in regards to the balance and regulation of the parasympathetic and sympathetic branches of the autonomic nervous system. The development of CAB and CAR has proven to be a vital step toward the conceptualization of cardiac autonomic activity, with both serving as valid and reliable indices of the dynamic between the parasympathetic and sympathetic nervous systems in several studies examining mental (Gump et al., 2011; Kreibig et al., 2012; Bylsma et al., 2015) and physical (Berntson et al., 2008; Singh et al., 2009; Vrijkotte et al., 2015) health. However, our data suggests that the calculation of these measures can be adjusted to build upon their efficacy as markers of psychophysiological health.

Additionally, with research showing that the health-related significance of various states of cardiac autonomic control of the heart can vary across different psychological stressors and pharmacological blockades (Carlsson et al., 1977; Stemmler, 1993; Berntson et al., 1994a), calculating CAB and CAR using cardiac autonomic measures with a shared functional foundation may be especially important in accurately classifying individuals and their respective cardiovascular states.

From a methodological perspective, calculating CAB and CAR using LVET may be beneficial for increasing their precision in predicting cardiovascular functioning. As previously mentioned, both parasympathetic and sympathetic influences can have differential effects on the heart depending on the effector tissue involved; even when both systems are active. For example, autonomic influences involved in the control of heart rate at the SA node (i.e., chronotropy) tend to be dependent on the level of background sympathetic activity, with higher levels of sympathetic activation resulting in greater decreases in heart rate associated with a given parasympathetic stimulus (a phenomenon known as accentuated antagonism; Levy and Zieske, 1969). Similarly, autonomic influences involved in cardiac contractility at the AV node (i.e., inotropy) are also dependent on the level of background sympathetic activity. While parasympathetic influence over contractility is negligible with low or no sympathetic activation, increases in sympathetic activity result in marked, non-algebraically additive decreases in contractility (Levy, 1997). Whereas studies tend to calculate CAB and CAR using different chronotropic measures such as HF (Singh et al., 2009), RMSSD (Williams et al., 2017), and respiratory sinus arrythmia (Kreibig et al., 2012), these and other studies almost exclusively use the inotropic measure of PEP to index sympathetic activity as opposed to more appropriate chronotropic sympathetic measures like LVET. Our results suggest that CAB and CAR show dependency when calculated using PEP irrespective of the HRV index used. Of course, this should not be the case, given that these measures reflect different states of cardiovascular functioning, albeit through similar modes of autonomic activity (e.g., coactivation of the parasympathetic and sympathetic branches). Therefore, it is possible that the results of studies that have used PEP-derived measures of CAB and CAR to capture autonomic activity may be limited in their accuracy or interpretation as they relate to cardiac autonomic activity, and our results suggest that using LVET in place of PEP may yield more appropriate results. Indeed, the association between CAB variables and CAR variables derived using different HRV measures and systolic time intervals show considerably high correlations (*r*'s between.6 and.8), however these correlations are far from perfect as one might expect, and thus can have a considerable impact on both data and results.

## Limitations and Future Directions

The current study is not without its limitations. The first limitation is that the sample largely consists of college students, and therefore the results may not be generalizable to all age groups. While we are confident that the present relationships seen between the various calculations of CAB and CAR would be present across all ages, future research should collect HRV and impedance data and conduct similar analyses to confirm this. To this end, when using consistent chronotropic measures to compute CAB and CAR in our young and healthy sample of individuals, true variation within autonomic space is revealed. In contrast, the Berntson et al. (2008) report showed similar variation using PEP and HF-HRV, however their sample of individuals were significantly older (ranging between 50 and 68 years) and some showed cardiovascular diseases (e.g., diabetes). Thus, it would be important to understand the differential impact of calculating CAB and CAR using LVET/PEP in both older and younger individuals, in addition in those who may show cardiovascular complications.

Additionally, although we did ask participants to smoke in the hours prior to the study, we did not ask if they were regular smokers, which may or may not have had an influence on general cardiovascular and respiratory functioning in select individuals. Another limitation is that we did not measure respiration via direct methods (e.g., using a transducer belt or counting thoracoabdominal movements) to ensure that participants had a breathing rate of at least nine respirations per minute, which could have influenced our current results. However, RMSSD has been shown to be resistant to respiratory influence following detrending and thus, results surrounding RMSSD are relatively free of respiratory influence (Lewis et al., 2012; Laborde et al., 2017). Lastly, our results may be limited by a lack of a pharmacological blockade to more accurately verify patterns of autonomic activity. However, as previously stated, sympathetic and parasympathetic influences on the heart differ based on the effector tissue involved, even when both branches are active. Thus, introducing a blockade to either one of these influences may effectively eliminate an important piece of the physiological puzzle. Additionally, we are not proposing a new method of indexing autonomic activity, but rather offering a more precise method of calculating CAB and CAR, which has already been verified via blockade studies (Berntson et al., 1994a; Cacioppo et al., 1994).

Future research aiming to replicate our research should also attempt to record HRV and impedance data over different or extended time periods, such as comparing CAB and CAR measures during the day and at night. Lastly, it may be beneficial for future studies to examine sex differences in the various calculations of CAB and CAR, as our current sample is predominantly female, and a recent meta-analysis on sex differences in HRV determined that women have higher vagal tone compared to males (Koenig and Thayer, 2016). Moreover, a recent investigation found that the association between HRV and heart rate was not equal between women and men, suggesting a differential influence of autonomic activity on heart chronotropy based on sex (Williams et al., in prep).

# CONCLUSION

As researchers continue to explore the physiological connection between the autonomic nervous system and cardiovascular functioning, special consideration should be given to how the dynamic between the parasympathetic and sympathetic branches are indexed and interpreted. Our data show that measures of CAR calculated from PEP significantly correlate with and predict measures of CAB calculated using PEP. Conversely, computed using LVET, CAR shows a significantly weaker association using HF-HRV and no association using RMSSD-HRV. The current study provides evidence suggesting that the chronotropic systolic time interval LVET provides better autonomic space compared to the inotropic measure PEP,

making it the superior index of sympathetic activity in the calculation of CAB and CAR.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ohio State University Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. Author order was agreed upon prior to the writing of the manuscript, with CW serving as first author and DW serving as senior author.

## FUNDING

This research was supported by funding from The Ohio State University College of Social and Behavioral Sciences, The Ohio State University Graduate School and The Ohio State University College of Social, Behavioral and Economic Sciences to the senior author (DW).

## REFERENCES

Alen, N. V., Deer, L. K., and Hostinar, C. E. (2020). Autonomic nervous system activity predicts increasing serum inflammatory cytokines in children. *Psychoneuroendocrinology* 119:104745. doi: 10.1016/j.psyneuen.2020.104745

Balocchi, R., Cantini, F., Varanini, M., Raimondi, G., Legramante, J. M., Macerata, A., et al. (2006). Revisiting the potential of time-domain indexes in short-term HRV analysis. *Biomed. Tech.* 51, 190–193. doi: 10.1515/bmt.2006.034

Berntson, G. G., Cacioppo, J. T., and Quigley, K. S. (1991). Autonomic determinism: the modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. *Psychol. Rev.* 98:459. doi: 10.1037/0033-295x.98.4.459

Berntson, G. G., Cacioppo, J. T., and Quigley, K. S. (1993). Cardiac psychophysiology and autonomic space in humans: empirical perspectives and conceptual implications. *Psychol. Bull.* 114:296. doi: 10.1037/0033-2909.114.2.296

Berntson, G. G., Cacioppo, J. T., Binkley, P. F., Uchino, B. N., Quigley, K. S., Fieldstone, A., et al. (1994a). Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. *Psychophysiology* 31, 599–608. doi: 10.1111/j.1469-8986.1994.tb02352.x

Berntson, G. G., Cacioppo, J. T., Quigley, K. S., and Fabro, V. T. (1994b). Autonomic space and psychophysiological response. *Psychophysiology* 31, 44–61. doi: 10.1111/j.1469-8986.1994.tb01024.x

Berntson, G. G., Lozano, D. L., Chen, Y. J., and Cacioppo, J. T. (2004). Where to Q in PEP. *Psychophysiology* 41, 333–337. doi: 10.1111/j.1469-8986.2004.00156.x

Berntson, G. G., Norman, G. J., Hawkley, L. C., and Cacioppo, J. T. (2008). Cardiac autonomic balance versus cardiac regulatory capacity. *Psychophysiology* 45, 643–652. doi: 10.1111/j.1469-8986.2008.00652.x

Bylsma, L. M., Yaroslavsky, I., Rottenberg, J., Jennings, J. R., George, C. J., Kiss, E., et al. (2015). Juvenile onset depression alters cardiac autonomic balance in response to psychological and physical challenges. *Biol. Psychol.* 110, 167–174. doi: 10.1016/j.biopsycho.2015.07.003

Cacioppo, J. T., Berntson, G. G., Binkley, P. F., Quigley, K. S., Uchino, B. N., Fieldstone, A., et al. (1994). Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. *Psychophysiology* 31, 586–598. doi: 10.1111/j.1469-8986.1994.tb02351.x

Carlsson, E., Dahlöf, C. G., Hedberg, A., Persson, H., and Tångstrand, B. (1977). Differentiation of cardiac chronotropic and inotropic effects of β-adrenoceptor agonists. *Naunyn Schmiedebergs. Arch. Pharmacol.* 300, 101–105. doi: 10.1007/bf00505039

Choi, J. B., Hong, S., Nelesen, R., Bardwell, W. A., Natarajan, L., Schubert, C., et al. (2006). Age and ethnicity differences in short-term heart-rate variability. *Psychosom. Med.* 68, 421–426. doi: 10.1097/01.psy.0000221378.09239.6a

Friedman, B. H., and Thayer, J. F. (1998). Autonomic balance revisited: panic anxiety and heart rate variability. *J. Psychosom. Res.* 44, 133–151. doi: 10.1016/s0022-3999(97)00202-x

Gump, B. B., MacKenzie, J. A., Bendinskas, K., Morgan, R., Dumas, A. K., Palmer, C. D., et al. (2011). Low-level Pb and cardiovascular responses to acute stress in children: the role of cardiac autonomic regulation. *Neurotoxicol. Teratol.* 33, 212–219. doi: 10.1016/j.ntt.2010.10.001

Hill, L. K., Hu, D. D., Koenig, J., Sollers, J. J. III., Kapuku, G., Wang, X., et al. (2015). Ethnic differences in resting heart rate variability: a systematic review and meta-analysis. *Psychosom. Med.* 77:16. doi: 10.1097/psy.0000000000000133

Hill, L. K., Siebenbrock, A., Sollers, J. J., and Thayer, J. F. (2009). Are all measures created equal? Heart rate variability and respiration. *Biomed. Sci. Instrum.* 45, 71–76.

Knottnerus, A., and Tugwell, P. (2008). STROBE–a checklist to Strengthen the Reporting of Observational Studies in Epidemiology. *J. Clin. Epidemiol.* 61:323. doi: 10.1016/j.jclinepi.2007.11.006

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Koenig, J., Jarczok, M. N., Warth, M., Ellis, R. J., Bach, C., Hillecke, T. K., et al. (2014). Body mass index is related to autonomic nervous system activity as measured by heart rate variability—a replication using short term measurements. *J. Nutr. Health Aging* 18, 300–302. doi: 10.1007/s12603-014-0022-6

Kreibig, S. D., Gendolla, G. H., and Scherer, K. R. (2012). Goal relevance and goal conduciveness appraisals lead to differential autonomic reactivity in emotional responding to performance feedback. *Biol. Psychol.* 91, 365–375. doi: 10.1016/j.biopsycho.2012.08.007

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research-recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213.

Levy, M. (1997). Neural control of cardiac function. *Ballieres. Clin. Neurosci.* 6, 227–244.

Levy, M. N., and Zieske, H. (1969). Autonomic control of cardiac pacemaker activity and atrioventricular transmission. *J. Appl. Physiol.* 27, 465–470. doi: 10.1152/jappl.1969.27.4.465

Lewis, G. F., Furman, S. A., McCool, M. F., and Porges, S. W. (2012). Statistical strategies to quantify respiratory sinus arrhythmia: Are commonly used metrics equivalent? *Biol. Psychol.* 89, 349–364. doi: 10.1016/j.biopsycho.2011.11.009

Li, Z., Snieder, H., Su, S., Ding, X., Thayer, J. F., Treiber, F. A., et al. (2009). A longitudinal study in youth of heart rate variability at rest and in response to stress. *Int. J. Psychophysiol.* 73, 212–217. doi: 10.1016/j.ijpsycho.2009.03.002

Licht, C. M., de Geus, E. J., and Penninx, B. W. (2013). Dysregulation of the autonomic nervous system predicts the development of the metabolic syndrome. *Int. J. Clin. Endocrinol. Metab.* 98, 2484–2493.' doi: 10.1210/jc.2012-3104

Lozano, D. L., Norman, G., Knox, D., Wood, B. L., Miller, B. D., Emery, C. F., et al. (2007). Where to B in dZ/dt. *Psychophysiology* 44, 113–119.

Malliani, A. (2005). Heart rate variability: from bench to bedside. *Eur. J. Intern. Med.* 16, 12–20. doi: 10.1016/j.ejim.2004.06.016

Malliani, A., Pagani, M., and Lombardi, F. (1994). *Vagal Control of the Heart: Experimental Basis and Clinical Implications*. Armonk, NY: Futura Publishing Co, 433–454.

Mitchell, J. C., Paulson, J., Cannarozzi, M., Neer, S. M., and Cassisi, J. E. (2017). Maladaptive Cardiac Autonomic Control during a Stress Reactivity Assessment Among Primary Care Patients with Metabolic Syndrome. *Appl. Psychophysiol. Biofeedback* 42, 97–105. doi: 10.1007/s10484-017-9355-3

Molfino, A., Fiorentini, A., Tubani, L., Martuscelli, M., Fanelli, F. R., Laviano, A., et al. (2009). Body mass index is related to autonomic nervous system activity as measured by heart rate variability. *Eur. J. Clin. Nutr.* 63, 1263–1265. doi: 10.1038/ejcn.2009.35

Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol.* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Lovallo, W. R., Van Doornen, L. J., et al. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology* 27, 1–23. doi: 10.1111/j.1469-8986.1990.tb02171.x

Singh, P., Hawkley, L. C., McDade, T. W., Cacioppo, J. T., and Masi, C. M. (2009). Autonomic tone and C-reactive protein: a prospective population-based study. *Clin. Auton. Res.* 19, 367–374. doi: 10.1007/s10286-009-0019-0

Sleight, P. (1997). The importance of the autonomic nervous system in health and disease. *Aust. N. Z. J. Med.* 27, 467–473. doi: 10.1111/j.1445-5994.1997.tb02220.x

Sollers, J. J., Buchanan, T. W., Mowrer, S. M., Hill, L. K., and Thayer, J. F. (2007). Comparison of the ratio of the standard deviation of the RR interval and the root mean squared successive differences (SD/rMSSD) to the low frequency-to-high frequency (LF/HF) ratio in a patient population and normal healthy controls. *Biomed. Sci. Instrum.* 43, 158–163.

Stemmler, G. (1993). Receptor antagonists as tools for structural measurements in psychophysiology. *Neuropsychobiology* 28, 47–53. doi: 10.1159/000118999

Stone, L. B., McCormack, C. C., and Bylsma, L. M. (2020). Cross system autonomic balance and regulation: associations with depression and anxiety symptoms. *Psychophysiology* 57:e13636.

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220.

Tarvainen, M. P., Ranta-Aho, P. O., and Karjalainen, P. A. (2002). An advanced detrending method with application to HRV analysis. *IEEE Trans. Med. Robot. Bionics.* 49, 172–175. doi: 10.1109/10.979357

Task Force of the European Society of Cardiology (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 3, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Thayer, J. F., and Brosschot, J. F. (2005). Psychosomatics and psychopathology: looking up and down from the brain. *Psychoneuroendocrinology* 30, 1050–1058. doi: 10.1016/j.psyneuen.2005.04.014

Thayer, J. F., and Friedman, B. H. (1997). "The heart of anxiety: a dynamical systems approach," in *The (non) expression of emotions in health and disease*, ed. A. Vingerhoets, (Amsterdam: Springer Verlag).

Thayer, J. F., and Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Ann. N. Y. Acad. Sci.* 1088, 361–372. doi: 10.1196/annals.1366.014

Thayer, J. F., and Uijtdehaage, S. H. (2001). Derivation of chronotropic indices of autonomic nervous system activity using impedance cardiography. *Biomed. Sci. Instrum.* 37, 331–336.

Thayer, J. F., Hansen, A. L., and Johnsen, B. H. (2010a). "The Non-invasive Assessment of Autonomic Influences on the Heart Using Impedance Cardiography and Heart Rate Variability," in *Handbook of behavioral medicine*. ed. A. Steptoe. (New York, NY: Springer).

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., Sollers, J. J., Ruiz-Padial, E., and Vila, J. (2002). Estimating respiratory frequency from autoregressive spectral analysis of heart period. *IEEE Trans. Med. Robot Bionics.* 21, 41–45. doi: 10.1109/memb.2002.1032638

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010b). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Uijtdehaage, S. H., and Thayer, J. F. (2000). Accentuated antagonism in the control of human heart rate. *Clin. Auton. Res.* 10, 107–110. doi: 10.1007/bf02278013

Voss, A., Schroeder, R., Heitmann, A., Peters, A., and Perz, S. (2015). Short-term heart rate variability—influence of gender and age in healthy subjects. *PLoS One* 10:e0118308. doi: 10.1371/journal.pone.0118308

Vrijkotte, T. G., van den Born, B. J. H., Hoekstra, C. M., Gademan, M. G., van Eijsden, M., de Rooij, S. R., et al. (2015). Cardiac autonomic nervous system activation and metabolic profile in young children: the ABCD study. *PLoS One* 10:e0138302. doi: 10.1371/journal.pone.0138302

Williams, D. P., Cash, C., Rankin, C., Bernardi, A., Koenig, J., and Thayer, J. F. (2015). Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. *Front. Psychol.* 6:261. doi: 10.3389/fpsyg.2015.00261

Williams, D. P., Wiley, C., Rahman, T., Barton, A., Gerardo, G. M., Hill, L. K., et al. (2017). Re-examining the relationship between low-to-high-frequency ratio and cardiac autonomic balance and regulation: a focus on systolic time intervals. *Biomed. Sci. Instrum.* 6:2017.

Wulsin, L., Herman, J., and Thayer, J. F. (2018). Stress, autonomic imbalance, and the prediction of metabolic risk: a model and a proposal for research. *Neurosci. Biobehavl. Rev.* 86, 12–20. doi: 10.1016/j.neubiorev.2017.12.010

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 13 May 2021
doi: 10.3389/fnins.2021.612566

# Gender Matters: Nonlinear Relationships Between Heart Rate Variability and Depression and Positive Affect

*Derek P. Spangler[1]*, Emily J. Dunn[2], Amelia Aldao[2], Nicole R. Feeling[2], Matthew L. Free[3], Brandon L. Gillie[4], Michael W. Vasey[2], DeWayne P. Williams[5], Julian Koenig[6,7] and Julian F. Thayer[2,5]*

[1] Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA, United States, [2] Department of Psychology, The Ohio State University, Columbus, OH, United States, [3] Anxiety and Behavioral Health Services, Worthington, OH, United States, [4] University of Pittsburgh Medical Center Sports Medicine Concussion Program, Department of Orthopedic Surgery, University of Pittsburgh, Pittsburgh, PA, United States, [5] Department of Psychological Science, University of California, Irvine, Irvine, CA, United States, [6] Section for Translational Psychobiology in Child and Adolescent Psychiatry, Department of Child and Adolescent Psychiatry, Heidelberg University, Heidelberg, Germany, [7] University Hospital of Child and Adolescent Psychiatry and Psychotherapy, University of Bern, Bern, Switzerland

Vagally mediated heart rate variability (vmHRV), a measure of the parasympathetic nervous system's control over the heart, is often negatively related to maladaptive emotional outcomes. Recent work suggests that quadratic relationships involving these factors may be present; however, research has not investigated gender differences in these nonlinear functions. To address this gap, the current study tested for quadratic relationships between resting vmHRV and depression and positive affect while investigating gender differences in these relationships. Significant quadratic effects were found between resting vmHRV and reports of both depression symptoms and positive affect in women but not men. Specifically, the lowest levels of depression and the highest levels of positive affect were found at moderate vmHRV in women. These results suggest that examinations of vmHRV's nonlinear associations require the consideration of gender. Our findings are interpreted based on proposed differential neuropsychological mechanisms of vmHRV in men versus women.

**Keywords: heart rate variability, nonlinear, gender, emotion, depression, positive affect, autonomic nervous system, gender differences**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Emilio Vanoli, University of Pavia, Italy*
*Uirassu Borges, German Sport University Cologne, Germany*

****Correspondence:***
*Derek P. Spangler*
*dpspang@gmail.com*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *30 September 2020*
***Accepted:*** *31 March 2021*
***Published:*** *13 May 2021*

***Citation:***
*Spangler DP, Dunn EJ, Aldao A, Feeling NR, Free ML, Gillie BL, Vasey MW, Williams DP, Koenig J and Thayer JF (2021) Gender Matters: Nonlinear Relationships Between Heart Rate Variability and Depression and Positive Affect. Front. Neurosci. 15:612566. doi: 10.3389/fnins.2021.612566*

## INTRODUCTION

Heart rate variability (HRV), or beat-to-beat variation in heart rate, is a non-invasive metric of autonomic nervous system activity (Berntson et al., 1997). Although there are many HRV metrics with different physiological interpretations, vagally mediated heart rate variability (vmHRV) has received special attention as a biomarker of adaptive functioning (Allen et al., 2007; Porges, 2007b; Thayer and Lane, 2009). VmHRV refers to high-frequency (∼0.25 Hz) oscillations in heart rate that index the parasympathetic nervous system's regulation of the heart via the vagus nerve (Saul, 1990; Malik et al., 1996). This is in contrast to metrics of overall HRV or low-frequency HRV which reflect a mixture of sympathetic and parasympathetic influences. The Neurovisceral Integration Model posits that the degree of resting vmHRV proxies the neural aspects of emotion regulation— that is, the degree of prefrontal cortex (PFC) inhibition over subcortical emotion circuits (Thayer and Lane, 2009; Thayer et al., 2012). Individuals with high relative to lower resting vmHRV are thought to exhibit greater regulation of maladaptive emotions. The Vagal Tank and Polyvagal Theories

similarly posit that relatively higher resting vmHRV indexes greater psychophysiological reserves for self-regulation (Porges, 2007a; Laborde et al., 2018). Consistent with these perspectives, higher resting vmHRV has been associated with lower depression (Kemp et al., 2010) and higher positive affect (PA; Kok and Fredrickson, 2010).

## Nonlinear Relationships Between VmHRV and Emotion

Most research on this topic has characterized vmHRV's relationships to emotion as strictly linear (for review, see Thayer and Lane, 2009). However, recent work has found support for a quadratic association between vmHRV and adaptive emotional outcomes in which moderate levels of vmHRV were associated with lower depression, higher life satisfaction (Kogan et al., 2013), and greater positive emotion (Kogan et al., 2014; Duarte and Pinto-Gouveia, 2017). In the same studies, participants with very low and very high levels of vmHRV reported less adaptive emotional outcomes (e.g., higher depression, less PA). These findings potentially shed light on a few studies reporting that vmHRV is *positively* (rather than negatively) related to maladaptive emotional outcomes such as depression (Rottenberg, 2007) and poor social competence (Eisenberg et al., 1995). Specifically, a positive and negative association involving vmHRV might exist within the same nonlinear function (Spangler and Friedman, 2017). Studies not testing quadratic terms may only characterize one linear piece of the larger nonlinear relationship between vmHRV and emotion.

One limitation of the research examining these quadratic functions is that it has not adequately considered gender: an important moderator of vmHRV (Koenig and Thayer, 2016) and its associations with brain activity (Nugent et al., 2011) and emotion (Verkuil et al., 2015). The quadratic vmHRV-emotion associations in prior studies (e.g., Kogan et al., 2013, 2014; Duarte and Pinto-Gouveia, 2017) might be unique to women. Consistently, some of these prior studies had either an exclusively female sample (Duarte and Pinto-Gouveia, 2017) or a mostly (64%) female sample (Kogan et al., 2014, Study 2). Other studies detecting similar nonlinear functions had samples with more balanced gender ratios. However, their samples were considerably older (Mean age = 40 in Kogan et al. (2014), Study 1 and Kogan et al., 2013)—corresponding to an age when gender differences in vmHRV decrease (Umetani et al., 1998). Whether or not nonlinear functions are unique to women requires clarification since prior studies did not examine gender as a moderator.

## Gender, VmHRV, and the Compensatory Hypothesis

Nonlinear relationships between vmHRV and depression/PA being limited to women has a basis in two evolutionary theories: *the tend-and-befriend theory and parental investment theory* (Trivers, 1972; Taylor et al., 2000). Both perspectives imply that the psychophysiological mechanisms of vagal responses differ between mammalian males and females. Below, we summarize both theories, which we then connect to the vmHRV literature in order to motivate our hypotheses regarding nonlinear functions and their gender differences.

According to the tend-and-befriend theory, female mammals exercise a larger role in caregiving relative to males and are therefore more likely to buffer themselves and their offspring from emotional distress (Taylor et al., 2000; Taylor and Master, 2011; Taylor, 2012). Therefore, in the face of high distress, women putatively exhibit heightened vagal activity and oxytocin reactivity—responses which are mounted to compensate for heightened distress and to promote affiliation. Males, in contrast, respond to distress with "fight-or-flight," which involves both vagal and social withdrawal. Similarly, in the parental investment theory, women are more strongly influenced by selection pressures to care for offspring and exercise cautious mate selection (Bjorklund and Kipp, 1996; Bjorklund and Shackelford, 1999). These pressures promote greater attempts at emotion regulation and inhibitory control in the face of context-appropriate responses, thus promoting the delay of impulses for the benefit of offspring. Tend-and-befriend makes a direct connection between parasympathetic (vagal) function and compensatory responses, which is consistent with the Neurovisceral Integration Model where vmHRV proxies emotion regulation capacity (Thayer and Lane, 2009). Similarly, the emotion regulation and inhibitory control functions cited in parental investment theory bear considerable overlap to the neurobehavioral functions proxied by resting vmHRV in Neurovisceral Integration.

Taken together, both evolutionary theories imply that high vmHRV in women, relative to men, more strongly represents a compensatory response—i.e., greater emotion regulation efforts in response to higher maladaptive emotion. High vmHRV in men may instead represent lower maladaptive emotion achieved by tonic inhibition of subcortical threat circuits (consistent with Neurovisceral Integration; Thayer and Lane, 2009). Importantly, depression can be conceptualized as a threat-related response because: (1) rumination is a key feature of depression and involves perseverative thinking about past threats to the self (Nolen-Hoeksema, 2000), and (2) enhanced neural responses to social threat signal heightened risk for depression (Chan et al., 2009; Kujawa et al., 2015). If women's high vmHRV indexes a compensatory response, then women should exhibit stronger positive relations of vmHRV to both threat-related responses (including depression) and emotion regulation. This is indeed consistent with prior evidence. First, higher vmHRV has been related to increased activity in the amygdala (a region implicated in threat processing) in women but decreased amygdala activity in men (Nugent et al., 2011). Second, relative to men, women exhibit stronger positive associations between vmHRV and emotion regulation ability (Williams et al., 2019). This compensatory response to threat and depressive symptoms has a neural basis in the PFC. According to Drevets et al. (2004, 2008), more severe depressive episodes are related to greater medial PFC activity (a region proxied by resting vmHRV; Thayer et al., 2012), and this heightened PFC activity is hypothesized to index a compensatory response to depressive episodes.

Evolutionarily based gender differences in vmHRV's mechanisms may explain positive associations between vmHRV

and depressive symptoms in females in contrast to consistently negative associations in males (Thayer et al., 1998; Verkuil et al., 2015; Jandackova et al., 2016; Jarczok et al., 2017, 2018). In these relationships, high vmHRV in women may represent an effortful compensatory emotion regulatory response (i.e., a "tend-and-befriend" response) to cope with greater depression (Thayer et al., 2003).

## Gender and Nonlinear Relationships Between vmHRV and Emotion

Positive associations may not fully characterize the broader relationship between vmHRV and depression in women. There are reports of negative associations between vmHRV and internalizing symptoms in women (Dishman et al., 2000; Henje Blom et al., 2010). The mixed associations between vmHRV and depression in women might be encapsulated by a larger nonlinear function that is not observed in men. If this is true, then the previously reported quadratic associations of vmHRV with depression and PA (a construct inversely related to depression and important for adaptive emotional function; Nutt et al., 2007) might be specific to women. Consistently, one study reported a significant negative association between vmHRV and depression in men and a null association in women with wider confidence limits, implying a nonlinear function in women (Jandackova et al., 2016).

Potential nonlinear vmHRV-emotion relationships in women but not men have grounding in evolutionary theory and previous studies. Gender differences are traditionally examined as average differences (e.g., greater depression in women than men overall; Feingold, 1994; Bianchin and Angrilli, 2012), However, evolved mechanisms more strongly affecting one gender (e.g., tend-and-befriend) are subject to phenotypic variation between persons (Archer, 2019). In this vein, a "tend-and-befriend" response to negative emotion (positive vmHRV-depression relationship) may represent only a subset of women for whom vagal reactions are pronounced in order to counteract high depression. In other women, there may be a more typical inverse relationship between vmHRV and depression-related measures, which may reflect fundamental links between very low vagal control and poor emotion regulation across genders (Thayer and Lane, 2009; Henje Blom et al., 2010).

In summary, the inverse relationship between vmHRV and depression may only appear in women from low to moderate vmHRV within a larger quadratic association. At higher levels of vmHRV (e.g., right side of function), greater vmHRV may represent a compensatory response to higher depression, emerging as a positive vmHRV-depression association. Supporting this possibility, clinical studies underscore exaggerated vagal activity as a compensatory response to stress which contributes to vasovagal syncope and emotional fainting—outcomes that are more common in women (van Lieshout et al., 1991; van Dijk and Sheldon, 2008; Alboni et al., 2021). In contrast to automatic emotion regulation strategies (e.g., Christou-Champi et al., 2015), we propose that the compensatory vagal response among women comprises more effortful regulation where interpersonal conflict is buffered via emotional and social labor (Hochschild, 1983; Wharton, 1999; Butler et al., 2006). Assuming the "tend-and-befriend" response does not influence men's vagal function, men's vmHRV-depression relationship should be strictly linear and negative. Since PA is inversely related to depression (Nutt et al., 2007), the aforementioned functions between vmHRV and PA might resemble those for depression except in the opposite direction.

Quadratic associations between vmHRV and emotion in women have grounding in Vagal Tank Theory (Laborde et al., 2018). In Vagal Tank Theory, relatively higher levels of vmHRV index greater psychophysiological resources (e.g., integration of neural, metabolic, and cognitive resources) that can be utilized for self-regulation and hence adaptive emotional responses. Through this lens, low vmHRV in women represents less self-regulatory resources which may lead to deficient emotion regulation and in turn higher depression and lower PA. Moderate vmHRV in women may represent greater self-regulatory resources that permit the context-appropriate emotion regulation and in turn less depression and higher PA. High vmHRV in women may represent even greater self-regulatory reserves that have been built up to deal with heightened depressive symptoms and low PA. Indeed, self-regulatory reserves—represented as emotion regulation capacity and resting vmHRV—can be built up over time through either increased exposure to stress or interventions targeting the integrity of stress-related neural pathways (e.g., physical exercise, mindfulness training, slow breathing) (Hansen et al., 2004; Gross et al., 2016; Wei et al., 2017; Hulbert and Anderson, 2018). Men's strictly linear vmHRV-emotion associations may be due to their vmHRV similarly indexing the degree of self-regulatory resources (e.g., higher vmHRV, more emotion regulation) without the existence of a compensatory response at high vmHRV.

## Current Study

The current study explicitly examined gender differences in the linear and quadratic relationships between resting vmHRV and depression symptoms and PA. We aimed to clarify the work of Kogan et al. (2013, 2014) and Duarte and Pinto-Gouveia (2017) by testing whether the quadratic relations of resting vmHRV with depression and PA are limited to women. As noted above, we hypothesized that vmHRV in women (but not men) would show a U-shaped function with depression symptoms and an inverted-U function with PA. In men, we predicted that vmHRV would exhibit strictly linear negative and positive associations with depression and PA, respectively.

# MATERIALS AND METHODS

## Participants

A total of 305 undergraduate students (58.69% female; mean age = 19.85, *SD* = 3.71; 70.82% Caucasian) participated in this study. After providing informed consent via a signed consent form, participants completed questionnaires. Participants were asked to abstain from alcohol, tobacco, caffeine, and vigorous exercise 6 h before the study. The university's Institutional Review

Board (Protocol #: 2014B0524) approved all study procedures, which were in accordance with the Declaration of Helsinki.

## Procedure

Upon entering the laboratory, participants were instructed on study procedures and then provided informed consent. Electrocardiography (ECG) leads were attached to disposable surface electrodes on the participant's thorax. Participants next completed a 5-min (eyes-open) resting baseline where they were asked to remain as still and quiet as possible while ECG was continuously collected. After the resting baseline, participants completed a series of self-report questionnaires pertaining to emotion and self-regulation. Many of the questionnaires are of distal relevance to the primary study; we therefore only describe the questionnaires measuring depression symptoms and PA (see below). The order of the questionnaires was randomly counterbalanced across participants. Participants next completed a thought suppression task (Grisham and Williams, 2009). Since this task is unrelated to the aims of the current paper, its results are not reported here. It is unlikely that the thought suppression task influenced the current results since the task occurred after our focal metrics (vmHRV, depression and PA) were collected.

## Self-Report Measures

### Depression

Depression was measured with the depression subscale of the Depression Anxiety Stress Scale (DASS-D), a valid and reliable measure of depression symptoms (Lovibond and Lovibond, 1995; in this sample: $\alpha = 0.95$). The DASS-D consists of 14 items that are rated on a four-point (0–3) Likert scale[1]. Ratings were summed across items to yield an overall depression score (Lovibond and Lovibond, 1995). Given their skew, DASS-D scores were normalized with an inverse transformation of percentile ranked scores (Templeton, 2011).

### Positive Affect

Trait PA was measured with the PA subscale of the Positive and Negative Affect Schedule (PANAS)—Trait form (Watson et al., 1988). The PANAS contains 20 items that were rated on a five-point (1–5) Likert scale, thus allowing participants to rate the extent to which they generally experienced 10 positive and 10 negative emotions. Ratings across the items were summed to index overall scores for positive and negative affect separately (Watson et al., 1988). The positive and negative affect subscales demonstrated good reliability: $\alpha = 0.89$ and $\alpha = 0.88$, respectively.

## VmHRV

Electrocardiography (ECG) was measured with three leads that were attached to adhesive spot electrodes on the thorax at a modified lead II configuration. ECG was digitally recorded (sample rate = 1,000 Hz) using the MindWare 2000D (MW2000D) Impedance Cardiograph package (MindWare Technologies, Ltd., Gahanna, OH). The ECG signal was processed in Mindware (HRV 2.51 Analysis software) in order to identify R-peaks and extract the interbeat interval (IBI) time series from the 5-min baseline. The IBI data were then entered into Kubios 2.0 software to calculate the vmHRV measures (Tarvainen et al., 2014). Prior to vmHRV estimation, IBI time series were detrended using smoothness priors in order to remove non-stationarities that could bias HRV estimates (Tarvainen et al., 2002). VmHRV was estimated across the entire 5-min baseline with the root mean square of successive differences (RMSSD), a well-established metric of vmHRV for both short-term (∼5 min) and long-term (∼24 h) recordings (Ali-Melkkila et al., 1991; Allen et al., 2007). In accord with standardized guidelines for vmHRV analysis, we natural logarithm transformed RMSSD in order to reduce its skew (denoted as lnRMSSD). VmHRV was also calculated as high-frequency HRV (HF-HRV). In further alignment with HRV analysis guidelines, HF-HRV was computed as mean absolute power ($ms^2$) in the HF band (0.15–0.4 Hz) using autoregressive spectral analysis (model order = 16). HF-HRV was natural logarithm transformed in order to reduce its positive skew (denoted as lnHF-HRV). LnHF-HRV was highly correlated with lnRMSSD, $r(303) = 0.96$, $p < 0.001$, and results were identical when analyzing lnHF-HRV. Prior reports indicate that RMSSD is less influenced by respiration and is more reliable than HF-HRV (Penttilä et al., 2001; Kuss et al., 2008). We therefore employed lnRMSSD as our primary metric of vmHRV, and all substantive results are reported with lnRMSSD.

The autoregressive spectral analysis was also used to compute the peak frequency (Hz) of the spectral power in the HF (0.15–0.4 Hz) band. This metric has been identified as a cost-effective and accurate proxy for respiration rate (Thayer et al., 2002). The peak frequency of HF power (natural log transformed to reduce skew; denoted as lnHFpeak) was entered as a covariate in statistical models in order to rule out the potentially confounding influence of respiration rate on vmHRV (Grossman et al., 1991).

## Statistical Analysis

Linear and quadratic effects of vmHRV in men and women were tested with a multiple regression approach, in accord with Cohen et al. (2003). Emotion variables (depression symptoms and PA) were entered as dependent measures in separate regression models. Linear relationships between vmHRV and emotion variables (depression symptoms and PA) were tested with a linear term of lnRMSSD which was grand-mean-centered. Nonlinear relationships were tested with a quadratic term of lnRMSSD which was created by squaring mean-centered lnRMSSD.

The first regression model included both men and women and predicted depression symptoms (normalized DASS-D scores) with the: (1) linear term of RMSSD, (2) quadratic term of lnRMSSD, (3) lnHFpeak, (4) Gender (factorial: 1 = men; 2 = women), (5) lnRMSSD X gender, and (6) $lnRMSSD^2$ X gender (Cohen et al., 2003). The regression model testing PA was also conducted across men and women and had the same

[1]Due to a technical error in collating the questionnaire materials, one item was missing from the DASS-D: "I felt that I had lost interest in just about everything." To alleviate this issue, we substituted the missing DASS-D item with a highly similar item from the Post-Traumatic Stress Disorder (PTSD) Checklist- Civilian Version (PCL-C; Weathers et al., 1994): "Loss of interest in things that you used to enjoy?" In order to place items on a common scale, scores were divided by the maximum possible score and then summed to compute an overall depression score. The depression findings presented in the paper include the aforementioned substitution. Results were identical when the DASS-D item was omitted ($p < 0.05$).

predictors as above (1–6); however, it instead included PA as the dependent measure.

To more clearly test gender differences in vmHRV effects, we re-estimated regression models predicting depression and PA separately for men and women. The predictors entered into the gender-separated models were: (1) linear RMSSD, (2) quadratic RMSSD, and (3) lnHFpeak.

All regression effects were reported as standardized regression coefficients with 95% confidence intervals. In order to judge associations based on effect size, regression coefficients were also reported as partial correlation coefficients alongside with 95% confidence intervals. Since hypotheses were directional in nature, we report one-tailed *p*-values (alpha = 0.05) and one-tailed confidence intervals for all regression coefficients and correlations[1]. The regression coefficients and partial correlations yielded the same results ($p < 0.05$). Importantly, depression models were re-estimated after Winsorizing one outlier for the normalized DASS-Depression variable (from z = 3.5 to z = 3 and Winsoried again to z = 2.5). In both cases, findings of Winsorized analyses were identical to those in the Results ($p < 0.05$). Other variables indicated no outliers ($z > |3|$). Furthermore, we re-estimated all regression models with untransformed variables (e.g., raw RMSSD), and the results remained unchanged ($p < 0.05$).

# RESULTS

**Table 1** contains descriptive statistics for study variables as well as independent samples *t*-tests examining gender differences in these variables. Our dependent measures—DASS-Depression scores and PA—were negatively correlated in the present sample, *Pearson product-moment* $r(303) = -0.37$, $p < 0.001$. Below, we report linear and nonlinear associations between vmHRV and emotion measures as a function of gender. These associations are depicted as partial correlations in **Table 2** and plotted in **Figure 1**.

## Depression

In the model predicting depression across men and women, both the linear (β = −0.17, $p = 0.200$, 95% CI [−0.49, 0.16]) and quadratic terms (β = −0.27, $p = 0.079$, 95% CI [−0.59, 0.05]) were not significant. However, there was an interaction of gender with the quadratic (β = 0.35, $p = 0.038$, 95% CI [0.03, 0.67]) but not the linear term of lnRMSSD (β = 0.11, $p = 0.285$, 95% CI [−0.21, 0.43]). To better understand the interaction between the quadratic term of lnRMSSD and gender, we re-estimated the models predicting depression separately for men and women.

### Men

In men, both the linear (β = −0.10, $p = 0.135$, 95% CI [−0.26, 0.05]) and quadratic (β = −0.07, $p = 0.236$, 95% CI [−0.22, 0.09]) effects of lnRMSSD on depression symptoms were not significant. These results indicate that there was no statistically significant relationship between vmHRV and depression in men. However, the negative direction of this linear effect is consistent with prior inverse linear relations between vmHRV and depressive symptoms in men but not women (e.g., Jandackova et al., 2016).

### Women

In women, the quadratic (β = 0.14, $p = 0.028$, 95% CI [0.02, 0.27]) but not the linear (β = −0.03, $p = 0.361$, 95% CI [−0.15, 0.10]) effect of lnRMSSD on depression was statistically significant. These results indicate that, among women, the relationship between resting vmHRV and depression was quadratic rather than linear. Inspection of **Figure 1** indicates that women's quadratic relationship was *U-shaped* where moderate levels of lnRMSSD were associated with the lowest depression symptoms. Taken together, the shape of this nonlinear function resembles functions reported by Kogan et al. (2013) and was observed in women but not men.

## Positive Affect

In the model predicting PA across men and women, the linear (β = 0.09, $p = 0.324$, 95% CI [−0.23, 0.40]) and quadratic terms (β = 0.29, $p = 0.059$, 95% CI [−0.02, 0.60]) of lnRMSSD were not statistically significant. Importantly, there was a significant interaction of gender with the quadratic (β = −0.43, $p = 0.012$, 95% CI [−0.74, −0.11]) but not the linear term of lnRMSSD (β = 0.14, $p = 0.223$, 95% CI [−0.17, 0.46]). The latter results indicate that the quadratic relationship between lnRMSSD and PA varies by gender. We next stratified regression analyses by gender to better understand this interaction.

### Men

Men evidenced a significant positive linear effect (β = 0.18, $p = 0.028$, 95% CI [0.03, 0.33]) but no significant quadratic effect of lnRMSSD (β = 0.04, $p = 0.341$, 95% CI [−0.11, 0.19]) on PA. These results indicate that, among men, higher resting vmHRV was significantly related to reports of higher PA, and this association was strictly linear.

### Women

Women evidenced a positive linear effect of lnRMSSD (β = 0.26, $p < 0.001$, 95% CI [0.14, 0.38]) on PA, but this effect was qualified by a significant quadratic term (β = −0.21, $p = 0.002$, 95% CI [−0.33, −0.09]). These results indicate that relationship between vmHRV and PA in women can be characterized as quadratic as opposed to strictly linear. Consistent with this interpretation, **Figure 1** revealed an inverted-U association between lnRMSSD and PA in women (but not men) where moderate levels of lnRMSSD were related to the highest levels of PA. Although only detected in women, this association is consistent with the functions of Kogan et al. (2014) and Duarte and Pinto-Gouveia (2017)[2].

# DISCUSSION

Consistent with Kogan et al. (2013), we found that resting vmHRV had a U-shaped quadratic relationship with depression symptoms where moderate levels of vmHRV were associated

[2]Separate models were conducted with PANAS-negative affect as the dependent measure. None of the linear or quadratic effects of lnRMSSD were significant in the entire sample, or when examining men and women separately (results not presented).

**TABLE 1 |** Descriptive statistics.

| | Entire sample (*n* = 305) | | Men (*n* = 125) | | Women (*n* = 180) | | Men vs. Women | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | | **t-test** | **Cohen's *d* [95% CI]** |
| Ethnicity (% Caucasian) | 71.1% | – | 68.0% | – | 73.3% | – | † | – |
| Age | 19.85 | 3.71 | 20.23 | 3.61 | 19.59 | 3.77 | 1.48 | 0.17 [−0.06, 0.40] |
| **Cardiac measures** | | | | | | | | |
| IBI | 804.54 | 126.89 | 825.95 | 128.79 | 789.68 | 123.74 | 2.46* | 0.29 [0.06, 0.52] |
| RMSSD | 45.58 | 27.30 | 44.81 | 26.53 | 46.11 | 27.88 | −0.41 | −0.05 [−0.28, 0.18] |
| lnRMSSD | 3.65 | 0.59 | 3.64 | 0.59 | 3.66 | 0.59 | −0.42 | −0.05 [−0.28, 0.18] |
| HF-HRV | 1252.92 | 1443.86 | 1143.01 | 1316.60 | 1329.24 | 1524.94 | −1.14 | −0.13 [−0.36, 0.10] |
| lnHF-HRV | 6.57 | 1.13 | 6.47 | 1.13 | 6.63 | 1.12 | −1.23 | −0.14 [−0.37, 0.09] |
| HFpeak | 0.22 | 0.06 | 0.20 | 0.06 | 0.24 | 0.06 | −5.31* | −0.62 [−0.85, −0.38] |
| LnHFpeak | −1.54 | 0.29 | −1.65 | 0.28 | −1.47 | 0.28 | −5.43* | −0.63 [−0.87, −0.40] |
| **Self-report measures** | | | | | | | | |
| DASS-D | 8.79 | 9.84 | 8.72 | 10.06 | 8.83 | 9.71 | −0.10 | −0.01 [−0.24, 0.22] |
| DASS-D (normalized) | 5.72 | 2.36 | 5.65 | 2.41 | 5.77 | 2.34 | −0.44 | −0.05 [−0.28, 0.18] |
| PANAS-PA | 33.89 | 6.97 | 33.94 | 6.84 | 33.86 | 7.07 | 0.11 | 0.01 [−0.22, 0.24] |
| PANAS-NA | 21.85 | 7.58 | 21.47 | 7.77 | 22.11 | 7.45 | −0.72 | −0.09 [−0.31, 0.14] |

*IBI, interbeat intervals (ms); RMSSD, root mean square of successive differences (ms); lnRMSSD, natural logarithm of RMSSD; HF-HRV, high-frequency heart rate variability; lnHF-HRV, natural logarithm of HF-HRV; DASS-D, Depression subscale of the Depression Anxiety Stress Scale; DASS-D (normalized), Depression scores in inverse normalized rank-order units with item substituted (see section "Materials and Methods"); PANAS-PA, Positive and Negative Affect Schedule- Positive Affect; PANAS-NA, Positive and Negative Affect Schedule- Negative Affect.*

*Two-tailed *p < 0.05 for t-tests. T-tests that are not noted with an asterisk are not statistically significant (two-tailed p > 0.05). T-tests indicate that mean resting IBI was shorter and mean resting respiration rate was faster in women relative to men. However, effect sizes (Cohen's d) suggest that compared to men, women had higher levels of vmHRV, depression, and negative affect, but lower levels of positive affect.*

†*Gender differences in ethnicity (proportion Caucasian) were tested with a chi-square test of independence. This test was not significant, $\chi^2 = 1.02$, two-tailed p = 0.312).*

**TABLE 2 |** Partial Pearson correlation coefficients [95% CI] representing relationships between vmHRV and emotional outcomes.

| | DV: DASS-depression | | | DV: PANAS-positive affect | | |
|---|---|---|---|---|---|---|
| | **Entire sample** | **Men** | **Women** | **Entire sample** | **Men** | **Women** |
| LnRMSSD-linear | −0.05 [−0.14, 0.04] | −0.10 [−0.24, 0.05] | −0.03 [−0.15, 0.10] | 0.03 [−0.06, 0.12] | 0.17 [0.03, 0.31]* | 0.26 [0.14, 0.37]* |
| LnRMSSD-quadratic | −0.08 [−0.17, 0.01] | −0.07 [−0.21, 0.08] | 0.14 [0.02, 0.26]* | 0.09 [−0.0007, 0.18] | 0.04 [−0.11, 0.18] | −0.22 [−0.33, −0.10]* |
| Sex*LnRMSSD-linear | 0.03 [−0.06, 0.13] | – | – | 0.04 [−0.05, 0.13] | – | – |
| Sex*LnRMSSD-quadratic | 0.10 [0.009, 0.19]* | – | – | −0.13 [−0.22, −0.04] | – | – |

*lnRMSSD, natural logarithm of the root mean square of successive differences (ms); DASS-D, Depression subscale of Depression Anxiety Stress Scale. Depression scores are in inverse normalized rank-order units with item substituted (see section "Materials and Methods"); PANAS-PA, Positive and Negative Affect Schedule- Positive Affect. LnRMSSD-linear represents the linear term of lnRMSSD. LnRMSSD-quadratic represents the square of the linear lnRMSSD term. Gender*LnRMSSD-linear represents the cross-product interaction between Gender (1 = men, 2 = women) and the linear lnRMSSD term. Gender*lnRMSSD-quadratic represents the product interaction between Gender (1 = men, 2 = women) and the quadratic lnRMSSD term.*

**p < 0.05 based on one-tailed tests. All p-values are identical to those testing analogous regression coefficients (see section "Results").*

with the lowest levels of depression. Aligned with Kogan et al. (2014) and Duarte and Pinto-Gouveia (2017), we also found that vmHRV was quadratically related to trait PA, such that moderate vmHRV was associated with the highest levels of PA. As hypothesized, these quadratic relationships were detected in women but not men. In men, vmHRV appeared to exhibit strictly linear associations with depression and PA (the relation for depression was not significant but in the expected direction). Our findings highlight the importance of examining gender differences in vmHRV's nonlinear associations with depression symptoms and PA. Depression involves a lack of PA, and, consistently, these constructs were negatively correlated in the current sample (Nutt et al., 2007). Therefore, our oppositely shaped quadratic relationship (inverted-U) between vmHRV and trait PA is a logical counterpoint to our depression findings in women. Taken together, these results suggest a broader relationship between vmHRV and adaptive emotional outcomes (Kogan et al., 2013). We speculate that prior research may have only detected linear relationships between vmHRV and emotional outcomes in women because they did not test for nonlinear associations.

## Gender Differences in VmHRV: Evolutionary Mechanisms

Extant theories of vagal function posit a strong link between resting vmHRV and emotion regulation capacity without directly positing a role of gender (Porges, 2007a,b; Thayer and Lane, 2009; Laborde et al., 2018). Expanding on these theories, the current study suggests that resting vmHRV in (some) women

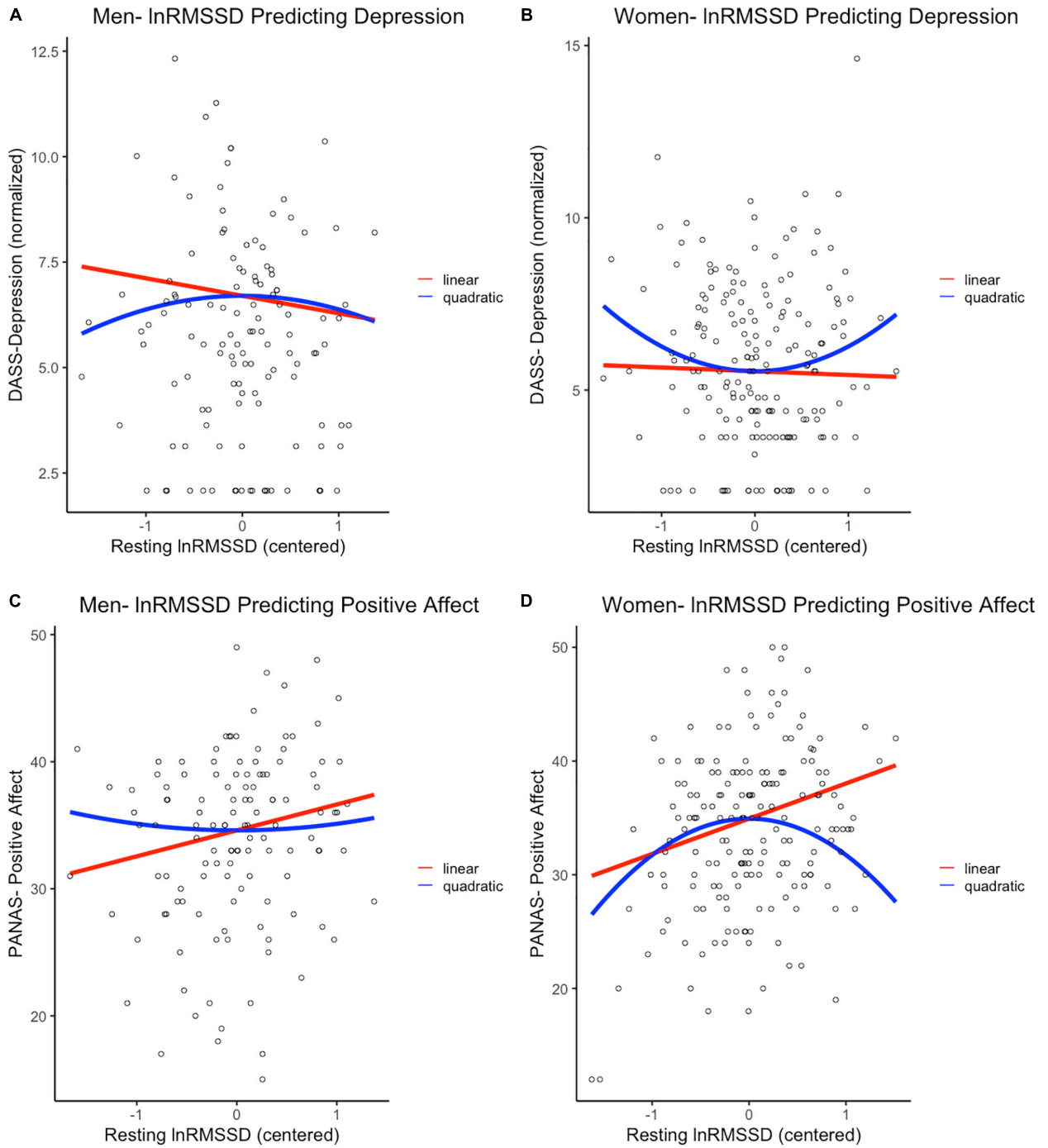



**FIGURE 1 |** First row: Linear and quadratic effects of resting lnRMSSD on DASS-Depression scores in **(A)** men and **(B)** women separately. Second row: Linear and quadratic effects of resting lnRMSSD on PANAS-positive affect scores in **(C)** men and **(D)** women separately. LnRMSSD = natural log of root mean square of successive differences (in the natural log of millisecond units). LnRMSSD is grand-mean centered; DASS = depression anxiety stress scale. DASS-Depression scores are in inverse normalized units to reduce skew for parametric analyses. All results were identical ($p < 0.05$, one-tailed) when analyzing raw DASS-Depression scores. Significant ($p < 0.05$, one-tailed) quadratic effects were only detected in women, as depicted in **(B,D)**.

may tap into emotion regulation mechanisms that are different from those in men. Specifically, our findings are consistent with theorized gender differences in emotion regulation that arose from evolutionary selection pressures related to caregiving. The tend-and-befriend and parental investment theories posit that, in the face of high maladaptive emotion, women are more likely than men to mount an emotion regulatory response (e.g., effort to acquire social support, inhibit expression of emotional behavior)

to divert resources to offspring (Bjorklund and Shackelford, 1999; Taylor et al., 2000). As suggested by tend-and-befriend, the compensatory response in women encompasses heighted parasympathetic activity (Taylor and Master, 2011). In this view, men are more likely to respond to maladaptive emotion with a prototypical fight-or-flight response involving vagal withdrawal and dis-inhibition of subcortical threat circuity.

The current linear associations between vmHRV and depression/PA in men (**Figures 1A,C**) reflect the "classic" mechanisms outlined in the Neurovisceral Integration Model, Vagal Tank Theory, and the "fight-or-flight" stress pattern. Here, relatively higher vmHRV reflects less threat responding achieved by automatic emotion regulation (i.e., tonic PFC-mediated inhibition of threat circuits) and, relatedly, greater self-regulatory resources (Thayer et al., 2012; Christou-Champi et al., 2015; Laborde et al., 2018). Critically, these strictly linear associations in males are consistent with prior reports (Carney et al., 1995; Thayer et al., 1998; Wang et al., 2013; Jarczok et al., 2017, 2018). In women, the same linear vmHRV-emotion relationships (positive for PA, negative for depression) were observed on the left side of the functions (**Figures 1B,D**). However, women with even higher levels of vmHRV (at the right side of the functions) exhibited reversal of these patterns, leading to: (i) a positive association between vmHRV and depression and (ii) a negative association between vmHRV and PA. These results are consistent with very high vmHRV representing a compensatory "tend-and-befriend" response to higher depression and lower PA (Thayer et al., 2003; Koenig and Thayer, 2016; Williams et al., 2019). In other terms, heightened parasympathetic function may reflect greater efforts to buffer maladaptive emotions that may otherwise impair tendencies related to caregiving and cautious mate selection (Bjorklund and Shackelford, 1999; Taylor et al., 2000; Taylor, 2012).

Very high levels of resting vmHRV at the right side of the function may also share key similarities with exaggerated vagal activity cited as a compensatory response in female vasovagal syncope patients (van Lieshout et al., 1991; Alboni et al., 2021). Furthermore, this greater compensatory effort at the right side of the function has been linked to augmentation in vmHRV among women (Butler et al., 2006). It may also encompass strenuous emotional labor that enhances women's risk for emotional burnout and depression (Hochschild, 1983; Wharton, 1999). Consistent with these accounts, the present findings are aligned with Vagal Tank Theory (Laborde et al., 2018). Through this lens, moderate relative to low vmHRV in women indexes a greater degree of self-regulatory resources or strength. These greater resources are adaptive because they are critical to active self-regulatory efforts that, in turn, inhibit depressive symptoms and up-regulate PA (Joormann and Quinn, 2014). At high vmHRV (i.e., right side of function) in women, such increased vagal activation may index even greater self-regulatory resources that have been accrued to support emotion regulatory efforts aimed at counteracting high depression and low PA. Consistent with Vagal Tank's view of vmHRV as an index of self-regulatory strength/reserves, resting vmHRV can be enhanced with training like a muscle. If this is true, then greater exposure to depression and low PA (for women with high vmHRV) may increasingly exercise emotion regulation circuits, in turn augmenting self-regulatory reserves and thus resting vmHRV (Troy and Mauss, 2011; Wei et al., 2017; Hulbert and Anderson, 2018). It should be noted that, like moderate vmHRV, high vmHRV in women may also represent an adaptive psychophysiological pattern that supports emotion regulation efforts. The particular emotion regulation mechanisms underlying very high vmHRV in women, although consistent with literature, are speculative and demand further investigation.

## Gender Differences and Potential Brain Mechanisms

The present findings might also reveal important central nervous system dynamics underlying gender differences in vmHRV and emotion regulation. For the entire functions in men and for the left side of the functions in women, relatively higher vmHRV may reflect greater tonic inhibition over subcortical circuits. In Neurovisceral Integration, such tonic inhibition is metabolically efficient, and it broadly supports context-appropriate emotional responses, expressed as less depression, higher PA, and other aspects of well-being (Thayer and Sternberg, 2006; Thayer and Lane, 2009; Geisler et al., 2010). At the right side of women's nonlinear functions of women (i.e., positive vmHRV-depression relation), the neural mechanisms of higher vmHRV may represent PFC inhibition that is more metabolic costly and more phasic in nature (Jacobs and D'Esposito, 2011). Here, amygdalar and other subcortical responses are perhaps first elicited as default stress responses, and PFC-mediated inhibition is built up over time to compensate for such maladaptive subcortical activity. These possibilities are supported by evidence across disparate studies. Depressive episodes are correlated with increased medial PFC activity, perhaps to compensate for (or inhibit) heightened amygdalar activity (Drevets et al., 2004, 2008). Similarly, vmHRV is positively correlated with medial PFC activity, and women but not men evidence a positive association between vmHRV and amygdalar activity (Nugent et al., 2011; Thayer et al., 2012).

## Implications

The presence of nonlinear relationships in women but not men has important implications for the literature on vmHRV and emotion. First, the current results pose constraints on the quadratic vmHRV-emotion associations reported by Kogan et al. (2013, 2014) and Duarte and Pinto-Gouveia (2017). These authors claim that high resting vmHRV, generally speaking, should be re-evaluated as an adaptive biomarker and that it may even reflect aberrant regulatory processes. However, we show that this nonlinear relationship is not general but rather limited to women. By limiting the nonlinear function to women, evolutionary and psychophysiological theories of gender must be considered. Interpreting the nonlinear function through these perspectives casts very high vmHRV (in the nonlinear function) not as a maladaptive process *per se* but rather as an to attempt to compensate for high levels of depression, for example. We must also note that the effect sizes of our nonlinear relationships, like those in prior reports, are small. Therefore, the notion that

a nonlinear function describes vmHRV's relationships to any emotional variable must be cautiously interpreted and replicated in future research.

As a second implication, our nonlinear effects clarify previous conflicting findings in women. The direction of women's vmHRV-depression associations is positive in some studies but negative in others (e.g., Thayer et al., 1998; Henje Blom et al., 2010). The current findings suggest that both the positive and negative relationships might exist within one nonlinear function. Third, the current nonlinear relations in women highlight how women and men differ in their neural regulation of specific maladaptive emotions: depression and low PA. These emotions are important because they are key risk factors in the etiology of clinical depression (Beaufort et al., 2017; de Jonge et al., 2017). Of course, depression and PA together cannot comprehensively assess maladaptive emotionality or other global constructs like adaptability. Anxiety and fear, which have also been related to vmHRV and psychopathological risk, could also elicit compensatory vagal reactions within a nonlinear function (Friedman, 2007). However, in the current study, there were no relationships (linear or nonlinear) between resting vmHRV and negative affect, suggesting some specificity in the emotional outcomes with which vmHRV is nonlinearly related.

### Limitations and Future Directions

As mentioned above, age is an important moderator of vmHRV and its relationships to gender. Our present sample was young (mean age = 19.85), so it is possible that the nonlinear relationships involving vmHRV would not replicate in an older sample. The magnitude of vmHRV and its gender differences have been shown to attenuate with increasing age (Umetani et al., 1998; Abhishekh et al., 2013). Similarly, both vagal and emotional function also appears to vary across the menstrual cycle, suggesting that present nonlinear relationships in women might be affected by female hormonal dynamics over time (Brar et al., 2015). Future studies should collect large samples with longitudinal designs in order to test this complex pattern of findings where vmHRV-emotion relationships and their gender differences vary across different timescales (e.g., menstrual cycle, age). The cross-sectional nature of our study also poses additional limitations. Notably, it is unclear whether the compensatory neural response represented by women's high vmHRV in initiated concurrently or subsequently to heighted distress (e.g., higher depression). Longitudinal designs with experimental methods are required to elucidate: (i) the temporal dynamics and lead-lag relationships among emotional reactivity and regulation, and (ii) the casual effects of heighted emotional reactivity on precipitating regulatory/compensatory efforts.

As aforementioned, we only focused on depressive symptoms and PA as facets of maladaptive emotionality. It is hence unclear whether the present findings are specific to these emotional constructs or are more general. Evolutionary theories cited in the current paper largely mention coarse affective constructs like stress and emotion, thus pointing to a need to refine whether gender differences in emotion are general or domain-specific (Bjorklund and Kipp, 1996; Taylor et al., 2000). Future research needs to further explore vmHRV's nonlinear relationships with other affective constructs.

## CONCLUSION

Despite limitations, we provide evidence that the nonlinear relations of vmHRV to depression and PA are limited to women. Within the nonlinear functions among women, very high vmHRV was related to greater depression and lower positive affect. These findings are consistent with exaggerated vmHRV indexing emotion regulatory efforts to cope with maladaptive emotionality. Taken together, the present findings further our understanding of the complex roles of vmHRV and gender in adaptive emotional outcomes.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ohio State University IRB. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

DS finalized analyses and wrote the manuscript with the assistance of JT. JK, DW, NF, MF, MV, and BG had a hand in designing the study and/or collecting and pre-processing the data. ED and AA assisted with analysis. All authors contributed to the article and approved the submitted version.

## REFERENCES

Abhishekh, H. A., Nisarga, P., Kisan, R., Meghana, A., Chandran, S., Raju, T., et al. (2013). Influence of age and gender on autonomic regulation of heart. *J. Clin. Monitor. Comput.* 27, 259–264. doi: 10.1007/s10877-012-9424-3

Alboni, P., Messop, A. C., Lauri, A., and Furlan, R. (2021). Are women really more affected by vasovagal syncope than men? *J. Cardiovasc. Med.* 22, 69–78. doi: 10.2459/JCM.0000000000001009

Ali-Melkkila, T., Kaila, T., Antila, K., Halkola, L., and Iisalo, E. (1991). Effects of glycopyrrolate and atropine on heart rate variability. *Acta Anaesthesiol. Scand.* 35, 436–441. doi: 10.1111/j.1399-6576.1991.tb03324.x

Allen, J. J., Chambers, A. S., and Towers, D. N. (2007). The many metrics of cardiac chronotropy: a pragmatic primer and a brief comparison of metrics. *Biol. Psychol.* 74, 243–262. doi: 10.1016/j.biopsycho.2006.08.005

Archer, J. (2019). The reality and evolutionary significance of human psychological sex differences. *Biol. Rev.* 94, 1381–1415. doi: 10.1111/brv.12507

Beaufort, I. N., De Weert-Van Oene, G. H., Buwalda, V. A., de Leeuw, J. R. J., and Goudriaan, A. E. (2017). The depression, anxiety and stress scale (DASS-21) as a screener for depression in substance use disorder inpatients: a pilot study. *Eur. Addict. Res.* 23, 260–268. doi: 10.1159/000485182

Berntson, G. G., Thomas Bigger, J. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Bianchin, M., and Angrilli, A. (2012). Gender differences in emotional responses: a psychophysiological study. *Physiol. Behav.* 105, 925–932. doi: 10.1016/j.physbeh.2011.10.031

Bjorklund, D. F., and Kipp, K. (1996). Parental investment theory and gender differences in the evolution of inhibition mechanisms. *Psychol. Bull.* 120:163. doi: 10.1037/0033-2909.120.2.163

Bjorklund, D. F., and Shackelford, T. K. (1999). Differences in parental investment contribute to important differences between men and women. *Curr. Direct. Psychol. Sci.* 8, 86–89. doi: 10.1111/1467-8721.00020

Brar, T. K., Singh, K. D., and Kumar, A. (2015). Effect of different phases of menstrual cycle on heart rate variability (HRV). *J. Clin. Diagnostic Res. JCDR* 9:CC01. doi: 10.7860/JCDR/2015/13795.6592

Butler, E. A., Wilhelm, F. H., and Gross, J. J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology* 43, 612–622. doi: 10.1111/j.1469-8986.2006.00467.x

Carney, R. M., Saunders, R. D., Freedland, K. E., Stein, P., Rich, M. W., and Jaffe, A. S. (1995). Association of depression with reduced heart rate variability in coronary artery disease. *Am. J. Cardiol.* 76, 562–564. doi: 10.1016/S0002-9149(99)80155-6

Chan, S. W., Norbury, R., Goodwin, G. M., and Harmer, C. J. (2009). Risk for depression and neural responses to fearful facial expressions of emotion. *Br. J. Psychiatry* 194, 139–145.

Christou-Champi, S., Farrow, T. F., and Webb, T. L. (2015). Automatic control of negative emotions: evidence that structured practice increases the efficiency of emotion regulation. *Cogn. Emot.* 29, 319–331. doi: 10.1080/02699931.2014.901213

Cohen, J., Cohen, P., West, S. G., and Aiken, L. S. (2003). *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*. 3rd Edn. Mahwah, NJ: Erlbaum.

de Jonge, M., Dekker, J. J. M., Kikkert, M. J., Peen, J., van Rijsbergen, G. D., and Bockting, C. L. H. (2017). The role of affect in predicting depressive symptomatology in remitted recurrently depressed patients. *J. Affective Disord.* 210, 66–71. doi: 10.1016/j.jad.2016.12.015

Dishman, R. K., Nakamura, Y., Garcia, M. E., Thompson, R. W., Dunn, A. L., and Blair, S. N. (2000). Heart rate variability, trait anxiety, and perceived stress among physically fit men and women. *Int. J. Psychophysiol.* 37, 121–133. doi: 10.1016/S0167-8760(00)00085-4

Drevets, W. C., Gadde, K., and Krishnan, K. R. R. (2004). "Neuroimaging studies of depression," in *The Neurobiological Foundation of Mental Illness*, 2nd Edn, eds D. S. Charney, E. J. Nestler, and B. S. Bunney (New York, NY: Oxford University Press).

Drevets, W. C., Price, J. L., and Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118. doi: 10.1007/s00429-008-0189-x

Duarte, J., and Pinto-Gouveia, J. (2017). Positive affect and parasympathetic activity: evidence for a quadratic relationship between feeling safe and content and heart rate variability. *Psychiatry Res.* 257, 284–289. doi: 10.1016/j.psychres.2017.07.077

Eisenberg, N., Fabes, R. A., Murphy, B., Maszk, P., Smith, M., and Karbon, M. (1995). The role of emotionality and regulation in children's social functioning: a longitudinal study. *Child Dev.* 66, 1360–1384. doi: 10.1111/j.1467-8624.1995.tb00940.x

Feingold, A. (1994). Gender differences in personality: a meta-analysis. *Psychol. Bull.* 116:429. doi: 10.1037/0033-2909.116.3.429

Friedman, B. H. (2007). An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone. *Biol. Psychol.* 74, 185–199. doi: 10.1016/j.biopsycho.2005.08.009

Geisler, F. C., Vennewald, N., Kubiak, T., and Weber, H. (2010). The impact of heart rate variability on subjective well-being is mediated by emotion regulation. *Pers. Individ. Differ.* 49, 723–728. doi: 10.1016/j.paid.2010.06.015

Grisham, J. R., and Williams, A. D. (2009). Cognitive control of obsessional thoughts. *Behav. Res. Ther.* 47, 395–402. doi: 10.1016/j.brat.2009.01.014

Gross, M. J., Shearer, D. A., Bringer, J. D., Hall, R., Cook, C. J., and Kilduff, L. P. (2016). Abbreviated resonant frequency training to augment heart rate variability and enhance on-demand emotional regulation in elite sport support staff. *Appl. Psychophysiol. Biofeedback* 41, 263–274.

Grossman, P., Karemaker, J., and Wieling, W. (1991). Prediction of tonic parasympathetic cardiac control using respiratory sinus arrhythmia: the need for respiratory control. *Psychophysiology* 28:2. doi: 10.1111/j.1469-8986.1991.tb00412.x

Hansen, A. L., Johnsen, B. H., Sollers, J. J., Stenvik, K., and Thayer, J. F. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur. J. Appl. Physiol.* 93, 263–272.

Henje Blom, E., Olsson, E. M., Serlachius, E., Ericson, M., and Ingvar, M. (2010). Heart rate variability (HRV) in adolescent females with anxiety disorders and major depressive disorder. *Acta Paediatr.* 99, 604–611. doi: 10.1111/j.1651-2227.2009.01657.x

Hochschild, A. R. (1983). *The Managed Heart*. Berkeley, CA: University of California Press.

Hulbert, J. C., and Anderson, M. C. (2018). What doesn't kill you makes you stronger: Psychological trauma and its relationship to enhanced memory control. *J. Exp. Psychol. Gen.* 147:1931.

Jacobs, E., and D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J. Neurosci.* 31, 5286–5293. doi: 10.1523/jneurosci.6394-10.2011

Jandackova, V. K., Britton, A., Malik, M., and Steptoe, A. (2016). Heart rate variability and depressive symptoms: a cross-lagged analysis over a 10-year period in the Whitehall II study. *Psychol. Med.* 46, 2121–2131.

Jarczok, M. N., Aguilar-Raab, C., Koenig, J., Kaess, M., Borniger, J. C., Nelson, R. J., et al. (2018). The Heart's rhythm 'n' blues: sex differences in circadian variation patterns of vagal activity vary by depressive symptoms in predominantly healthy employees. *Chronobiol. Int.* 35, 896–909. doi: 10.1080/07420528.2018.1439499

Jarczok, M. N., Koenig, J., Shively, C. A., and Thayer, J. F. (2017). Behavioral depression is associated with increased vagally mediated heart rate variability in adult female cynomolgus monkeys (*Macaca fascicularis*). *Int. J. Psychophysiol.* 131, 139–143. doi: 10.1016/j.ijpsycho.2017.11.004

Joormann, J., and Quinn, M. E. (2014). Cognitive processes and emotion regulation in depression. *Depression Anxiety* 31, 308–315. doi: 10.1002/da.22264

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Kogan, A., Gruber, J., Shallcross, A. J., Ford, B. Q., and Mauss, I. B. (2013). Too much of a good thing? Cardiac vagal tone's nonlinear relationship with well-being. *Emotion* 13, 599–604. doi: 10.1037/a0032725

Kogan, A., Oveis, C., Carr, E. W., Gruber, J., Mauss, I. B., Shallcross, A., et al. (2014). Vagal activity is quadratically related to prosocial traits, prosocial emotions, and observer perceptions of prosociality. *J. Pers. Soc. Psychol.* 107:1051. doi: 10.1037/a0037509

Kok, B. E., and Fredrickson, B. L. (2010). Upward spirals of the heart: Autonomic flexibility, as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biol. Psychol.* 85, 432–436. doi: 10.1016/j.biopsycho.2010.09.005

Kujawa, A., MacNamara, A., Fitzgerald, K. D., Monk, C. S., and Phan, K. L. (2015). Enhanced neural reactivity to threatening faces in anxious youth: evidence from event-related potentials. *J. Abnorm. Child Psychol.* 43, 1493–1501.

Kuss, O., Schumann, B., Kluttig, A., Greiser, K. H., and Haerting, J. (2008). Time domain parameters can be estimated with less statistical error than frequency domain parameters in the analysis of heart rate variability. *J. Electrocardiol.* 41, 287–291. doi: 10.1016/j.jelectrocard.2008.02.014

Laborde, S., Mosley, E., and Mertgen, A. (2018). Vagal tank theory: the three rs of cardiac vagal control functioning–resting, reactivity, and recovery. *Front. Neurosci.* 12:458. doi: 10.3389/fnins.2018.00458

Lovibond, P. F., and Lovibond, S. H. (1995). The structure of negative emotional states: comparison of the depression anxiety stress scales (DASS) with the beck depression and anxiety inventories. *Behav. Res. Ther.* 33, 335–343. doi: 10.1016/0005-7967(94)00075-U

Malik, M., Bigger, J. T., Camm, A. J., Kleiger, R. E., Malliani, A., Moss, A. J., et al. (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Nolen-Hoeksema, S. (2000). The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. *J. Abnorm. Psychol.* 109:504.

Nugent, A. C., Bain, E. E., Thayer, J. F., Sollers, J. J., and Drevets, W. C. (2011). Sex differences in the neural correlates of autonomic arousal: a pilot PET study. *Int. J. Psychophysiol.* 80, 182–191. doi: 10.1016/j.ijpsycho.2011.03.001

Nutt, D., Demyttenaere, K., Janka, Z., Aarre, T., Bourin, M., Canonico, P. L., et al. (2007). The other face of depression, reduced positive affect: the role of catecholamines in causation and cure. *J. Psychopharmcol.* 21, 461–471. doi: 10.1177/0269881106069938

Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol. Funct. Imaging* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Porges, S. W. (2007b). The polyvagal perspective. *Biol. Psychol.* 74, 116–143. doi: 10.1016/j.biopsycho.2006.06.009

Porges, S. W. (2007a). A phylogenetic journey through the vague and ambiguous Xth cranial nerve: a commentary on contemporary heart rate variability research. *Biol. Psychol.* 74, 301–307. doi: 10.1016/j.biopsycho.2006.08.007

Rottenberg, J. (2007). Cardiac vagal control in depression: a critical analysis. *Biol. Psychol.* 74, 200–211. doi: 10.1016/j.biopsycho.2005.08.010

Saul, J. P. (1990). Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *Physiology* 5, 32–37. doi: 10.1152/physiologyonline.1990.5.1.32

Spangler, D. P., and Friedman, B. H. (2017). A little goes a long way: low working memory load is associated with optimal distractor inhibition and increased vagal control under anxiety. *Front. Hum. Neurosci.* 11:43. doi: 10.3389/fnhum.2017.00043

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comp. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Tarvainen, M. P., Ranta-Aho, P. O., and Karjalainen, P. A. (2002). An advanced detrending method with application to HRV analysis. *IEEE Trans. Biomed. Eng.* 49, 172–175. doi: 10.1109/10.979357

Taylor, S. E. (2012). "Tend and befriend theory," in *Handbook of Theories of Social Psychology*, eds P. A. M. Van Lange, A. W. Kruglanski, and E. T. Higgins (London: Sage Publications Ltd), 32–49. doi: 10.4135/9781446249215.n3

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., and Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol. Rev.* 107:411. doi: 10.1037/0033-295X.107.3.411

Taylor, S. E., and Master, S. L. (2011). Social responses to stress: the tend-and-befriend model. *Handb. Stress Sci. Biol. Psychol. Health* 101:109.

Templeton, G. F. (2011). A two-step approach for transforming continuous variables to normal: implications and recommendations for IS research. *Commun. Assoc. Inform. Syst.* 28:1.

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2009). Claude bernard and the heart–brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Rossy, L. A., Ruiz-Padial, E., and Johnsen, B. H. (2003). Gender differences in the relationship between emotional regulation and depressive symptoms. *Cognit. Ther. Res.* 27, 349–364. doi: 10.1023/A:1023922618287

Thayer, J. F., Smith, M., Rossy, L. A., Sollers, J. J., and Friedman, B. H. (1998). Heart period variability and depressive symptoms: gender differences. *Biol. Psychiatry* 44, 304–306. doi: 10.1016/S0006-3223(98)00008-0

Thayer, J. F., Sollers, J. J., Ruiz-Padial, E., and Vila, J. (2002). Estimating respiratory frequency from autoregressive spectral analysis of heart period. *IEEE Eng. Med. Biol. Mag.* 21, 41–45. doi: 10.1109/MEMB.2002.1032638

Thayer, J. F., and Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Ann. N. Y. Acad. Sci.* 1088, 361–372. doi: 10.1196/annals.1366.014

Trivers, R. (1972). "Parental investment and sexual selection," in *Sexual Selection & the Descent of Man*, ed. B. Campbell (New York, NY: Aldine de Gruyter), 136–179.

Troy, A. S., and Mauss, I. B. (2011). Resilience in the face of stress: emotion regulation as a protective factor. *Resilience Ment. Health Challenges Across Lifespan* 1, 30–44.

Umetani, K., Singer, D. H., McCraty, R., and Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. Coll. Cardiol.* 31, 593–601. doi: 10.1016/S0735-1097(97)00554-8

van Dijk, J. G., and Sheldon, R. (2008). Is there any point to vasovagal syncope? *Clin. Autonomic Res.* 18:167. doi: 10.1007/s10286-008-0484-x

van Lieshout, J. J., Wieling, W., Karemaker, J. M., and Eckberg, D. L. (1991). The vasovagal response. *Clin. Sci.* 81, 575–586. doi: 10.1042/cs0810575

Verkuil, B., Brosschot, J. F., Marques, A. H., Kampschroer, K., Sternberg, E. M., and Thayer, J. F. (2015). Gender differences in the impact of daily sadness on 24-h heart rate variability. *Psychophysiology* 52, 1682–1688. doi: 10.1111/psyp.12541

Wang, Y., Zhao, X., O'Neil, A., Turner, A., Liu, X., and Berk, M. (2013). Altered cardiac autonomic nervous function in depression. *BMC Psychiatry* 13:187. doi: 10.1186/1471-244X-13-187

Watson, D., Clark, L. A., and Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54:1063. doi: 10.1037/0022-3514.54.6.1063

Weathers, F. W., Litz, B. T., Huska, J. A., and Keane, T. M. (1994). *PTSD Checklist—Civilian Version*. Boston, MA: National Center for PTSD, Behavioral Science Division.

Wei, C., Han, J., Zhang, Y., Hannak, W., Dai, Y., and Liu, Z. (2017). Affective emotion increases heart rate variability and activates left dorsolateral prefrontal cortex in post-traumatic growth. *Sci. Rep.* 7:16667.

Wharton, A. S. (1999). The psychosocial consequences of emotional labor. *Ann. Am. Acad. Political Soc. Sci.* 561, 158–176. doi: 10.1177/000271629956100111

Williams, D. P., Tracy, L. M., Gerardo, G. M., Rahman, T., Spangler, D. P., Koenig, J., et al. (2019). Sex moderates the relationship between resting heart rate variability and self-reported difficulties in emotion regulation. *Emotion* 19:992. doi: 10.1037/emo0000500

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 17 May 2021
doi: 10.3389/fnins.2021.617698

# Age-Related Changes in Cardiac Autonomic Modulation and Heart Rate Variability in Mice

*Chiara Piantoni[1,2†], Luca Carnevali[3†], David Molla[1], Andrea Barbuti[1], Dario DiFrancesco[1,4], Annalisa Bucchi[1] and Mirko Baruscotti[1]**

[1] Department of Biosciences, The PaceLab and "Centro Interuniversitario di Medicina Molecolare e Biofisica Applicata", Università degli Studi di Milano, Milan, Italy, [2] Institute of Neurophysiology, Hannover Medical School, Hanover, Germany, [3] Stress Physiology Lab, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy, [4] IBF-CNR, University of Milano Unit, Milan, Italy

**Objective:** The aim of this study was to assess age-related changes in cardiac autonomic modulation and heart rate variability (HRV) and their association with spontaneous and pharmacologically induced vulnerability to cardiac arrhythmias, to verify the translational relevance of mouse models for further in-depth evaluation of the link between autonomic changes and increased arrhythmic risk with advancing age.

**Methods:** Heart rate (HR) and time- and frequency-domain indexes of HRV were calculated from Electrocardiogram (ECG) recordings in two groups of conscious mice of different ages (4 and 19 months old) (i) during daily undisturbed conditions, (ii) following peripheral β-adrenergic (atenolol), muscarinic (methylscopolamine), and β-adrenergic + muscarinic blockades, and (iii) following β-adrenergic (isoprenaline) stimulation. Vulnerability to arrhythmias was evaluated during daily undisturbed conditions and following β-adrenergic stimulation.

**Results:** HRV analysis and HR responses to autonomic blockades revealed that 19-month-old mice had a lower vagal modulation of cardiac function compared with 4-month-old mice. This age-related autonomic effect was not reflected in changes in HR, since intrinsic HR was lower in 19-month-old compared with 4-month-old mice. Both time- and frequency-domain HRV indexes were reduced following muscarinic, but not β-adrenergic blockade in younger mice, and to a lesser extent in older mice, suggesting that HRV is largely modulated by vagal tone in mice. Finally, 19-month-old mice showed a larger vulnerability to both spontaneous and isoprenaline-induced arrhythmias.

**Conclusion:** The present study combines HRV analysis and selective pharmacological autonomic blockades to document an age-related impairment in cardiac vagal modulation in mice which is consistent with the human condition. Given their short life span, mice could be further exploited as an aged model for studying the trajectory of vagal decline with advancing age using HRV measures, and the mechanisms underlying its association with proarrhythmic remodeling of the senescent heart.

**Keywords: heart rate variability (HRV), aging, mouse model, autonomic cardiac modulation, arrhythmias (cardiac)**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Beth A. Habecker, Oregon Health and Science University, United States*
*Emilio Vanoli, University of Pavia, Italy*

****Correspondence:***
*Mirko Baruscotti*
*mirko.baruscotti@unimi.it*

*† These authors have contributed equally to this work and shared first authorship*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *15 October 2020*
***Accepted:*** *20 April 2021*
***Published:*** *17 May 2021*

***Citation:***
*Piantoni C, Carnevali L, Molla D, Barbuti A, DiFrancesco D, Bucchi A and Baruscotti M (2021) Age-Related Changes in Cardiac Autonomic Modulation and Heart Rate Variability in Mice. Front. Neurosci. 15:617698. doi: 10.3389/fnins.2021.617698*

## INTRODUCTION

This study explores the translational relevance of mouse models for the investigation of the link between cardiac autonomic changes and increased arrhythmic risk with advancing age. In humans, the natural process of aging is associated with a progressive structural and functional remodeling of the heart that predisposes elderly people to higher vulnerability to both brady- and tachyarrhythmias, causing substantial morbidity and mortality (Lakatta et al., 2001; Yazdanyar and Newman, 2009; Chow et al., 2012; Chadda et al., 2018; Curtis et al., 2018). Susceptibility to arrhythmogenesis is enhanced in the senescent heart even in the absence of apparent structural abnormalities, and this instability may depend on age-dependent modifications of both the electrical profile of cardiac cells (i.e., ion currents balance), and of cardiac autonomic nervous system (ANS) function (Jovanovic, 2006; Chow et al., 2012; Jeevaratnam et al., 2017; Chadda et al., 2018; Curtis et al., 2018). For example, basal plasma norepinephrine levels increase with age (Pfeifer et al., 1983), suggesting that sympathetic nervous activity may be elevated in elderly people and affect the electrical stability of both atria and ventricles (Shen and Zipes, 2014; Kalla et al., 2016; Curtis et al., 2018). Moreover, an age-related impairment in cardiac vagal modulation has been documented in studies reporting (i) a decline in the vagal component of heart rate variability (HRV) – a surrogate measure of ANS function – and (ii) diminished heart rate (HR) responses to blockade of muscarinic acetylcholine receptors with advancing age (Korkushko et al., 1991; Poller et al., 1997; Umetani et al., 1998; Antelmi et al., 2004; De Meersman and Stein, 2007). Therefore, the aim of this study is to assess whether the aging process in mice is characterized by similar changes in cardiac autonomic modulation and HRV, and whether these changes lead to increased vulnerability to cardiac arrhythmias.

Indeed, low vagally mediated HRV has been proposed as a prognostic marker of increased mortality and propensity to lethal ventricular arrhythmias in post-myocardial infarction and chronic heart failure patients (La Rovere et al., 2003; Frenneaux, 2004; Huikuri and Stein, 2012), and associated with increased Cardiovascular Disease (CVD) morbidity and mortality in the elderly population (Tsuji et al., 1994).

Further support to this view comes from the observation that increased vagally mediated HRV has been described in centenarians compared with old adults (Piccirillo et al., 1998; Paolisso et al., 1999), suggesting that the maintenance of a sound vagal tone may be crucial for successful aging and longevity (Hernandez-Vicente et al., 2020). It must be noted, however, that other studies have shown that increased vagally mediated HRV may also predict cardiac mortality in elderly populations (Dekker et al., 1997; de Bruyne et al., 1999). These contrasting reports may find an explanation in the fact that vagal stimulation is antiarrhythmic in the ventricles but proarrhythmic in the atria (Shen and Zipes, 2014), and may hint at a more complex interplay between patterns of cardiac ANS activity and the electrical stability of the myocardium during the aging process (Chadda et al., 2018; Winter et al., 2018).

Animal models could therefore be useful to shed light on the relation between aging of the cardiac ANS, decline in vagally mediated HRV, and proarrhythmic electrical remodeling of the heart. For example, a longitudinal study in rats reported a gradual increase in the occurrence of different types of spontaneous arrhythmias with aging, which was coupled with a decline in vagally mediated HRV and a progressive alteration of the specialized conducting system (Rossi et al., 2014). More recently, resting measures of vagally mediated HRV were found to predict vulnerability to pharmacologically induced ventricular arrhythmias in healthy adult rats (Carnevali et al., 2019), and to correlate with the severity of spontaneous ventricular tachyarrhythmias in transgenic, aged (10-month-old) mice overexpressing the β2 adrenoceptors (He et al., 2020). Previous attempts to characterize age-related changes in HRV in mice seem to suggest a decrease of HRV with aging; however, their interpretation in the context of a proper animal model for human aging presents some limitations. Indeed, one (Yaniv et al., 2016) identifies this decrease, but was carried out under anesthesia, while the other (Axsom et al., 2020), carried out in conscious and unrestrained animals maintained at laboratory temperature, reports a significant decrease of the time-domain parameters, but not of parameters of the frequency domain. The conclusion of these studies is therefore yet incomplete, and before addressing mechanistic hypothesis it is of foremost importance to ascertain whether mouse models are suitable for the cardiac ANS changes that characterize the natural process of aging in humans. Moreover, while HRV in humans is thought to be predominantly modulated by vagal influences (Goldstein et al., 2011; Billman, 2013; Reyes del Paso et al., 2013; Laborde et al., 2017), a discussion of the specificity of commonly used time- and frequency-domain indexes of HRV to capture features of cardiac ANS modulation in mice is still incomplete. This is relevant also in light of the much higher HR and respiratory rate that characterize mice (Carnevali et al., 2013) compared to humans, which might affect the proper computation of HRV. Finally, to the best of our knowledge, no studies have investigated the relationship between changes in cardiac ANS modulation and arrhythmia vulnerability with advancing age in mice.

On the basis of these considerations, the purposes of this study are threefold. First, to investigate the relative contribution of sympathetic and vagal components on HR modulation in two groups of conscious mice of different age. To this end, we measured HR and HRV parameters from Electrocardiogram (ECG) recordings performed during daily undisturbed conditions, and following exposure to pharmacological autonomic challenges. We hypothesized that resting HR would be predominantly modulated by sympathetic influences in older mice and that this would be reflected by (i) reduced HRV, (ii) larger HR responses (i.e., decreases) to sympathetic block with atenolol (β-adrenergic receptor antagonist), and (iii) lower HR responses (i.e., increases) to vagal block with methylscoplamine (muscarinic receptor antagonist) compared to younger mice. Second, to discuss the specificity of time- and frequency-domain HRV indexes to reliably reflect these autonomic changes. Anticipating that HRV would be largely modulated by vagal influences in mice, as in humans

(Laborde et al., 2017), we hypothesized that both time- and frequency-domain HRV indexes would be reduced following muscarinic, but not β-adrenergic, block in younger mice, and to a lesser extent in older mice. Third, to evaluate age-related changes in the vulnerability to both spontaneous and pharmacologically induced cardiac arrhythmias. We expected a larger incidence of cardiac arrhythmias both under undisturbed resting conditions and following potent sympathetic stimulation with isoprenaline (non-selective β-adrenergic agonist) in older mice.

# MATERIALS AND METHODS

## Animals

C57BL/6J mice (JAX from Charles River Europe) of two different ages (4-month-old and 19-month-old) were individually housed, kept in rooms with controlled temperature (22 ± 2°C) and humidity (60 ± 10%), and maintained in a 12/12 h light/dark cycle (light on from 7:00 to 19:00 h), with food and water *ad libitum*.

Experiments were performed in accordance with the European Community Council Directive 2010/63/UE and approved by the Italian legislation on animal experimentation (D.L. 04/04/2014, n. 26, authorization n. 141/2016). All efforts were made to reduce sample size and minimize animal suffering.

## Surgery: Transmitter Implantation

Mice were anesthetized using inhaled isoflurane (Isoflurane-VET, Merial). Anesthesia was induced by spontaneous breathing of 5% isoflurane in 100% oxygen at a flow rate of 1 L/min and then maintained at 1.5-3% isoflurane in 100% oxygen at a flow rate of 1 L/min; all animals received the analgesic Rymadil (5 mg/kg, Pfizer) and antibiotic Baytril (5.8 mg/kg, Bayer) immediately prior to transmitter implantation. Mice were then implanted with radiotelemetric transmitters (TA10ETA-F20, Data Sciences Int., St. Paul, MN, United States) for recordings of ECG (sampling frequency 2000 Hz), and locomotor activity (LOC, expressed as counts/minute) signals. The transmitter was placed in the abdominal cavity; one electrode was fixed to the dorsal surface of the xiphoid process and another electrode was placed in the anterior mediastinum close to the right atrium, according to a previously described procedure (Sgoifo et al., 1996). This electrode location guarantees high-quality ECG recordings, even during vigorous somatomotor activity. Body heat was maintained both during and immediately after surgery. Animals were given food and water post-surgery and were housed individually. Rymadil and Baytril were given to the animals for five consecutive days after surgery. Prior to the start of experimental recordings, mice were allowed to recover two weeks and re-establish normal daily rhythms of HR and LOC.

## Daily Recordings of ECG and LOC Signals

After recovery from surgery, ECG and LOC signals were recorded for 120 s every 30 min for six consecutive days, with the animals left undisturbed in their home cages. ECG and LOC signals were picked up by platform receivers (RPC-1, Data Sciences Int., St. Paul, MN, United States) and acquired via Dataquest A.R.T. (TM) Gold 4.3 acquisition system (Data Sciences Int., St. Paul, MN, United States).

## Pharmacological Autonomic Blockades

Mice were injected intraperitoneally on different days, following a rotational design, with: (i) 1 ml/kg saline solution (0.9% NaCl; control condition); (ii) 0.1 mg/kg methylscopolamine (muscarinic receptor antagonist, Sigma-Aldrich); (iii) 1 mg/kg atenolol (β1-adrenergic receptor antagonist, Sigma-Aldrich); (iv) methylscopolamine + atenolol (at the same above-indicated doses; for both vagal and sympathetic blockade). Each injection was separated by at least a 2-day washout period. Drug doses were selected on the basis of previous studies (Statello et al., 2017). ECG recordings were performed during the hour that preceded (baseline condition) and the 2 h that followed each injection. All injections were done between 14.00 and 15.00 h.

## Pharmacological Sympathetic Stimulation

Mice were injected intraperitoneally with isoprenaline (non-selective β-adrenergic agonist, Sigma-Aldrich, at a dose of 0.2 mg/kg for mimicking potent sympathetic stimulation). ECG recordings were performed during the hour that preceded (baseline condition) and the hour that followed the injection. All injections were done between 14.00 and 15.00 h.

## ECG Analysis

Initially, each raw ECG signal was visually inspected to ensure that all R-waves were correctly detected. Those parts of ECG recordings which exhibited recording artifacts were discarded without substitution and excluded from further analysis. Heart rate (reported in beats per minute, bpm) and time- and frequency-domain parameters of HRV were quantified using ChartPro 5.0 software (ADInstruments, Sydney, Australia), following the guidelines suggested by Thireau and colleagues (Thireau et al., 2008) for the assessment of HRV parameters in mice. Time-domain measures included the standard deviation of the time between normal-to-normal beats (SDNN) and the root mean squared of successive beat-to-beat interval differences (RMSSD) (No authors listed, 1996). For spectral (frequency-domain) analysis of HRV, a power spectrum was obtained with a fast Fourier transform-based method (Welch's periodogram: 256 points, 50% overlap, and Hamming window). We considered: (i) the total power of the spectrum (TP, $ms^2$), (ii) the power ($ms^2$) of the low frequency band (LF, 0.15–1.5 Hz) and (HF, 1.5–5.0 Hz) bands in absolute values ($ms^2$) and normalized units (n.u.), and (iii) the low frequency/high frequency ratio (LF/HF).

## Data Analysis

### Daily Recordings of ECG and LOC Signals

Separate estimates of HR, HRV, and LOC were initially generated for each 2-min recording period and subsequently averaged as mean values of the 12 h-light and 12 h-dark phase of each recording day. These parameters were then further averaged to

obtain light phase and dark phase means of the six recording days. Subsequently, to test for differences between the two groups of mice, a series of two-way ANOVAs for repeated measures were applied on HR and HRV data, with "group" as the between-subject factor (two levels: 4-month-old and 19-month-old mice) and "phase" as the within-subject factor (two levels: light and dark phases). Follow-up *post hoc* analyses were conducted using Fisher's LSD.

### Pharmacological Autonomic Manipulations

Each recording period was split in 2-min epochs (0–2 min, 2–4, etc.). For each epoch, separate estimates of HR and HRV were generated. Initially, to test the effects of pharmacological autonomic manipulations on HR values within each group, ΔHR values were calculated as the differences between each 2-min post-injection period and the respective mean baseline value, and then averaged for the 60-min period corresponding to the second hour that followed each injection [min 60–120; i.e., when animals had completely re-established baseline HR and HRV following stress associated with handling and injection, as shown in the saline (control) condition]. For each group, ΔHR and ΔHRV values were analyzed by means of one-way ANOVAs for repeated measures, with "autonomic manipulation" as the within subject factor [4 levels: control (saline) condition, vagal blockade, sympathetic blockade, double blockade]. Follow-up analyses were conducted using Student's *t*-tests, with a Fisher's correction for multiple comparisons.

### Incidence of Cardiac Arrhythmias

Lastly, the occurrence of spontaneous and pharmacologically (isoprenaline)-induced cardiac arrhythmias was determined and quantified off-line as in Surawicz and Knilans (2008), Curtis et al. (2013), Carnevali et al. (2015). We identified and quantified the separate occurrence of sinus pauses, atrioventricular blocks, and supraventricular (SV) and ventricular (V) ectopic beats and the total number of arrhythmic events [reported as number of events per length (hours) of analyzed ECG recording] during daily undisturbed conditions and following pharmacological sympathetic stimulation with isoprenaline. Age-related changes in arrhythmia vulnerability were assessed by means of unpaired Student's *t*-tests.

All data in figures and tables are presented as means ± SEM. All statistical analyses were performed using SPSS 24 software package (SPSS Inc. Chicago, IL) and OriginPro 2020 (OriginLab Corporation, Northampton, MA). Statistical significance was set at $p < 0.05$.

# RESULTS

## Daily Recordings

Basal HR and HRV indexes were evaluated in 4 and 19 month-old freely moving mice to identify possible aging associated changes.

We initially tested for the presence of a circadian oscillation of HR and to this aim we collected 2 min-long ECG traces every 30 min for six consecutive days. Heart rate values measured during light and dark phases of equal duration of the daily cycle are shown for each mouse in **Figure 1A**, while collective mean ± SEM data are shown in panel B (top, left) and presented in **Supplementary Table 1**. As expected, heart rates during the dark/active phase of the daily cycle were significantly higher than those during the light/inactive phase for each age group; comparison between age-groups in the light and dark conditions did not reveal significant differences, thus confirming that aging does not impact on basal heart rate, an observation that is consistent with literature data on humans and mice. Also, no difference in the locomotor activity was detected between the groups (**Supplementary Table 1**). HRV indexes, extracted from the same ECG recordings, are plotted in **Figure 1B** (data in **Supplementary Table 1**). Despite the similarity in basal HR, the 19-month-old group showed significantly lower values of SDNN, RMSSD, total power, and HF power during both phases of the daily cycle, while LF power was reduced only during the light phase. No significant differences were instead observed when the LF and HF bands were expressed in normalized units as well as in the LF/HF ratio.

## HR Responses to Pharmacological Autonomic Blockades

In order to investigate the influences of the Autonomic Nervous System on HR and dissect the relative contribution of each autonomic arm in the two age conditions, we evaluated the HR responses to selective pharmacological blockades of the parasympathetic and sympathetic systems (**Figure 2**) by i.p. injection of methylscopolamine and/or atenolol. Since HR and HRV indexes are greatly influenced by the experimental handling of the mouse, we first identified a protocol where this stress-induced experimental artifacts could be eliminated. To this aim we injected the mice with a saline solution and verified that complete recovery to the basal level (defined as the mean value measured during 1 h preceding the treatments) was attained after 1 h from the injection time. All the analyses were then carried on the data recorded during the following hour (squared dashed box in the figure). The time-courses of HR changes (ΔHR) following each autonomic manipulation are shown overlapped separately for the two age groups (**Figure 2**, left). Mean ± SEM ΔHR values are presented in the right panels of **Figure 2** and are listed in **Supplementary Table 2**.

Within-group statistics yielded a significant effect of "autonomic manipulation" both in 4-month-old and 19-month-old mice. Specifically, *post hoc* analysis revealed that in 4-month-old mice vagal blockade with methylscopolamine provoked a significant increase in HR compared with the control (i.e., saline) condition ($p < 0.05$), whereas sympathetic blockade with atenolol provoked a significant decrease in HR compared with the control condition ($p < 0.05$). No significant changes in HR were observed in 4-month-old mice after double autonomic blockade (methylscopolamine + atenolol) compared with the control condition. In the 19-month-old group a significant decrease in HR was observed during sympathetic blockade ($p < 0.05$), while an increase, albeit not significant, was observed during parasympathetic deprivation. Quite interestingly, in the

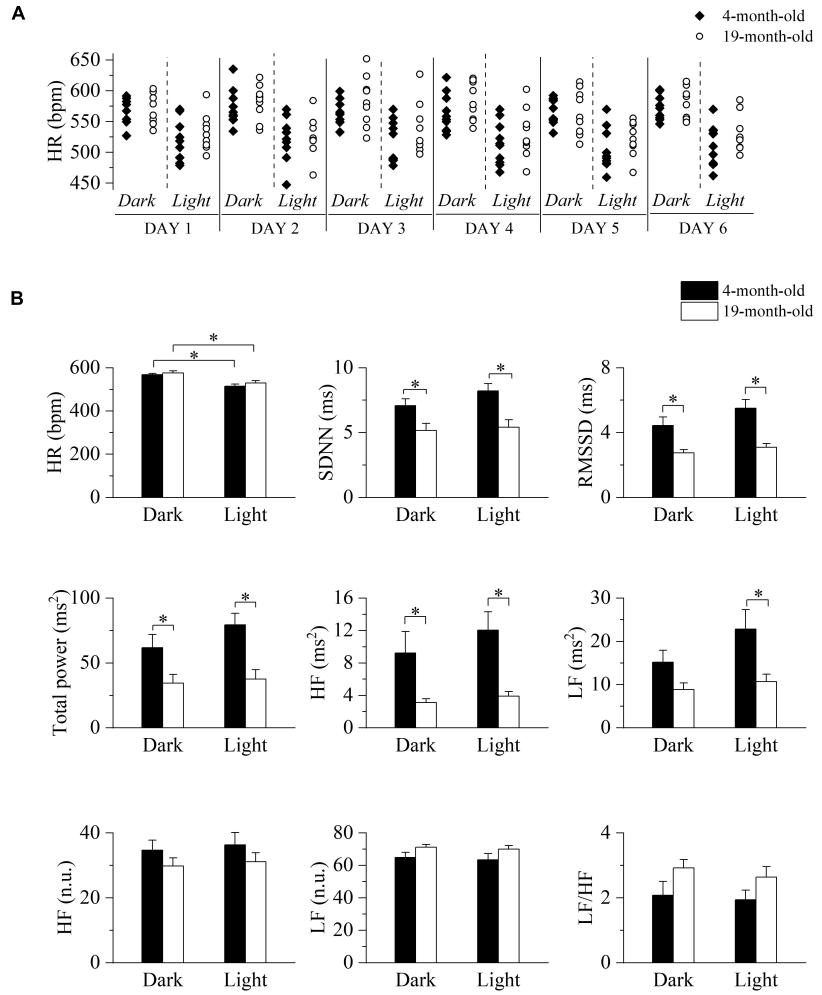



**FIGURE 1 |** Long-term evaluation of HR and HRV in 4- and 19-month-old freely moving mice. **(A)** Time course of heart rates (HR) recorded during the 12-h light and 12-h dark phases of six consecutive days in 4-month-old ($n$ = 10, filled diamonds) and 19-month-old ($n$ = 9, empty circles) freely moving mice. **(B)** Mean heart rate and heart rate variability parameters evaluated from ECG traces recorded from 4-month-old and 19-month-old mice during the daily cycle (dark and light phases). Data are reported as mean ± SEM. SDNN, standard deviation of beat-to-beat intervals; RMSSD, root mean square of successive beat-to-beat interval differences; LF, low frequency; HF, high frequency; LF (n.u.), low frequency in normalized units; HF (n.u.), high frequency in normalized units. *$p < 0.05$, two-way ANOVA for repeated measures followed by Fisher's LSD *post hoc* test.

older group the double autonomic blockade caused a significant decrease of HR, thus revealing a substantial difference between the intrinsic and basal rate.

We also further proceeded to verify whether the effect of autonomic blockade could reveal aging associated differences. To this aim we compared heart rate differences (ΔHR) during each specific pharmacological manipulation and confirmed that both vagal and double autonomic blockade were different between the two age groups (Student's $t$-test, $p < 0.05$), while no difference was identified during sympathetic deprivation.

## HRV Responses to Pharmacological Autonomic Blockades

Basal heart rate results from the complex balance of at least three main elements: intrinsic heart rate, sympathetic and parasympathetic drive. Results obtained in previous figures clearly illustrate that 4 and 19 month-old mice have similar basal heart rates, but the underlying relative contribution of the above-mentioned elements is different. To shed light on this point and better characterize the differences, HRV analysis was carried out on the ECG traces collected during selective autonomic blockades (**Figures 3**, **4**). In this case, we calculated differences in the HRV indexes measured during each autonomic manipulation or saline injection versus those calculated in Basal condition (prior to treatments). In 4-month-old mice (**Figure 3** and **Supplementary Table 3**), vagal blockade (methylscopolamine) and double autonomic block (methylscopolamine + atenolol) significantly reduced ($p < 0.05$) SDNN, RMSSD, total power, HF, and LF values compared with the control (saline) condition. No differences were observed in the LF/HF ratio. Interestingly, sympathetic blockade alone did not result in significant changes in any index.

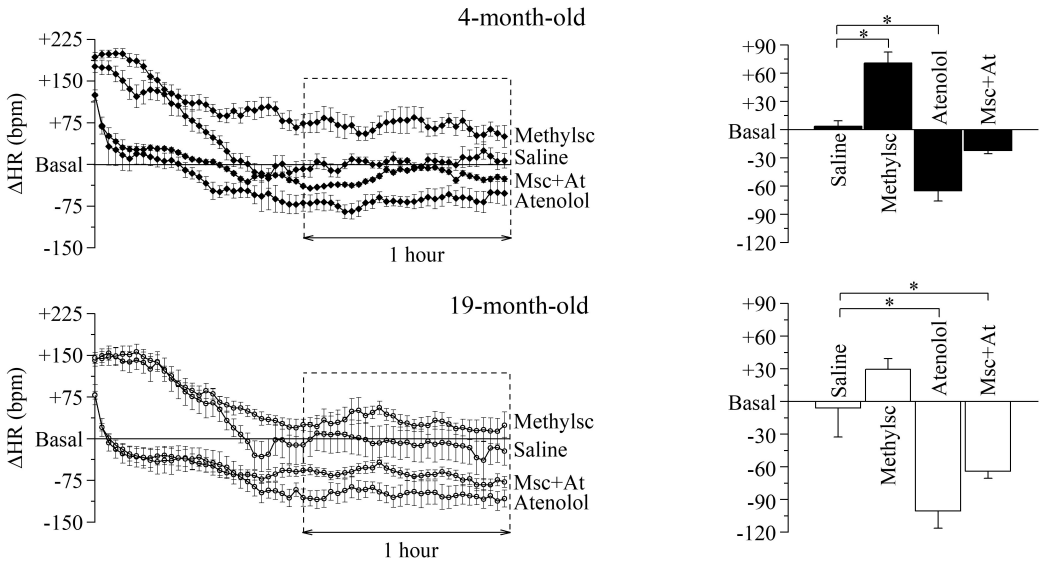



**FIGURE 2 |** Age-related differences in the autonomic modulation of heart rate. Heart rate changes (ΔHR) after i.p. injection of vehicle (Saline) and autonomic modulator drugs (Methylscopolamine, Atenolol and Methylscopolamine + Atenolol) in 4 month-old ($n$ = 10) and 19 month-old ($n$ = 9) freely moving mice. The left-hand panels represent the time courses of ΔHR recorded from the moment of the injection; each point represents the mean ΔHR of a 2-min period. Saline was employed to identify the time span which is needed by the animals to recover from the handling-induced stress (60 min after the injection the HR returned similar to the mean basal HR recorded for 60 min before drugs administration, Basal). The right-hand panels report mean ΔHR values recorded during the second hour post-injection. Data are reported as mean ± SEM. *$p < 0.05$, one-way ANOVA for repeated measures followed by Fisher's LSD *post hoc* test.

In 19-month-old mice (**Figure 4** and **Supplementary Table 4**), unexpectedly, vagal or sympathetic blockade alone did not result in significant changes in SDNN, RMSSD, total power, HF, and LF. However, significant reductions ($p < 0.05$) of all HRV parameters were observed under double autonomic blockade. In addition, differences LF/HF values were identified during sympathetic blockades, respectively.

Taken together the result of **Figures 3**, **4** point to a lower level of rate variability in aged mice, and this appear to be mostly associated with a decreased vagal component.

## Vulnerability to Cardiac Arrhythmias

A more rigid chronotropism (i.e., a reduction of HRV) is a pro-arrhythmic marker in humans; therefore, based on our previous findings, and with the overall aim to test whether the mouse is a reasonable experimental model to study cardiac aging, we further proceeded to verify the propensity to arrhythmias in the two age groups.

In **Figure 5** representative recordings (panel A) and mean ± SEM cumulative data (panel B) of the arrhythmic events observed in the mice are shown. During undisturbed daily recordings (**Figure 5B**, top), the overall incidence of cardiac arrhythmias was significantly larger in 19-month-old-mice compared to 4-month-old mice ($p < 0.05$), without specific age-related differences in the incidence of the various arrhythmic subtypes.

Since an increased adrenergic background drive is also a well-known pro-arrhythmic condition, we repeated the analysis of arrhythmic events in the presence of adrenergic stimuli with isprenaline, and a similar larger overall incidence of cardiac arrhythmias was found in 19-month-old mice (**Figure 5B**, bottom). Of note, HR response to maximal ISO stimulation, measured as ΔHR was significantly smaller ($p < 0.05$ Student's $t$-test) in 19-month-old (171.4 ± 16.0 bpm, $n = 7$) compared with 4-month-old mice (226.8 ± 14.3 bpm, $n = 8$).

# DISCUSSION

The present study combines HRV analysis and selective pharmacological autonomic challenges to demonstrate a relative lower contribution of the vagal component on HR modulation in 19-month-old mice compared with 4-month-old mice. This age-related cardiac autonomic effect is (i) observed both during the light and dark phases of the daily cycle in conscious and freely moving mice, (ii) independent from somatomotor activity levels, (iii) compensated by a reduction in intrinsic HR, leading to a stable basal resting HR with advancing age, and (iv) associated with a larger vulnerability to both spontaneous and pharmacologically induced arrhythmias. In the next sections, we discuss the age-related changes in cardiac ANS modulation, the utility of commonly used time- and frequency-domain indexes of HRV to capture these autonomic changes in mice, and their association with arrhythmia susceptibility.

## Age-Related Changes in Cardiac ANS Modulation in Mice

Specific patterns of cardiac ANS modulation may vary across species, with possible species-typical sympathovagal balance (Evans et al., 1990; Japundzic et al., 1990;

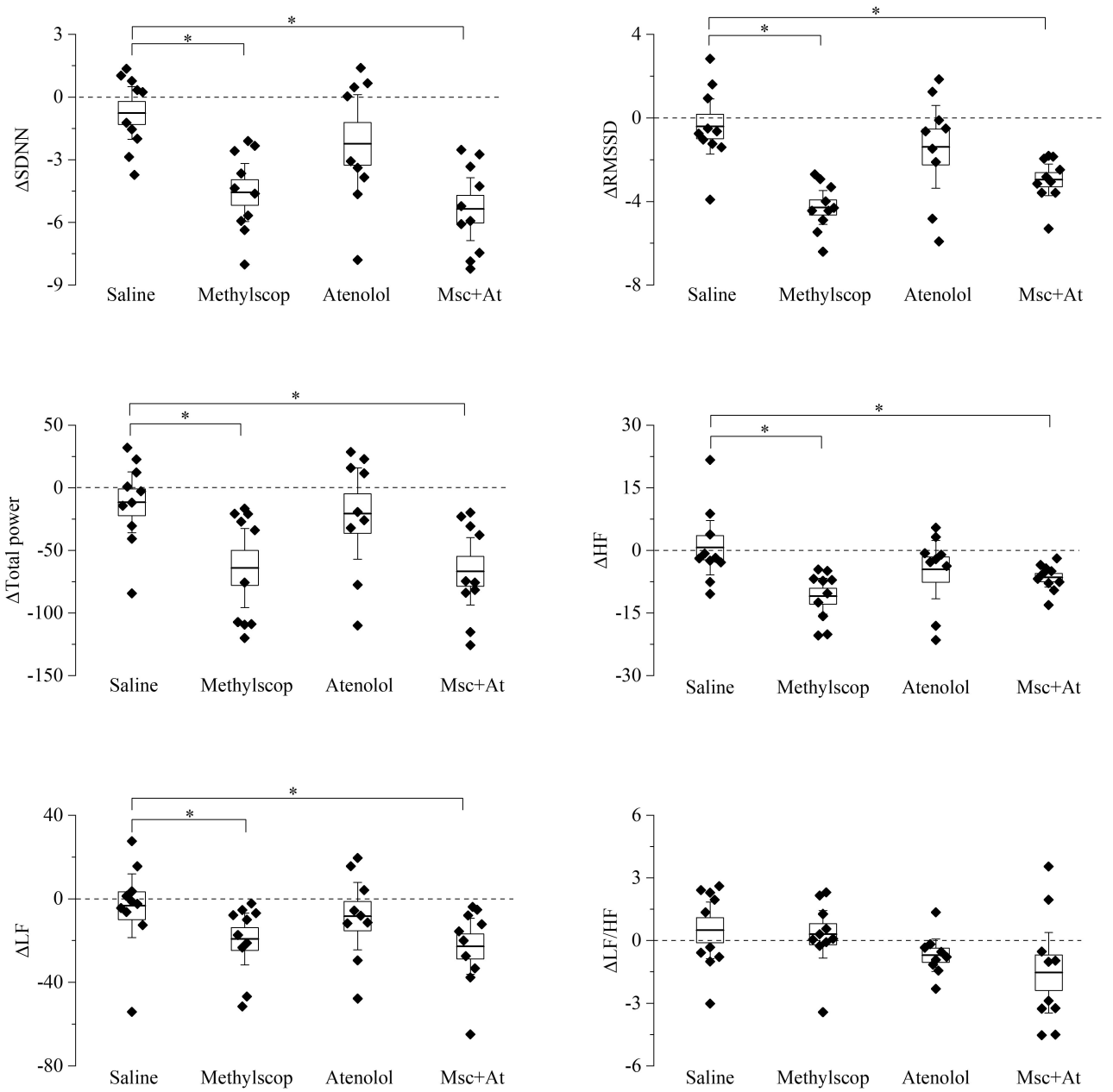



**FIGURE 3 |** Heart rate variability parameters response to pharmacological autonomic blockade in 4-month-old mice. Changes in the heart rate variability parameters calculated from ECG traces recorded from 60 to 120 min after i.p. injection of vehicle (saline) and autonomic modulator drugs (Methylscopolamine, Atenolol, Methylscopolamine + Atenolol) in 4-month-old freely moving mice ($n$ = 10). The dotted line (reference level) indicates mean basal HRV indexes recorded 60 min before drug administration. In the box chart, middle line indicates the mean value, delimiting lines of the box represent SEM and whiskers are confidence intervals at 95%. *$p < 0.05$, one-way ANOVA with repeated measures followed by Fisher's LSD *post hoc* test.

Carnevali and Sgoifo, 2014). For example, resting HR is characterized by a relatively larger vagal prevalence in humans (Tan et al., 2009) while it is generally believed that mice have a sympathetically dominated resting HR (Ishii et al., 1996; Gehrmann et al., 2000; Axsom et al., 2020), thus questioning the translational relevance of the murine model for the study of cardiac ANS-related conditions. For example, a recent study suggested that mice operate under high sympathetic drive in order to maintain a normal core temperature in standard laboratory conditions (20°C), and that when mice are acclimated to their thermoneutral zone (30°C) for 3 days, sympathetic input to the heart is greatly reduced (Axsom et al., 2020). Based on these findings, the authors conclude that in the context of a translational model that recapitulates human physiology, it is vital that mice be housed under thermoneutral conditions to allow for normal autonomic regulation (Axsom et al., 2020).

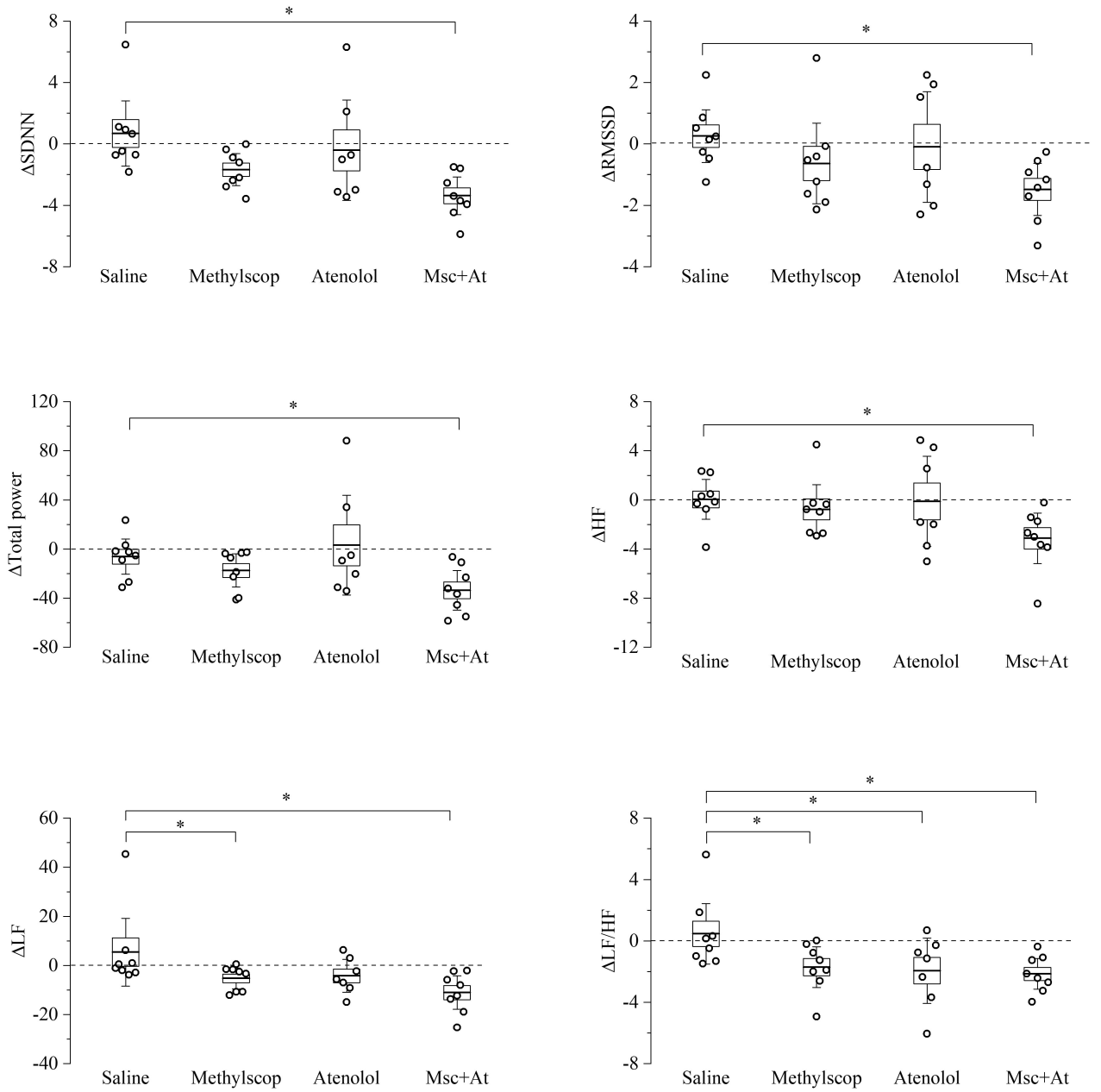



**FIGURE 4 |** Heart rate variability parameters response to pharmacological autonomic blockade in 19-month-old mice. Changes in the heart rate variability parameters calculated from ECG traces recorded from 60 to 120 min after i.p. injection of vehicle (saline) and autonomic modulator drugs (Methylscopolamine, Atenolol, Methylscopolamine + Atenolol) in 19-month-old freely moving mice ($n$ = 9). The dotted line (reference level) indicates mean basal HRV indexes recorded 60 min before drug administration. In the box chart, middle line indicates the mean value, delimiting lines of the box represent SEM and whiskers are confidence intervals at 95%. *$p < 0.05$, one-way ANOVA with repeated measures followed by Fisher's LSD *post hoc* test.

However, one may argue that patterns of ANS modulation at 30°C might represent a transient physiological response to environmental alterations in mice that were born and raised at ∼20°C. Moreover, it must be noted that previous attempts to characterize age-related ANS changes in mice were performed under anesthesia (which may affect ANS function) or during short-term recordings (which do not take into account potential circadian effects on ANS function) (Yaniv et al., 2016; Axsom et al., 2020; He et al., 2020). Therefore, in our study we sought to overcome these limitations by assessing age-related changes in cardiac autonomic modulation using two different and complementary approaches: via HRV analysis of ECG signals obtained during daily recordings at standard laboratory temperature conditions (∼22°C) in conscious mice of different age (4 and 19 months old), and by evaluating HR responses to selective pharmacological autonomic blockades.

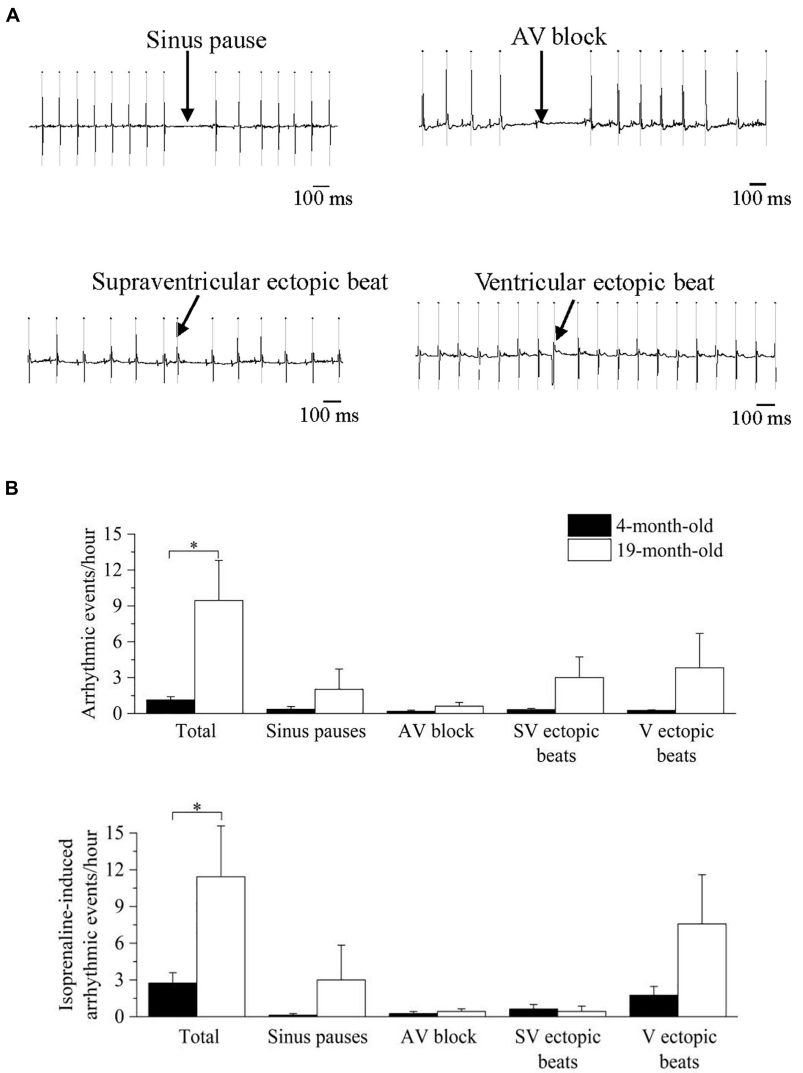



**FIGURE 5 |** Analysis and classification of spontaneous and induced arrhythmic events occurred in 4- and 19-month-old mice. **(A)** sample ECG traces of four different types of arrhythmic events: sinus pause, atrioventricular block, supraventricular ectopic beat and ventricular ectopic beat. **(B)** mean values of the spontaneous (top) and isoprenaline-induced (bottom) total and type-specific (sinus pauses, atrioventricular block, supraventricular ectopic beats and ventricular ectopic beats) arrhythmic events. Data are reported as mean ± SEM. Comparisons were evaluated by Student's *t*-test, $^*p < 0.05$.

We found an age-related reduction in time- (SDNN, RMSSD) and frequency-domain (total and HF power values) indexes of HRV during both phases of the daily cycle (**Figure 1B**), which recapitulates human findings (Jandackova et al., 2016). Power values of the LF index was instead reduced only during daytime analysis. Importantly, we provide evidence that age-related changes in HRV are not ascribable to changes in somatomotor activity levels. Relatedly, we observed a significant increment in HR values under vagal blockade with methylscopolamine in 4-month-old, but not in 19-month-old, mice compared with the control (saline) condition (**Figure 2**). Taken together, these data (see below our discussion on HRV indexes) are indicative of a decline in resting cardiac vagal modulation with advancing age that typically characterizes humans (De Meersman and Stein, 2007). On the other hand, adrenergic receptor blockade with atenolol led to a decrease in the average HR in both groups with a larger, albeit not significant effect observed in the 19 month-old group. Notably, these age-related ANS changes were not reflected by concomitant changes in resting measures of HR. A potential explanation lies in the fact that the intrinsic HR (i.e., HR under double autonomic blockade) was significantly lower in 19-month-old mice compared to the younger group. It has been suggested that changes in the mechanisms intrinsic to heart pacemaker cells may compensate for the aging-associated shift in the sympathovagal balance toward a sympathetic prevalence to preserve a stable resting HR (De Meersman and Stein, 2007).

In interpreting our findings from a translational point of view, we must underline similarities and differences with the human condition. First, the fact that muscarinic receptor blockade with methylscopolamine was associated with a significant increase of

the average HR in the younger group suggests that in young adult mice vagal modulation plays a significant role in the regulation of resting HR. It must be acknowledged, however, that the intrinsic HR in the young group was similar to the average resting HR, confirming that, contrary to young adult humans (Tan et al., 2009), resting HR in young adult mice is not predominantly modulated by vagal influences. Most importantly, our data indicate that mice exhibit age-related changes in cardiac ANS regulation (i.e., decline in vagal functioning) and automaticity of cardiac pacemaker cells (i.e., reduced intrinsic HR) that closely recapitulate those observed in human populations (Jose and Collison, 1970; Korkushko et al., 1991; Poller et al., 1997; Umetani et al., 1998; Antelmi et al., 2004; De Meersman and Stein, 2007). Moreover, we also found that, similar to humans (White et al., 1994), maximal β-adrenergic receptor stimulation with isoprenaline provoked a smaller HR response in the older group. This result is in agreement with a previous study documenting a reduced sensitivity of the average heart beating intervals to β-adrenergic receptor stimulation in the isolated SAN tissue of older mice (Yaniv et al., 2016). However, the correct evaluation of the response to maximal adrenergic stimuli should likely consider as a starting point the intrinsic HR and not basal HR since in this last condition adrenergic receptors may be already partly occupied. If this consideration is taken into account the maximal adrenergic excursions observed in our mice are + 224 ± 4 bpm in 4 month-old mice and + 238 ± 14 bpm in 19-month-old mice; these similar accelerations likely suggest a fully maintained adrenergic modulation of rate. Interstingly, Peters et al. (2020), also report that the maximal heart rate attainable during adrenaline stimulation decreases with age and this reduction is mainly determined by a lower intrinsic heart rate, albeit a modest decline in the sympathetic nervous function is also present. However, in another study carried out in isolated innervated hearts of aged female mice (Francis Stuart et al., 2018), the authors suggested that the decreased HR response to isoprenaline is primarily due to a decreased β−adrenergic responsiveness since, upon sympathetic nerve stimulation, atrial noradrenaline content was similar in aged and young hearts, thus suggesting preserved sympathetic nerve density with advancing age (Francis Stuart et al., 2018). In contrast, decreased responsiveness to sympathetic nerve stimulation in the ventricles of aged female mice seemed to be primarily due to nerve degeneration, since β−adrenergic responsiveness was preserved, but sympathetic nerve density and noradrenaline content were reduced (Francis Stuart et al., 2018).

## Specificity of HRV Indexes to Capture Age-Related Changes in Cardiac ANS Modulation in Mice

HRV can be separated into various components, reflecting ANS influence on cardiac control, and its analysis has become a popular approach in several investigational domains, both in human and animal research (Berntson et al., 1997; Rowan et al., 2007; Laborde et al., 2017). In humans, the SDNN is thought to reflect all the cyclic components responsible for variability, and the SDNN squared is equivalent to the variance (total power) when viewed in the frequency domain (Shaffer and Ginsberg, 2017). The RMSSD and the HF component of HRV detect quick beat-to-beat fluctuations in a heart period time series, primarily reflecting vagal modulation (Laborde et al., 2017). In this study, we confirm the reliability of RMSSD and HF power values to assess vagally mediated HRV since in 4 month-old mice we observe a substantial reduction of these indexes following muscarinic receptor blockade with methylscopolamine (**Figure 3**). Relatedly, vagal blockade, but not sympathetic blockade, modulates SDNN and total power values, suggesting that a prevalent vagal influence mediates HRV in mice. This finding well correlates with the evidence that vagal block in young significantly modifies heart rate in this age group. Notably, the effect of vagal blockade on HRV indexes was significant in 4-month-old but not in 19-month-old mice (**Figures 3**, **4**) in agreement with the above discussed decline in resting cardiac vagal functioning in the older group. Furthermore, although the LF component of HRV has been theorized to represent both sympathetic and vagal influences (Goldstein et al., 2011; Reyes del Paso et al., 2013), findings of reduced LF values following vagal, but not sympathetic, blockade in the young group suggest that the LF band [0.15–1.5 Hz in this study (Thireau et al., 2008)] captures predominantly vagal influences in mice. This finding has two important implications for the use of HRV in mice: (i) normalized LF and HF units, which report frequency power proportional to the total observed power, may not discriminate between different levels of resting cardiac vagal modulation, (ii) the LF to HF ratio should not be interpreted as an index of sympathovagal balance in mice, as already pointed out in humans (Heathers, 2012; Billman, 2013; Laborde et al., 2017). On the other hand, differences in resting measures of RMSSD and HF power values between the two age groups well support our pharmacological evidence of reduced cardiac vagal modulation with advancing age.

In the light of these considerations, we advocate the use of vagally mediated HRV indexes (i.e., RMSSD and HF power values) for capturing the decline in cardiac vagal modulation in mouse models of aging. However, it must be noted that while HRV alterations are often ascribed solely to changes in ANS signaling, age-related changes in HRV in mice have also been associated with deterioration of autonomic neuronal receptor signaling and mechanisms intrinsic to heart pacemaker cells (Yaniv et al., 2016).

## Age-Related Changes in the Vulnerability to Cardiac Arrhythmias

Reduced vagally mediated HRV has been proposed as a prognostic marker of increased mortality and propensity to lethal ventricular arrhythmias in cardiac patients (La Rovere et al., 2003; Frenneaux, 2004; Huikuri and Stein, 2012), and associated with increased CVD morbidity and mortality in the elderly (Tsuji et al., 1994).

In this study 19-month-old mice exhibited, alongside reduced vagally mediated HRV, a larger vulnerability to both spontaneous and pharmacologically-(isoprenaline-) induced cardiac arrhythmias compared to 4-month-old mice (**Figure 5**). Arrhythmias were both of supraventricular (e.g., sinus pauses,

supraventricular ectopic beats) and ventricular (ventricular ectopic beats) origin, with no clear chamber prevalence. It must be acknowledged that the incidence of arrhythmias was relatively modest even in the older group. In fact, one of the major limitations of this study is that we used only two age groups, with the older group likely representing an early phase of the aging process in this mouse strain. Therefore, we cannot exclude that arrhythmogenesis would be more evident in older mice. Interestingly, a previous study reported a higher incidence of ventricular arrhythmias in aged female mice following rapid ventricular pacing, but not after sympathetic nerve stimulation (Francis Stuart et al., 2018). Therefore, age−related sex differences in arrhythmia vulnerability with advancing age is an interesting area for future work. Notably, in humans premature atrial and ventricular complexes are frequently present in the healthy elderly population, even in the absence of apparent structural abnormalities (Chow et al., 2012; Chadda et al., 2018; Curtis et al., 2018). For example, in the Cardiovascular Health Study (Manolio et al., 1994), 24-h ambulatory monitoring in 60- to 85-year-old healthy individuals showed that 86% of patients had premature atrial complexes, with 26% having > 36 premature atrial complexes/h. Premature ventricular complexes were found in 82% of elderly subjects, including runs of non-sustained ventricular tachycardia. Importantly, a high burden of premature ventricular complexes was associated with increased left ventricular systolic dysfunction, incident heart failure, and mortality among the participants of this study (Dukes et al., 2015). However, despite promising and preliminary results on the utility of vagally mediated HRV indexes for predicting arrhythmic risk in rodents (Carnevali et al., 2019; He et al., 2020), we believe it is premature to assume that decreased vagally mediated HRV in aged mice can be regarded as a negative prognostic indicator of CVD risk. Nevertheless, the preliminary association found in this study between decline in cardiac vagal functioning and increased vulnerability to arrhythmias with advancing age encourages future mouse research aimed at investigating the causal role of age-related perturbations in ANS in the proarrhythmic electrical remodeling of the heart.

## CONCLUSION

The present study documents an age-related impairment in cardiac vagal modulation and HRV in mice, which is coupled with a larger vulnerability to cardiac arrhythmias. These results should be interpreted within the context of their limitations. Besides the use of only two age groups, we must acknowledge that HRV is influenced by several factors that were not considered here, including respiratory activity and blood pressure. For example, in rodents, the respiratory pattern consists of normal (eupnoea) or rapid (tachypnoea) breathing, intermingled with periods of sniffing of variable intensity and duration (Carnevali et al., 2013). While controlling for respiration is a long debated issue within human HRV research (Laborde et al., 2017), it would be interesting to test the extent to which different respiratory behaviors influence HRV indexes in mice.

Furthermore, an important component of short- and long-term blood pressure regulation is the arterial baroreflex, which plays a central role particularly in modulating sympathetic and vagal control of the heart and peripheral vasculature to respond to shifting metabolic demands and maintenance of a stable blood pressure (Guyenet, 2006; Reyes del Paso et al., 2006). Given that aging in humans is associated with decreased cardiovagal baroreflex sensitivity (Monahan, 2007), future mouse studies should combine HRV and blood pressure monitoring to obtain a more complete picture of the cardiac autonomic changes that characterize the aging process in mice. Importantly, while one of the major advantages of using mice as an aged model is their short lifespan, available research, including the present investigation, is mostly cross-sectional (Yaniv et al., 2016; Bennett et al., 2018). Consequently, these results warrant future longitudinal studies adopting HRV measures to investigate the trajectory of vagal decline with advancing age and its causal relationship with cardiac proarrhythmic remodeling in conscious mice.

Lastly, since autonomic dysfunction can contribute to the physiological decline of cognitive functions linked to the aging process (Murman, 2015; Forte et al., 2019), it would be interesting to evaluate the extent to which mouse models can contribute to the development of theoretical models established in humans with HRV that connect the heart and brain via the vagus nerve (Thayer et al., 2009; Smith et al., 2017).

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

All animal procedures performed in this study were carried out in accordance with the guidelines of the care and use of laboratory animals established by the Italian and UE laws (D. Lgs no. 2014/26, 2010/63/UE), the experimental protocols were approved by the Animal Welfare Committee of the Universitá degli Studi di Milano and by the Italian Minister of Health (protocol number 141/2016).

## AUTHOR CONTRIBUTIONS

CP, LC, and MB: conceived and designed the research. CP and LC: acquired the data. CP, LC, DM, and MB: performed statistical analysis and drafted the manuscript. CP, LC, DM, ABu, DD, ABa, and MB: made critical revision of the manuscript for key intellectual content. DD and MB: handled funding and supervision. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the grant to DD from the CARIPLO Foundation (ACROSS 2014-0728).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.617698/full#supplementary-material

## REFERENCES

Antelmi, I., de Paula, R. S., Shinzato, A. R., Peres, C. A., Mansur, A. J., and Grupi, C. J. (2004). Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. *Am. J. Cardiol.* 93, 381–385. doi: 10.1016/j.amjcard.2003.09.065

Axsom, J. E., Nanavati, A. P., Rutishauser, C. A., Bonin, J. E., Moen, J. M., and Lakatta, E. G. (2020). Acclimation to a thermoneutral environment abolishes age-associated alterations in heart rate and heart rate variability in conscious, unrestrained mice. *Geroscience* 42, 217–232. doi: 10.1007/s11357-019-00126-7

Bennett, B. A., Spannhake, E. W., Rule, A. M., Breysse, P. N., and Tankersley, C. G. (2018). The acute effects of age and particulate matter exposure on heart rate and heart rate variability in mice. *Cardiovasc. Toxicol.* 18, 507–519. doi: 10.1007/s12012-018-9461-3

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26.

Carnevali, L., and Sgoifo, A. (2014). Vagal modulation of resting heart rate in rats: the role of stress, psychosocial factors, and physical exercise. *Front. Physiol.* 5:118.

Carnevali, L., Sgoifo, A., Trombini, M., Landgraf, R., Neumann, I. D., and Nalivaiko, E. (2013). Different patterns of respiration in rat lines selectively bred for high or low anxiety. *PLoS One* 8:e64519. doi: 10.1371/journal.pone.0064519

Carnevali, L., Statello, R., and Sgoifo, A. (2019). Resting heart rate variability predicts vulnerability to pharmacologically-induced ventricular arrhythmias in male rats. *J. Clin. Med.* 8:655. doi: 10.3390/jcm8050655

Carnevali, L., Vacondio, F., Rossi, S., Callegari, S., Macchi, E., Spadoni, G., et al. (2015). Antidepressant-like activity and cardioprotective effects of fatty acid amide hydrolase inhibitor URB694 in socially stressed Wistar Kyoto rats. *Eur. Neuropsychopharmacol.* 25, 2157–2169. doi: 10.1016/j.euroneuro.2015.07.015

Chadda, K. R., Ajijola, O. A., Vaseghi, M., Shivkumar, K., Huang, C. L., and Jeevaratnam, K. (2018). Ageing, the autonomic nervous system and arrhythmia: from brain to heart. *Ageing Res. Rev.* 48, 40–50. doi: 10.1016/j.arr.2018.09.005

Chow, G. V., Marine, J. E., and Fleg, J. L. (2012). Epidemiology of arrhythmias and conduction disorders in older adults. *Clin. Geriatr. Med.* 28, 539–553. doi: 10.1016/j.cger.2012.07.003

Curtis, A. B., Karki, R., Hattoum, A., and Sharma, U. C. (2018). Arrhythmias in patients >/=80 years of age: pathophysiology, management, and outcomes. *J. Am. Coll. Cardiol.* 71, 2041–2057. doi: 10.1016/j.jacc.2018.03.019

Curtis, M. J., Hancox, J. C., Farkas, A., Wainwright, C. L., Stables, C. L., Saint, D. A., et al. (2013). The Lambeth conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. *Pharmacol. Ther.* 139, 213–248. doi: 10.1016/j.pharmthera.2013.04.008

de Bruyne, M. C., Kors, J. A., Hoes, A. W., Klootwijk, P., Dekker, J. M., Hofman, A., et al. (1999). Both decreased and increased heart rate variability on the standard 10-second electrocardiogram predict cardiac mortality in the elderly: the Rotterdam study. *Am. J. Epidemiol.* 150, 1282–1288. doi: 10.1093/oxfordjournals.aje.a009959

De Meersman, R. E., and Stein, P. K. (2007). Vagal modulation and aging. *Biol. Psychol.* 74, 165–173. doi: 10.1016/j.biopsycho.2006.04.008

Dekker, J. M., Schouten, E. G., Klootwijk, P., Pool, J., Swenne, C. A., and Kromhout, D. (1997). Heart rate variability from short electrocardiographic recordings predicts mortality from all causes in middle-aged and elderly men. the Zutphen study. *Am. J. Epidemiol.* 145, 899–908. doi: 10.1093/oxfordjournals.aje.a009049

Dukes, J. W., Dewland, T. A., Vittinghoff, E., Mandyam, M. C., Heckbert, S. R., Siscovick, D. S., et al. (2015). Ventricular ectopy as a predictor of heart failure and death. *J. Am. Coll. Cardiol.* 66, 101–109. doi: 10.1016/j.jacc.2015.04.062

Evans, J. M., Randall, D. C., Funk, J. N., and Knapp, C. F. (1990). Influence of cardiac innervation on intrinsic heart rate in dogs. *Am. J. Physiol.* 258(4 Pt 2), H1132–H1137.

Forte, G., Favieri, F., and Casagrande, M. (2019). Heart rate variability and cognitive function: a systematic review. *Front. Neurosci.* 13:710.

Francis Stuart, S. D., Wang, L., Woodard, W. R., Ng, G. A., Habecker, B. A., and Ripplinger, C. M. (2018). Age-related changes in cardiac electrophysiology and calcium handling in response to sympathetic nerve stimulation. *J. Physiol.* 596, 3977–3991. doi: 10.1113/jp276396

Frenneaux, M. P. (2004). Autonomic changes in patients with heart failure and in post-myocardial infarction patients. *Heart* 90, 1248–1255. doi: 10.1136/hrt.2003.026146

Gehrmann, J., Hammer, P. E., Maguire, C. T., Wakimoto, H., Triedman, J. K., and Berul, C. I. (2000). Phenotypic screening for heart rate variability in the mouse. *Am. J. Physiol. Heart Circ. Physiol.* 279, H733–H740.

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Guyenet, P. G. (2006). The sympathetic control of blood pressure. *Nat. Rev. Neurosci.* 7, 335–346.

He, M., Zhao, W. B., Nguyen, M. N., Kiriazis, H., Li, Y. Q., Hu, H., et al. (2020). Association between heart rate variability indices and features of spontaneous ventricular tachyarrhythmias in mice. *Clin. Exp. Pharmacol. Physiol.* 47, 1193–1202. doi: 10.1111/1440-1681.13275

Heathers, J. A. (2012). Sympathovagal balance from heart rate variability: an obituary. *Exp. Physiol.* 97:556. doi: 10.1113/expphysiol.2011.063867

Hernandez-Vicente, A., Hernando, D., Santos-Lozano, A., Rodriguez-Romo, G., Vicente-Rodriguez, G., Pueyo, E., et al. (2020). Heart rate variability and exceptional longevity. *Front. Physiol.* 11:566399.

Huikuri, H. V., and Stein, P. K. (2012). Clinical application of heart rate variability after acute myocardial infarction. *Front. Physiol.* 3:41.

Ishii, K., Kuwahara, M., Tsubone, H., and Sugano, S. (1996). Autonomic nervous function in mice and voles (*Microtus arvalis*): investigation by power spectral analysis of heart rate variability. *Lab. Anim.* 30, 359–364. doi: 10.1258/002367796780739880

Jandackova, V. K., Scholes, S., Britton, A., and Steptoe, A. (2016). Are changes in heart rate variability in middle-aged and older people normative or caused by pathological conditions? findings from a large population-based longitudinal cohort study. *J. Am. Heart Assoc.* 5:e002365.

Japundzic, N., Grichois, M. L., Zitoun, P., Laude, D., and Elghozi, J. L. (1990). Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J. Auton Nerv. Syst.* 30, 91–100. doi: 10.1016/0165-1838(90)90132-3

Jeevaratnam, K., Chadda, K. R., Salvage, S. C., Valli, H., Ahmad, S., Grace, A. A., et al. (2017). Ion channels, long QT syndrome and arrhythmogenesis in ageing. *Clin. Exp. Pharmacol. Physiol.* 44(Suppl. 1), 38–45. doi: 10.1111/1440-1681.12721

Jose, A. D., and Collison, D. (1970). The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc. Res.* 4, 160–167. doi: 10.1093/cvr/4.2.160

Jovanovic, A. (2006). Ageing, gender and cardiac sarcolemmal K(ATP) channels. *J. Pharm. Pharmacol.* 58, 1585–1589. doi: 10.1211/jpp.58.12.0004

Kalla, M., Herring, N., and Paterson, D. J. (2016). Cardiac sympatho-vagal balance and ventricular arrhythmia. *Auton. Neurosci.* 199, 29–37. doi: 10.1016/j.autneu.2016.08.016

Korkushko, O. V., Shatilo, V. B., and Kaukenas, J. K. (1991). Changes in heart rhythm power spectrum during human aging. *Aging* 3, 177–179. doi: 10.1007/bf03324001

La Rovere, M. T., Pinna, G. D., Maestri, R., Mortara, A., Capomolla, S., Febo, O., et al. (2003). Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation* 107, 565–570. doi: 10.1161/01.cir.0000047275.25795.17

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and Cardiac Vagal Tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213.

Lakatta, E. G., Zhou, Y. Y., Xiao, R. P., and Boluyt, M. (2001). "Aging of the cardiovascular system," in *Heart Physiology and Pathophysiology*, 4th Edn, ed. N. Sperelakis, (San Dieego, CA: Academic Press).

Manolio, T. A., Furberg, C. D., Rautaharju, P. M., Siscovick, D., Newman, A. B., Borhani, N. O., et al. (1994). Cardiac arrhythmias on 24-h ambulatory electrocardiography in older women and men: the Cardiovascular health study. *J. Am. Coll. Cardiol.* 23, 916–925. doi: 10.1016/0735-1097(94)90638-6

Monahan, K. D. (2007). Effect of aging on baroreflex function in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R3–R12.

Murman, D. L. (2015). The impact of age on cognition. *Semin. Hear.* 36, 111–121. doi: 10.1055/s-0035-1555115

No authors listed, (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European society of cardiology and the North American Society of pacing and electrophysiology. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Paolisso, G., Manzella, D., Barbieri, M., Rizzo, M. R., Gambardella, A., and Varricchio, M. (1999). Baseline heart rate variability in healthy centenarians: differences compared with aged subjects (>75 years old). *Clin. Sci.* 97, 579–584. doi: 10.1042/cs19990101

Peters, C. H., Sharpe, E. J., and Proenza, C. (2020). Cardiac pacemaker activity and aging. *Annu. Rev. Physiol.* 82, 21–43. doi: 10.1146/annurev-physiol-021119-034453

Pfeifer, M. A., Weinberg, C. R., Cook, D., Best, J. D., Reenan, A., and Halter, J. B. (1983). Differential changes of autonomic nervous system function with age in man. *Am. J. Med.* 75, 249–258.

Piccirillo, G., Bucca, C., Bauco, C., Cinti, A. M., Michele, D., Fimognari, F. L., et al. (1998). Power spectral analysis of heart rate in subjects over a hundred years old. *Int. J. Cardiol.* 63, 53–61. doi: 10.1016/s0167-5273(97)00282-9

Poller, U., Nedelka, G., Radke, J., Ponicke, K., and Brodde, O. E. (1997). Age-dependent changes in cardiac muscarinic receptor function in healthy volunteers. *J. Am. Coll. Cardiol.* 29, 187–193. doi: 10.1016/s0735-1097(96)00437-8

Reyes, del Paso, G. A., Hernandez, J. A., and Gonzalez, M. I. (2006). Differential evaluation of the baroreceptor cardiac reflex effectiveness as a function of sequence length. *Int. J. Psychophysiol.* 59, 91–96. doi: 10.1016/j.ijpsycho.2005.02.006

Reyes del Paso, G. A., Langewitz, W., Mulder, L. J., van Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Rossi, S., Fortunati, I., Carnevali, L., Baruffi, S., Mastorci, F., Trombini, M., et al. (2014). The effect of aging on the specialized conducting system: a telemetry ECG study in rats over a 6 month period. *PLoS One* 9:e112697. doi: 10.1371/journal.pone.0112697

Rowan, W. H., Campen, M. J., Wichers, L. B., and Watkinson, W. P. (2007). Heart rate variability in rodents: uses and caveats in toxicological studies. *Cardiovasc. Toxicol.* 7, 28–51. doi: 10.1007/s12012-007-0004-6

Sgoifo, A., Stilli, D., Medici, D., Gallo, P., Aimi, B., and Musso, E. (1996). Electrode positioning for reliable telemetry ECG recordings during social stress in unrestrained rats. *Physiol. Behav.* 60, 1397–1401. doi: 10.1016/s0031-9384(96)00228-4

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258.

Shen, M. J., and Zipes, D. P. (2014). Role of the autonomic nervous system in modulating cardiac arrhythmias. *Circ. Res.* 114, 1004–1021. doi: 10.1161/circresaha.113.302549

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Statello, R., Carnevali, L., Paterlini, S., Gioiosa, L., Bertocchi, I., Oberto, A., et al. (2017). Reduced NPY Y1 receptor hippocampal expression and signs of decreased vagal modulation of heart rate in mice. *Physiol. Behav.* 172, 31–39. doi: 10.1016/j.physbeh.2016.07.017

Surawicz, B. K., and Knilans, T. K. (2008). *Chou's Electrocardiograhy in Clinical Practice*, 6th Edn. Philadelphia: Saunders Elsevier.

Tan, C. O., Cohen, M. A., Eckberg, D. L., and Taylor, J. A. (2009). Fractal properties of human heart period variability: physiological and methodological implications. *J. Physiol.* 587(Pt 15), 3929–3941. doi: 10.1113/jphysiol.2009.169219

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thireau, J., Zhang, B. L., Poisson, D., and Babuty, D. (2008). Heart rate variability in mice: a theoretical and practical guide. *Exp. Physiol.* 93, 83–94. doi: 10.1113/expphysiol.2007.040733

Tsuji, H., Venditti, F. J., Manders, E. S., Evans, J. C., Larson, M. G., Feldman, C. L., et al. (1994). Reduced heart rate variability and mortality risk in an elderly cohort. the Framingham heart study. *Circulation* 90, 878–883. doi: 10.1161/01.cir.90.2.878

Umetani, K., Singer, D. H., McCraty, R., and Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J. Am. Coll. Cardiol.* 31, 593–601. doi: 10.1016/s0735-1097(97)00554-8

White, M., Roden, R., Minobe, W., Khan, M. F., Larrabee, P., Wollmering, M., et al. (1994). Age-related changes in beta-adrenergic neuroeffector systems in the human heart. *Circulation* 90, 1225–1238. doi: 10.1161/01.cir.90.3.1225

Winter, J., Tipton, M. J., and Shattock, M. J. (2018). Autonomic conflict exacerbates long QT associated ventricular arrhythmias. *J. Mol. Cell Cardiol.* 116, 145–154. doi: 10.1016/j.yjmcc.2018.02.001

Yaniv, Y., Ahmet, I., Tsutsui, K., Behar, J., Moen, J. M., Okamoto, Y., et al. (2016). Deterioration of autonomic neuronal receptor signaling and mechanisms intrinsic to heart pacemaker cells contribute to age-associated alterations in heart rate variability in vivo. *Aging Cell* 15, 716–724. doi: 10.1111/acel.12483

Yazdanyar, A., and Newman, A. B. (2009). The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. *Clin. Geriatr. Med.* 25, 563–577. doi: 10.1016/j.cger.2009.07.007

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 17 May 2021
doi: 10.3389/fnins.2021.600574

# Different Impact of Heart Rate Variability in the Deep Cerebral and Central Hemodynamics at Rest: An *in silico* Investigation

*Stefania Scarsoglio*[1]* *and Luca Ridolfi*[2]

[1] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy, [2] Department of Environmental, Land and Infrastructure Engineering, Politecnico di Torino, Torino, Italy

**Background:** Heart rate variability (HRV), defined as the variability between consecutive heartbeats, is a surrogate measure of cardiac vagal tone. It is widely accepted that a decreased HRV is associated to several risk factors and cardiovascular diseases. However, a possible association between HRV and altered cerebral hemodynamics is still debated, suffering from HRV short-term measures and the paucity of high-resolution deep cerebral data. We propose a computational approach to evaluate the deep cerebral and central hemodynamics subject to physiological alterations of HRV in an ideal young healthy patient at rest.

**Methods:** The cardiovascular-cerebral model is composed by electrical components able to reproduce the response of the different cardiovascular regions and their features. The model was validated over more than thirty studies and recently exploited to understand the hemodynamic mechanisms between cardiac arrythmia and cognitive deficit. Three configurations (baseline, increased HRV, and decreased HRV) are built based on the standard deviation (SDNN) of RR beats. For each configuration, 5,000 RR beats are simulated to investigate the occurrence of extreme values, alteration of the regular hemodynamics pattern, and variation of mean perfusion/pressure levels.

**Results:** In the cerebral circulation, our results show that HRV has overall a stronger impact on pressure than flow rate mean values but similarly alters pressure and flow rate in terms of extreme events. By comparing reduced and increased HRV, this latter induces a higher probability of altered mean and extreme values, and is therefore more detrimental at distal cerebral level. On the contrary, at central level a decreased HRV induces a higher cardiac effort without improving the mechano-contractile performance, thus overall reducing the heart efficiency.

**Conclusions:** Present results suggest that: (i) the increase of HRV *per se* does not seem to be sufficient to trigger a better cerebral hemodynamic response; (ii) by accounting for both central and cerebral circulations, the optimal HRV configuration is found at baseline.

OPEN ACCESS

**Edited by:**
*Sylvain Laborde, German Sport University Cologne, Germany*

**Reviewed by:**
*Dorota Zyśko, Wroclaw Medical University, Poland*
*Ingrid Tonhajzerova, Comenius University, Slovakia*

***Correspondence:**
*Stefania Scarsoglio*
*stefania.scarsoglio@polito.it*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

**Received:** *30 August 2020*
**Accepted:** *19 April 2021*
**Published:** *17 May 2021*

**Citation:**
*Scarsoglio S and Ridolfi L (2021) Different Impact of Heart Rate Variability in the Deep Cerebral and Central Hemodynamics at Rest: An in silico Investigation. Front. Neurosci. 15:600574. doi: 10.3389/fnins.2021.600574*

Given the relation inversely linking HRV and HR, the presence of this optimal condition can contribute to explain why the mean HR of the general population settles around the baseline value (70 bpm).

**Keywords: heart rate variability, cardiovascular modeling, cerebral circulation, computational hemodynamics, time-series analysis**

## 1. INTRODUCTION

Heart rate variability (HRV), defined as the variability between successive RR heartbeats, has grown as *hot topic*, with a simple "Heart rate variability" topic search currently listing more than 44,000 results on Web of Science. The great interest elicited within the scientific community ranges from psychophysiology (Laborde et al., 2017) to exercise (Michael et al., 2017), cardiovascular risk factors (Tsuji et al., 1996), and chronic fatigue syndrome (Meeus et al., 2013). This increasing and wide interest is also due to the fact that HRV measurements are easy to perform, quite reliable, and non-invasive. In particular, HRV can be considered a surrogate measure of the cardiac vagal tone (Laborde et al., 2017), although there are controversial aspects in using HRV to determine cardiac vagal tone independently from other factors, such as age, physical condition, and the presence of cardiac pathologies (Boyett et al., 2019). In this paper, we refer to the parasympathetic activity within cardiac regulation as cardiac vagal tone. HRV parameters in time and frequency domain able to reflect cardiac vagal tone (Malik et al., 1996; Berntson et al., 1997; Laborde et al., 2017) show that an increased cardiac vagal tone is associated to a higher HRV, while a reduction of the cardiac vagal tone response is linked to a HRV reduction (Hayano et al., 1991; Singh et al., 2018). Moreover, it has recently being recognized that HRV (as measured by means of different metrics, such as SDNN, RMSSD, pvRSA, and HF), inversely correlates with the heart rate (HR), and this relation should be taken into account when dealing with increased/reduced HRV (Monfredi et al., 2014; Shaffer and Ginsberg, 2017; Boyett et al., 2019; de Geus et al., 2019). The inverse relation between HR and HRV can be explained considering that a faster HR reduces the interval between successive beats and the opportunity for the RR beating to vary, lowering the HRV. On the contrary, a slower HR increases the cardiac interval and enhances the chance for RR to vary, raising HRV (Shaffer and Ginsberg, 2017).

From an overall cardiovascular point of view, a HRV increase in the physiological range seems beneficial at rest, while a reduced HRV is symptomatic of a stress action, can be predictor of cardiovascular risk factors and is correlated to higher morbidity and mortality (Tsuji et al., 1996; Dekker et al., 2000; Meeus et al., 2013). Thus, the clinical interest has been mainly focused on the prognostic significance of HRV related to the risk factors of cardiovascular pathologies. There is substantial evidence that a decreased HRV enhances several risk factors and is associated to cardiovascular diseases, such as left ventricle hypertrophy (Acharya et al., 2006), sudden cardiac death (Sessa et al., 2018), and myocardial infarction (Buccelletti et al., 2009). On the contrary, lower risk factor profiles are associated with increased HRV (Thayer et al., 2009b). The HRV scenario is opposite under physical effort: for increasing moderate-to-vigorous exercise intensity HRV decreases (Tulppo et al., 1998; Michael et al., 2017). During exercise a HRV decrease is beneficial and forced by an increased HR, however there is an immediate post-exercise recovery of HRV (Michael et al., 2017) and exercise training can increase resting HRV (Billman and Kukielka, 2006).

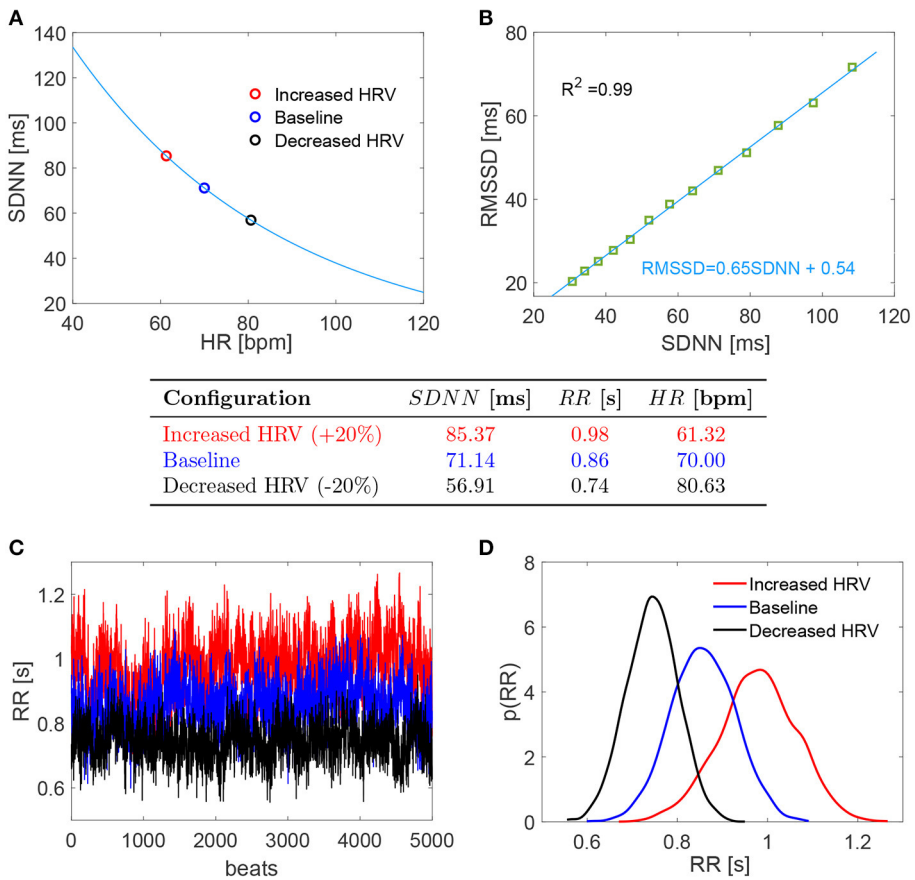
Although the overall HRV impact on the central hemodynamics is quite clear, especially related to cardiac disease conditions, its influence on the cerebral hemodynamics is barely known so far. In fact, definitive association between HRV and cerebral circulation is still missing and mainly involve pathologic conditions, such as chronic fatigue syndrome (Meeus et al., 2013; Boissoneault et al., 2019) and stroke (Fyfe-Johnson et al., 2016). Attention has recently grown about the possible association between increased HRV and improved cognitive performance. Results are conflicting, as some studies have demonstrated the association (Kim et al., 2006; Shah et al., 2011; Schaich et al., 2020), while some others observed that a reduced HRV does not contribute to cognitive impairment or even dementia (Allan et al., 2005; Britton et al., 2008; Mahinrad et al., 2016; Hazzouri et al., 2017). To understand this controversy it should be kept in mind that, among many influencing factors, such as the sample and the objectives of the study, most of the literature is based on short term measures, which have lower prognostic value than 24-h HRV (Malik et al., 1996; Nunan et al., 2010; Shaffer and Ginsberg, 2017).

Though a possible association is widely debated, the underlying hemodynamic mechanisms are so far only hypothesized and mostly unclear (Schaich et al., 2020). It is presently unknown whether HRV is able to influence the normal pressure and flow rate pattern at distal-capillary level, to alter the mean perfusion and pressure levels, and to enhance the occurrence of extreme values. Detailed information are lacking since current non-invasive techniques, such as transcranial doppler ultrasonography, are not able to offer high-resolution data in terms of pressure and flow rate beyond the circle of Willis. Therefore, in the deep cerebral circulation hemodynamic measurements are unreliable or almost absent. Thus, it can be of great significance understanding and predicting from a computational point of view how deep cerebral hemodynamics is affected by HRV at rest. Despite the intrinsic limits, computational hemodynamics and cardiovascular modeling are becoming increasingly important to isolate specific mechanisms and investigate processes where clinical data are not yet feasible, accurate, or easily measurable.

We propose an *in-silico* study where, through a validated combined cardiovascular-cerebral model, we evaluated the deep cerebral hemodynamics subject to physiological alterations of HRV in an ideal young healthy patient at rest, thereby comparing

the cerebral to central hemodynamics responses to HRV changes. With deep cerebral circulation we refer to the microcirculation beyond the circle of Willis (i.e., distal and capillary-venous circulation), while the set of parameters defined through cardiac variables and central arterial pressure characterizes the central hemodynamics. The complete lumped-parameter model is composed by a suitable combination of electrical counterparts (resistances, compliances/elastances, inertances), accounting for the arterial and venous circuits of both systemic and pulmonary circulations, an active representation of the four cardiac chambers, an accurate valve motion description, and a short-term baroreceptor mechanism. The cerebral circulation is divided into three main regions: large arteries, distal arterial circulation, and capillary/venous circulation. Cerebrovascular control mechanisms of autoregulation and $CO_2$ reactivity are taken into account. The present computational approach has been validated and recently exploited in a wide area of applications, such as the hemodynamic response to exercise in atrial fibrillation (Anselmino et al., 2017), the linking mechanisms between cardiac arrythmia and cognitive impairment (Anselmino et al., 2016; Scarsoglio et al., 2017a,b; Saglietto et al., 2019), and the cardiovascular deconditioning emerging in altered gravity conditions (Gallo et al., 2020).

We considered three HRV configurations (baseline, increased HRV, decreased HRV), each of them analyzed over 5,000 RR beats, so that our results are not affected by transient behavior and are statistically significant and stable. HRV variations were assessed through SDNN, which is the standard deviation of all RR beats and allowed us to define in a straightforward and univocal way the RR stochastic beating extraction. The model is able to provide the whole pressure and flow rate time-series from proximal to distal circulation as well as beat-to-beat values. The focus was on the possible occurrence of extreme values, such as hypertensive events, alteration of the regular hemodynamics pattern, and variation of mean perfusion/pressure levels. All these aspects are to date mostly unexplored, but extremely useful to understand the role of HRV on the cerebral circulation and how, in turn, the hemodynamic alteration can impact the cognitive sphere. The present work can offer precious insights by: (i) isolating the net basic mechanisms induced by HRV changes into the cerebral circulation in healthy resting conditions, and (ii) quantifying how differently HRV acts on the cerebral hemodynamics with respect to the central hemodynamics,




| Configuration | $SDNN$ [ms] | $RR$ [s] | $HR$ [bpm] |
|---|---|---|---|
| Increased HRV (+20%) | 85.37 | 0.98 | 61.32 |
| Baseline | 71.14 | 0.86 | 70.00 |
| Decreased HRV (-20%) | 56.91 | 0.74 | 80.63 |

**FIGURE 1 |** (Top) **(A,B)** Relation SDNN (HR) and RMSSD (SDNN). (Middle) Table with the chosen values of the three configurations. (Bottom) **(C,D)** RR series and probability density functions of the three configurations. Red: increased HRV; blue: baseline; black: decreased HRV.

thus fostering necessary future clinical measurements within this topic.

## 2. MATERIALS AND METHODS

### 2.1. HRV Configurations

We considered three HRV configurations: baseline condition (reported in the following sections with blue color), increased HRV (in red color) and decreased HRV (in black color). To assess HRV variations we adopted the simplest HRV metric, i.e., SDNN—which is the standard deviation of all RR beats. SDNN belongs to the time-domain analyses, which are computationally simpler and easier to apply than frequency-domain analyses (Michael et al., 2017). Since we focused on resting configurations lasting hours, we chose SDNN because it is the gold standard for long-term measurements (Malik et al., 1996; Shaffer and Ginsberg, 2017). Moreover, the SDNN adoption allows us to define in a straightforward and univocal way the RR beating extraction. In terms of SDNN, HRV was found to be dependent on the circadian day/night cycle (Shaffer and Ginsberg, 2017), e.g., 93.06 (ms) day, 121.31 (ms) night, 101.71 (ms) 24-h (Talib et al., 2005)—and to decrease with age in adulthood (van den Berg et al., 2018). In general, SDNN recording was lower in resting supine condition [65 (ms) (Lehavi et al., 2019); 49 (ms) (Nunan et al., 2010)] than in non-resting or upright position [e.g., 93.06 (ms) (Talib et al., 2005); 127 (ms) (Genovesi et al., 2007)]. It was recently observed that HRV inversely correlates with HR through an exponential function (Monfredi et al., 2014; Kazmi et al., 2016; Shaffer and Ginsberg, 2017; van den Berg et al., 2018; de Geus et al., 2019), and this relation should be taken into account when dealing with increased/reduced HRV.

To consider SDNN for a healthy young person in resting supine condition during day (awake) and account for the interplay between SDNN and HR, we adopted the exponential relation obtained by de Geus et al. (2019) in leisure baseline condition:

$$\mathrm{SDNN} = 309.4\mathrm{e}^{-0.021\mathrm{HR}} \tag{1}$$

The baseline configuration was set at HR = 70 bpm and SDNN = 71.14 (ms) was obtained from the above relation. Recalling that the beating period is RR = 60/HR (s), we define the coefficient of variation as $c_v$ = SDNN/RR. The resulting $c_v$ is

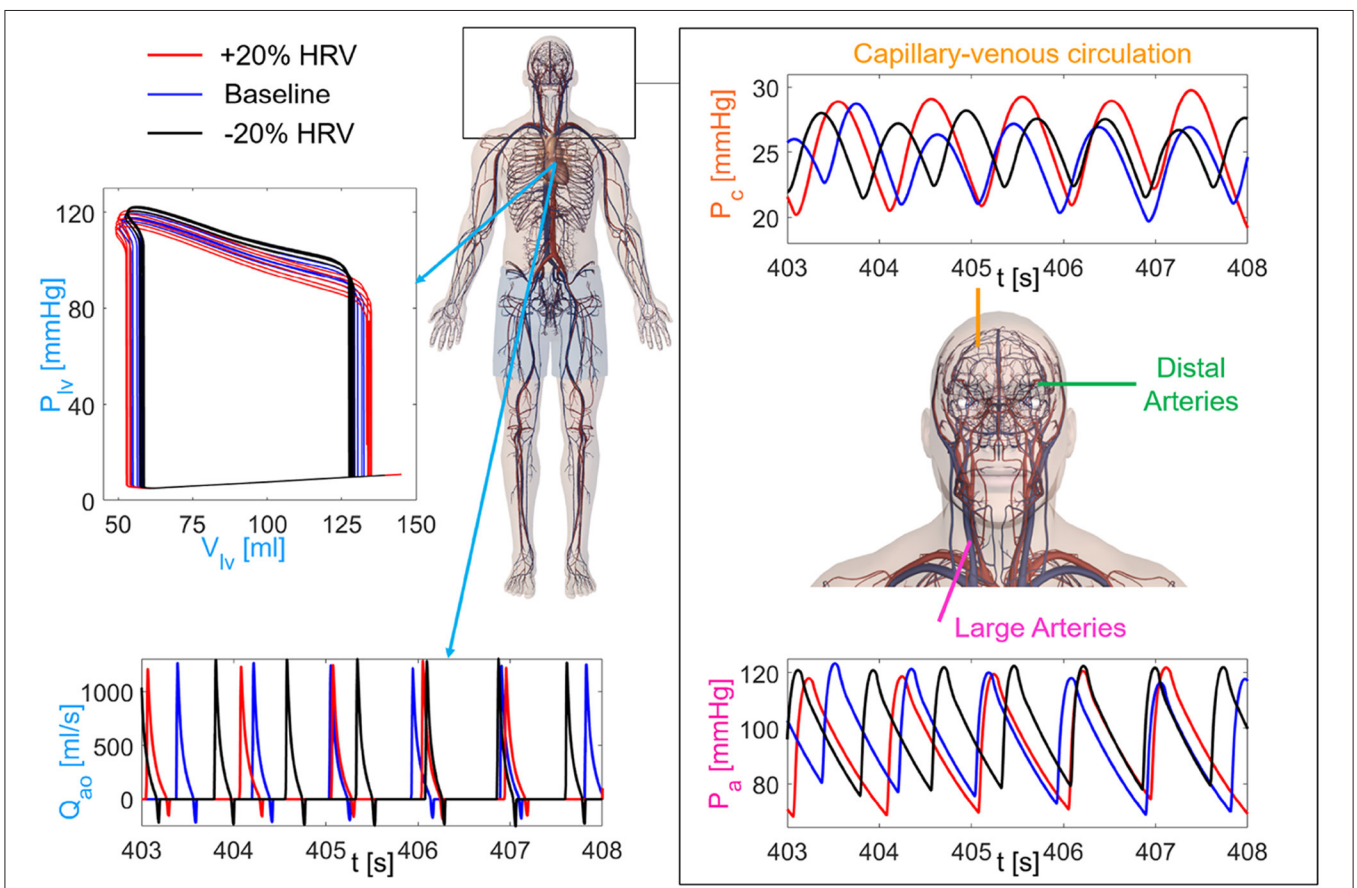



**FIGURE 2 |** Sketch of the cardiovascular-cerebral model for the three HRV configurations (red: increased HRV, blue: baseline, black: decreased HRV). (Left) Central and systemic-pulmonary circulation with representative time-series of the aortic flow rate ($Q_{ao}$) and left ventricle PV loops (5 s length). (Right) Cerebral circulation with the three main regions (large arteries, distal arteries, capillary-venous circulation), together with examples of the systemic arterial pressure, $P_a$ (Bottom), and cerebral capillary pressure, $P_c$ (Top), time-series.

0.08 which well represents normal sinus rhythm daily values in resting supine condition [e.g., $c_v = 0.05$ (Pikkujämsä et al., 2001); $c_v = 0.07$ (Lehavi et al., 2019); $c_v = 0.06$ (Nussinovitch et al., 2011)]. Moreover, $c_v = 0.08$ as baseline value is close to the one adopted by our group ($c_v = 0.07$) to simulate resting supine sinus rhythm (Scarsoglio et al., 2014). Increased and decreased HRV configurations were achieved by changing SDNN by +20 and −20%, respectively. This threshold represents a significant variation within a range of physiological values. The corresponding HRs were individuated again using Equation (1) as proposed by de Geus et al. (2019). **Figure 1A** reports the SDNN(HR) curve in resting condition introduced by de Geus et al. (2019) with the values here chosen and below a summarizing table of the three cases.

For each configuration, we evaluated 5,000 RR beats to have statistically significant and stationary results. RR beatings were extracted from an *in silico* pink-correlated Gaussian distribution, which well reproduces the typical beating features of normal sinus rhythm recorded *in vivo* (Hayano et al., 1997; Pikkujämsä et al., 2001; Hennig et al., 2006; Scarsoglio et al., 2014), having mean and standard deviation values identified as above. In the range SDNN ∈ [30, 100] ms, we also evaluated the RMSSD (i.e., the root mean square of successive RR interval differences) for the RR series extracted with the adopted time-correlation features and composed by $10^8$ beats. A high linear correlation value ($R^2 = 0.99$) was found between RMSSD and SDNN, with fitting law RMSSD = 0.65 SDNN + 0.54, as reported in **Figure 1B**. Although RMSSD and SDNN don't have the same physiological origin and only RMSSD can be assumed to fully reflect cardiac vagal activity (Malik et al., 1996; Berntson et al., 1997; Laborde et al., 2017), SDNN is a reliable proxy of RMSSD for the considered RR time series. Bottom panels of **Figure 1** display the RR series (panel C) and the corresponding probability density functions (PDFs) for the three HRV cases here investigated (panel D).

## 2.2. Cardiovascular and Cerebral Circulation Modeling

Following RR extraction, the cardiovascular-cerebral model was run to obtain the hemodynamic cerebral signals. The complete lumped model is composed by electrical components able to reproduce the response of the different (cardiac and vascular) regions and their features, in terms of resistance $R$ (diffusive effects), inertance $L$ (inertial effects), and compliance $C$ or elastance $E$ (elasticity/contractility effects). The cardiovascular dynamics includes the arterial and venous circuits of both systemic and pulmonary circulations, an active representation of the four cardiac chambers, and an accurate valve motion

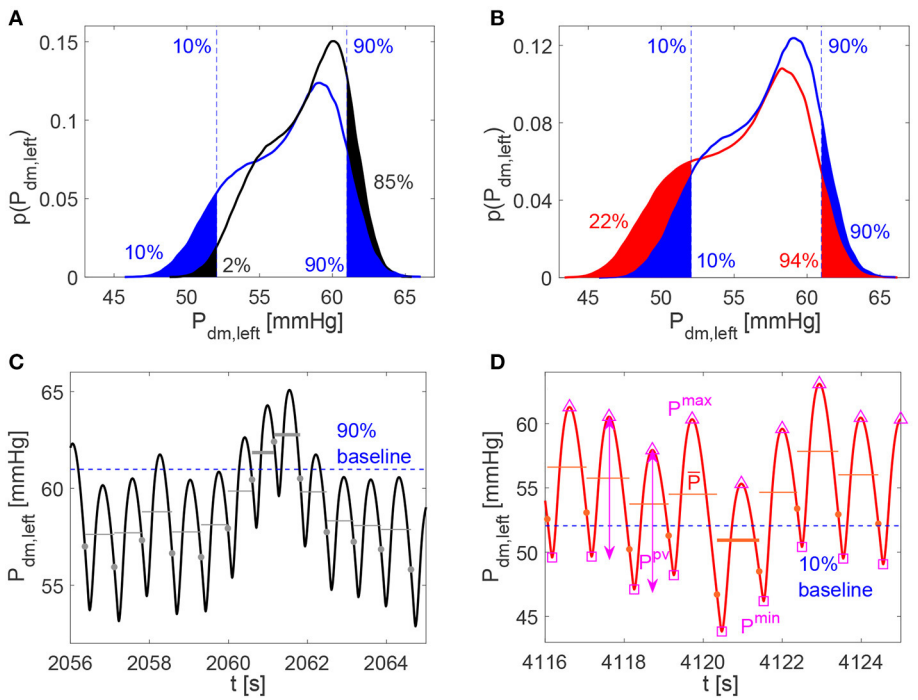



**FIGURE 3 | (A,B)** Examples of percentile evaluation for $P_{dm,left}$ ($p(P_{dm,left})$ is the probability density function). Ten and 90% blue dashed lines individuate the 10th and 90th percentiles in the baseline configuration (**A,B**, blue areas), while they correspond to the 2nd and 85th percentiles in the decreased HRV configuration (**A**, black areas), and to the 22nd and 94th percentiles in the increased HRV configuration (**B**, red areas). **(C,D)** $P_{dm,left}$ examples of hypertensive event lasting two beats in the decreased HRV configuration (average pressure per beat, $\overline{P}$, is represented by gray horizontal lines and hypertensive events are marked with bold gray lines) and hypotensive event in the increased HRV configuration ($\overline{P}$ is represented by red horizontal lines and hypothensive events are marked with bold red lines). The 10th and 90th percentile baseline thresholds are displayed through the dashed blue horizontal lines, while full circles indicate the heartbeat extremes. In **(D)** examples of $P^{max}$ (△), $P^{min}$ (□), and $P^{pv}$ (↔) values per beat are evidenced for $P_{dm,left}$.

description. A short-term baroreceptor mechanism is also modeled, accounting for the inotropic effect of both ventricles, as well as the control of the systemic vasculature (peripheral arterial resistances, unstressed volume of the venous system, and venous compliance). The chronotropic effects due to the heart rate regulation are intrinsically taken into account by the RR extraction. The cerebral circulation is divided into three principal regions: large arteries, distal arterial circulation, and capillary/venous circulation. Cerebrovascular control mechanisms of autoregulation and $CO_2$ reactivity are taken into account. The model is expressed in terms of pressures, $P$, flow rates, $Q$, volumes, $V$, and valve opening angles, $\theta$.

The cardiovascular model has been proposed and validated over more than thirty studies to check the consistency of the hemodynamic response during AF: extensive details of this evaluation are reported in Scarsoglio et al. (2014). Then, the model has been exploited to study the impact of atrial fibrillation on the cardiovascular system (Anselmino et al., 2015, 2017; Scarsoglio et al., 2016a,b). The complete cardiovascular-cerebral model has been used to understand the hemodynamic mechanisms between atrial fibrillation and cognitive deficit (Anselmino et al., 2016; Scarsoglio et al., 2017a,b; Saglietto et al., 2019). A full description of the governing equations and model parameters is given in the **Supplementary Material**.

We here focused—in terms of pressure $P$ and flow rate $Q$—on the left internal carotid artery-middle cerebral artery (ICA-MCA) pathway, which already turned out to be representative of the hemodynamics from proximal to distal cerebral districts (Anselmino et al., 2016; Scarsoglio et al., 2017a,b; Saglietto et al., 2019). The left ICA-MCA path starts at the internal carotid level ($P_a$: systemic arterial pressure; $Q_{ICA,left}$: left internal carotid flow rate), goes through the middle cerebral artery ($P_{MCA,left}$: left middle cerebral artery pressure; $Q_{MCA,left}$: left middle cerebral artery flow rate), includes the middle distal regions ($P_{dm,left}$: left middle distal pressure; $Q_{dm,left}$: left middle distal flow rate), and ends with capillary-venous districts ($P_c$: cerebral capillary pressure; $Q_{pv}$: proximal venous flow rate). A schematic representation of the systemic-pulmonary circulation and ICA-MCA pathway is reported in **Figure 2**, together with examples of left ventricle PV loops, aortic flow rate ($Q_{ao}$), systemic arterial pressure ($P_a$), and cerebral capillary pressure ($P_c$) time-series for different HRV configurations.

## 2.3. Variable Definition and Data Analysis

We recall the definition of mechano-energetic and oxygen consumption indexes, which will be used to describe the central hemodynamics. End-systolic left ventricular volume, $V_{lves}$ [ml], is the left ventricle volume at the closure of the aortic valve, while end-diastolic left ventricular volume, $V_{lved}$ [ml], corresponds to the closure of the mitral valve. Stroke volume is defined as $SV = (V_{lved} - V_{lves})$ [ml], ejection fraction is $EF = V/V_{lved} \cdot 100$ [%]. Cardiac output is $CO = SV \cdot HR$ [l/min], stroke work per minute, $SW/min$ [J/min], is measured as the area within the left ventricle pressure-volume loop per beat. Oxygen consumption is evaluated through three estimates (Westerhof et al., 2010): (i) the rate pressure product, $RPP = P_{a,syst} \cdot HR$ [mmHg/min], where $P_{a,syst}$ is the aortic systolic pressure; (ii) the tension time index per minute, $TTI/min = \overline{P}_{lv} \cdot RR \cdot HR$ [mmHg s/min], where the symbol $\overline{f}$ indicates the mean value of the generic hemodynamic variable $f$ averaged over a RR beat (in this case $f = P_{lv}$); and (iii) the pressure volume area per minute, $PVA/min = (PE+SW) \cdot HR$, where $PE = [P_{lves} \cdot (V_{lves} - V_{lv,un})/2 - P_{lved} \cdot (V_{lved} - V_{lv,un})/4]$ is the elastic potential energy ($V_{lv,un}$ = 5 ml is the unstressed left ventricle volume), $SW$ is the stroke work, $P_{lves}$ and $P_{lved}$ are the end-systolic and end-diastolic left ventricle pressures. The left ventricular efficiency $LVE$ is defined by the ratio $SW/PVA$.

The cerebral hemodynamics is assessed by means of two main approaches: (i) analysis of the continuous time-series, where the signal is continuous and defined by all the temporal instants of the whole time-series, and (ii) beat-to-beat analysis, where the signal was discretized and one-per-beat data were obtained. In so doing, beat-to-beat signals are composed by 5,000 values, corresponding to the 5,000 RR beats simulated.

For the analysis of the continuous time-series, extremely high or low cerebral hemodynamic values were evaluated through the percentile analysis of the hemodynamic signals. We adapted the definition used for studying atrial fibrillation (Anselmino et al., 2016; Saglietto et al., 2019), to assess the possibility of extreme events related to HRV changes. For the increased and decreased HRV cases, we estimated to which percentile the baseline (10th and 90th) reference thresholds

**TABLE 1 |** Basic statistics (mean: $\mu$, standard deviation: $\sigma$, coefficient of variation: $c_v$) of the continuous time-series along the ICA-MCA pathway: $P_a$, $Q_{ICA,left}$, $P_{MCA,left}$, $Q_{MCA,left}$, $P_{dm,left}$, $Q_{dm,left}$, $P_c$, and $Q_{pv}$.

| Configuration | Pressure P [mmHg] | | | Flow rate Q [ml/s] | | |
|---|---|---|---|---|---|---|
| | $\mu$ | $\sigma$ | $c_v$ | $\mu$ | $\sigma$ | $c_v$ |
| | $P_a$ | | | $Q_{ICA,left}$ | | |
| Decreased HRV (−20%) | 99.30 | 13.40 | 0.13 | 4.74 | 1.68 | 0.35 |
| Baseline | 96.91 | 14.42 | 0.15 | 4.73 | 1.83 | 0.39 |
| Increased HRV (+20%) | 94.41 | 15.40 | 0.16 | 4.72 | 1.99 | 0.42 |
| | $P_{MCA,left}$ | | | $Q_{MCA,left}$ | | |
| Decreased HRV (−20%) | 96.60 | 12.51 | 0.13 | 3.75 | 1.22 | 0.33 |
| Baseline | 94.22 | 13.44 | 0.14 | 3.74 | 1.35 | 0.36 |
| Increased HRV (+20%) | 91.72 | 14.32 | 0.16 | 3.72 | 1.48 | 0.40 |
| | $P_{dm,left}$ | | | $Q_{dm,left}$ | | |
| Decreased HRV (−20%) | 58.11 | 2.79 | 0.05 | 3.75 | 0.43 | 0.11 |
| Baseline | 56.91 | 3.38 | 0.06 | 3.74 | 0.51 | 0.14 |
| Increased HRV (+20%) | 55.66 | 3.97 | 0.07 | 3.72 | 0.60 | 0.16 |
| | $P_c$ | | | $Q_{pv}$ | | |
| Decreased HRV (−20%) | 25.02 | 1.85 | 0.07 | 12.49 | 1.52 | 0.12 |
| Baseline | 25.00 | 2.33 | 0.09 | 12.46 | 1.87 | 0.15 |
| Increased HRV (+20%) | 24.97 | 2.85 | 0.11 | 12.43 | 2.24 | 0.18 |

correspond, by quantifying the probability of assuming rare values. Top panels of **Figures 3A,B** provide a representative graphical representation of the percentile analysis for $P_{dm,left}$. The 10th and 90th percentiles individuated in baseline correspond to the 2nd and 85th percentiles in the decreased HRV case (panel A), while the 10th and 90th baseline percentiles correspond to the 22nd and 94th percentiles in the increased HRV case (panel B). In so doing, we quantify whether HRV changes

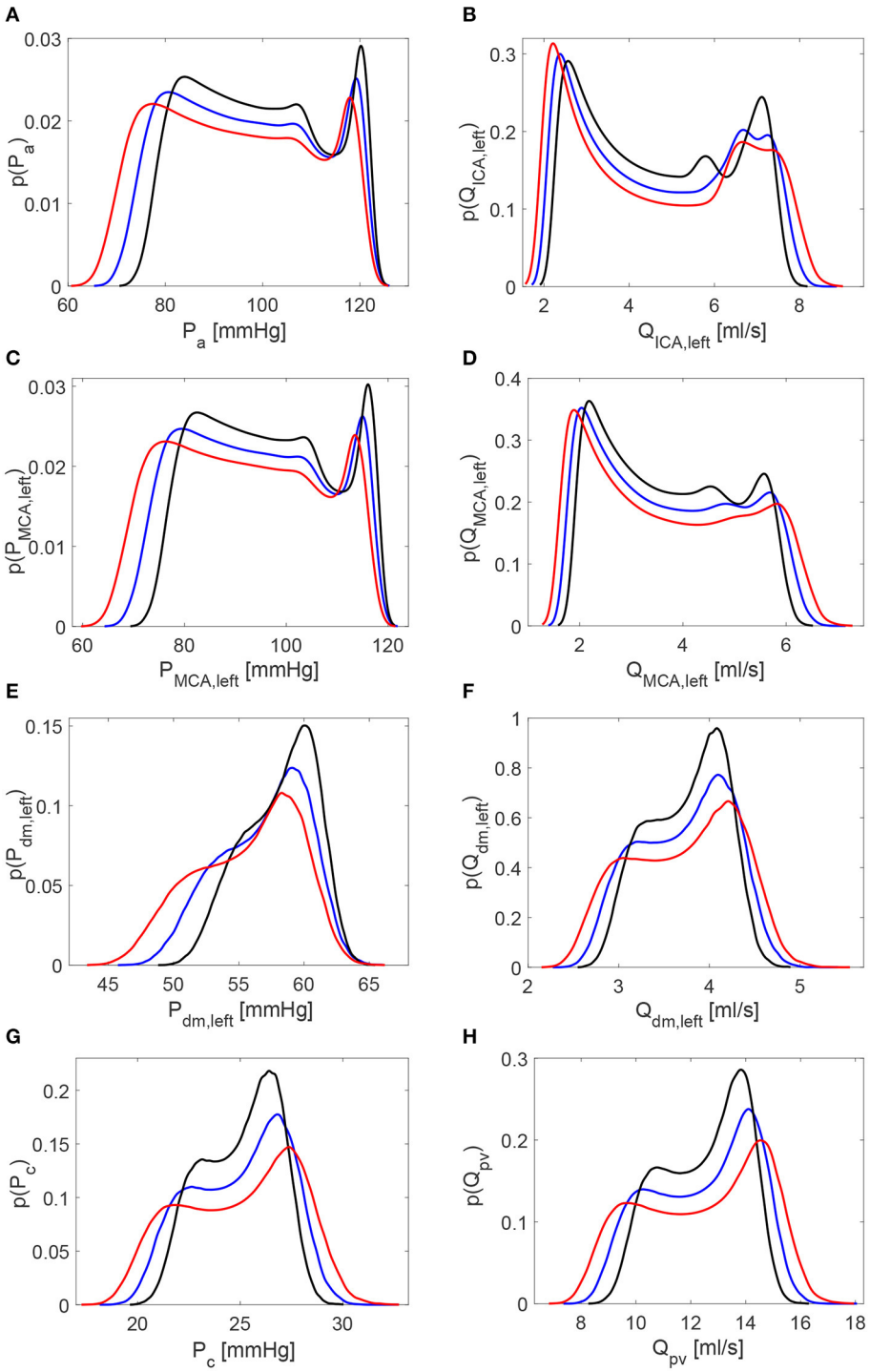



**FIGURE 4 |** Probability density functions of the continuous hemodynamic time-series along the ICA-MCA pathway. **(A,C,E,G)** Pressures. **(B,D,F,H)** Flow rates. Black: decreased HRV; blue: baseline; red: increased HRV.

are able to make extreme values more frequently reached with respect to the baseline case (e.g., in **Figure 3B** the 10th baseline percentile corresponds to the 22nd percentile for increased HRV).

For the beat-to-beat analysis, the $i$-th element of the discretized series may contain the average value ($\overline{Q}$ and $\overline{P}$), the maximum ($Q^{max}$ and $P^{max}$) and minimum ($Q^{min}$ and $P^{min}$) values, as well as the pulse value ($Q^{pv}$ and $P^{pv}$, defined as the difference between maximum and minimum values) of the related hemodynamic variables computed over the $i$-th beat. In **Figure 3D** examples of $\overline{P}$, $P^{max}$, $P^{min}$, and $P^{pv}$ are represented for $P_{dm,left}$.

The average values per beat, $\overline{P}$ and $\overline{Q}$, were still exploited to enrich the beat-to-beat analysis, by accounting for the persistence of extreme values over the whole beat (Anselmino et al., 2016; Saglietto et al., 2019), and not only the occurrence of instantaneous peak values. We introduce hypoperfusions (or hypotensive events), which take place when the mean flow rate per beat $\overline{Q}$ (or mean pressure per beat $\overline{P}$) is below the 10th percentile referred to the whole baseline signal. On the contrary, hyperperfusions (or hypertensive events) emerge when $\overline{Q}$ (or $\overline{P}$) is above the 90th percentile of the baseline condition. Note that, by definition, extreme events cannot emerge in the baseline configuration. Bottom panels of **Figures 3C,D** show examples of hypertensive and hypotensive events for $P_{dm,left}$.

# 3. RESULTS

## 3.1. Continuous *P* and *Q* Time-Series Analysis

**Table 1** reports the main statistics (mean $\mu$, standard deviation $\sigma$, and coefficient of variation $c_v$) of the continuous time-series of the hemodynamic variables along the ICA-MCA pathway. **Figure 4** shows probability density functions (PDFs) of the whole hemodynamic time-series for the three configurations. Considering the continuous hemodynamic signals, HRV influenced much more pressure than flow rate series. In fact, mean values were overall maintained (see **Table 1**) and PDFs revealed self-similar features (see **Figure 4**) for flow rates $Q$, while HRV significantly affected mean values and PDFs of pressures $P$ (only $P_c$ at the end of the pathway regained a self-similar shape for the three configurations). At a given district, HRV impacted $\sigma$ relative variations with respect to baseline similarly for both pressure and flow rate, while $c_v$ relative variations induced by HRV with respect to baseline were more evident toward the distal-capillary circulation.

It is then useful to evaluate how extreme values of the continuous time-series $P(t)$ and $Q(t)$ distribute as HRV changes, by considering the percentile analysis (as represented in **Figures 3A,B**). By assessing to which percentile the baseline reference thresholds correspond in the configurations with altered HRV, we can quantify whether HRV is able to modify the probability of reaching extremely high/low values (see **Figure 5**).

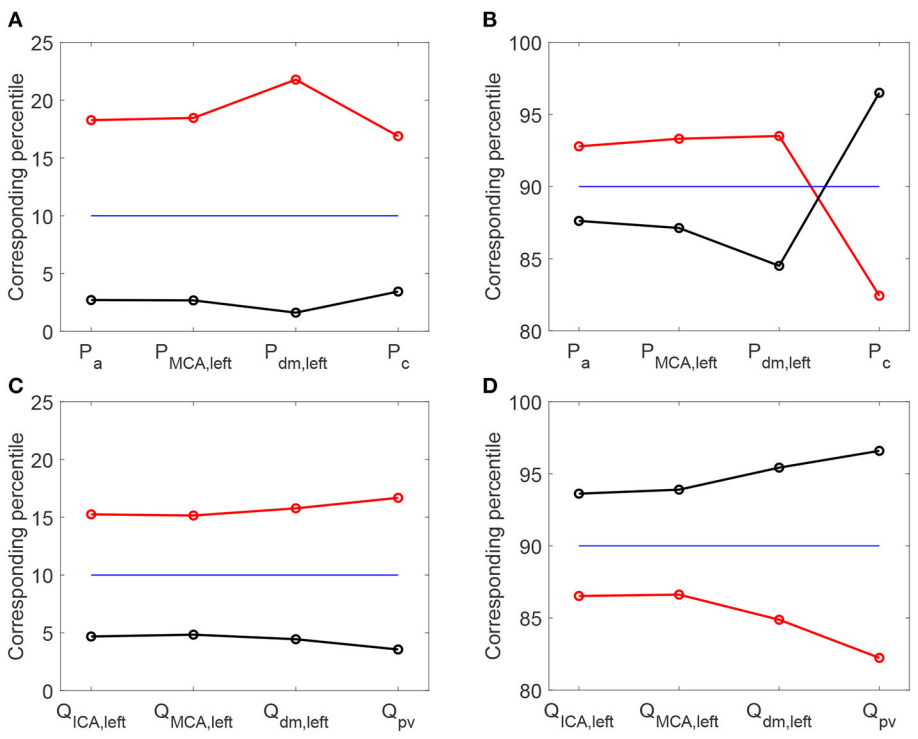



**FIGURE 5 |** Percentile values in increased (red) and decreased (black) HRV to which the 10th **(A,C)** and 90th **(B,D)** percentiles in baseline configuration correspond. Pressure **(A,B)** and flow rate **(C,D)** along the ICA-MCA pathway.

**TABLE 2 |** Mean and standard deviation (in brackets) values for the minimum ($P^{min}$ and $Q^{min}$), average ($\overline{P}$ and $\overline{Q}$), maximum ($P^{max}$ and $Q^{max}$), and pulse ($P^{pv}$ and $Q^{pv}$) values per beat of $P$ and $Q$ along the ICA-MCA pathway: $P_a$, $Q_{ICA,left}$, $P_{MCA,left}$, $Q_{MCA,left}$, $P_{dm,left}$, $Q_{dm,left}$, $P_c$, and $Q_{pv}$.

| Configuration | Min value | Mean value | Max value | Pulse value |
|---|---|---|---|---|
| | $P_a^{min}$ | $\overline{P}_a$ | $P_a^{max}$ | $P_a^{pv}$ |
| Decreased HRV (−20%) | 78.43 (2.65) | 99.42 (1.86) | 121.26 (1.36) | 42.82 (1.56) |
| Baseline | 74.52 (3.02) | 97.06 (2.19) | 120.45 (1.64) | 45.93 (1.72) |
| Increased HRV (+20%) | 70.63 (3.31) | 94.59 (2.47) | 119.54 (1.82) | 48.91 (1.89) |
| | $P_{MCA,left}^{min}$ | $\overline{P}_{MCA,left}$ | $P_{MCA,left}^{max}$ | $P_{MCA,left}^{pv}$ |
| Decreased HRV (−20%) | 77.08 (2.58) | 96.71 (1.83) | 117.11 (1.36) | 40.03 (1.44) |
| Baseline | 73.27 (2.94) | 94.36 (2.16) | 116.16 (1.63) | 42.89 (1.58) |
| Increased HRV (+20%) | 69.48 (3.24) | 91.90 (2.43) | 115.11 (1.81) | 45.63 (1.73) |
| | $P_{dm,left}^{min}$ | $\overline{P}_{dm,left}$ | $P_{dm,left}^{max}$ | $P_{dm,left}^{pv}$ |
| Decreased HRV (−20%) | 53.72 (1.56) | 58.16 (1.30) | 61.04 (1.25) | 7.33 (0.82) |
| Baseline | 51.59 (1.84) | 56.97 (1.52) | 60.54 (1.50) | 8.95 (1.02) |
| Increased HRV (+20%) | 49.34 (2.05) | 55.73 (1.65) | 60.05 (1.61) | 10.71 (1.21) |
| | $P_c^{min}$ | $\overline{P}_c$ | $P_c^{max}$ | $P_c^{pv}$ |
| Decreased HRV (−20%) | 22.05 (0.74) | 25.03 (0.66) | 27.12 (0.78) | 5.07 (0.64) |
| Baseline | 21.29 (0.90) | 25.01 (0.77) | 27.70 (0.93) | 6.41 (0.81) |
| Increased HRV (+20%) | 20.45 (0.99) | 24.98 (0.83) | 28.37 (1.07) | 7.92 (0.99) |
| | $Q_{ICA,left}^{min}$ | $\overline{Q}_{ICA,left}$ | $Q_{ICA,left}^{max}$ | $Q_{ICA,left}^{pv}$ |
| Decreased HRV (−20%) | 2.32 (0.16) | 4.75 (0.14) | 7.30 (0.24) | 4.99 (0.23) |
| Baseline | 2.14 (0.15) | 4.74 (0.17) | 7.53 (0.28) | 5.40 (0.29) |
| Increased HRV (+20%) | 1.98 (0.15) | 4.73 (0.18) | 7.80 (0.33) | 5.81 (0.34) |
| | $Q_{MCA,left}^{min}$ | $\overline{Q}_{MCA,left}$ | $Q_{MCA,left}^{max}$ | $Q_{MCA,left}^{pv}$ |
| Decreased HRV (−20%) | 1.96 (0.13) | 3.75 (0.13) | 5.76 (0.21) | 3.81 (0.20) |
| Baseline | 1.81 (0.14) | 3.74 (0.15) | 5.96 (0.26) | 4.16 (0.25) |
| Increased HRV (+20%) | 1.66 (0.14) | 3.73 (0.16) | 6.17 (0.29) | 4.51 (0.29) |
| | $Q_{dm,left}^{min}$ | $\overline{Q}_{dm,left}$ | $Q_{dm,left}^{max}$ | $Q_{dm,left}^{pv}$ |
| Decreased HRV (−20%) | 3.06 (0.17) | 3.75 (0.15) | 4.23 (0.18) | 1.17 (0.13) |
| Baseline | 2.91 (0.19) | 3.74 (0.18) | 4.33 (0.22) | 1.42 (0.16) |
| Increased HRV (+20%) | 2.75 (0.20) | 3.73 (0.19) | 4.44 (0.24) | 1.68 (0.19) |
| | $Q_{pv}^{min}$ | $\overline{Q}_{pv}$ | $Q_{pv}^{max}$ | $Q_{pv}^{pv}$ |
| Decreased HRV (−20%) | 9.98 (0.52) | 12.49 (0.43) | 14.26 (0.53) | 4.28 (0.46) |
| Baseline | 9.41 (0.62) | 12.47 (0.48) | 14.70 (0.60) | 5.29 (0.56) |
| Increased HRV (+20%) | 8.82 (0.66) | 12.44 (0.51) | 15.19 (0.68) | 6.37 (0.66) |

Distal-capillary regions were the most affected by HRV: the 10th percentile for baseline $P_{dm,left}$ corresponds to over the 20th percentile for increased HRV, while the 90th percentile for baseline $P_c$ corresponds to about the 82th percentile for the reduced HRV configuration (see top panels of **Figure 5**). It is worth noting that a similar scenario was found for flow rates as well. The increased HRV enhanced extreme values of the continuous time-series toward the venous circulation: the 10th percentile corresponds to the 17th, while the 90th to the 82th (see bottom panels of **Figure 5**).

## 3.2. Beat-to-Beat Analysis

The scenario of higher impact of HRV on cerebral pressures than flow rates was also confirmed in the beat-to-beat analysis. **Table 2** displays mean and standard deviation values for the minimum ($P^{min}$ and $Q^{min}$), average ($\overline{P}$ and $\overline{Q}$), maximum ($P^{max}$ and $Q^{max}$), and pulse ($P^{pv}$ and $Q^{pv}$) values per beat of pressures $P$ and flow rates $Q$ as computed over 5,000 RR beats. PDFs of $\overline{P}$ and $\overline{Q}$ along the ICA-MCA pathway are depicted in **Figure 6**. The altered HRV did not substantially modify $\overline{Q}$ values, with relative variations of increased and decreased HRV <0.1% with respect to baseline, see the $\overline{Q}$ column in **Table 2** and the corresponding PDFs (see **Figure 6**, right panels). Relative variations of minimum ($Q^{min}$) and maximum ($Q^{max}$) values per beat were within 8%. $\overline{P}$ values as well as the corresponding PDFs were instead much more affected by HRV: only at the capillary level, similar $\overline{P}_c$ values were recovered for the three HRV configurations (with relative variations <0.1%, see bottom left panel of **Figure 6** and the mean value column of **Table 2**), while in all the other regions relative $\overline{P}$ variations were within 3%. Relative variations of maximum ($P^{max}$) and minimum ($P^{min}$) values per beat did not exceed 5% with respect to the baseline configuration (see **Table 2**).

The different impact of HRV change on pressure and flow rate can be explained noting that cerebral control mechanisms act on the continuous flow rate values at the distal level only, by modifying pial arterial-arteriolar compliances and resistances (Ursino and Giannessi, 2010). Thanks to the autoregulation effects, mean values of the continuous flow rate time-series (**Table 1**) and beat-to-beat $\overline{Q}$ recordings (**Table 2**) were preserved unvaried in the three configurations, while the same was not true for cerebral pressure. Despite cerebral autoregulation mechanisms are concentrated in the distal district solely, their effects involve both upstream and downstream regions. In fact, the mean flow rate is guaranteed as constant for the three configurations throughout the ICA-MCA pathway (i.e., from $Q_{ICA,left}$ to $Q_{pv}$, see $Q$ mean values in **Table 1** and $\overline{Q}$ in **Table 2**).

Based on what emerged so far, we expect a higher occurrence of extreme events in the beat-averaged pressure, $\overline{P}$, than beat-averaged flow rate, $\overline{Q}$. **Table 3** reports the total number of rare one-beats hypotensive, hypertensive, hypoperfusion, and hyperperfusion events. We recall that these events, by definition, cannot occur in the baseline configuration, which is taken to set the reference thresholds (10th and 90th percentiles). For the other configurations, one of these events happens at a specific district if the mean value per beat for $\overline{P}$ or $\overline{Q}$ reaches extremely high (above the 90th percentile of the baseline configuration) or low (below the 10th percentile of the baseline configuration) values. In particular, out of 5,000 RR beats we counted 42 hypotensive events at distal level for the increased HRV configuration, and 69 hypertensive events for reduced HRV. No hypoperfusions were found, while other more occasional events, such as nine

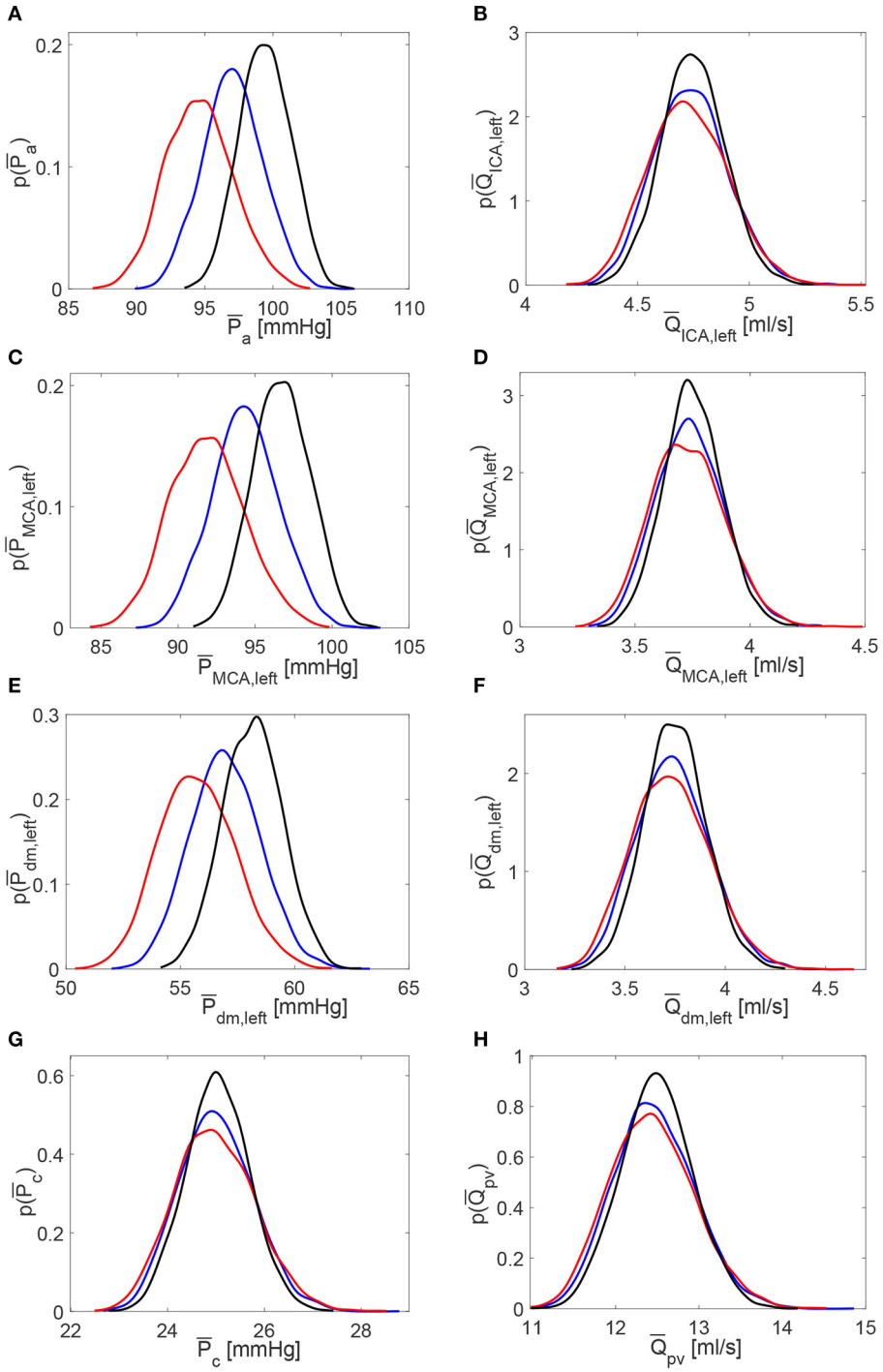



**FIGURE 6 |** Probability density functions of pressure and flow rate values averaged per beat, $\overline{P}$ and $\overline{Q}$, along the ICA-MCA pathway. **(A,C,E,G)** Pressures $\overline{P}_a$, $\overline{P}_{MCA,left}$, $\overline{P}_{dm,left}$, $\overline{P}_c$. **(B,D,F,H)** Flow rates $\overline{Q}_{ICA,left}$, $\overline{Q}_{MCA,left}$, $\overline{Q}_{dm,left}$, $\overline{Q}_{pv}$. Black: decreased HRV; blue: baseline; red: increased HRV.

distal hyperperfusion events, six capillary hypertensive events, and four distal hypertensive events, were induced by the higher HRV configuration. The reason of the low occurrence of extreme perfusive events can be understood by observing that the widening of the PDFs for the continuous flow rate time-series caused by increased HRV (see the red curves in right panels of **Figure 4**) is related to instantaneous peak values rather than enduring events in time. Thus, increased HRV was not able to trigger significant extreme perfusive events over a whole beat. In fact, PDFs of $\overline{Q}$ values were quite coincident for the three

**TABLE 3 |** Total number of one-beat extreme events (out of 5,000 RR beats) along the ICA-MCA pathway for the two decreased and increased HRV configurations.

| Configuration | Pressure $\overline{P}$ | | | | Flow rate $\overline{Q}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | $\overline{P}_a$ | $\overline{P}_{MCA,left}$ | $\overline{P}_{dm,left}$ | $\overline{P}_c$ | $\overline{Q}_{ICA,left}$ | $\overline{Q}_{MCA,left}$ | $\overline{Q}_{dm,left}$ | $\overline{Q}_{pv}$ |
| | **Hypotensive events** | | | | **Hypoperfusion events** | | | |
| Decreased HRV (−20%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Increased HRV (+20%) | 0 | 0 | 42 | 0 | 0 | 0 | 0 | 0 |
| | **Hypertensive events** | | | | **Hyperperfusion events** | | | |
| Decreased HRV (−20%) | 0 | 0 | 69 | 0 | 0 | 0 | 0 | 0 |
| Increased HRV (+20%) | 0 | 0 | 4 | 6 | 0 | 0 | 9 | 0 |

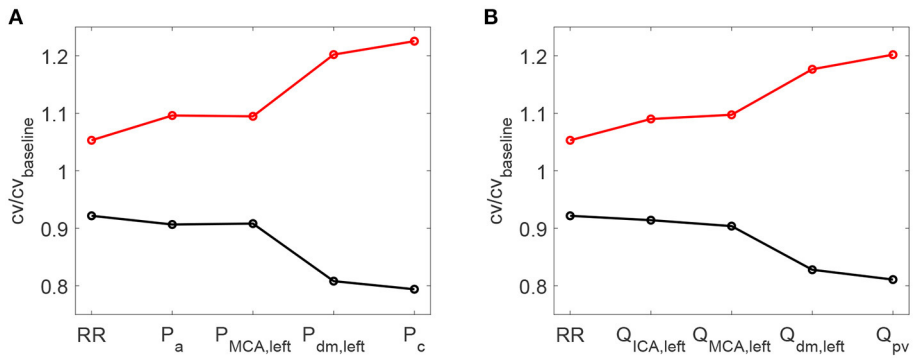



**FIGURE 7 |** Ratio between the coefficient of variation $c_v$ in increased/decreased (red/black) HRV cases and the coefficient of variation in baseline condition. Ratios for RR time-series and pressure/flow rate **(A,B)** continuous time-series along the ICA-MCA pathaway are shown.

configurations (right panels of **Figure 6**), leading to very sporadic hyperperfusion events and no hypoperfusion events at all. It should be also recalled that HRV variations were taken in a physiological range (±20% of the baseline value), therefore it is reasonable not expecting a large number of extreme events based on averaged-beat variables.

## 4. DISCUSSION

The present study aims at computationally investigating the role of HRV on the deep cerebral and central hemodynamics. SDNN has been adopted as a proxy measure for HRV and, although it does not have the same physiological origin reflecting cardiac vagal activity as RMSSD, it is highly correlated with the latter and more suited to the proposed computational approach. HRV seems to have overall a stronger impact on $\overline{P}$ than $\overline{Q}$ values, due to autoregulation effects acting to maintain an adequate average cerebral perfusion. However, in terms of extreme (minimum and maximum) values and percentile distributions, HRV similarly altered both pressure and flow rate. By comparing reduced and increased HRV, this latter induced a higher probability of altered mean values and extreme values (the only exception was the $P_{dm,left}$ case with reduced HRV and hypertensive events), and is therefore more detrimental at distal cerebral level.

As a further confirmation of the worsening linked to a higher HRV, it is observed that starting with an increased HRV, the variability of hemodynamic variables increased from proximal to distal regions along the left ICA-MCA pathway. On the contrary, starting with a reduced HRV, the hemodynamic variability decreased toward the deep cerebral circulation. This behavior is evident for the continuous signals in terms of $c_v$ ratio (as reported in **Figure 7**), as well as at beat-to-beat level, through relative variations of pulse values, $P^{pv}$ and $Q^{pv}$ (see **Figure 8**). It should be noted that the relative variations of $P^{pv}$ and $Q^{pv}$ showed this increasing/decreasing trend, although $P^{min}$, $P^{max}$, $Q^{min}$, and $Q^{max}$ did not singularly present, as mentioned before, a similar behavior: relative variations of $P^{min}$ and $P^{max}$ ($Q^{min}$ and $Q^{max}$) with respect to baseline did not exceed 5% (8%) along the ICA-MCA pathway (please refer to **Table 2**, columns with minimum and maximum values). Moreover, as displayed by **Figures 7**, **8**, the behavior was analogously found for both pressure and flow rate.

Even though all results fell within a range of physiological values, increased HRV seems more deleterious than reduced HRV along the proximal-to-distal cerebral pathway. An unsafe increased pulsatility of the hemodynamic signals was found especially at capillary-venous levels, and can promote extremely high/low pressure and flow rate values.

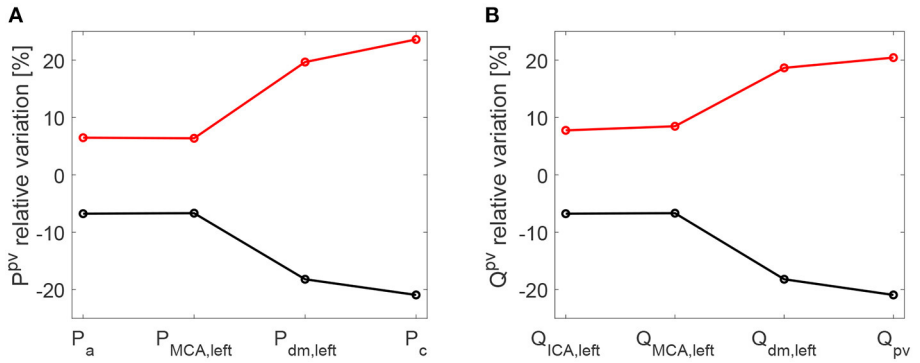



**FIGURE 8 |** Relative variations [%] of pulse values per beat with respect to baseline: pressure $(P^{pv} - P^{pv}_{baseline})/P^{pv}_{baseline} \cdot 100$ **(A)** and flow rate $(Q^{pv} - Q^{pv}_{baseline})/Q^{pv}_{baseline} \cdot 100$ **(B)**. Pulse values, $P^{pv}$ and $Q^{pv}$, are evaluated at each site of the ICA-MCA pathway. Black: decreased HRV/baseline. Red: increased HRV/baseline.

This result can be explained recalling what observed for the cerebral hemodynamics during atrial fibrillation (Scarsoglio et al., 2017b). The intrinsic structural latency revealed by the cerebral microcirculation and combined with a sequence of in-series irregular RR beats produced an alteration of the cerebral circulation. Indeed, due to the delay related to mechanical and structural properties, the inertia of the system increased toward the microvasculature.

Atrial fibrillation represents a succession of disturbances in terms of RR beating, which are then transmitted from the carotid level to the deep cerebral circulation. Each of these perturbations singularly produced an alteration of the cerebral circulation. The continuous sequence of transient perturbations at the carotid entrance represented by the fibrillated beating did not allow the system to recover the physiological state before another disturbance arrives. When this disturbance chain spread throughout the cerebral vessel network, the distal and capillary regions remained altered for longer. The behavior is similar to a system of springs in series and parallel, which is externally forced at one end: the stiffness of each spring combines with the others and the oscillation survives even if the external perturbation is ceased. It is important to note that this mechanism occurred regardless of whether the beat in atrial fibrillation is accelerated or not. In fact, the comparison between NSR and atrial fibrillation (Scarsoglio et al., 2017b) was proposed at the same mean heart rate, highlighting the role of RR variability in promoting the observed hemodynamic alteration.

With due proportions, the basic mechanism detected in atrial fibrillation seems to be similar in the case of increased HRV. Clearly, fibrillated outcomes were much more exacerbated and pathological since variability was much higher, and the RR beating was uncorrelated too. However, also here the component of increased variability seems to plausibly combine with the intrinsic latency of the system, causing the observed altered scenario.

The hemodynamic framework is inverted if we focus at central level, in terms of cardiac contractility, efficiency end energetic indexes (see **Table 4**). The decreased HRV configuration showed an increase of oxygen consumption indexes (+16% *RPP*, +6% *TTI*/*min*, +11% *PVA*/*min*) and cardiac work (+9% *EW*/*min*), in front of a reduction of the ejection fraction (−5%) and a limited increase of cardiac output (+6%). The reduction of the left ventricle efficiency *LVE*, though slight (−2%), confirms that a higher cardiac effort did not translate into a better mechanical and contractile cardiac performance. Moreover, variations of mean central flow rate (in terms of *CO*) did not entail average changes of the flow rate in the deep cerebral circulation (see cerebral mean flow rates in **Tables 1**, **2**), due to the autoregulation mechanisms at the distal cerebral level. In other words, the greater central energy expenditure did not produce any gain in terms of mean flow rate in the cerebral microcirculation.

To extend the discussion, we considered a configuration of heart failure as representative of a very different condition from the healthy state and often present as a co-morbidity in the elderly population. With respect to the baseline heart failure condition, we analyzed two HRV configurations also here, increased (+20% SDNN) and decreased (−20%

**TABLE 4 |** Cardiac parameters and oxygen consumption indexes at central level: mean (std).

| Variable | Decreased HRV (−20%) | Baseline | Increased HRV (+20%) |
|---|---|---|---|
| $V_{lved}$ [ml] | 126.99 (1.71) | 130.87 (1.79) | 134.43 (1.95) |
| $V_{lves}$ [ml] | 58.80 (1.16) | 56.73 (1.30) | 54.78 (1.38) |
| *SV* [ml] | 68.18 (2.77) | 74.14 (2.97) | 79.65 (3.22) |
| *EF* [%] | 53.67 (1.49) | 56.63 (1.53) | 59.23 (1.58) |
| *CO* [l/min] | 5.52 (0.30) | 5.21 (0.32) | 4.91 (0.31) |
| *SW*/*min* [J/min] | 75.44 (4.84) | 69.46 (5.01) | 63.72 (4.98) |
| *RPP* [mmHg/min] | 9837.29 (835.45) | 8494.19 (786.38) | 7393.16 (733.68) |
| *TTI*/*min* [mmHgs/min] | 2775.53 (100.87) | 2622.53 (102.08) | 2488.01 (101.07) |
| *PVA*/*min* [J/min] | 101.85 (7.15) | 91.98 (7.26) | 82.93 (7.13) |
| *LVE* | 0.74 (0.01) | 0.76 (0.01) | 0.77 (0.01) |

*$V_{lved}$, end-diastolic left ventricular volume; $V_{lves}$, end-systolic left ventricular volume; SV, stroke volume; EF, ejection fraction; CO, cardiac output; SW/min, stroke work per minute; RPP, rate pressure product; TTI/min, tension time index per minute; PVA/min, pressure volume area per minute; LVE, left ventricle efficiency.*

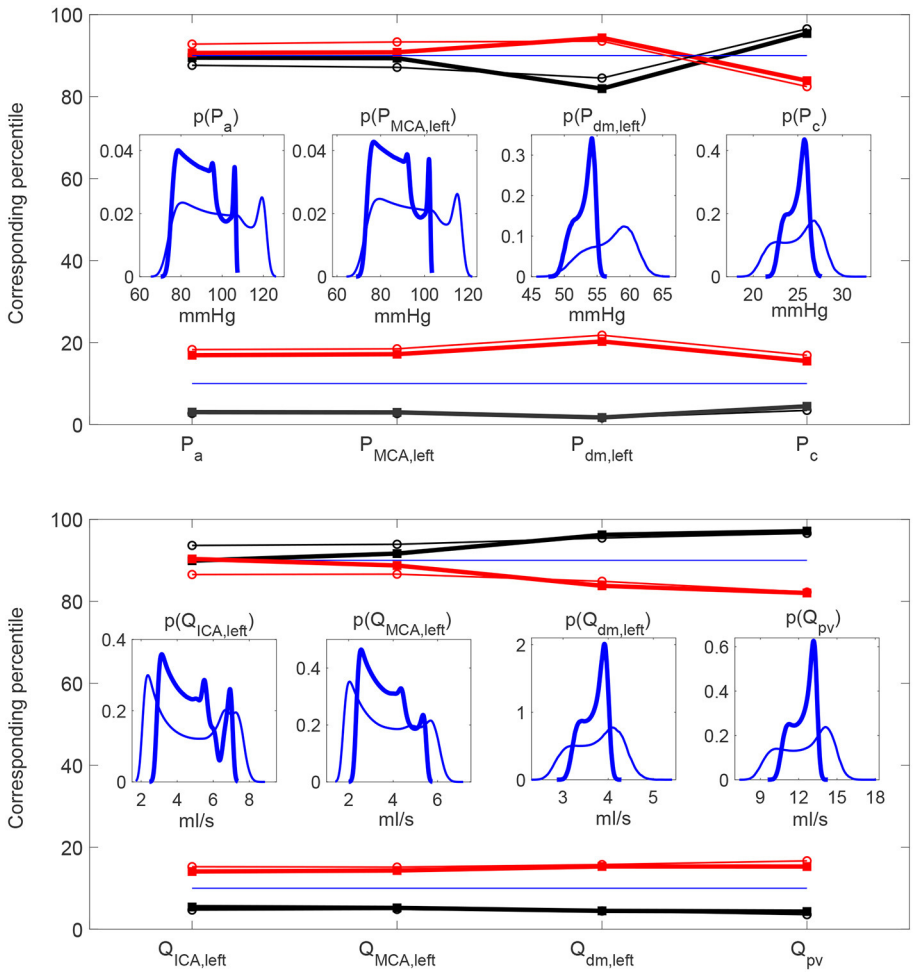



**FIGURE 9 |** Heart failure (thick) vs. healthy (thin) percentile analyses. Percentile values in increased (red) and decreased (black) HRV to which the 10th and 90th percentiles in baseline configuration correspond. Pressure (Top) and flow rate (Bottom) along the ICA-MCA pathway. Inset panels represent the PDFs of the continuous hemodynamic time-series.

SDNN) HRV. Details of the heart failure configurations are provided in the **Supplementary Material**. For the deep cerebral hemodynamics, the percentile analysis and the PDFs of the continuous hemodynamic time-series in the heart failure case are reported and compared with the healthy case in **Figure 9**. Although the values achieved are different from the case healthy (see the blue thick and thin curves), the HRV variations with respect to the baseline are quite close to the healthy case (see the red/black thick and thin curves), especially in the distal regions. **Table 5** shows the values of the central hemodynamic parameters in the heart failure case. Compared to the healthy case, the values achieved are very different and show a rather compromised hemodynamic framework (e.g., at baseline $CO = 3.55$ l/min, $EF = 16.41\%$, $LVE = 0.31$). However, the HRV variations compared to baseline are in agreement with the healthy case (decreased HRV: +12% $RPP$, +3% $TTI/min$, +10% $PVA/min$, +4% $EW/min$, −8% $EF$, +3% $CO$, −5% $LVE$).

For both healthy and heart failure cases, at central level there is a qualitative agreement with what has been widely observed in literature, that is an increased HRV is beneficial for the cardiac performance and efficiency. At cerebral level, where instead results are still debated, an increase of HRV only—regardless any other variation, compensatory mechanism or pathological condition—seems to be associated to a worse hemodynamic response. A plausible interpretation of these findings with respect to the possible association between HRV and cognitive performance observed in some literature studies is that we only considered the net contribution of HRV alterations, without any complementary and further variation of the sympathetic system. In fact, changes of the sympathetic activity are hardly quantifiable and out of the objectives of the present study. According to the neurovisceral integration model vagally-mediated HRV metrics, such as RMSSD and HF, are expected to be linked to cognitive performance, but not SDNN (Thayer et al., 2009a). Based on our outcomes the increase of HRV, measured by means of SDNN,

**TABLE 5 |** Heart failure.

| Variable | Decreased HRV (−20%) | Baseline | Increased HRV (+20%) |
|---|---|---|---|
| $V_{lved}$ [ml] | 251.07 (1.16) | 253.01 (1.11) | 254.51 (1.01) |
| $V_{lves}$ [ml] | 213.19 (0.81) | 211.47 (1.17) | 209.43 (1.56) |
| *SV* [ml] | 37.88 (1.87) | 41.54 (2.19) | 45.08 (2.47) |
| *EF* [%] | 15.08 (0.68) | 16.41 (0.80) | 17.71 (0.90) |
| *CO* [l/min] | 3.64 (0.17) | 3.55 (0.17) | 3.46 (0.18) |
| *SW/min* [J/min] | 39.22 (1.95) | 37.55 (1.97) | 35.98 (1.99) |
| *RPP* [mmHg/min] | 10243.26 (699.45) | 9116.87 (691.62) | 8189.49 (672.01) |
| *TTI/min* [mmHgs/min] | 2709.65 (55.37) | 2621.87 (58.59) | 2543.39 (61.17) |
| *PVA/min* [J/min] | 132.13 (7.64) | 120.59 (7.81) | 110.73 (7.71) |
| *LVE* | 0.30 (0.01) | 0.31 (0.01) | 0.33 (0.01) |

*Cardiac parameters and oxygen consumption indexes at central level: mean (std). $V_{lved}$, end-diastolic left ventricular volume; $V_{lves}$, end-systolic left ventricular volume; SV, stroke volume; EF, ejection fraction; CO, cardiac output; SW/min, stroke work per minute; RPP, rate pressure product; TTI/min, tension time index per minute; PVA/min, pressure volume area per minute; LVE, left ventricle efficiency.*

does not seem to be sufficient *per se* to trigger a better cerebral hemodynamic response.

### 4.1. Limitations

The present computational approach has some limiting aspects related to the modeling hypotheses. We considered the same young healthy resting subject as forced through three different HRV configurations. In particular, the three configurations only differ by the entrance inputs, the beating sequence RR, while the remaining hemodynamic framework is set as in baseline condition, regardless of other compensatory mechanisms that may occur or altered sympathetic activity. Cerebral autoregulation and autonomic regulation are not fully coupled, but this interplay is present in one-way direction only: through the baroreceptor mechanisms acting on the systemic arterial pressure, autonomic regulation influences the cerebral autoregulation, but there are no feedbacks from cerebral blood flow to autonomic control. Moreover, coronary circulation was not included into the model. In the end, we evaluated a monitoring temporal window of about 1 h (5,000 RR beats), thus no long-term remodeling effects related to increased or decreased HRV were taken into account.

## 5. CONCLUSIONS

In conclusion, by recalling the relation inversely linking SDNN and HR (Monfredi et al., 2014; Shaffer and Ginsberg, 2017; de Geus et al., 2019), at deep cerebral level a higher SDNN is worse than a higher HR. On the contrary, at central level a higher HR is more negatively impacting than a higher SDNN. From an overall point of view which contemporarily accounts for both central and deep cerebral circulations, present results suggested that the optimal HRV configuration is found at baseline. In fact, an increased HRV (lower HR) is detrimental for the cerebral circulation causing possible hypertensive events and extreme pressure and flow rate values, while a decreased HRV (higher HR) induces a higher cardiac effort without improving the mechano-contractile performance, thus overall reducing the heart efficiency. The presence of this optimal condition is the main finding and can contribute to explain why the mean HR of the general population settles around 70 bpm: the baseline value is a good compromise able to guarantee an adequate hemodynamic response for both central and deep cerebral regions, preserving from extreme cerebral hemodynamic events and at the same time maintaining satisfactory cardiac efficiency.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

SS performed the experiments and wrote the paper. All authors conceived and designed the experiments, analyzed the data, contributed the reagents, materials, analysis tools, reviewed, and approved the final version of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.600574/full#supplementary-material

## REFERENCES


Acharya, U. R., Joseph, K. P., Kannathal, N., Lim, C. M., and Suri, J. S. (2006). Heart rate variability: a review. *Med. Biol. Eng. Comput.* 44, 1031–1051. doi: 10.1007/s11517-006-0119-0

Allan, L. M., Kerr, S. R. J., Ballard, C. G., Allen, J., Murray, A., McLaren, A. T., et al. (2005). Autonomic function assessed by heart rate variability is normal in Alzheimer's disease and vascular dementia. *Dement. Geriatr. Cogn. Disord.* 19, 140–144. doi: 10.1159/000082885

Anselmino, M., Scarsoglio, S., Camporeale, C., Saglietto, A., Gaita, F., and Ridolfi, L. (2015). Rate control management of atrial fibrillation: may a mathematical model suggest an ideal heart rate? *PLoS ONE* 10:e0119868. doi: 10.1371/journal.pone.0119868

Anselmino, M., Scarsoglio, S., Saglietto, A., Gaita, F., and Ridolfi, L. (2016). Transient cerebral hypoperfusion and hypertensive events during atrial fibrillation: a plausible mechanism for cognitive impairment. *Sci. Rep.* 6:28635. doi: 10.1038/srep28635

Anselmino, M., Scarsoglio, S., Saglietto, A., Gaita, F., and Ridolfi, L. (2017). A computational study on the relation between resting heart rate and atrial fibrillation hemodynamics under exercise. *PLoS ONE* 12:e0169967. doi: 10.1371/journal.pone.0169967

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Billman, G. E., and Kukielka, M. (2006). Effects of endurance exercise training on heart rate variability and susceptibility to sudden cardiac death: protection is

not due to enhanced cardiac vagal regulation. *J. Appl. Physiol.* 100, 896–906. doi: 10.1152/japplphysiol.01328.2005

Boissoneault, J., Letzen, J., Robinson, M., and Staud, R. (2019). Cerebral blood flow and heart rate variability predict fatigue severity in patients with chronic fatigue syndrome. *Brain Imaging Behav*. 13, 789–797. doi: 10.1007/s11682-018-9897-x

Boyett, M., Wang, Y., and D'Souza, A. (2019). Crosstalk opposing view: heart rate variability as a measure of cardiac autonomic responsiveness is fundamentally flawed. *J. Physiol.* 597, 2599–2601. doi: 10.1113/JP277501

Britton, A., Singh-Manoux, A., Hnatkova, K., Malik, M., Marmot, M. G., and Shipley, M. (2008). The association between heart rate variability and cognitive impairment in middle-aged men and women–the whitehall ii cohort study. *Neuroepidemiology* 31, 115–121. doi: 10.1159/000148257

Buccelletti, E., Gilardi, E., Scaini, E., Galiuto, L., Persiani, R., Biondi, A., et al. (2009). Heart rate variability and myocardial infarction: systematic literature review and metanalysis. *Eur. Rev. Med. Pharmacol. Sci.* 13, 299–307.

de Geus, E. J. C., Gianaros, P. J., Brindle, R. C., Jennings, J. R., and Berntson, G. G. (2019). Should heart rate variability be "corrected" for heart rate? Biological, quantitative, and interpretive considerations. *Psychophysiology* 56:e13287. doi: 10.1111/psyp.13287

Dekker, J. M., Crow, R. S., Folsom, A. R., Hannan, P. J., Liao, D., Swenne, C. A., et al. (2000). Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the aric study. Atherosclerosis risk in communities. *Circulation* 102, 1239–1244. doi: 10.1161/01.CIR.102.11.1239

Fyfe-Johnson, A. L., Muller, C. J., Alonso, A., Folsom, A. R., Gottesman, R. F., Rosamond, W. D., et al. (2016). Heart rate variability and incident stroke: the atherosclerosis risk in communities study. *Stroke* 47, 1452–1458. doi: 10.1161/STROKEAHA.116.012662

Gallo, C., Ridolfi, L., and Scarsoglio, S. (2020). Cardiovascular deconditioning during long-term spaceflight through multiscale modeling. *NPJ Microgravity* 6:27. doi: 10.1038/s41526-020-00117-5

Genovesi, S., Zaccaria, D., Rossi, E., Valsecchi, M. G., Stella, A., and Stramba-Badiale, M. (2007). Effects of exercise training on heart rate and qt interval in healthy young individuals: are there gender differences? *Europace* 9, 55–60. doi: 10.1093/europace/eul145

Hayano, J., Sakakibara, Y., Yamada, A., Yamada, M., Mukai, S., Fujinami, T., et al. (1991). Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. *Am. J. Cardiol.* 67, 199–204. doi: 10.1016/0002-9149(91)90445-Q

Hayano, J., Yamasaki, F., Sakata, S., Okada, A., Mukai, S., and Fujinami, T. (1997). Spectral characteristics of ventricular response to atrial fibrillation. *Am. J. Physiol. Heart Circ. Physiol.* 273, H2811–H2816. doi: 10.1152/ajpheart.1997.273.6.H2811

Hazzouri, A. Z. A., Elfassy, T., Carnethon, M. R., Lloyd-Jones, D. M., and Yaffe, K. (2017). Heart rate variability and cognitive function in middle–age adults: the coronary artery risk development in young adults. *Am. J. Hypertens*. 31, 27–34. doi: 10.1093/ajh/hpx125

Hennig, T., Maass, P., Hayano, J., and Heinrichs, S. (2006). Exponential distribution of long heart beat intervals during atrial fibrillation and their relevance for white noise behaviour in power spectrum. *J. Biol. Phys*. 32, 383–392. doi: 10.1007/s10867-006-9022-z

Kazmi, S. Z. H., Zhang, H., Aziz, W., Monfredi, O., Abbas, S. A., Shah, S. A., et al. (2016). Inverse correlation between heart rate variability and heart rate demonstrated by linear and nonlinear analysis. *PLoS ONE* 11:e0157557. doi: 10.1371/journal.pone.0157557

Kim, D. H., Lipsitz, L. A., Ferrucci, L., Varadhan, R., Guralnik, J. M., Carlson, M. C., et al. (2006). Association between reduced heart rate variability and cognitive impairment in older disabled women in the community: women's health and aging study I. *J. Am. Geriatr. Soc.* 54, 1751–1757. doi: 10.1111/j.1532-5415.2006.00940.x

Laborde, S., Mosley, E., and Thayer, J. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lehavi, A., Golomb, N., Leiba, R., Katz, Y. S., and Raz, A. (2019). One-minute heart rate variability – an adjunct for airway obstruction identification. *Physiol. Rep.* 7:e13948. doi: 10.14814/phy2.13948

Mahinrad, S., Jukema, J. W., van Heemst, D., Macfarlane, P. W., Clark, E. N., de Craen, A. J. M., et al. (2016). 10-second heart rate variability and cognitive function in old age. *Neurology* 86, 1120–1127. doi: 10.1212/WNL.0000000000002499

Malik, M., Camm, A. J., Breithardt, A. G., Bigger, J. T., Cerutti, S., Cohen, R. J., et al. (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Meeus, M., Goubert, D., DeBacker, F., Struyf, F., Hermans, L., Coppieters, I., et al. (2013). Heart rate variability in patients with fibromyalgia and patients with chronic fatigue syndrome: a systematic review. *Semin. Arthritis Rheum*. 43, 279–287. doi: 10.1016/j.semarthrit.2013.03.004

Michael, S., Graham, K., and Oam, G. (2017). Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals–a review. *Front. Psychol.* 8:301. doi: 10.3389/fphys.2017.00301

Monfredi, O., Lyashkov, A. E., Johnsen, A. B., Inada, S., Schneider, H., Wang, R., et al. (2014). Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. *Hypertension* 64, 1334–1343. doi: 10.1161/HYPERTENSIONAHA.114.03782

Nunan, D., Sandercock, G. R. H., and Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing Clin. Electrophysiol.* 33, 1407–1417. doi: 10.1111/j.1540-8159.2010.02841.x

Nussinovitch, U., Elishkevitz, K. P., Katz, K., Nussinovitch, M., Segev, S., Volovitz, B., et al. (2011). Reliability of ultra-short ECG indices for heart rate variability. *Ann. Noninvasive Electrocardiol.* 16, 117–122. doi: 10.1111/j.1542-474X.2011.00417.x

Pikkujämsä, S. M., Mäkikallio, T. H., Airaksinen, K. E., and Huikuri, H. V. (2001). Determinants and interindividual variation of R-R interval dynamics in healthy middle-aged subjects. *Am. J. Physiol. Heart Circ. Physiol.* 280, H1400–H1406. doi: 10.1152/ajpheart.2001.280.3.H1400

Saglietto, A., Scarsoglio, S., Ridolfi, L., Gaita, F., and Anselmino, M. (2019). Higher ventricular rate during atrial fibrillation relates to increased cerebral hypoperfusions and hypertensive events. *Sci. Rep.* 9:3779. doi: 10.1038/s41598-019-40445-5

Scarsoglio, S., Camporeale, C., Guala, A., and Ridolfi, L. (2016a). Fluid dynamics of heart valves during atrial fibrillation: a lumped parameter-based approach. *Comput. Methods Biomech. Biomed. Engin.* 19, 1060–1068. doi: 10.1080/10255842.2015.1094800

Scarsoglio, S., Cazzato, F., and Ridolfi, L. (2017a). From time-series to complex networks: application to the cerebrovascular flow patterns in atrial fibrillation. *Chaos* 27:093107. doi: 10.1063/1.5003791

Scarsoglio, S., Guala, A., Camporeale, C., and Ridolfi, L. (2014). Impact of atrial fibrillation on the cardiovascular system through a lumped-parameter approach. *Med. Biol. Eng. Comput.* 52, 905–920. doi: 10.1007/s11517-014-1192-4

Scarsoglio, S., Saglietto, A., Anselmino, M., Gaita, F., and Ridolfi, L. (2017b). Alteration of cerebrovascular haemodynamic patterns due to atrial fibrillation: an *in silico* investigation. *J. R. Soc. Interface* 14:20170180. doi: 10.1098/rsif.2017.0180

Scarsoglio, S., Saglietto, A., Gaita, F., Ridolfi, L., and Anselmino, M. (2016b). Computational fluid dynamics modelling of left valvular heart diseases during atrial fibrillation. *PeerJ* 4:e2240. doi: 10.7717/peerj.2240

Schaich, C. L., Malaver, D., Chen, H., Shaltout, H. A., Hazzouri, A. Z. A., Herrington, D. M., et al. (2020). Association of heart rate variability with cognitive performance: the multi–ethnic study of atherosclerosis. *J. Am. Heart Assoc*. 9:e013827. doi: 10.1161/JAHA.119.013827

Sessa, F., Anna, V., Messina, G., Cibelli, G., Monda, V., Marsala, G., et al. (2018). Heart rate variability as predictive factor for sudden cardiac death. *Aging* 10, 166–177. doi: 10.18632/aging.101386

Shaffer, F., and Ginsberg, J. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shah, A. J., Su, S., Veledar, E., Bremner, J. D., Goldstein, F. C., Lampert, R., et al. (2011). Is heart rate variability related to memory performance in middle-aged men? *Psychosom. Med*. 73, 475–482. doi: 10.1097/PSY.0b013e3182227d6a

Singh, N., Moneghetti, K., Christle, J., Hadley, D., Plews, D., and Froelicher, V. (2018). Heart rate variability: an old metric with new meaning in the era of using mhealth technologies for health and exercise training guidance.

Part one: physiology and methods. *Arrhythm. Electrophysiol. Rev*. 7, 193–198. doi: 10.15420/aer.2018.27.2

Talib, S. H., Mulay, P. Y., and Patil, A. N. (2005). Twenty-four hour ambulatory holter monitoring and heart rate variability in healthy individuals. *JIACM* 6, 136–141.

Thayer, J. F., Hansen, A., Saus-Rose, E., and Johnsen, B. (2009a). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med*. 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2009b). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol*. 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Tsuji, H., Larson, M., Venditti, F., Manders, E., Evans, J., Feldman, C., et al. (1996). Impact of reduced heart rate variability on risk for cardiac events – the framingham heart study. *Circulation* 94, 2850–2855. doi: 10.1161/01.CIR.94.11.2850

Tulppo, M. P., Mäkikallio, T. H., Seppänen, T., Laukkanen, R. T., and Huikuri, H. V. (1998). Vagal modulation of heart rate during exercise: effects of age and physical fitness. *Am. J. Physiol*. 274, H424–H429. doi: 10.1152/ajpheart.1998.274.2.H424

Ursino, M., and Giannessi, M. (2010). A model of cerebrovascular reactivity including the circle of Willis and cortical anastomoses. *Ann. Biomed. Eng*. 38, 955–974. doi: 10.1007/s10439-010-9923-7

van den Berg, M. E., P R Rijnbeek and, M. N. N., Hofman, A., van Herpen, G., Bots, M. L., Hillege, H., et al. (2018). Normal values of corrected heart-rate variability in 10-second electrocardiograms for all ages. *Front. Physiol*. 9:424. doi: 10.3389/fphys.2018.00424

Westerhof, N., Stergiopulos, N., and Noble, M. I. M. (2010). *Snapshots of Hemodynamics, 2nd Edn*. Berlin: Springer. doi: 10.1007/978-1-4419-6363-5

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 19 May 2021
doi: 10.3389/fnins.2021.619171

# The Cardiovascular Conundrum in Ethnic and Sexual Minorities: A Potential Biomarker of Constant Coping With Discrimination

*Fausta Rosati[1†], DeWayne P. Williams[2†], Robert-Paul Juster[3], Julian F. Thayer[2], Cristina Ottaviani[4,5*] and Roberto Baiocco[1]*

[1] Department of Developmental and Social Psychology, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy, [2] Department of Psychological Science, University of California, Irvine, Irvine, CA, United States, [3] Department of Psychiatry and Addiction, University of Montreal, Montreal, QC, Canada, [4] Department of Psychology, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy, [5] Functional Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy

OPEN ACCESS

**Edited by:**
Sylvain Laborde,
German Sport University Cologne, Germany

**Reviewed by:**
Stefan Duschek,
Private University for Health Sciences, Medical Informatics and Technology (UMIT), Austria
Simone Messerotti Benvenuti,
University of Padua, Italy
Patrick R. Steffen,
Brigham Young University, United States

***Correspondence:**
Cristina Ottaviani
cristina.ottaviani@uniroma1.it

[†] These authors have contributed equally to this work

**Specialty section:**
This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience

**Received:** 19 October 2020
**Accepted:** 26 April 2021
**Published:** 19 May 2021

**Citation:**
Rosati F, Williams DP, Juster R-P, Thayer JF, Ottaviani C and Baiocco R (2021) The Cardiovascular Conundrum in Ethnic and Sexual Minorities: A Potential Biomarker of Constant Coping With Discrimination. Front. Neurosci. 15:619171. doi: 10.3389/fnins.2021.619171

**Background:** A paradoxical profile of greater elevated sympathetic vasoconstriction (increased total peripheral resistance, TPR) and increased vagally-mediated heart rate variability (HRV) -the so-called Cardiovascular Conundrum- has been reported in African Americans (AAs) both at rest and in response to orthostasis. Whereas some authors have attributed this pattern to genetic factors, others have pointed to the potential role of coping with repeated racial discrimination.

**Objective:** To disentangle between these alternative explanations, we have examined the hemodynamic profile of another population that is likely to be exposed to episodes of discrimination, i.e., sexual minorities.

**Methods:** The first study was conducted on a sample of AAs and European Americans (EAs) with the aim of replicating previous results on the Cardiovascular Conundrum. In the second study, lesbian, gay, and bisexual (LGB) people, matched by age and sex with heterosexual participants, underwent a hemodynamic and autonomic assessment at rest and during an emotional (in the experimental group, both LGB-related and non LGB related), and a cognitive stressor.

**Results:** The first study confirmed a pattern of higher resting HRV, paired with higher TPR, in AAs compared to EAs. In the second study, compared to heterosexuals, the LGB group showed the Cardiovascular Conundrum pattern, characterized by greater HRV and higher TPR at baseline and a more vascular hemodynamic profile and prominent compensation deficit in response to both tasks, and particularly during the LGB-related emotional task. However, in LGB only, the vascular response was negatively correlated with perceived discrimination.

**Conclusion:** Present preliminary results are discussed in terms of maladaptive physiological consequences of exposure to chronic stress and the chronic use of dysfunctional emotion regulation strategies such as suppression.

**Keywords: cardiovascular conundrum, minorities, discrimination, heart rate variability, hemodynamic profile**

## INTRODUCTION

Despite overwhelming evidence that African Americans have greater hypertension (Go et al., 2014) and related mortality and morbidity (Pool et al., 2017) than European Americans, the ethnic difference in hypertension still remains unexplained. Based on the reactivity hypothesis (Obrist, 1981), positing higher cardiovascular risk for physiological hyper-reactors, one would assume to find chronic sympathetic nervous system hyperactivation and parasympathetic withdrawal in African American. In the past decade, however, a series of studies have found a completely opposite picture, with African Americans being characterized by tonically higher heart rate variability (HRV), a measurement of parasympathetic autonomic function, compared to European Americans (reviewed in Hill and Thayer, 2019). This is surprising, given that low (not high) tonic HRV has been associated with a number of established and emerging modifiable and non-modifiable cardiovascular risk factors, including hypertension (e.g., Thayer et al., 2010). To make the picture even more complicated, this is also associated with higher total peripheral resistance (TPR; a measure of the amount of force affecting resistance to blood flow throughout the circulatory system) at rest (Brownlow et al., 2020 for a meta-analysis) and a more vascular (compared to myocardial) hemodynamic profile in response to stressors (e.g., Carnevali et al., 2019) in African American. The term "hemodynamic profile" describes the relationship between cardiac output (CO; a measure of the amount of blood the heart pumps in a minute) and TPR in the homeostatic regulation of blood pressure (Gregg et al., 2002). More vascular reactors respond to a stressor by increasing TPR more than CO, with the latter being predominantly increased by myocardial reactors, with the same ending point of increased blood pressure.

Considering that the baroreflex regulates both cardiac vagal tone and vascular resistance, such a pattern of both high HRV and higher TPR in African Americans represents a physiological enigma. Indeed, this has been referred to as the Cardiovascular Conundrum (Hill et al., 2015, 2017). Recent findings point to the role of constant effortful emotion regulation engaged by African Americans in response to daily discrimination (Hill et al., 2017; Thayer et al., 2020). A systematic review of the literature has suggested that perceived racial discrimination is linked with hypertensive status, being associated in particular with increased nighttime ambulatory blood pressure (BP), especially among African Americans (Dolezsar et al., 2014). In general, greater self-reported discrimination is coupled with lower resting HRV in African Americans (Hill et al., 2017), and this association appears to be moderated by rumination (Williams et al., 2017). For example, a previous study has shown that African Americans who expressed their anger had lower HRV and lower HRV recovery from a racially charged debate compared to their European Americans counterparts and compared to African Americans who inhibited their anger, which is considered a more socially appropriate response (Dorr et al., 2007). It has to be noted that while high tonic HRV is a measure of robust parasympathetic control on the heart and of the ability to engage in context-appropriate responses, phasic HRV suppression represents the withdrawal of cardiac vagal control and the activation of the defensive systems (Park et al., 2014). On the other hand, in a large, pooled dataset of 452 European Americans and 102 African Americans, greater use of reappraisal and suppression of anger were associated with greater HRV in African Americans but not in European Americans. Moreover, anger expression correlated with HRV in African Americans only (Thayer and Koenig, 2019). Notably, Thayer and Koenig (2019) found that cerebral blood flow in the anterior cingulate cortex was negatively associated with HRV in African Americans, whereas the opposite pattern emerged for European Americans. The authors conclude that the use of these habitual emotion regulation strategies may be associated with altered autonomic and central nervous systems coupling in African Americans (Thayer and Koenig, 2019). In a large sample of African American women ($N$ = 208), only those reporting active coping with racism were characterized by a positive association between daily discrimination and hypertension, whereas the opposite pattern emerged for those characterized by low levels of active coping (Michaels et al., 2019). A number of recent studies provide support for the association between perceived discrimination or racism and poorer cardiovascular health in African Americans (e.g., metabolic syndrome in Cardel et al., 2020; several stress markers in Cedillo et al., 2020; urinary catecholamines in Homandberg and Fuller-Rowell, 2020).

Overall, the reviewed evidence suggests that the Cardiovascular Conundrum might emerge from the need to exert constant control over one's anger (either with the use of rumination, suppression or reappraisal) in response to discrimination (Thayer et al., 2020). To investigate this hypothesis and rule out the contribution of the genetics of hypertension in African Americans (for a recent review see Zilbermint et al., 2019), one should investigate the same physiological pattern in a White population that is similarly exposed to discrimination. In this way, it would be possible to determine whether discrimination represents a key factor underlying ethnic disparities in cardiovascular functioning, regardless of the presence of "at risk" genetic polymorphisms putatively associated with specific ethnicities.

Sexual minorities appear to be the optimal population to study with this regard, as they are characterized by increased risk of stigma and prejudice (Herek, 2004). Through the *minority stress model,* Meyer (2003) conceptualized stigma as a source of psychosocial stress that is additive to the other stressors that are experienced by the majority of people and chronic because it depends on quite stable social and cultural structures. Minority stress processes range from distal objective stressors (e.g., discrimination, harassment, and victimization) to more proximal subjective stressors (e.g., expectations of rejections, vigilance, and internalized sexual stigma).

Based on the sexual minority stress model, several studies have shown the detrimental impact of sexual minority stress and stigma on lesbian, gay, and bisexual (LGB) people's mental health (Kuyper and Fokkema, 2011; Lehavot and Simoni, 2011; Dürrbaum and Sattler, 2019; Baiocco et al., 2021). Only a few studies, however, examined the impact of sexual minority stress on outcomes such as cardiovascular function,

and highlighted potential increased risk for health in sexual minorities when compared to the heterosexual population (Mays et al., 2018; Caceres et al., 2019). To date, none of these studies have assessed HRV. With a few exceptions, most studies assessed both minority stressors and health outcomes through interview or self-report methods, which made the results partially unclear. An exception is represented by the study of Cook and colleagues, who found that sexual orientation moderates the association between parental overprotection and stress biomarker profiles of acute and chronic stress responses, assessed by stress reactive cortisol and allostatic load (indexed using several neuroendocrine, immune, metabolic and cardiovascular biomarkers), respectively, suggesting underlying differential profiles of physiological stress processes among LGB and heterosexual individuals (Cook et al., 2018).

The present study had two interrelated objectives. The first was to replicate previous findings on the Cardiovascular Conundrum in African Americans, and we hypothesized to find a pattern of higher HRV associated with higher TPR at rest. The second was to examine if LGB people, who are similarly exposed to unfair treatment, show the same Cardiovascular Conundrum pattern as repeatedly found in African Americans. Specifically, we hypothesized (i) to find a more vascular HP in LGB compared to heterosexual people, particularly during reactivity to and recovery from the LGB-related task and that (ii) this pattern of more vascular profile as well as higher resting HRV would be positively associated with self-reported day-to-day minority stress (i.e., scores on the DHEQ).

# MATERIALS AND METHODS

Hemodynamic profiles and HRV were assessed in two stigmatized social groups to better define the physiological concomitants of dealing with discrimination. Study 1 involved physiological profiling at rest in African American participants and focused on racial discrimination; Study 2 involved physiological profiles in response to social and cognitive stressors in LGB participants and focused on discrimination based on sexual stigma.

## Study 1

### Participants

Participants were recruited via two methods: (1) an introductory level psychology course research pool for partial class credit; and (2) cash compensation for individuals' participation outside of the research pool at The Ohio State University. Participants were recruited for the purposes of a larger study; however, results from these data have not been published elsewhere. Fifty-eight individuals (30 AAs and 28 EAs) were available for analyses. Participants were between the ages of 18–30, with an average age of 19.83 years old ($SD$ = 2.2 years). Body Mass Index (BMI) for the full sample ranged from 19.25 to 47.27 Kg/m$^2$ ($M$ = 26.22, $SD$ = 5.74). For African Americans, BMI ranged from 19.92 to 47.26 Kg/m$^2$ ($M$ = 27.47, $SD$ = 6.68) and for European Americans, BMI ranged from 19.25 to 43.76 Kg/m$^2$ ($M$ = 24.96, $SD$ = 4.39). Exclusion criteria, assessed via self-report questionnaires, were diagnosis of hypertension, heart disease, psychiatric disorder or habitual intake of drugs/medications affecting the cardiovascular system.

The Ohio State Institutional Review Board approved the study, and all participants signed written informed consent.

### Procedure

We asked all participants not to smoke, undergo vigorous physical activity, or drink caffeine during the 6 h prior to the experiment. Participants then completed a 5 min baseline period, in which they sat in a resting position (spontaneous breathing) with the television displaying a blank, gray screen, and were instructed not to move or fall asleep.

### Physiological Assessment

Beat-to-beat BP and HR were recorded from the non-dominant middle finger by using finger photoplethysmography (Finometer Pro, FMS, Finapres Measurement Systems, Amsterdam, The Netherlands; sampling rate: 200 Hz), tested against a mercury sphygmomanometer. During physiological assessment, participants were in a seated position with their non-dominant hand lying on a table. Raw pulse wave files were converted to text files by Beatscope Easy. Text files were then processed using a custom-made LabVIEW software (LabVIEW8.5, National Instruments, Austin, Texas, United States) to calculate mean indices of systolic BP (SBP), root mean square of successive difference (RMSSD), as well as TPR, and stroke volume (SV) derived using the Modelflow method. RMSSD reflects vagal regulation of HR (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and is less susceptible to respiratory influences compared to frequency-domain measures of HRV (Penttilä et al., 2001).

### Data Analysis

All statistical tests were conducted using SPSS (ver. 20, IBM Chicago, IL, United States). Independent samples $t$-tests were conducted to analyze differences in baseline physiological variables between AAs and EAs. Group differences in age, BMI, and sex were also examined. RMSSD was natural log-transformed to fit assumptions of linear analyses. Given extensive literature and meta-analyses (e.g., Hill et al., 2015; Brownlow et al., 2020) on baseline physiological differences between AAs and EAs, all tests were one-tailed and significance levels were evaluated using an alpha of 0.05. Effect sizes are reported as Cohen's $d$.

# RESULTS

AAs and EAs did not significantly differ on BMI [$t(56) = 1.69$, $p = 0.099$], sex ($\chi^2 = 2.01$, $p = 0.184$), or age [$t(56) = 1.31$, $p = 0.196$].

See **Table 1A** for all resting cardiovascular and hemodynamic parameters in AAs and EAs. AAs showed significantly greater SBP [$t(56) = -1.87$, $d = 0.50$, $p = 0.035$], RMSSD [$t(56) = -1.87$, $d = 0.49$, $p = 0.033$], and TPR [$t(56) = -2.36$, $d = 0.65$ $p = 0.011$] compared to EAs. It is important to note that these results remain

**TABLE 1 |** Mean and standard deviations of resting cardiovascular and hemodynamic parameters in African Americans (AAs) compared to European Americans (EAs) (study 1) and in Lesbian, Gay and Bisexuals (LGB) compared to heterosexual (HS) participants (study 2).

| Table 1A—study 1 | AAs ($n$ = 30) | EAs ($n$ = 28) | $t$ | $p$ |
|---|---|---|---|---|
| MAP (mmHg) | 70 ± 10 | 64 ± 5 | −2.51 | **0.015*** |
| SBP (mmHg) | 122 ± 12 | 116 ± 10 | −1.87 | **0.035*** |
| IBI (ms) | 840.99 ± 91.33 | 828.46 ± 141.11 | −0.40 | 0.344 |
| HR (bpm) | 72.13 ± 7.67 | 74.49 ± 12.82 | 0.853 | 0.397 |
| RMSSD (ms \| natural log) | 3.98 ± 0.40 | 3.76 ± 0.50 | −1.87 | **0.033*** |
| SV (ml) | 91.62 ± 25.32 | 95.43 ± 20.58 | 0.63 | 0.267 |
| CO (l/min) | 6.50 ± 1.52 | 7.00 ± 1.51 | 1.26 | 0.213 |
| TPR (PRU) | 806.69 ± 265.31 | 673.33 ± 143 | −2.36 | **0.011*** |
| **Table 2A—study 2** | **LGB ($n$ = 19)** | **HS ($n$ = 20)** | $t$ | $p$ |
| MAP (mmHg) | 106 ± 28 | 89 ± 10 | 2.45 | **0.019*** |
| SBP (mmHg) | 142 ± 33 | 123 ± 12 | 2.39 | **0.022*** |
| HR (bpm) | 76.99 ± 8.4 | 81.58 ± 14.2 | 1.22 | 0.231 |
| RMSSD (ms) | 41.44 ± 10.8 | 33.2 ± 13.9 | 2.05 | **0.047*** |
| CO (l/min) | 5.15 ± 1.5 | 5.42 ± 1.2 | 0.61 | 0.549 |
| TPR (PRU) | 1519.63 ± 778.5 | 600.91 ± 634.4 | 4.05 | **<0.001*** |

*SBP, systolic blood pressure; IBI, inter-beat-intervals; RMSSD, root mean square of successive differences; SV, stroke volume; TPR, Total Peripheral Resistance; PRU, Peripheral Resistance Units; MAP, Mean Arterial Pressure; HR, Heart Rate; CO, Cardiac Output.*
*Significant p-values bolded. *$p < 0.05$.*

significant if ANOVAs controlling for BMI as a covariate are performed instead of *t*-tests.

# Study 2

## Participants

Recruitment occurred through the snowball sample technique, as well as using a flyer posted on social media and the "Be as you are" research and clinical center (Baiocco and Pistella, 2019). Inclusion criteria for the study were: (1) self-identifying as LGB; (2) not having diagnosis of hypertension, heart disease, and psychiatric diagnosis; and (3) not using any drugs/medications that might affect cardiovascular function. Of the 40 people who took part, 1 was excluded due to Portapres device malfunction. A total of 19 LGB (mean age: 35.42 (10.88) years; BMI: 25.33 (5.45) Kg/m$^2$) and 20 heterosexual individuals (mean age of 35.26 (10.14) years, BMI: 25.78 (3.42) Kg/m$^2$) participated in the study. The LGB group was composed by 8 cisgender women who self-identified as lesbians ($n$ = 6) or bisexuals ($n$ = 2) and 11 cisgender men who self-identified as gay ($n$ = 10) or bisexual ($n$ = 1). To discount the effects of genetics (and potentially of diet/lifestyle) on the conundrum in African Americans, all participants were Caucasians. Most participants were of middle socio-economic status, 2 participants of low- and 2 of high-socio-economic status. The educational level varied from high school diploma ($n$ = 15) to bachelor's ($n$ = 9) or higher degree ($n$ = 15).

## Procedure

To ensure data accuracy, participants were asked not to drink coffee and alcohol, not to smoke cigarettes and to avoid strenuous exercise for 2 h prior to the appointment. The experiment took place at the premises of the "Be as you are" clinical service of the Department of Developmental and Social Psychology, Sapienza University of Rome, in a quiet room with a closed door. After informed consent procedures, the continuous BP cuff was attached on the index finger of participants' dominant hand, while participants were in a seated position with their hands lying on a table. The experimental protocol started with a baseline period of 2 min followed by three experimental conditions: (1) a cognitive task, consisting in performing simple arithmetic operations and verbally report the results; (2) an emotional stress induction task, consisting in asking participants to verbally report a stressful episode of their life; and -for the LGB group only- (3) a sexual minority emotional stress induction task, consisting in asking participants to verbally report a stressful episode related to their LGB identity. The order of the stressful tasks was randomized for each participant. The cognitive task lasted 2 min, while each of the emotional tasks lasted 5 min. Each task was followed by a recovery period of 5 min, during which participants were invited to remain silent and just leaf through a content-neutral magazine (Jennings et al., 1992).

## Experimental Task Instructions

The instructions for the cognitive task were: "*Now I will ask you to count backwards out loud by subtracting 5 units from the number that I will tell you. As an example: The starting number is 285. The following numbers are: 280, 275, 270, 265, and so on. Is everything clear? I will tell you when to stop. Now it is your turn. The starting number is 253*."

The instructions for the emotional stress induction task were: "*Now I ask you to tell me about a stressful episode in your life* (for the LGB participants we specified that the stressful episodes should not be related to their sexual minority identity). *Let's take an example. You could tell about a job interview that went wrong or a theft you suffered, a difficult time from an economic point of view. Take your time to think about it. Tell me when you are ready*."

The instructions for the sexual minority emotional stress induction task were: "*Now I ask you to tell me about a stressful episode in your life which is related to your LGB identity. Let's take an example. You could talk about an episode of discrimination at work, mockery or exclusion by your peers because you are LGB. Take your time to think about it. Tell me when you are ready*."

## Questionnaires

### *Sociodemographic Variables*

The survey included several sociodemographic questions, to obtain information such as age, gender identity, sexual orientation, ethnicity, socio-economic status, and educational level. Exclusion criteria, assessed via self-report questionnaires, were diagnosis of hypertension, heart disease, psychiatric disorder or habitual intake of drugs/medications affecting the cardiovascular system.

### *Minority Stress Experiences*

The *Daily Heterosexist Experiences Questionnaire* (DHEQ; Balsam et al., 2013) was used to measure day-to-day minority stress experienced by participants. For the purpose of this

study, we used 5 of the 10 subscales of the questionnaire, assessing negative experiences related to gender expression (e.g., Being harassed in bathrooms because of your gender expression), vigilance (e.g., Watching what you say and do around heterosexual people), discrimination (e.g., Being treated unfairly in stores or restaurants because you are LGB), victimization (e.g., Being punched, hit, kicked, or beaten because you are LGB), and isolation (e.g., Difficulty finding a partner because you are LGB). Participants were invited to answer the following question: "How much has this problem distressed or bothered you during the past 12 months?" by using a 6-point Likert scale ranging from 0 (did not happen/not applicable to me) to 5 (it happened, and it bothered me extremely). Previous studies conducted in the Italian context showed good internal reliability of the scales (*blinded for peer review*).

#### *Depressive Rumination*

The Ruminative Response Scale (RRS; Nolen-Hoeksema and Morrow, 1991) was administered to exclude group differences in trait rumination, given the well-established effects of this trait on HRV and hemodynamic parameters (Ottaviani et al., 2016, 2017). The RRS measures how often people engage in responses to depressed mood that are self-focused (e.g., I think "Why do I react this way?"), symptom-focused (e.g., I think about how hard it is to concentrate), and focused on the possible consequences and causes of one's mood (e.g., I think "I won't be able to do my job if I don't snap out of this").

### Physiological Assessment

Noninvasive continuous measurement of beat-to-beat BP was obtained throughout the study with the Portapres II (FMS; The Netherlands; sampling rate: 200 Hz) device, tested against a mercury sphygmomanometer. The BeatScope® software, which is based on the Modelflow® algorithm and corrects for age, height, and weight, was used to derive heart rate (HR), inter-beat intervals (IBIs), mean arterial pressure (MAP), cardiac output (CO), and TPR. Following the orthogonal, physiologically grounded model proposed by Gregg and colleagues for both reactivity and recovery periods, participants are described using two independent parameters: the way in which they respond, i.e., "more vascular or more myocardial" (hemodynamic profile; HP) and "the extent to which an increase of TPR compensates for an increase in CO and vice versa" (compensation deficit; CO) (Gregg et al., 2002). The model: (i) takes into account the multiplicative relationship between CO and TPR (Guyton, 1987); (ii) is based on the assumption that HP and CD are orthogonal; and (iii) uses ratio scores instead of difference scores of reactivity (see James et al., 2012 for a meta-analysis). The equation used to address the concept of hemodynamic profile was: $\log(\mathrm{CO})\mathrm{r} + log(TPR)_r = log(MAP)_r$, where "*r*" in the equation refers to the ratio of task to baseline values for reactivity and to the ratio of resting to baseline for recovery.

Then, a 45° rotation of the two-dimensional space formed by the cardiac output and total peripheral resistance reactivity dimensions was performed to achieve the orthogonal relationship between HP and CD (see Gregg et al., 2002 for further methodological details). The outcome of the model is a continuous variable in which greater HP values correspond to more vascular responses and greater CD values indicate that increased TPR is not compensated by a proportionate decrease in CO (see Gregg et al., 2002 for methodological details).

HRV was assessed by computing the RMSSD: IBIs were transferred to Kubios HRV software for RMSSD analysis and artifact detection (Tarvainen et al., 2014). Ectopic beats were corrected using the "automatic correction" function, in which artifacts are detected from a time series consisting of differences between successive RR intervals.

### Data Analysis

All data are expressed as means (SD). Differences at $p < 0.05$ were regarded as significant. Data processing was performed with the software modules of SPSS 25 (IBM). MAP, HR, RMSSD, CO, and TPR, HP, CD, and scores on RRS were treated as continuous variables.

Assumptions for normality were tested for all continuous variables using the Shapiro-Wilk test. Differences between the two groups (LGB vs. heterosexuals) in age, sex distribution, BMI, years of education, economic status, and levels of dispositional depressive rumination were analyzed by *t*-tests and $\chi^2$-tests. The variables that differed significantly between groups were included as covariates in all the subsequent analyses.

To test for group differences in reactivity and recovery for the shared two experimental conditions, a series of 2 (task: emotional vs. cognitive) ×2 (group: LGB vs. heterosexuals) general linear models (GLMs) were performed on HP and CD during reactivity and recovery. For the LGB group only, further analyses were conducted to test for differences in hemodynamics between the LGB-related and LGB-unrelated emotional task. To do so, a 2 (task: LGB-related vs. LGB-unrelated) ×2 (time: reactivity vs. recovery) GLMs were executed on HP and CD.

Lastly, Spearman's correlations between scores on the DHEQ, resting RMSSD, and HP and CD during reactivity to and recovery from the LGB-related task (LGB group only) were performed.

### Results

Each dependent variable was normally distributed. No group differences due to potential confounders (i.e., age, sex distribution, BMI, years of education, economic status, and levels of dispositional depressive rumination) emerged (all *p*s > 0.05). In the LGB group, scores on the DHEQ ranged from 1.07 to 2.33 ($M = 1.55$, $SD = 0.35$), and scores on the RRS ranged from 1.09 to 2.68 ($M = 1.85$, $SD = 0.45$).

Table 2A depicts resting cardiovascular and hemodynamic parameters in LGB and heterosexual participants. The two groups were significantly different in terms of MAP, TPR, and RMSSD at baseline with LGB having a higher MAP ($t = 2.45$, $p = 0.019$; $d = 0.78$), TPR ($t = 4.05$, $p < 0.0001$; $d = 1.29$) and RMSSD ($t = 2.05$, $p = 0.047$; $d = 0.66$) compared to heterosexuals. No differences emerged for HR and CO. This replicated the Cardiovascular Conundrum pattern of higher RMSSD and higher TPR found in AAs in Study One.

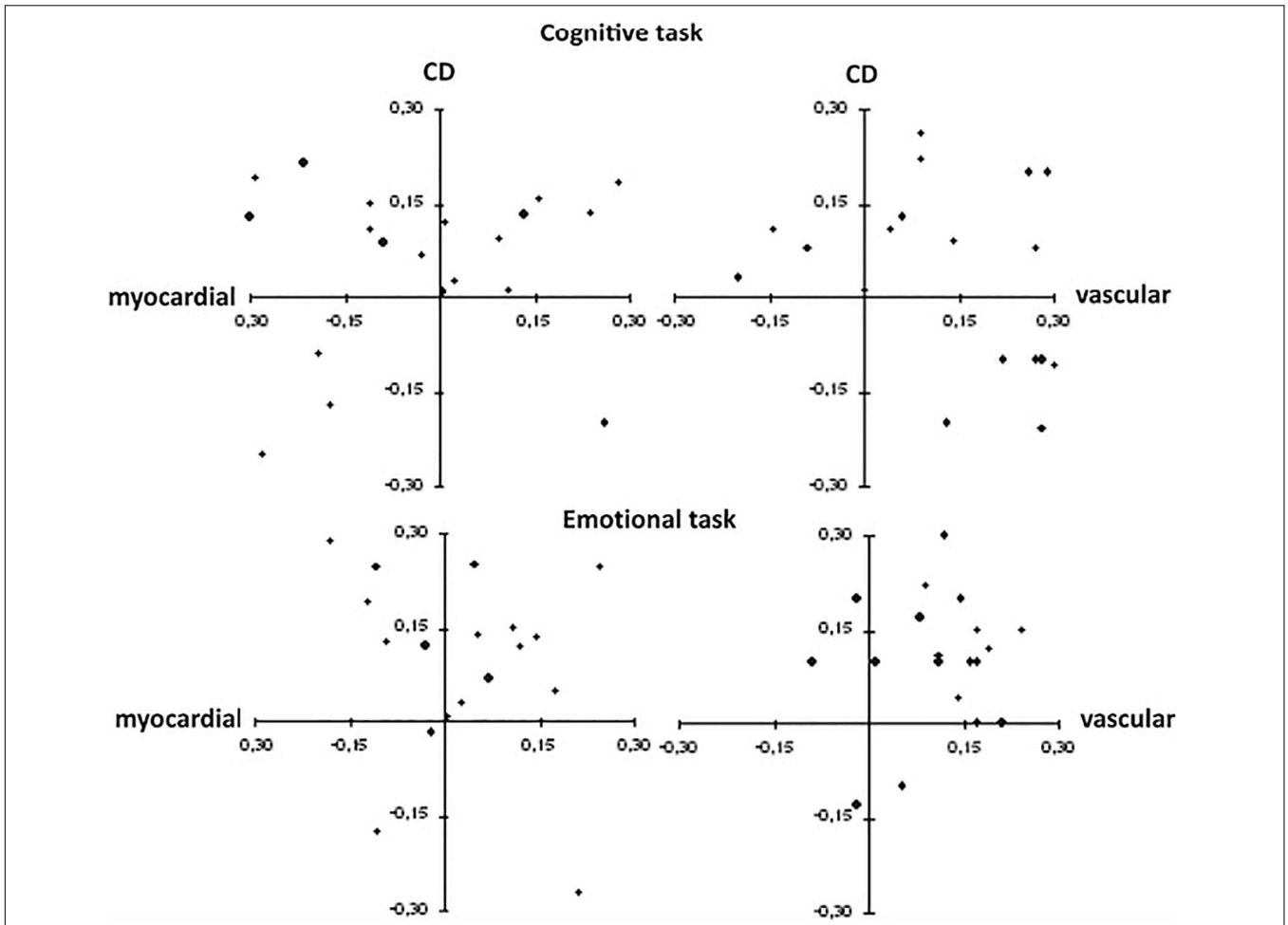



**FIGURE 1 |** Scatterplots for hemodynamic profile and compensation deficit during each task in heterosexual (right panel) and lesbian, gay and bisexual (left panel) participants. Each point represents each participant's hemodynamic profile (HP) and compensation deficit (CD). A "more vascular" profile is associated with more positive values along the *x*-axis and a "more myocardial" profile is associated with more negative values along the *x*-axis. A "higher deficit" in compensating is associated with more positive values on the *y*-axis and a "lower deficit" in compensating is associated with more negative values on the *y*-axis.

**Figure 1** shows the relationship between hemodynamic profile and compensation deficit scores in the two groups for the different tasks. As for HP reactivity, main effects of Task, $F(1, 37) = 6.26$, $p = 0.017$; $\eta_p^2 = 0.15$, and Group, $F(1, 37) = 5.69$, $p = 0.022$; $\eta_p^2 = 0.13$ emerged, while the Task X Group interaction was not statistically significant. Pre-planned comparisons showed that, irrespective of task, LGB had a more vascular HP compared to heterosexual participants and that the emotional task was characterized by a more vascular pattern of response compared to the cognitive task.

A similar pattern of results emerged when CD was considered. The GLM yielded main effects of Task, $F(1, 37) = 20.39$, $p < 0.0001$; $\eta_p^2 = 0.36$, and Group, $F(1, 37) = 4.30$, $p = 0.045$; $\eta_p^2 = 0.10$ but no significant Task X Group interaction. As shown by pre-planned comparisons, LGB had a "higher deficit" in compensating compared to heterosexual participants and the emotional task was characterized by a higher CD compared to the cognitive task.

The GLMs having HP and CD during recovery from the tasks as outcomes did not yield any significant main effect or interaction.

When the difference between LGB-related vs. LGB-unrelated emotional task in HP and CD for the LGB group was considered, the GLM yielded a marginally significant Time x Task interaction for HP, $F(1, 18) = 4.04$, $p = 0.06$; $\eta_p^2 = 0.18$. Pre-planned comparisons showed that a more vascular hemodynamic response emerged for reactivity to the LGB-related (see **Figure 2**) compared to the LGB-unrelated task ($t = 5.30$; $p < 0.0001$), whereas no differences emerged for the recovery phase.

Spearman's correlations yielded significant inverse associations between scores on the day-to-day minority stress (DHEQ) and reactivity to the LGB-related task for both HP ($rho = -0.59$; $p = 0.008$) and CD ($rho = -0.48$; $p = 0.037$). Higher RMSSD at rest was also marginally associated with "higher deficits" in compensating during the LGB-related task ($rho = 0.41$; $p = 0.07$). No other significant correlations emerged.

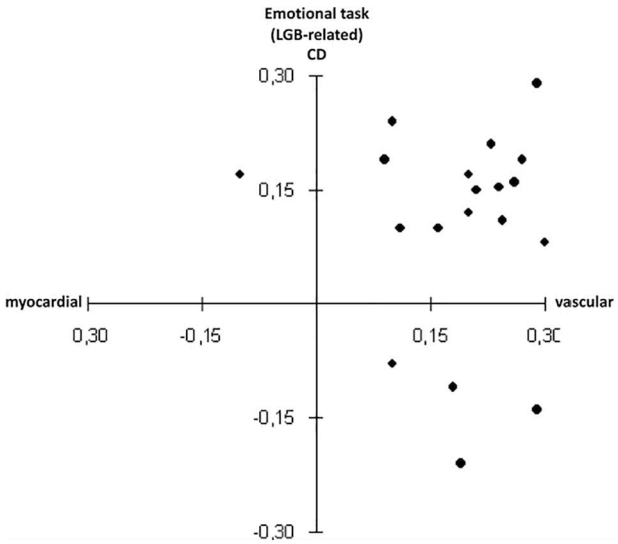



**FIGURE 2 |** Scatterplots for hemodynamic profile and compensation deficit during the lesbian, gay and bisexual (LGB)-related task in the LGB group.

## DISCUSSION

The aim of this study was to preliminarily investigate whether the dysfunctional physiological profile found in African Americans is seen also in other minority groups subjected to discrimination, such as LGB. First, study 1 replicated previous reports on the presence of a so-called Cardiovascular Conundrum in African Americans, that is a pattern of high resting HRV coupled with high TPR, that is likely due to a dysfunction at the baroreflex level (Hill et al., 2015 for a meta-analysis of ethnic differences in HRV and Brownlow et al., 2020 for a meta-analysis on ethnic differences in TPR). Study 1 did not include a stressor because the Conundrum was originally found at rest, however, the fact that resting high HRV is also associated with a more prominent vascular hemodynamic reactivity to physical (i.e., orthostasis) and emotional (i.e., anger recall) stressors in African Americans has been previously reported (Carnevali et al., 2019).

As hypothesized by Thayer and Koenig (2019), we looked at whether this Cardiovascular Conundrum could be due to chronic exposure to a psychosocial stressor such as discrimination. Remarkably, when we looked at sexual minorities instead of ethnic minorities, the pattern was the same both at baseline and during confrontation with an emotional task. First, present data preliminarily suggest that when compared to heterosexual individuals, LGB people are characterized by higher HRV and higher TPR at rest after controlling for a number of potential confounders. Despite showing greater HRV at baseline, the LGB group showed a prominent vascular hemodynamic profile and compensation deficit during emotional tasks such as the recall of personal (both LGB- and non LGB-related) episodes, but not during a mathematical task. These results replicate and extend our prior findings of the Cardiovascular Conundrum pattern in African Americans compared to European Americans (Hill et al., 2015; Carnevali et al., 2019; Brownlow et al., 2020). Furthermore, they suggest that genetic factors are unlikely to account for this unique cardiovascular pattern.

To date, findings on cardiovascular health disparities in LGB are inconsistent. And yet, a recent review concluded that sexual minorities are at increased risk for cardiovascular disease (Caceres et al., 2017). As previously described with regards to ethnic minorities, this has been ascribed to hyperreactivity to stressors (see for example Juster et al., 2019 for differences between men and women). The present study took a step further, looking at the underlying hemodynamic profiles of cardiovascular reactivity and found that -compared to that of heterosexuals- the pattern shown by LGB participants was more vascular in nature. This is crucial information, given that elevated BP driven by TPR, compared to CO, has been linked to increased risk of cardiac events and mortality (Julius, 1988).

The associations between discrimination, reports or lack thereof of such discrimination and psychophysiological responses is complex. Whereas some studies have reported that discrimination is associated with deleterious physiological responses such as greater BP (Pascoe and Richman, 2009; Dolezsar et al., 2014), others have found that reports of discrimination associated with salubrious physiological responses such as greater HRV (Kemp et al., 2016). In addition, some studies have reported that fewer reports of discrimination are associated with greater physiological responses such as greater BP (Krieger and Sidney, 1996).

Notably, in the present study, a more vascular pattern during the recall of LGB-related episodes emerged in those who reported fewer daily experiences of discrimination, victimization, isolation, and so on (i.e., scores on the DHEQ). While counterintuitive, this result is not uncommon to find in the literature. For example, Christian and colleagues also found an inverse association between incidences of discrimination and TPR in pregnant African Americans, while this was not true for European Americans (Christian et al., 2020). In a larger study, working-class African American adults ($N$ = 1,974) who typically accepted unfair treatment and had reported no experience of racial discrimination had higher systolic BP compared to those who challenged unfair treatment and reported experiencing racial discrimination (Krieger and Sidney, 1996). Similarly, in studying another minority ethnic group in the United States, Rodriguez and colleagues have found that a dysfunctional pattern of nocturnal non-dipping BP was particularly present in black-Hispanic participants with low perceived racism compared to those with high perceived racism (Rodriguez et al., 2016). The authors interpreted this result in terms of internalization of racism, where those with higher perceived racism may be "more proactive against discrimination and possibly less likely to internalize the associated stress" (Rodriguez et al., 2016). Interestingly, Frost et al. (2015) found that only externally rated minority stressors such as prejudice events -but not self-appraised self-exposure- predicted physical health problems, such as hypertension in LGB people. Such current and reviewed findings highlight the complex relationships among discrimination, reports of discrimination, and psychophysiological responses such that deleterious effects and salubrious effects may co-occur

in the same individuals. Future studies are needed to further explicate these associations as effects may be non-linear or compensatory in nature.

*John Henryism* is considered a high-effort strategy to cope with discrimination (James et al., 1983). Current results suggest that such strategy is likely to involve processes that take place outside the individual's awareness. It is mandatory to conduct further studies examining whether the Cardiovascular Conundrum is associated with a specific coping strategy that is peculiar of individuals who are subject to discrimination without being fully aware of it.

There are some limitations that need to be mentioned. In the second study, the number of participants was limited and *post-hoc* power analysis pointed to some of the analyses being underpowered ($1 - \beta > 0.60$); thus, replication with a larger sample is warranted before we can answer the research question "Is the Cardiovascular Conundrum a marker of constant coping with discrimination?" Moreover, the present study focused on the experience of being potentially subjected to discrimination without specifically assessing subjective levels of stress or coping strategies in response to it such as reappraisal and anger suppression. Based on prior work, however, we can speculate that suppression would be associated with higher HRV in minority groups only, therefore playing an important role in the evolution of the Cardiovascular Conundrum (Thayer and Koenig, 2019). Also, the cognitive and emotional tasks used in Study 2 had different durations (2 vs. 5 min) which may have biased the results. Lastly, it has to be noted that although estimation of HRV derived from photoplethysmographic technique is not considered the golden standard, studies have shown that HRV measures can be accurately derived using such technique in healthy subjects under ideal conditions (e.g., Lu et al., 2009).

Limitations notwithstanding, the current results may inform the interpretation of previous research on the Cardiovascular Conundrum in African Americans, pointing to the presence of this physiological pattern in other populations and suggesting a plausible underlying mechanism related to emotion regulation as opposed to genetic factors. Large scale data are needed to draw causal inferences on this complex and intriguing phenomenon.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies were reviewed and approved by the Ohio State Institutional Review Board at The Ohio State University (Study 1) and Research Ethics Committee of the Department of Developmental and Social Psychology, Sapienza University of Rome, Rome, Italy (Study 2). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JT, CO, DW, and RB contributed to the conceptualization of the study. FR and DW conducted the study and analyzed the data. CO wrote the initial draft of the manuscript. DW also contributed to writing elements of the manuscript. All authors contributed to the interpretation of the results, provided critical feedback, helped shape the analysis and manuscript, and approved the submitted manuscript.

## FUNDING

The publication fee was covered by grant no. RG11916B56464-AED (Progetto Grande di Ateneo 2019: "Coming out to Siblings, Best Friends, and Parents in an Italian Sample of Lesbian, Gay, Bisexual People and those of all other sexual orientations").

## ACKNOWLEDGMENTS

We were grateful to Sara Cannavó for her valuable contribution to the conduction of Study 2 and to Dr. Giovanni Calcagnini for kindly helping us with the use of the Portapres device.

## REFERENCES

Baiocco, R., and Pistella, J. (2019). "Be as You Are" Clinical Research Center at the Sapienza University of Rome. *J. Gay Lesbian Ment. Health* 23, 376–379. doi: 10.1080/19359705.2019.1644572

Baiocco, R., Scandurra, C., Rosati, F., Pistella, J., Ioverno, S., Bochicchio, V., et al. (2021). Minority stress, resilience, and health in Italian and Taiwanese LGB+ people: a cross-cultural comparison. *Curr. Psychol.* doi: 10.1007/s12144-021-01387-2

Balsam, K. F., Beadnell, B., and Molina, Y. (2013). The daily heterosexist experiences questionnaire: measuring minority stress among lesbian, gay, bisexual, and transgender adults. *Meas. Eval. Couns. Dev.* 46, 3–25. doi: 10.1177/0748175612449743

Brownlow, B. N., Williams, D. P., Kapuku, G., Vasey, M. W., Anderson, N. B., Koenig, J., et al. (2020). Ethnic differences in resting total peripheral resistance: a systematic review and meta-analysis. *Psychosom. Med.* 82, 548–560. doi: 10.1097/PSY.0000000000000820

Caceres, B. A., Brody, A., Luscombe, R. E., Primiano, J. E., Marusca, P., Sitts, E. M., et al. (2017). A Systematic review of cardiovascular disease in sexual minorities. *Am. J. Public Health* 107:570. doi: 10.2105/AJPH.2016.303630a

Caceres, B. A., Makarem, N., Hickey, K. T., and Hughes, T. L. (2019). Cardiovascular disease disparities in sexual minority adults: an examination of the behavioral risk factor surveillance system (2014-2016). *Am. J. Health Promot.* 33, 576–585.

Cardel, M. I., Chi, X., Min, Y. I., Sims, M., Musani, S. K., Dulin, A., et al. (2020). Experiences of discrimination are associated with worse metabolic syndrome severity among African Americans in the Jackson Heart Study. *Ann. Behav. Med.* 55, 266–279. doi: 10.1093/abm/kaaa050

Carnevali, L., Ottaviani, C., Williams, D., Kapoor, G., Thayer, J., and Hill, L. B. (2019). Hemodynamic profile and compensation deficit in African and European Americans during physical and mental stress. *Biol. Psychol.* 141, 17–24. doi: 10.1016/j.biopsycho.2018.12.003

Cedillo, Y. E., Lomax, R. O., Fernandez, J. R., and Moellering, D. R. (2020). Physiological significance of discrimination on stress markers, obesity, and

LDL oxidation among a European American and African American Cohort of Females. *Int. J. Behav. Med.* 27, 213–224. doi: 10.1007/s12529-020-09850-3

Christian, L. M., Koenig, J., Williams, D. P., Kapuku, G., and Thayer, J. F. (2020). Impaired vasodilation in pregnant African Americans: preliminary evidence of potential antecedents and consequences. *Psychophysiology* 58:e13699. doi: 10.1111/psyp.13699

Cook, S. H., Pruessner, J. C., Lupien, S. J., and Juster, R. (2018). Sexual orientation moderates the association between parental overprotection and stress biomarker profiles. *Psychol. Sex.* 9, 204–220. doi: 10.1080/19419899.2018.1470105

Dolezsar, C. M., McGrath, J. J., Herzig, A. J. M., and Miller, S. B. (2014). Perceived racial discrimination and hypertension: a comprehensive systematic review. *Health Psychol.* 33, 20–34. doi: 10.1037/a0033718

Dorr, N., Brosschot, J. F., Sollers, J. J. I. I. I., and Thayer, J. F. (2007). Damned if you do, damned if you don't: the differential effect of expression and inhibition of anger on cardiovascular recovery in black and white males. *Int. J. Psychophysiol.* 66, 125–134. doi: 10.1016/j.ijpsycho.2007.03.022

Dürrbaum, T., and Sattler, F. A. (2019). Minority stress and mental health in lesbian, gay male, and bisexual youths: a meta-analysis. *J. LGBT Youth* 17, 298–314. doi: 10.1080/19361653.2019.1586615

Frost, D. M., Lehavot, K., and Meyer, I. H. (2015). Minority stress and physical health among sexual minority individuals. *J. Behav. Med.* 38, 1–8. doi: 10.1007/s10865-013-9523-8

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Blaha, M. J., et al. (2014). Heart disease and stroke statistics–2014 update: a report from the American Heart Association. *Circulation* 129:e28–e292. doi: 10.1161/01.cir.0000441139.02102.80

Gregg, M. E., Matyas, T. A., and James, J. E. (2002). A new model of individual differences in hemodynamic profile and blood pressure reactivity. *Psychophysiology* 39, 64–72. doi: 10.1111/1469-8986.3910064

Guyton, A. (1987). *Human Physiology and Mechanisms Of Disease*, 4th Edn. Philadelphia, PA: W.B. Saunders.

Herek, G. M. (2004). Beyond "homophobia": thinking about sexual stigma and prejudice in the twenty-first century. *Sex. Res. Soc. Policy* 1, 6–24. doi: 10.1525/srsp.2004.1.2.6

Hill, L. K., Hoggard, L. S., Richmond, A. S., Gray, D. L., Williams, D. P., and Thayer, J. F. (2017). Examining the association between perceived discrimination and heart rate variability in African Americans. *Cultur. Divers. Ethnic Minor. Psychol.* 23, 5–14. doi: 10.1037/cdp0000076

Hill, L. K., Hu, D. D., Koenig, J., Sollers, J. J. III, Kapuku, G., Wang, X., et al. (2015). Ethnic differences in resting heart rate variability: a systematic review and meta-analysis. *Psychosom. Med.* 77, 16–25. doi: 10.1097/PSY.0000000000000133

Hill, L. K., and Thayer, J. F. (2019). The autonomic nervous system and hypertension: ethnic differences and psychosocial factors. *Curr. Cardiol. Rep.* 21:15. doi: 10.1007/s11886-019-1100-5

Homandberg, L. K., and Fuller-Rowell, T. E. (2020). Experiences of discrimination and urinary catecholamine concentrations: longitudinal associations in a college student sample. *Ann. Behav. Med.* 54, 843–852. doi: 10.1093/abm/kaaa033

James, J. E., Gregg, M. E., Matyas, T. A., Hughes, B. M., and Howard, S. (2012). Stress reactivity and the hemodynamic profile-compensation deficit (HP-CD) model of blood pressure regulation. *Biol. Psychol.* 90, 161–170. doi: 10.1016/j.biopsycho.2012.02.021

James, S. A., Hartnett, S. A., and Kalsbeek, W. D. (1983). John Henryism and blood pressure differences among black men. *J. Behav. Med.* 6, 259–278.

Jennings, J. R., Kamarck, T., Stewart, C., Eddy, M., and Johnson, P. (1992). Alternate cardio- vascular baseline assessment techniques: vanilla or resting baseline. *Psychophysiology* 29, 742–750.

Julius, S. (1988). Transition from high cardiac output to elevated vascular resistance in hypertension. *Am. Heart J.* 116, 600–606.

Juster, R. P., Doyle, D. M., Hatzenbuehler, M. L., Everett, B. G., DuBois, L. Z., and McGrath, J. J. (2019). Sexual orientation, disclosure, and cardiovascular stress reactivity. *Stress* 22, 321–331. doi: 10.1080/10253890.2019.1579793

Kemp, A. H., Koenig, J., Thayer, J. F., Bittencourt, M. S., Pereira, A. C., Santos, I. S., et al. (2016). Race and resting-state heart rate variability in Brazilian civil servants and the mediating effects of discrimination: an ELSA-Brasil cohort study. *Psychosom. Med.* 78, 950–958. doi: 10.1097/PSY.0000000000000359

Krieger, N., and Sidney, S. (1996). Racial discrimination and blood pressure: the CARDIA Study of young black and white adults. *Am. J. Public Health* 86, 1370–1378. doi: 10.2105/ajph.86.10.1370

Kuyper, L., and Fokkema, T. (2011). Minority stress and mental health among Dutch LGBs: examination of differences between sex and sexual orientation. *J. Couns. Psychol.* 58, 222–233. doi: 10.1037/a0022688

Lehavot, K., and Simoni, J. M. (2011). The impact of minority stress on mental health and substance use among sexual minority women. *J. Consult. Clin. Psychol.* 79, 159–170. doi: 10.1037/a0022839

Lu, G., Yang, F., Taylor, J. A., and Stein, J. F. (2009). A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J. Med. Eng. Technol.* 33, 634–641. doi: 10.3109/03091900903150998

Mays, V. M., Juster, R. P., Williamson, T. J., Seeman, T. E., and Cochran, S. D. (2018). Chronic physiologic effects of stress among lesbian, gay, and bisexual adults: results from the National Health and Nutrition Examination Survey. *Psychosom. Med.* 80, 551–563. doi: 10.1097/PSY.0000000000000600

Meyer, I. H. (2003). Prejudice, social stress, and mental health in lesbian, gay, and bisexual populations: conceptual issues and research evidence. *Psychol. Bull.* 129, 674–697. doi: 10.1037/0033-2909.129.5.674

Michaels, E. K., Reeves, A. N., Thomas, M. D., Price, M. M., Hasson, R. E., Chae, D. H., et al. (2019). Everyday racial discrimination and hypertension among midlife African American women: disentangling the role of active coping dispositions versus active coping behaviors. *Int. J. Environ. Res. Public Health* 16:4759. doi: 10.3390/ijerph16234759

Nolen-Hoeksema, S., and Morrow, J. (1991). A prospective study of depression and distress following a natural disaster: the 1989 Loma Prieta earthquake. *J. Pers. Soc. Psychol* 61, 115–121. doi: 10.1037/0022-3514.61.1

Obrist, P. (1981). *Cardiovascular Psychophysiology: A Perspective*. New York, NY: Plenum Press.

Ottaviani, C., Brosschot, J. F., Lonigro, A., Medea, B., Van Diest, I., and Thayer, J. F. (2017). Hemodynamic profiles of functional and dysfunctional forms of repetitive thinking. *Ann. Behav. Med.* 51, 261–271. doi: 10.1007/s12160-016-9851-3

Ottaviani, C., Thayer, J. F., Verkuil, B., Lonigro, A., Medea, B., Couyoumdjian, A., et al. (2016). Physiological concomitants of perseverative cognition: a systematic review and meta-analysis. *Psychol. Bull.* 142, 231–259. doi: 10.1037/bul0000036

Park, G., Vasey, M. W., Van Bavel, J. J., and Thayer, J. F. (2014). When tonic cardiac vagal tone predicts changes in phasic vagal tone: the role of fear and perceptual load. *Psychophysiology* 51, 419–426. doi: 10.1111/psyp.12186

Pascoe, E. A., and Richman, L. S. (2009). Perceived discrimination and health: a meta-analytic review. *Psychol. Bull.* 135, 531–554. doi: 10.1037/a0016059

Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometri- cal and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol.* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Pool, L. R., Ning, H., Lloyd-Jones, D. M., and Allen, N. B. (2017). Trends in racial/ethnic disparities in cardiovascular health among US adults from 1999-2012. *J. Am. Heart Assoc.* 6:e006027. doi: 10.1161/JAHA.117.006027

Rodriguez, C. J., Gwathmey, T. M., Jin, Z., Schwartz, J., Beech, B. M., Sacco, R. L., et al. (2016). Perceived discrimination and nocturnal blood pressure dipping among Hispanics: the Influence of Social Support and Race. *Psychosom. Med.* 78, 841–850. doi: 10.1097/PSY.0000000000000341

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Prog. Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Thayer, J. F., Carnevali, L., Sgoifo, A., and Williams, D. P. (2020). Angry in America: psychophysiological responses to unfair treatment. *Ann. Behav. Med.* 54, 924–931. doi: 10.1093/abm/kaaa094

Thayer, J. F., and Koenig, J. (2019). Resting cerebral blood flow and ethnic differences in heart rate variability: links to self-reports of affect and

affect regulation. *Neuroimage* 202:116154. doi: 10.1016/j.neuroimage.2019.116154

Thayer, J. F., Yamamoto, S. S., and Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Williams, D. P., Pandya, K. D., Hill, L. K., Kemp, A. H., Way, B. M., Thayer, J. F., et al. (2017). Rumination moderates the association between resting high-frequency heart rate variability and perceived ethnic discrimination. *J. Psychophysiol.* 33, 13–21. doi: 10.1027/0269-8803/a000201

Zilbermint, M., Hannah-Shmouni, F., and Stratakis, C. A. (2019). Genetics of hypertension in African Americans and others of African descent. *Int. J. Mol. Sci.* 20:1081. doi: 10.3390/ijms20051081

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer SB declared a past publication with one of the author JT to the handling editor.

frontiers in Neuroscience

OPINION
published: 08 June 2021
doi: 10.3389/fnins.2021.564159

# Wireless Heart Rate Variability in Assessing Community COVID-19

*Robert L. Drury*[1]*, *Marc Jarczok*[2], *Andrew Owens*[3] and *Julian F. Thayer*[4]

[1] Canary Systems, Bainbridge Island, WA, United States, [2] Clinic for Psychosomatic Medicine and Psychotherapy, University Clinic Ulm, Ulm, Germany, [3] Department of Old Age Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [4] Psychological Sciences Faculty, University of California, Irvine, Irvine, CA, United States

**Keywords: heart rate variability, COVID-19 pandemic, community prevalence, networked hardware/software systems, digital epidemiology, Internet of Healthy Things (IoHT)**

## INTRODUCTION

According to the Johns Hopkins Coronavirus Resources Center, the number of confirmed COVID-19 cases exceeds 170,558,922 worldwide today with more than 3,546,881 fatalities (2021). The pandemic's massive health and well-being issues have already impacted the lives of millions globally, including spikes in mortality and morbidity. Many nations were unprepared for these significant consequences, revealing the critical need for standard public health principles of population assessment, intervention, and treatment. This editorial will address an innovative use of Heart Rate Variability (HRV), which is a non-invasive, inexpensive, and sensitive measure of inflammatory processes and immunomodulation (Kovatchev et al., 2003; Ahmad et al., 2009; Leitzke et al., 2020; Owens, 2020), among other health and well-being parameters. Specifically, the vagus nerve maintains tonic inhibitory control of proinflammatory cytokines via acetylcholine release into the reticuloendothelial system (e.g., spleen, gastrointestinal tract, heart, liver), mediating the inflammatory reflex through the cholinergic anti-inflammatory pathway (Dantzer and Kelley, 2007). HRV has been described in this special HRV Horizons 2030 Frontiers series as follows: "HRV offers insights into humoral, neural, and neurovisceral processes in health and disorders of brain, body, and behavior but has yet to be fully potentiated in the digital age" (Owens, 2020). Building on a growing body of HRV data (Rangon et al., 2020; Whitelaw et al., 2020; Hirten et al., 2021), we propose use of a wearable high fidelity Oura sensor ring (https://ouraring.com/blog/category/research-validation/) to acquire HRV, in addition to other physiological indicators, to track both pre-illness longitudinal baseline and an ongoing longitudinal Community assessment of those indicators associated with COVID-19 using algorithmic analysis and actionable feedback. While various aspects of this proposal have been used by investigators producing promising results at UC San Francisco (Smarr et al., 2020), UC San Diego/Scripps Research (Whitelaw et al., 2020), Stanford School of Medicine (Perez et al., 2019), Mt. Sinai's Icahn School of Medicine (Hirten et al., 2021), and others (Chung, 2020; Hasty et al., 2020), we propose a synthetic approach that incorporates the advantages of the most promising, actionable and practical elements to elucidate how HRV can act as a predictor of COVID19 infection. The use of longitudinal HRV data acquired by a personal device, transferred by smart phone application and analyzed by high throughput cloud-based machine learning algorithm represents an innovative, inexpensive, easily deployable, and scalable method for both individual use for health behavior maintenance and for communication and decision support with clinical and public health professionals in communities and larger jurisdictions.

OPEN ACCESS

***Edited by:***
*Vitor Engracia Valenti, São Paulo State University, Brazil*

***Reviewed by:***
*Luiz Carlos Marques Vanderlei, São Paulo State University, Brazil*
*Vlasta Bari, IRCCS Policlinico San Donato, Italy*

***Correspondence:**
*Robert L. Drury*
*rl.drury@gmail.com*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *20 May 2020*
***Accepted:*** *07 May 2021*
***Published:*** *08 June 2021*

***Citation:***
*Drury RL, Jarczok M, Owens A and Thayer JF (2021) Wireless Heart Rate Variability in Assessing Community COVID-19. Front. Neurosci. 15:564159. doi: 10.3389/fnins.2021.564159*

HRV research has produced extensive literature, with a recent PubMed search of the term "heart rate variability" producing more than 50,000 citations (Malik and Camm, 2004; Shaffer and Ginsberg, 2019), with rapidly evolving neuroscientific HRV studies (Holzman and Bridgett, 2017). Major theoretical contributions have been made by Porges' Polyvagal Theory (Porges, 2011); Grossman's biobehavioral studies of cardiac vagal tone (Grossman and Taylor, 2007); Owens, Critchley and associates studies of HRV as a remote digital biomarker (Owens et al., 2018); and Thayer's neurovisceral integration approach (Thayer and Lane, 2020), all show the important role of HRV as a physiological indicator of inflammatory and immune system activity. Briefly, HRV is the instantaneous variation in the inter-beat interval (IBI) of the electrocardiogram. HRVs relation to many disease states and human psychophysiological functions has been studied extensively. Perhaps counterintuitively, greater variability in IBI, measured as the time between adjacent R to R peaks in the ECG, is positively correlated to fewer and/or lesser negative health or well-being consequences in many diseases and conditions. These constant allostatic variations can be seen as analogous to the over 22,000 course corrections necessary for Apollo 8 to land on the moon (McEwen, 2017). Recent reviews have described the wide variety of applications of HRV in both medical and psychosocial settings (Drury et al., 2019). In particular, the Thayer group showed that HRV is related to inflammatory processes in humans (Williams et al., 2019; Jarczok et al., 2021) and identified an HRV related cholinergic anti-inflammatory pathway (Thayer and Fischer, 2009). Investigators have explored the use of HRV in medical conditions, including infectious and immune related disorders, in both human and animal studies showing various HRV parameters to be related to infection and immune system function (Fairchild, 2013; Herry et al., 2016; Pavlov and Tracey, 2019; Pavlov et al., 2020).

Based on this body of basic and applied HRV research, we wish to urgently propose using HRV monitoring as an element of a larger framework of truly personalized health (Drury, 2019; Hood et al., 2019). HRV screening, analysis and feedback can be applied immediately to the present COVID-19 pandemic. A recent report by Jarczok et al. (2019) has presented proof of concept of HRV as a marker of health risks in human adults. We propose applying the Jarczok et al. method to IBI data obtained from personal devices such as the Oura ring, Apple Watch, Fitbit, and the Polar strap, among others, facilitating scalability, accessibility, economy and high fidelity data acquisition. The Apple Heart Study conducted by Stanford University's School of Medicine demonstrated the feasibility of using wearable technology, specifically the Apple Watch, to examine cardiovascular data for atrial fibrillation. They point out "this is just the beginning, as this study opens the door to further research into wearable technologies and how they might be used to prevent disease before it strikes—a key goal of precision health" (Perez et al., 2019). Validating this point is the report of Hirten et al. (2021) which used Apple Watches to acquire and process HRV and circadian HRV analysis to detect COVID-19 symptoms up to 7 days before they emerge clinically. We propose an iP4 model of healthcare, an approach that Integrates: Personalized, Prescriptive, Participatory, and Preventive variables in modeling human health and function (Drury, 2019; Hood et al., 2019) and is similar to the precision medicine concept recently advocated by NIH Director Collins (Denny and Collins, 2021).

An HRV measure sensitive to the inflammatory processes in viral infection is the root mean square of successive differences between normal heart beats (RMSSD). Jarczok et al. (2019) developed an algorithm that converts IBI to RMSSD, which is then associated with appropriate risk factor values. We propose an developing a smart phone app containing the algorithm made widely available, which would obtain IBI from a suitable personal device (e.g., Oura Ring, Apple Watch, etc.). Data is analyzed on the app, allowing an assessment of a HRV-derived COVID-19 risk factor in addition to other physiological measures, and subjective symptom and demographic data assessment using the COVID-19 Symptom Tracker (Chan et al., 2020). This algorithm would prompt appropriate health behaviors, including judiciously seeking medical help if the risk threshold is reached. With informed consent, the information would be encrypted and transferred to the appropriate local health authority for tracking community COVID-19 prevalence. This approach could be rapidly deployed. As recently as 2015, 64% of the overall US population and 82% of those aged 18-49 own app-enabled smart phones (Smith et al., 2015). The most recent data available from Statistica[1] on smart phone deployment shows there are 3.8 billion app enabled smart phones worldwide (2020). Apple alone reports more than 22 million Apple Watches shipments in 2018, and several other smart watches and devices with IBI capability exist, such as Elite HRV CorSense, Fitbit, Polar straps, and Garmin monitors.

The current problems with widespread assessment of COVID-19 prevalence are urgent with high case incidence, intensive care unit occupancy and case fatalities. The US life span has declined by 1 year in the first 6 months of 2020, the biggest decline since World War 2 (Arias et al., 2021). We have proposed here an integrated systems approach to acquiring relevant individual data, analyzing it algorithmically, and producing health prompts based on those analyses. Our algorithm will be refined by including HRV and other physiological parameters such as temperature, respiration, SPO2, and activity level. Optimal complementary HRV parameters will also be explored by the use of sophisticated data analytic platforms such as Kubios (Tarvainen et al., 2014). This would allow community monitoring of likely COVID-19 status, which would be subject to further investigation and intervention by public health authorities as indicated. Participants would receive data driven health information including prompts for appropriate health behavior. Beyond this critical application, proactive use of the proposed approach would also be relevant for public health and clinical medical application. Now that most responsible national, state, and local governments have recognized the threat posed by the COVID-19 pandemic, the HRV monitoring proposed here can also be incorporated into digital epidemiology (Wolfe, 2011) processes to study population health and patterns of outbreak, as well as facilitate crucial contact tracing (Colizza et al., 2021). Murray and Piot (2021)

[1] Statista.com/statistics/330695/number-of-smartphone-users-worldwide/.

emphasize the importance of aligning surveillance and public health response. Beyond this key role of state and local public health authorities, optimized longitudinal HRV monitoring combined with other remotely obtained data can be used in clinical practice as a highly sensitive vital sign indicating exacerbation of a patient's chronic condition or the onset of a new condition. While not currently available commercially, the rapid development of printed self-powered wearable sensors (Hai et al., 2021; Sempianatto et al., 2021) will further enable HRV related health innovations described in this proposal. Other technological innovations such as Shi et al.'s (2021) use of radar interferometry and LSTM networking eliminate the need for physical contact with the individual. Given the popularity of pursuing positive health and well-being, unobtrusive HRV monitoring can assist individuals in pursuing health maintenance and optimal performance goals. Beyond health and wellness applications, contributions to performance optimization in education, business and military/operational settings are feasible. But the immediate and, for some, vital imperative is to make the assessment and treatment of COVID 19 as accessible and useful as possible. Our project proposes longitudinal, wireless HRV monitoring to assist in the digital/algorithmic management and treatment of the COVID-19 pandemic in addition to other health conditions, using scalable, unobtrusive, and accurate technology.

## AUTHOR CONTRIBUTIONS

RD and JT participated in the conceptualization and preparation of this opinion. All authors contributed to the article and approved the submitted version.

## ACKNOWLEDGMENTS

We gratefully acknowledge E.O. Wilson, the intellectual examples of leaders in evolutionary and psychological science including Stephen Porges, Francisco Varela, Fritjof Capra, Wasyl Malyj and the emotional support of our significant relations.

## REFERENCES

Ahmad, S., Ramsay, T., Huebsch, L., Flanagan, S., McDiarmid, S., Batkin, I., et al. (2009). Continuous multi-parameter heart rate variability analysis heralds onset of sepsis in adults. *PLoS ONE* 4:e6642. doi: 10.1371/journal.pone.0006642

Arias, E., Tejada-Vera, B., and Ahmad, F. (2021). *Provisional Life Expectancy Estimates for January through June, 2020*. Vital Statistics Rapid Release; no. 10. Hyattsville, MD: National Center for Health Statistics. doi: 10.15620/cdc:100392

Chan, A. T., Drew, D. A., Nguyen, L. H., Joshi, A. D., Ma, W., Guo, C. G., et al. (2020). *Harvard/Zoe COVID 19 Symptom Study*. Available online at: https://covid.joinzoe.com/us/blog (accessed May 22, 2021).

Chung, Y. T. (2020). Continuous temperature monitoring by a wearable device for early detection of febrile events in the SARS-CoV-2 outbreak in Taiwan, 2020. *J. Microbiol. Immunol. Infect*. 53, 503–504. doi: 10.1016/j.jmii.2020.04.005

Colizza, V., Grill, E., Mikolajczyk, R., Cattuto, C., Kucharski, A., Riley, S., et al. (2021). Time to evaluate COVID-19 contact-tracing apps. *Nat. Med*. 27, 361–362. doi: 10.1038/s41591-021-01236-6

Dantzer, R., and Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain Behav. Immun.* 21, 153–160. doi: 10.1016/j.bbi.2006.09.006

Denny, J., and Collins, F. (2021). Precision medicine in 2030 -seven ways to transform healthcare. *Cell*. 184, 1415–1419. doi: 10.1016/j.cell.2021.01.015

Drury, R. (2019). "HRV in an integrated hardware/software system using artificial intelligence to provide assessment, intervention and performance optimization," in *Autonomic Nervous System Monitoring*, ed T. Aslanidis, 1–11. doi: 10.5772/intechopen.89042

Drury, R. L., Porges, S., Thayer, J. F., and Ginsberg, J. (eds.) (2019). Heart rate variability, health and well-being: a systems perspective. *Front. Public Health* 7:323. doi: 10.3389/fpubh.2019.00323

Fairchild, D. (2013). Predictive monitoring for early detection of sepsis in neonatal ICU patients. *Curr. Opin. Pediatr.* 25, 172–179. doi: 10.1097/MOP.0b013e32835e8fe6

Grossman, P., and Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsycho.2005.11.014

Hai, L., Song, H., Long, M., Saeeda, G., and Lim, S. (2021). Mortise–tenon joint structured hydrophobic surface-functionalized barium titanate/polyvinylidene fluoride nanocomposites for printed self-powered wearable sensors. *Nanoscale* 13, 2542–2555. doi: 10.1039/D0NR07525F

Hasty, F., García, G., Dávila, C. H., Wittels, S. H., Hendricks, S., and Chong, S. (2020). Heart rate variability as a possible predictive marker for acute inflammatory response in COVID-19 patients. *Military Med.* 186, e34–e38. doi: 10.1093/milmed/usaa405

Herry, C. L., Cortes, M., Wu, H. T., Durosier, L. D., Cao, M., Burns, P., et al. (2016). Temporal patterns in sheep fetal heart rate variability correlate to systemic cytokine inflammatory response: a methodological exploration of monitoring potential using complex signals bioinformatics. *PLoS ONE* 11:e0153515. doi: 10.1371/journal.pone.015351

Hirten, R. P., Danieletto, M., Tomalin, L., Choi, K. H., Zweig, M., Golden, E., et al. (2021). Use of Physiological Data From a Wearable Device to Identify SARS-CoV-2 Infection and Symptoms and Predict COVID-19 Diagnosis: Observational Study. *J. Med. Inter. Res*. 23:e26107. doi: 10.2196/26107

Holzman, J. B., and Bridgett, D. J. (2017). Heart rate variability indices as bio-markers of top-down self-regulatory mechanisms: a meta-analytic review. *Neurosci. Biobehav. Rev.* 74(Pt A), 233–255. doi: 10.1016/j.neubiorev.2016.12.032

Hood, L., Dean, K. R., Hammamieh, R., Mellon, S. H., Abu-Amara, D., Flory, J. D., et al. (2019). Multi-omic biomarker identification and validation for diagnosing warzone-related post-traumatic stress disorder. *Mol. Psychiatry* 25, 3337–3349. doi: 10.1038/s41380-019-0496-z

Jarczok, M. N., Koenig, J., and Thayer, J. F. (2021). Lower values of a novel index of Vagal-Neuroimmunomodulation are associated to higher all-cause mortality in two large general population samples with 18 year follow up. *Sci Rep.* 11:2554. doi: 10.1038/s41598-021-82168-6

Jarczok, M. N., Koenig, J., Wittling, A., Fischer, J. E., and Thayer, J. F. (2019). First evaluation of an index of low vagally mediated heart rate variability as a marker of health risks in human adults: proof of concept. *J. Clin. Med.* 8:E1940. doi: 10.3390/jcm8111940

Kovatchev, B. P., Farhy, L. S., Cao, H., Griffin, M. P., Lake, D. E., and Moorman, J. R. (2003). Sample asymmetry analysis of heart rate characteristics with application to neonatal sepsis and systemic inflammatory response syndrome. *Pediatr Res.* 54, 892–898. doi: 10.1203/01.PDR.0000088074.97781.4F

Leitzke, M., Stefanovic, D., Meyer, J. J., Schimpf, S., and Schönknecht, P. (2020). Autonomic balance determines the severity of COVID-19 courses. *Bioelectron. Med.* 6:22. doi: 10.1186/s42234-020-00

Malik, M., and Camm, A. J. (2004). *Dynamic Electrocardiology*. New York, NY: Blackwell. doi: 10.1093/oxfordjournals.eurheartj.a014868

McEwen, B. (2017). Allostasis and the epigenetics of brain and body health over the life course. *JAMA Psychiatry* 74, 551–552. doi: 10.1001/jamapsychiatry.2017.0270

Murray, C. J. L., and Piot, P. (2021). The potential future of the COVID-19 pandemic. *JAMA* 325, 1249-1250. doi: 10.1001/jama.2021.2828

Owens, A. (2020). The role of heart rate variability in the future of remote digital biomarkers. *Front. Neurosci.* 14:582145. doi: 10.3389/fnins.2020.582145

Owens, A. P., Friston, K. J., Low, D. A., Mathias, C. J., and Critchley, H. D. (2018). Investigating the relationship between cardiac interoception and autonomic cardiac control using a predictive coding framework. *Auton. Neurosci. Basic Clin.* 210, 65–71. doi: 10.1016/j.autneu.2018.01.001

Pavlov, V. A., Chavan, S. S., and Tracey, K. J. (2020). Bioelectronic medicine: from preclinical studies on the inflammatory reflex to new approaches in disease diagnosis and treatment. *Cold Spring Harbor. Perspect. Med.* 10:a034140. doi: 10.1101/cshperspect.a034140

Pavlov, V. A., and Tracey, K. J. (2019). Bioelectronic medicine:updates, challenges and paths forward. *Bioelectron. Med.* 5:1. doi: 10.1186/s42234-019-0018-y

Perez, M. V., Mahaffey, K. W., Hedlin, H., Rumsfeld, J. S., Garcia, A., Ferris, T., et al. (2019). Large-scale assessment of a smartwatch to identify atrial fibrillation. *N. Engl. J. Med.* 381, 1909–1917. doi: 10.1056/NEJMoa1901183

Porges, S. (2011). *The Polyvagal Theory.* New York, NY: Norton.

Rangon, C. M., Krantic, S., Moyse, E., and Fougère, B. (2020). The vagal autonomic pathway of COVID-19 at the crossroad of Alzheimer's disease and aging: a review of knowledge. *J. Alzheimers Dis. Rep.* 4, 537–551. doi: 10.3233/ADR-200273

Sempianatto, J., Lin, M., Yin, L., De la paz, E., Pei, K., Sonsa-ard, T., et al. (2021). An epidural patch for the simultaneous monitoring of hemodynamic and metabolic biomarkers. *Nat. Biomed. Eng.* 172:112750 doi: 10.1038/s41551-021-00685-1

Shaffer, F., and Ginsberg, J. (2019). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shi, K., Steigleder, T., Schellenberger, S., Michler, F., Malessa, A., Lurz, F., et al. (2021). Contactless analysis of heart rate variability during cold pressor test. *Sci Rep.* 11:3025. doi: 10.1038/s41598-021-81101-1

Smarr, B. L., Aschbacher, K., Fisher, S. M., Chowdhary, A., Dilchert, S., Puldon, K., et al. (2020). Feasibility of continuous fever monitoring using wearable devices. *Sci. Rep.* 10:21640. doi: 10.1038/s41598-020-78355-6

Smith, C., Gold, J., Ngo, T. D., Sumpter, C., and Free, C. (2015). Mobile phone-based interventions for improving contraception use. *Cochrane Database Syst. Rev.* 2015:CD011159. doi: 10.1002/14651858.CD011159.pub2

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalaine, P. A. (2014). Kubios HRV – heart rate variability analysis software. *Comput. Methods Progr. Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Thayer, J. F., and Fischer, J. E. (2009). Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. *J. Intern. Med.* 265, 439–447. doi: 10.1111/j.1365-2796.2008.02023.x

Thayer, J. F., and Lane, R. D. (2020). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Dis.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Whitelaw, S., Mamas, M. A., Topol, E., and Van Spall, H. G. C. (2020). Applications of digital technology in COVID-19 pandemic planning and response. *Lancet Digit. Health* 2, e435–e40. doi: 10.1016/S2589-7500(20)30142-4

Williams, D. P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M. N., Sternberg, E. M., et al. (2019). Heart rate variability and inflammation: a meta-analysis of human studies. *Brain Behav. Immun.* 80:219226. doi: 10.1016/j.bbi.2019.03.009

Wolfe, N. (2011). *The Viral Storm*. New York, NY: St. Martins.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 10 June 2021
doi: 10.3389/fnins.2021.577418

# No Difference in Arousal or Cognitive Demands Between Manual and Partially Automated Driving: A Multi-Method On-Road Study

*Monika Lohani[1]*, Joel M. Cooper[2], Gus G. Erickson[2], Trent G. Simmons[2], Amy S. McDonnell[2], Amanda E. Carriero[2], Kaedyn W. Crabtree[2] and David L. Strayer[2]*

*[1] Department of Educational Psychology, University of Utah, Salt Lake City, UT, United States, [2] Department of Psychology, University of Utah, Salt Lake City, UT, United States*

OPEN ACCESS

**Edited by:**
*Sylvain Laborde, German Sport University Cologne, Germany*

**Reviewed by:**
*Dorota Zyśko, Wrocław Medical University, Poland*
*Riender Happee, Delft University of Technology, Netherlands*
*Joonwoo Son, Daegu Gyeongbuk Institute of Science and Technology (DGIST), South Korea*

***Correspondence:**
*Monika Lohani*
*Monika.Lohani@utah.edu*

**Specialty section:**
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

**Received:** *29 June 2020*
**Accepted:** *03 May 2021*
**Published:** *10 June 2021*

**Citation:**
*Lohani M, Cooper JM, Erickson GG, Simmons TG, McDonnell AS, Carriero AE, Crabtree KW and Strayer DL (2021) No Difference in Arousal or Cognitive Demands Between Manual and Partially Automated Driving: A Multi-Method On-Road Study. Front. Neurosci. 15:577418. doi: 10.3389/fnins.2021.577418*

**Introduction:** Partial driving automation is not always reliable and requires that drivers maintain readiness to take over control and manually operate the vehicle. Little is known about differences in drivers' arousal and cognitive demands under partial automation and how it may make it difficult for drivers to transition from automated to manual modes. This research examined whether there are differences in drivers' arousal and cognitive demands during manual versus partial automation driving.

**Method:** We compared arousal (using heart rate) and cognitive demands (using the root mean square of successive differences in normal heartbeats; RMSSD, and Detection Response Task; DRT) while 39 younger ($M$ = 28.82 years) and 32 late-middle-aged ($M$ = 52.72 years) participants drove four partially automated vehicles (Cadillac, Nissan Rogue, Tesla, and Volvo) on interstate highways. If compared to manual driving, drivers' arousal and cognitive demands were different under partial automation, then corresponding differences in heart rate, RMSSD, and DRT would be expected. Alternatively, if drivers' arousal and cognitive demands were similar in manual and partially automated driving, no difference in the two driving modes would be expected.

**Results:** Results suggest no significant differences in heart rate, RMSSD, or DRT reaction time performance between manual and partially automated modes of driving for either younger or late-middle-aged adults across the four test vehicles. A Bayes Factor analysis suggested that heart rate, RMSSD, and DRT data showed extreme evidence in favor of the null hypothesis.

**Conclusion:** This novel study conducted on real roads with a representative sample provides important evidence of no difference in arousal and cognitive demands. Younger and late-middle-aged motorists who are new to partial automation are able to maintain arousal and cognitive demands comparable to manual driving while using the partially automated technology. Drivers who are more experienced with partially automated technology may respond differently than those with limited prior experience.

**Keywords: heart rate, heart rate variability, detection response task, partial driving automation, applied cognition**

## INTRODUCTION

Some commercially available vehicles with partial vehicle automation can support safe driving (e.g., Tesla-Autopilot, Nissan-ProPilot, Volvo-Pilot Assist, and Cadillac-Supercruise). However, partial vehicle automation is not always reliable, requiring drivers to maintain readiness to take over vehicle control at all times (SAE, 2016). If drivers' cognitive demands during partially automated driving are different from manual driving mode, it may raise concerns about drivers' cognitive readiness to take over, should automation fail. In studying cognitive demands during manual and partially automated driving, it is important to consider driver's arousal (Yerkes and Dodson, 1908; Hebb, 1955; Broadhurst, 1959; Wekselblatt and Niell, 2015). The concern with partially automated technology is that it may lead to suboptimal arousal levels and cognitive demands resulting in poor driving performance. This motivated the study's research question: Are there differences in drivers' arousal and cognitive demands during manual versus partially automated driving? A comparison of physiological arousal and cognitive demands during the two modes of driving would help understand potential differences (or lack thereof) in cognitive demands and subsequent driving performance. The aim of the current study was to investigate whether there are differences in drivers' arousal and cognitive demands during manual versus partial automation driving on real highways.

Only limited research is currently available to understand potential differences in drivers' cognitive demands under partial vehicle automation. Based on classic cognitive models of attention (Kahneman, 1973; Wickens, 2002), *driving-related cognitive demands* are defined as all cognitive and mental processing resources required to perform a driving task. Some studies have suggested that there may be significant cognitive demands (e.g., workload) during automated driving compared to manual driving (Solís-Marcos et al., 2018; Kim et al., 2020). However, other research has found cognitive demands during partially automated driving may actually be reduced (Biondi et al., 2018; Heikoop et al., 2019; Zhai and Lu, 2019). By contrast, other research suggests little or no difference (Sibi et al., 2017; Stapel et al., 2019; Calvi et al., 2020; Várhelyi et al., 2020). Note that Stapel et al. (2019) found no difference in automation for inexperienced drivers; however, lower workload was found with experienced drivers. A variety of factors can help explain these inconsistent findings, including small sample sizes limiting the likelihood of detecting a true effect; testing only a single vehicle raising questions of ecological validity; and use of self-reports administered after driving raising concerns of retrospective report biases. Furthermore, most of the research on this topic has been restricted to samples of younger adults that limits the applicability of findings to the general public. Thus, a clear understanding of whether and how partially automated technology influences drivers' arousal and cognitive demands are still lacking.

*Arousal* is a heterogeneous construct that involves general autonomic activation (e.g., Robbins and Everitt, 1995; Satpute et al., 2019). Heart rate is a biomarker of arousal and they are positively related (Berntson et al., 2007; Mauss and Robinson, 2010; Satpute et al., 2019). Heart rate is commonly used to measure driving-related arousal changes (Lohani et al., 2019) and it was used to operationalize arousal in the current study. Moreover, in real-world driving (for a review see, Lohani et al., 2019), multiple overlapping constructs dynamically change simultaneously and interdependently (e.g., workload, stress, boredom, distraction) resulting in net cognitive demands experienced by a motorist that can be broadly categorized along a low-to-high spectrum. Low cognitive demands may be represented by a combination of constructs such as drowsiness and boredom, while high cognitive demands may be represented by mental workload and stress (Lohani et al., 2019). Cognitive demands are associated with *cardiac vagal control*, which represents the influence of the vagus nerve on heart functioning (for a review, see Berntson et al., 2007; Laborde et al., 2017). Cardiac vagal control can be indexed by vagally-mediated *heart rate variability* (HRV), i.e., the temporal variability in adjacent heartbeats (Malik, 1996; Berntson et al., 2007). According to the neurovisceral integration model, the neural circuitry for cognitive and autonomic regulation has an overlapping neurovisceral mechanism (see Thayer et al., 2009; Smith et al., 2017), which can explain the coupling between cognitive demands and vagally-mediated HRV. Thus, vagally-mediated HRV can measure changes in cognitive demands, such as mental effort, workload, and attention (e.g., Mulder and Mulder, 1981; Mulder, 1985; Thayer et al., 2009).

Indeed, vagally-mediated HRV has been shown to provide a near-real-time objective measure of dynamic changes in cognitive demands, making it a suitable measure for applied driving research without disrupting the driving task (e.g., Lee et al., 2007; Liang et al., 2009; Mehler et al., 2011; Noda et al., 2015; Sugie et al., 2016; Heine et al., 2017; Piotrowski and Szypulska, 2017; Cisler et al., 2019; Lohani et al., 2019). Some of these studies found that cognitive state detection while driving was better when vagally-mediated HRV was utilized in addition to behavioral measures (e.g., Lenneman and Backs, 2009). Even though a variety of measures of vagally-mediated HRV have been used in applied research, the root mean square of successive differences in normal heartbeats (RMSSD) has been found to change more systematically with driving-related cognitive demands (Mehler et al., 2011; Heine et al., 2017), and thus was a suitable measure for the current study. In particular, RMSSD decreases with an increase in cognitive demands while driving (Mehler et al., 2011) due to the links between vagally-mediated HRV and neural activity associated with cognitive regulation (e.g., Thayer et al., 2009). Furthermore, a standard behavioral method to assess cognitive demands in driving research is the Detection Response Task (DRT; International Organization for Standardization [ISO], 2016). Performance on the DRT (International Organization for Standardization [ISO], 2016) is a measure of driving-related cognitive demands due to visual demands and driving difficulty (Bengler et al., 2012; Bruyas and Dumont, 2013; Cooper et al., 2016). An increase in driving-related cognitive demands is associated with increased reaction time performance and decreased hit-rate on the DRT (Young et al., 2013). At the same time, drowsiness and fatigue also increase reaction time on this task (Gershon et al., 2009). DRT

was included as a behavioral measure of cognitive demands in the current study.

In prior research, we conducted a pilot study with 28 young drivers ($M_{age}$ = 29.29 years) new to partial automation who drove three partially automated vehicles on a flat and straight section of interstate highway (Lohani et al., 2020). In this pilot study, there were no differences between manual and partially automated driving modes across outcomes: heart rate, RMSSD, electroencephalogram (EEG) alpha, and theta power, and DRT performance. The current study is a new follow-up study designed to replicate and extend the earlier findings with a larger and more representative sample of younger and late-middle-aged drivers, partially automated vehicles (Cadillac, Nissan Rogue, Tesla, and Volvo), and sections of roadway. The current research measured heart rate to compare arousal and RMSSD and DRT performance to compare cognitive demands under manual versus partially automated driving.

Based on previous work, the current study considered three alternative hypotheses. First, if partially automated driving leads to high arousal and high cognitive demands (e.g., workload and stress; Solís-Marcos et al., 2018; Kim et al., 2020), then, a significant increase in heart rate, a decrease in RMSSD, and an increase in the DRT reaction time rate would be expected when compared to manual driving. Second, if partially automated driving leads to low arousal and low cognitive demands (e.g., drowsiness and boredom; Biondi et al., 2018; Heikoop et al., 2019; Zhai and Lu, 2019), then a significant decrease in heart rate, an increase in RMSSD, and an increase in DRT reaction time would be expected when compared to manual driving. Finally, if arousal and cognitive demands are similar under manual and partially automated driving, then no differences in the outcome measures would be expected (e.g., Sibi et al., 2017; Stapel et al., 2019; Calvi et al., 2020; Lohani et al., 2020; Várhelyi et al., 2020). This prediction of the null hypothesis has a compelling rationale and a meaningful interpretation that would imply that manual and partially automated modes are comparable in arousal and cognitive demands. However, some limitations (e.g., small sample size, a non-representative sample of people and vehicles, and inadequate statistics) can hamper the ability to interpret evidence for a null hypothesis adequately. Therefore, we designed the current study to allow for a fair test of the null hypothesis by testing a larger and more representative sample of drivers and vehicles. We bolstered the interpretation by conducting the classic null-hypothesis significance testing and a Bayesian alternative (Kruschke, 2011) to meaningfully interpret whether the current dataset supported the null or the alternative hypothesis.

# MATERIALS AND METHODS

## Participants

A total of 71 adults with no prior experience with partially automated vehicles participated in this study. 39 participants were younger (21–42 age range, $M_{age}$ = 28.82 years, $SD_{age}$ = 6.41, 13 females). 32 participants were late-middle-aged (43–64 age range, $M_{age}$ = 52.72 years, $SD_{age}$ = 6.33, 12 females). The study protocol was in accordance with the Institutional Review Board at the University of Utah. Participants had no previous experience with partial automation, had a valid driver's license, no at-fault accidents in the past 2 years, drove at least an average of 10 h per month, had no history of neurological disorders or heart conditions, and were not pregnant. In addition, participants were required to pass a 20 min online defensive driving course and certification test. Upon arrival in the lab, eligible participants were allowed to participate in the study only if they had slept at least 6 h the previous night and had their blood alcohol level at 0.0%, which was verified using a BACtrac breathalyzer.

## Measures

Psychophysiological data was continuously sampled at 2,000 Hz using a portable wireless physiology system (Smart Center, Biopac System Inc., United States) and Acqknowledge software (Biopac System Inc., United States). This setup allowed real-time data quality monitoring while participants drove on the highway.

### Electrocardiography

The electrical activity of the heart was recorded by using an electrocardiogram (ECG). After cleaning the site, standard disposable electrodes were placed on the right collar bone and the left and right end of the ribcage (Lead II configuration; Berntson et al., 2007). During data collection, the ECG was monitored by a research assistant who sat in the front passenger seat. Any noticeable movements that could add artifacts (e.g., sneezes and itch) were marked.

Standardized methods in accordance with recommended guidelines for ECG data were followed (e.g., Malik, 1996; Berntson et al., 1997, 2007; Peltola, 2012; Shaffer et al., 2014; Laborde et al., 2017). Post data collection, the ECG data was processed using AcqKnowledge software (Biopac System Inc., United States). The raw data was bandpass filtered at 1 and 35 Hz cutoffs. The software was used to detect R-wave peaks. All R-wave peaks were visually inspected for accurate detection and manually corrected if the software marked improbable values. This included any artifacts generated by facial or head movements (e.g., yawns or checking blind spots while driving). After data cleaning, data were processed to calculate RMSSD and heart rate for manual and partially automated driving tasks for each vehicle operated by each participant. Based on the recommended guidelines (Malik, 1996; Berntson et al., 1997; Laborde et al., 2017), RMSSD was calculated using the same length epochs (1 min) for the pre-condition baseline and the main-condition periods (see the procedure for details). The epochs were then averaged over the entire period to get average RMSSD values during the pre-condition and main-condition periods. For RMSSD and heart rate data, any values that exceeded three standard deviations from the mean of normal distribution were removed before analyses.

### Detection Response Task (DRT)

To probe drivers' cognitive demand, participants were asked to perform a vibrotactile detection task, DRT. In line with the ISO 17488 guidelines (2016), a vibrotactor (a small vibration motor) emitted a small vibration stimulus, similar to a vibrating cell

phone. This stimulus was presented pseudo-randomly every 3–5 s. Participants wore a vibrotractor that was taped to their left biceps. A microswitch was attached to either the index or middle finger of the left hand, which participants could press against the steering wheel to respond to the onset of the stimulus. Instead of the standard left collarbone placement, the left bicep was used to avoid any potential interference with the ECG signal. A similar approach has been successfully used in past work (e.g., Lohani et al., 2020). Participants' goal was to respond to the stimulus onset as quickly as possible while driving (with priority always given to safe driving practices). Response time in milliseconds was recorded for each stimulus. The vibration stimulus was set up to turn off after 1 s.

The average reaction time performance on DRT was calculated for each participant in each vehicle in manual and partially automated driving tasks. Any values that exceeded three standard deviations from the mean of normal distribution were removed before analyses. The hit-rate performance for the current sample was at a ceiling level (∼95% and above). The average proportion of hit-rate during manual driving was 96% ($SE$ = 0.003) and during partially automated driving was 95% ($SE$ = 0.004). Because of a lack of variance in performance, the hit-rate was not further analyzed.

### Self-Reported Experiences With Automation

After driving partially automated vehicles, participants responded to a list of questions about their experiences and attitudes about partially automated vehicles. An example item was "I was anxious and nervous when the automated driving systems were on." Participants indicated their agreement to the following statements using a 5-point scale anchored by completely disagree to completely agree.

## Vehicles

We examined a representative sample of commercially available vehicles for this study. A 2018 Cadillac CT6, 2019 Nissan Rogue, 2018 Tesla Model 3, and a 2018 Volvo XC90 were used in this study. Each of these vehicles was equipped with the partial vehicle automation that centered the vehicle within the lane (e.g., Lane Centering) and maintained the following distance and speed (e.g., Adaptive Cruise Control). These features, when activated together, meet the definition of partial automation (SAE, 2016).

## Procedure

Participants were sent a training document and a short video about the partial automation features of the vehicle they would drive for the day of the visit. Upon arrival in the lab, after completing the consent form and inclusion criteria testing, participants were set up for ECG data collection. Next, a research assistant directed the participant to the designated vehicle in the parking lot close to the lab. Participants were instructed that they were driving commercially available vehicles, and we were interested in examining the vehicular systems on real roads. They were instructed to operate the vehicle as they would usually drive on the road.

Before driving the vehicle, participants would familiarize themselves with the vehicle and get trained on steps to activate its partial automation features. They engaged the partially automated systems during the training phase, and only when they were comfortable operating the vehicle was the main part of the study started. Participants were asked to keep their hands on the wheel and monitor the road (as recommended by most vehicle manufacturers). In the partial automation condition, participants always had the automation engaged. In rare instances, participants took control of the vehicle to pull over for emergency vehicles, debris on the road, and construction. Participants drove on the same road in manual and partial automation (counterbalanced). As soon as possible, partial automation was re-engaged. It is important to note that participants drove on the same road in both manual and partial automation (counterbalanced), so driving conditions were equated as best as possible. Moreover, any section of the drive where the driver had to deal with an event (e.g., pulling over for an emergency vehicle) were excluded from the analyses (in both manual and partial automation) to ensure a fair comparison.

Participants were also fitted with the DRT equipment and trained on how to perform the DRT task while driving. ECG data were monitored for quality. Next, they drove on a training route with the research assistant in the passenger seat to ensure that the participant could engage and disengage partial automation, change lanes, and control the vehicle's speed. After confirming that participants understood how to use the partial automation and the DRT task while driving safely, the study's testing phase was initiated.

For the testing phase, all participants drove comparable distances on two highways (I-15 and I-80) in two modes (manual and partial automation), with the order partially counterbalanced across participants (leading to 4 experimental sessions in each vehicle). The Average Annual Daily Travel (AADT) for I-15 (N/S bound 4–5 lanes in each direction separated by a median) is 175,000 vehicles and for I-80 (E-W bound 2–3 lanes in each direction separated by a median) is 19,000 vehicles (Utah Department of Transportation). Because there were no significant differences in outcomes between the two highways, the data were averaged for the two highways. The manual and partially automated conditions were also counterbalanced to control for any unsystematic differences.

Each of the four experimental sessions was about 20 min and began once the vehicle was at the posted speed limit. The first 2 min of each session were used as the *pre-condition baseline* measurement. The following 18 min of the experimental session were the *main condition* during which the DRT task was performed while participants drove in manual or partial automation conditions (depending on the order). Participants received a short rest break between each of the four experimental sessions. The average value of RMSSD (calculated by averaging 1 min epochs) in the pre-condition baseline period was subtracted from the average value of the main condition to account for any baseline differences within participants. The average heart rate in the pre-condition baseline was subtracted from the main condition. After accounting for baseline differences and collapsing across the two highways, there were two average values for each vehicle driven in manual and partial automation.

## Data Analysis Plan

The primary research focus was to examine how automation (manual versus partial automation) affects driver arousal and cognitive demand. To address this question, two analytical approaches were adopted – linear mixed-effects models and Bayes Factor analysis. For each of the outcome measures, a linear mixed-effects model was run with three fixed factors, automation (manual and partially automated), age (younger or late-middle-aged), and vehicle (Cadillac, Nissan Rogue, Tesla, and Volvo) and participants as the random intercept. Note that preliminary analyses were performed to determine if time should be included as a factor in the model. We found that including time as a factor in the model did not explain any significant variability. In contrast to automation condition and age, we had no theoretical or empirical rationale to include time in the models. Thus, to keep a parsimonious model, time was not included in the model because it did not explain significant variability in the outcome variables.

Next, Bayes Factor analysis was conducted to adopt a Bayesian alternative to evaluate how meaningful a significant difference or a lack of significant difference was between the manual and partial automation for each of the outcome variables. This was done by comparing a full model with automation, age, vehicle as predictors to a restricted model without automation (e.g., Kruschke, 2011). The Bayes Factor value is the ratio of the marginal likelihoods of the full model and the restricted model (Lee and Wagenmakers, 2014). The resulting Bayes factor value was interpreted using the classification scheme such that a value higher than 1 is interpreted as evidence in favor of the alternative hypothesis. A Bayes Factor value between 1 and 3 provides anecdotal evidence, 3–10 provides moderate evidence, 10–30 provides strong evidence, 30–100 provides very strong evidence, and a value more than 100 is extreme evidence in favor of the alternative hypothesis. By contrast, a value lower than 1 is interpreted as evidence in favor of the null hypothesis. Correspondingly, a value of 1–0.33 provides anecdotal evidence, 0.33–0.1 provides moderate evidence, 0.1–0.03 provides strong evidence, 0.03–0.01 provides very strong evidence, and a value less than 0.01 provides extreme evidence in favor of the null hypothesis (Lee and Wagenmakers, 2014; Quintana and Williams, 2018). All analyses were done by using the R language for statistical computing (R Core Team, 2020). Mixed models were fit using the *lme4* package (Bates et al., 2015), and Bayes Factor values were calculated via the *BayesFactor* package (Morey et al., 2018).

# RESULTS

See **Table 1** for descriptive statistics for the three outcome variables as a function of age and vehicle. **Figures 1–3** present individual data points to complement the descriptive tables. These include violin plots that are similar to box plots, but in addition, they have the kernel probability density of all the observed data. The mean values are also indicated by a dot in the center of each distribution.

Age differences and gender differences were examined during the pre-condition baseline and main condition. In the pre-condition baseline values of RMSSD, there were significant gender and age-related differences with higher mean values for males ($M$ = 29.5, $SE$ = 1.76) than females ($M$ = 22.7, $SE$ = 2.27), $t(69) = 2.38$, $p = 0.02$, and higher RMSSD values for younger ($M$ = 30.2, $SE$ = 1.96) than late-middle-aged ($M$ = 21.9, $SE$ = 2.10) drivers, $t(69) = 2.89$, $p = 0.01$. However, after accounting for pre-condition values (average main condition – average pre-condition baseline values), neither gender differences ($p = 0.19$) nor age differences ($p = 0.89$) were significant. We did not have specific hypotheses for gender-related differences in driving manual and partially automated vehicles. Thus, gender was not included as a factor in the models to test the study's research questions.

## The Effect of Automation on Heart Rate

A linear mixed-effects model was run with a baseline-corrected heart rate as the outcome. Neither the main effect of automation, $F(1, 320.48) = 0.05$, $p = 0.82$, age, $F(1, 60.71) = 0.69$, $p = 0.41$, nor vehicle, $F(3, 355.24) = 0.42$, $p = 0.74$ were significant. Likewise, none of the 2-way or 3-way interactions were significant.

## The Effect of Automation on RMSSD

A linear mixed-effects model examined how RMSSD varied as a function of automation (manual and partially automated), age (young or late-middle-aged), and vehicle (Cadillac, Nissan Rogue, Tesla, and Volvo), and participants as the random intercept. No main effect of automation was found, $F(1, 321.99) = 1.76$, $p = 0.19$. In addition, no main effect of age, $F(1, 60.96) = 0.04$, $p = 0.84$, or vehicle was found, $F(3, 358.84) = 1.45$,

**TABLE 1 |** Means (and Standard Error) for heart rate, RMSSD, and DRT reaction time as a function of automation (manual and partial automation), age (younger or older), and vehicle (Cadillac, Nissan Rogue, Tesla, and Volvo).

| Measure | Automation | Young | | | | Old | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cadillac | Nissan | Tesla | Volvo | Cadillac | Nissan | Tesla | Volvo |
| **Heart rate** | Manual | 0.49 (0.32) | 0.37 (0.32) | 0.76 (0.34) | 0.81 (0.33) | 0.58 (0.34) | −0.11 (0.34) | 0.38 (0.33) | 0.60 (0.34) |
| | Partial automation | 0.26 (0.33) | 1.05 (0.32) | 0.38 (0.33) | 0.48 (0.33) | 0.30 (0.34) | 0.27 (0.33) | 0.33 (0.34) | 0.55 (0.34) |
| **RMSSD** | Manual | −0.45 (0.96) | −0.28 (0.96) | 0.07 (1.01) | −2.02 (0.98) | 0.68 (1.02) | −0.64 (0.98) | −2.72 (0.96) | −0.96 (1.00) |
| | Partial automation | −0.49 (0.97) | −1.90 (0.96) | −0.66 (0.99) | −2.36 (0.97) | −1.83 (1.02) | −0.25 (0.98) | −1.81 (1.0) | −1.71 (1.0) |
| **DRT reaction time** | Manual | 437 (28.5) | 443 (28.9) | 455 (29.2) | 480 (28.9) | 498 (31.1) | 495 (30.4) | 498 (30.5) | 523 (31.1) |
| | Partial automation | 459 (28.5) | 463 (28.9) | 468 (29.2) | 489 (28.9) | 506 (31.4) | 529 (30.4) | 508 (30.5) | 518 (31.1) |

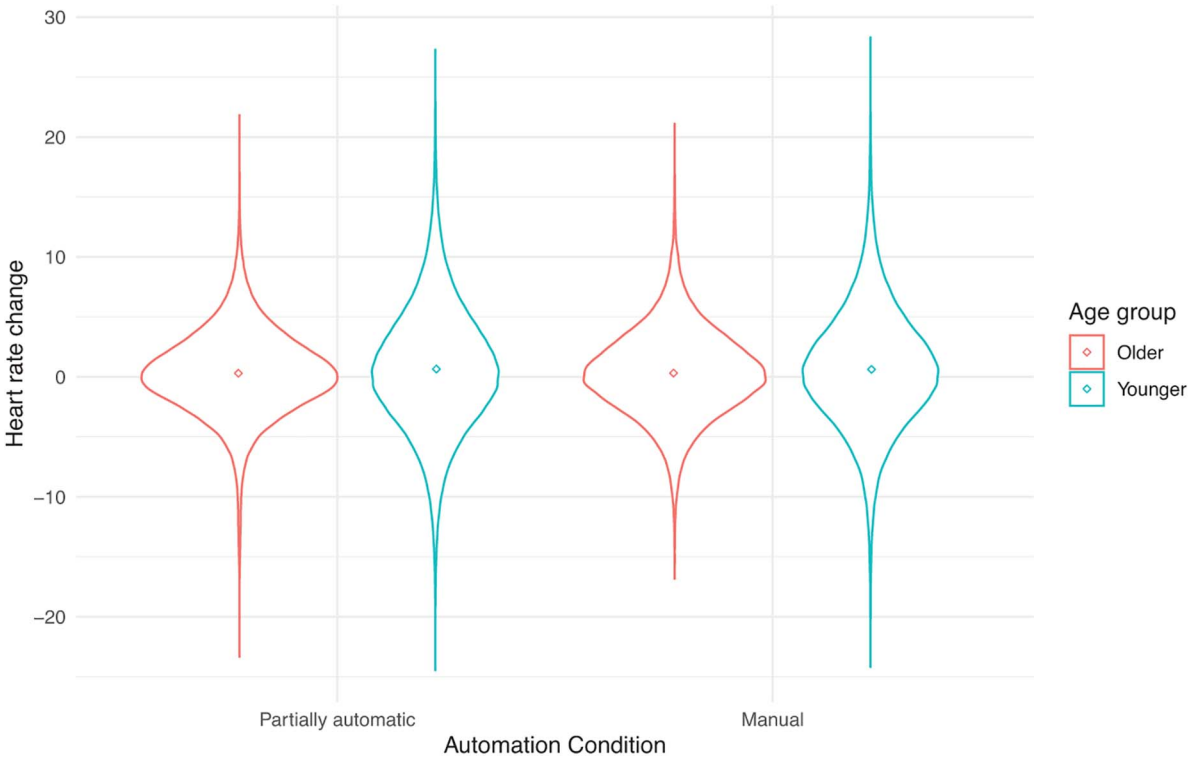



**FIGURE 1 |** Heart rate change from baseline as a function of automation (manual and partial automation) and age (younger or older).

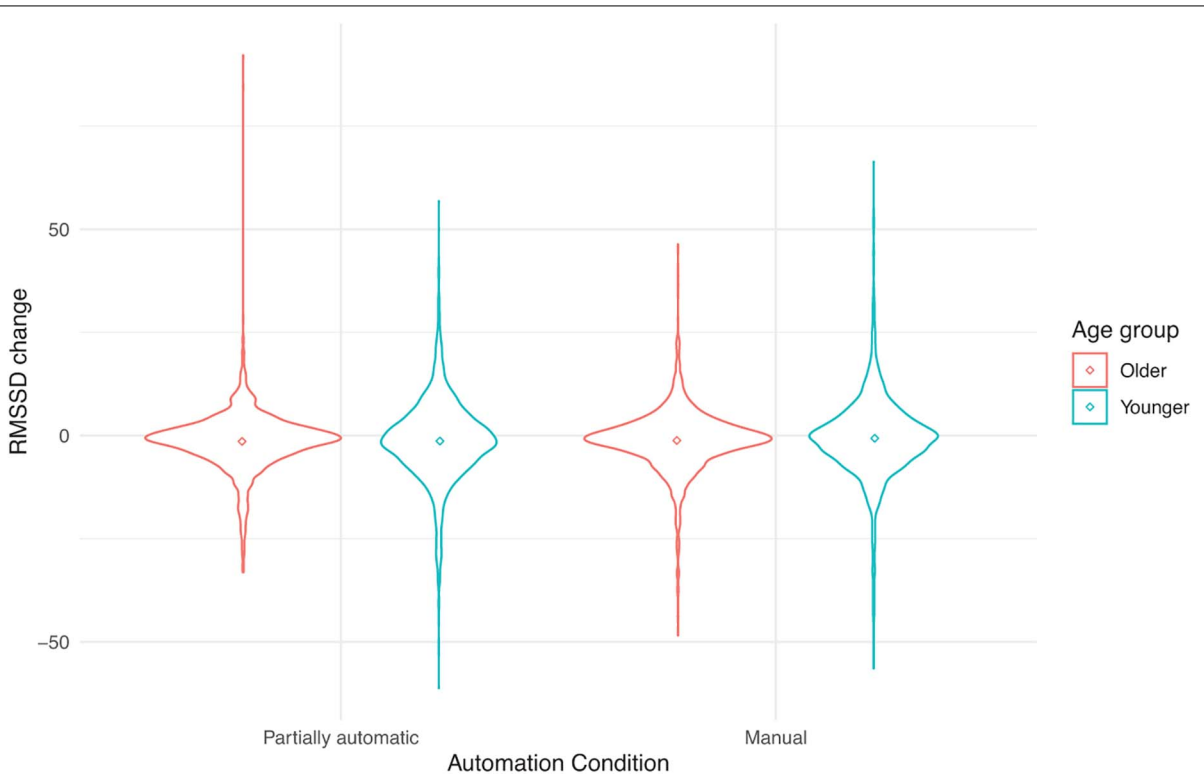



**FIGURE 2 |** RMSSD change from baseline as a function of automation (manual and partial automation) and age (younger or older).

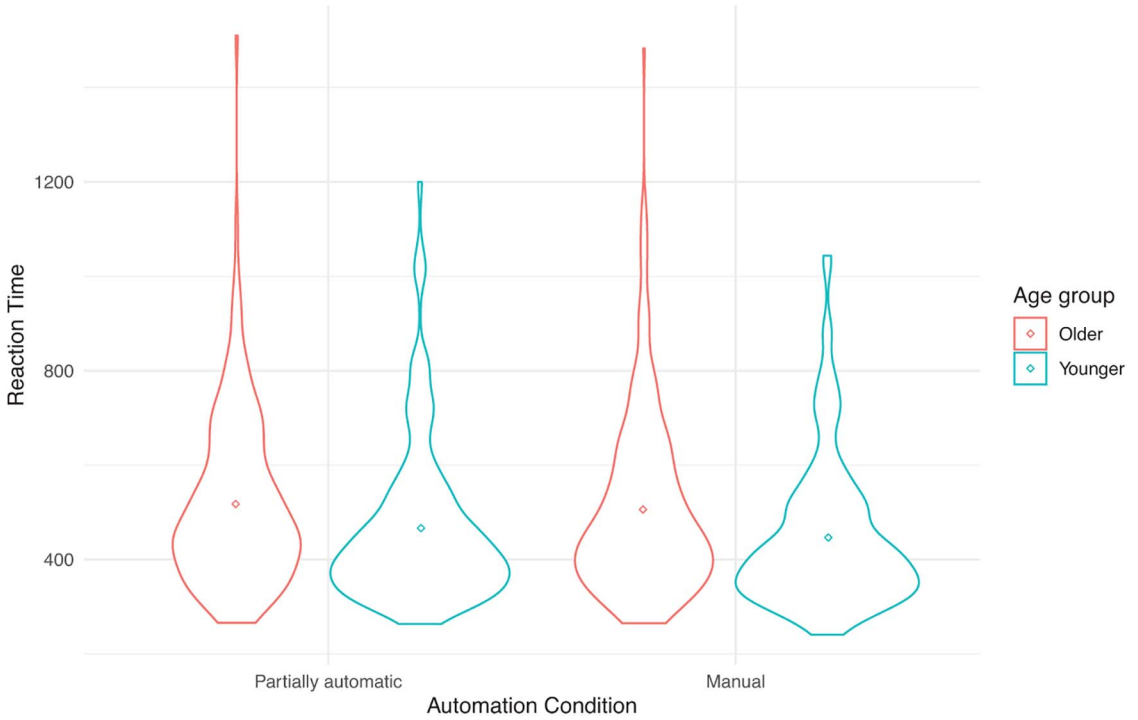



**FIGURE 3 |** Reaction time (in ms) performance on the Detection Response Task as a function of automation (manual and partial automation) and age (younger or older).

$p = 0.23$. Neither the automation by age by vehicle interaction nor any of the 2-way or 3-way interactions were significant.

## The Effect of Automation on DRT Reaction Time

A linear mixed-effects model with reaction time as the outcome did not have a significant main effect of automation, $F(1, 325.77) = 2.76$, $p = 0.10$. Similarly, neither the main effects of age, $F(1, 73.77) = 1.77$, $p = 0.19$, nor vehicle, $F(3, 337.02) = 1.67$, $p = 0.17$ were significant. The 2-way and 3-way interactions were also not significant.

## Bayes Factor Analyses

In order to examine the effect of automation on RMSSD, heart rate, and DRT reaction time, for each of these outcomes, a Bayes factor analysis was conducted by running a full model with main effects and interactions of automation, age, vehicle, and participants as the random intercept. Next, a restricted model was run without automation with main and interaction effects of age and vehicles as predictors and participants as the random intercept. These full and restricted models were compared to calculate a Bayes Factor of 0.0002, 0.0004, and 0.0002 for heart rate, RMSSD, and DRT reaction time, respectively (see **Figure 4**). According to the Lee and Wagenmakers (2014) classification scheme for interpreting Bayes factors, these values suggest extreme evidence that favors the null hypothesis for the effect of automation on heart rate, RMSSD, and DRT reaction time.

## Self-Reported Driving Experience

All participants drove in the partially automated conditions and their experiences on a 5-point scale (completed disagree to completely agree) were analyzed by comparing the responses to the midpoint. The results are reported in **Table 2**. Participants could relax, but relative to manual driving, they were neither less stressed nor bored when the automated driving systems were activated. Participants reported not engaging in unrelated activities while driving the automated systems, such as daydreaming. Participants were well-calibrated in their trust in the automated features of the vehicles. On the one hand, they believed that it made traveling safer and enjoyable, and they were not more anxious or nervous while driving it relative to manual driving. At the same time, they showed restraint when the driving conditions were challenging (e.g., curvy/hilly roads and heavy traffic).

# DISCUSSION

## Lack of Differences Between Manual and Partially Automated Modes

In order to examine the possible impact of partially automated technology on drivers' arousal and cognitive demand, it is necessary to have sensitive near real-time measures that can detect changes in real-world driving tasks. The current study used heart rate (an arousal measure), RMSSD (a heart rate variability based cognitive demands measure), and DRT (a

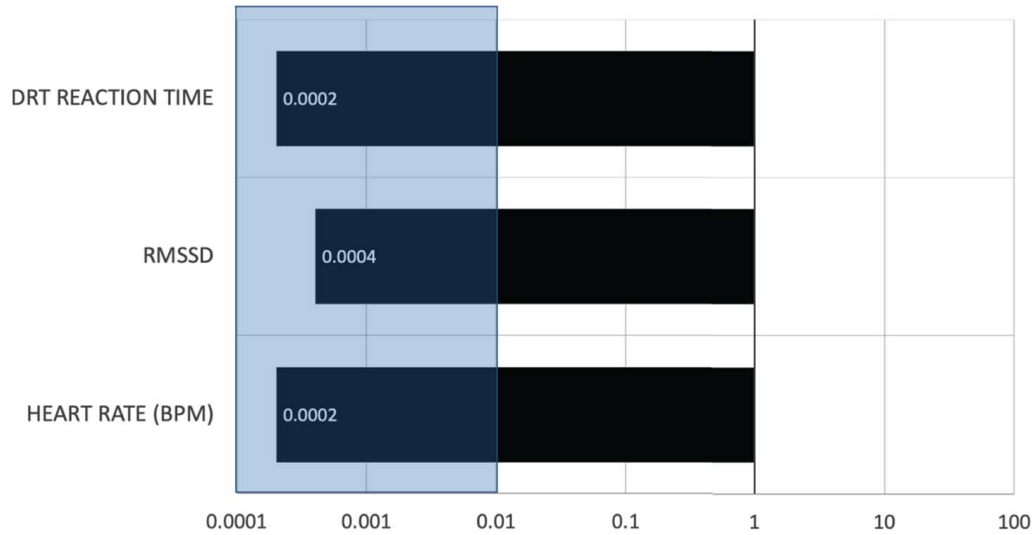



**FIGURE 4 |** The Bayesian values were plotted for the three outcome variables. A value less than 0.01 (shaded region) provides extreme evidence in favor of the null hypothesis (Lee and Wagenmakers, 2014).

**TABLE 2 |** Results from the driving experiences questionnaire.

| Items | Finding | Statistic | Mean (SE) |
|---|---|---|---|
| **The driving experiences related questions** | | | |
| I was able to relax when the automated driving systems were on | Agree | $t(210) = 5.32$, $p < 0.001$ | 3.44 (0.08) |
| I was able to engage in more activities unrelated to driving when the automated driving systems were on | Disagree | $t(210) = 6.35$, $p < 0.001$ | 2.43 (0.09) |
| The automated driving system made traveling boring for me | Disagree | $t(210) = 8.95$, $p < 0.001$ | 2.26 (0.08) |
| The automated driving systems made traveling safer | Agree | $t(210) = 5.46$, $p < 0.001$ | 3.39 (0.07) |
| I was anxious and nervous when the automated driving systems were on | Disagree | $t(210) = 5.69$, $p < 0.001$ | 2.51 (0.09) |
| The automated driving system made traveling more enjoyable | Agree | $t(210) = 6.14$, $p < 0.001$ | 3.47 (0.08) |
| The automated driving system took the fun out of driving | Disagree | $t(210) = 5.59$, $p < 0.001$ | 2.49 (0.09) |
| The automated driving system allowed me to think and daydream | Disagree | $t(210) = 4.53$, $p < 0.001$ | 2.61 (0.09) |
| I was uncomfortable relinquishing control of the vehicle to the automated driving system on curvy and hilly roads | Agree | $t(210) = 5.31$, $p < 0.001$ | 3.48 (0.09) |
| I was uncomfortable relinquishing control of the vehicle to the automated driving systems in heavier traffic | Agree | $t(210) = 3.08$, $p = 0.002$ | 3.28 (0.09) |
| I was concerned that the automated driving systems would shut off unexpectedly | Not sig. | $t(210) = 0.10$, $p = 0.919$ | 2.99 (0.09) |
| The automated driving system reduced the stress of driving | Not sig. | $t(210) = 1.84$, $p = 0.067$ | 3.16 (0.07) |
| **Intentions to use and purchase automated driving systems** | | | |
| I would not feel comfortable using automated driving systems on most roads | Disagree | $t(210) = 7.31$, $p < 0.001$ | 2.39 (0.08) |
| If I was tired or distracted, I would rely heavily on automated driving systems | Disagree | $t(210) = 2.46$, $p = 0.015$ | 2.78 (0.09) |
| I would utilize the automated driving systems in a vehicle as much as possible | Agree | $t(210) = 8.41$, $p < 0.001$ | 3.68 (0.08) |
| I would not feel comfortable using the automated driving systems in a vehicle without monitoring it closely | Agree | $t(210) = 13.16$, $p < 0.001$ | 4.00 (0.08) |
| If I can afford it, I am going to buy or lease a car with automated driving systems | Agree | $t(210) = 7.84$, $p < 0.001$ | 3.67 (0.09) |
| I am going to make sure that the next car I buy, or lease has automated driving systems | Not sig. | $t(210) = 1.84$, $p = 0.067$ | 3.15 (0.08) |

behavioral performance task based cognitive demands measure) to compare differences in drivers' arousal and cognitive demands during manual versus partially automated driving. To our knowledge, this study is the first effort to examine the partial vehicle automation on arousal and cognitive demands with a representative sample of younger and late-middle-aged drivers and vehicles on real highways while their psychophysiological and behavioral responses were assessed in real-time.

The results suggested that there were no differences in heart rate and its variability or DRT reaction time performance between manual and partially automated modes of driving either in younger or late-middle-aged adults across the four test vehicles. A Bayes Factor analysis on heart rate, RMSSD, and DRT reaction time data showed *extreme evidence in favor of the null hypothesis,* suggesting that drivers new to partial automation maintain comparable levels of arousal and cognitive demands during manual and partially automated driving as no evidence of under or over-arousal and cognitive demands was found. In another forthcoming paper (McDonnell et al., in review), EEG alpha power and frontal theta (a central physiology-based

index of visual engagement and mental workload) were also found to show no significant difference between manual and partially automated modes. Past research on potential cognitive differences between manual and partial automation has been mixed. Various methodological limitations can explain these mixed findings, such as limited sample size, use of only self-report measures, and the use of driving simulators or a single-vehicle. The current study allowed for a fair test of the null hypothesis by testing a large representative sample of drivers and vehicles with sufficient sample size and a combination of reliable physiological and behavioral measures.

The current findings support and extend previous research that has found no difference between manual and partial automation (e.g., Sibi et al., 2017; Stapel et al., 2019; Calvi et al., 2020; Lohani et al., 2020; Várhelyi et al., 2020). Moreover, the pilot study that examined young drivers operating three vehicles on the highway also showed a similar pattern (Lohani et al., 2020). Similar to the current study, the pilot study found no differences across outcomes (heart rate, RMSSD, EEG alpha and theta power, and DRT performance). Bayes Factor analysis had suggested that there was strong evidence that arousal and cognitive demands did not differ during manual and partially automated driving (Lohani et al., 2020). With a single measure, null results are harder to interpret, but replicable effects with multiple reliable outcomes provide a more convincing interpretation of the null hypothesis. Taken together, these findings provide strong evidence that arousal and cognitive demands are similar during manual versus partially automated conditions for drivers who are new to partially automated technology.

A lack of differences between manual and partially automated modes also suggests that the cognitive demands imposed during manual driving are comparable to those imposed by monitoring partial automation. Based on the Neurovisceral Integration model (Thayer et al., 2009), these findings would imply that neural activity associated with cognitive functioning during manual and partially automated driving is comparable. Self-reports from participants suggested that participants were cognitively engaged in the driving process. This may be because participants still have to monitor ongoing traffic conditions and maintain cognitive readiness to take control of the vehicle. Interestingly, the age group did not lead to any differences in heart rate and RMSSD (after accounting for pre-condition baseline) or DRT reaction time. This implies that late-middle-aged drivers are able to use partially automated technology similar to their younger counterparts and that they can benefit from assistance provided by partially automated technology without additional cognitive costs. While this study investigated drivers between 21 and 64 years of age, future research should examine potential differences in teenage drivers and drivers older than 64 years of age to better understand driving partial automation vehicles across the lifespan.

## Limitations and Outstanding Questions

There are a few limitations of the current study. First, this study focused on drivers with no prior experience with partial driving automation. Drivers that are more experienced with partially automated technology may respond differently to automated vehicles than those with limited prior experience. The perceived workload was reduced for automation-experienced drivers, while it did not change from the manual mode for inexperienced drivers (Stapel et al., 2019). Motorists may learn to accept and trust the automation technology with additional driving experience (Beggiato et al., 2015). Experienced drivers may get better at calibrating their trust after understanding the automation system's limitations (Walker et al., 2018). However, it is also possible that more trusting participants increase their reliance on automation (Walker et al., 2019), resulting in poor readiness to switch from automated to manual driving safely. More work is needed to explore any associated risks.

Second, we had a research assistant sit in the passenger seat next to the participant for safety reasons, and we cannot rule out that this presence may have impacted drivers' performance and physiology. However, any confounding effects of social presence were constant in both manual and partial automation conditions across all the participants. Third, this study was not explicitly designed to test gender differences. Factors such as the different phases of the menstrual cycle were not recorded or accounted for and could affect the RMSSD data. Future work is needed to understand the effect this may have on RMSSD. Fourth, this study did not examine traffic conditions, and low and high traffic demands could moderate the outcomes. Finally, it is possible that constantly transitioning between manual and partially automated driving modes could be a demanding task for some motorists. For instance, one study found that older adults were slower at switching between manual and partially automated modes (Wu et al., 2019). Future research should examine transition-related driving demands on motorists while driving partially automated vehicles.

## Benefits and Challenges of Adopting HRV Indices in Driving Safety

With advances in methodological developments, it is now possible to collect high-quality psychophysiological data outside traditional lab settings from research-grade equipment at a low cost. This study conducted on real roads highlights the applicability of heart rate variability to real-world automation driving research. However, some caution is warranted while interpreting HRV measurement. As is true for most psychophysiological measures, HRV does not have a one-to-one correspondence with a single psychological construct (e.g., Cacioppo and Tassinary, 1990). HRV may be sensitive to many cognitive factors that may occur in real-world driving contexts. For example, over time, drivers may become more relaxed or disengaged with the driving task and both scenarios would lead to a lower-arousal and a corresponding increase in HRV indices (Jasper et al., 2016). Furthermore, there may be other factors (e.g., Laborde et al., 2017) while driving that can influence HRV measurements, such as driving task-related factors (e.g., bad traffic), driver-related factors (e.g., a driver has irregular heartbeats or is on psychotropic medications), or concurrent activities (talking, smoking, or caffeine intake). Because such

factors may influence HRV indices and may vary across driving conditions; thoughtful analyses of such effects in conjunction with other measures (such as behavioral performance) is crucial for accurate interpretations.

A challenge with HRV measurement is that it is susceptible to artifacts that can result in inaccurate values. Some analytic approaches have adopted shorter segments (e.g., 30 s) of data to detect momentary effects of driving-related workload (Stuiver et al., 2014). While preliminary solutions to handle noise and artifacts have been proposed (e.g., Nowara et al., 2018; van Gent et al., 2019; Brown et al., 2020), more work is needed to develop near-real-time artifact detection methods that are amenable to driving task-related changes. As in the current study, we argue that a multi-method approach provides a more accurate interpretation of cognitive demands in applied settings. Several efforts in driving research have proposals to assess cognitive states using a multi-method approach that seems promising (Fu et al., 2016; Brouwer et al., 2017; Aricò et al., 2018; Haouij et al., 2018; Paredes et al., 2018; Rastgoo et al., 2018). The current findings imply that RMSSD is a suitable measure that could be utilized in multi-method studies to evaluate drivers' cognitive demand.

## CONCLUSION

This investigation was conducted on real roads with a large and representative sample of younger and late-middle-aged motorists to compare manual and partially automated driving. The current findings show important evidence of no difference in arousal and cognitive demands (extreme evidence in favor of the null hypothesis), suggesting that drivers maintain similar levels of arousal and cognitive demands during manual and partially automated driving. These theoretically relevant results imply that younger and late-middle-aged motorists who are new to partial automation may maintain arousal and cognitive levels comparable to manual driving. This research extends a growing literature highlighting the applicability of heart rate variability to real-world automation research.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board, University of Utah. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

DS and JC conceived the study idea and design. ML, GE, TS, AC, and KC were involved in data collection and processing. ML analyzed the data and wrote the first draft of the manuscript. DS, JC, and AM provided suggestions to improve the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

Support for this work was provided by a grant from AAA Foundation for Traffic Safety.

## REFERENCES

Aricò, P., Borghini, G., Di Flumeri, G., Sciaraffa, N., and Babiloni, F. (2018). Passive BCI beyond the lab: current trends and future directions. *Physiol. Meas.* 39:08TR02. doi: 10.1088/1361-6579/aad57e

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Beggiato, M., Pereira, M., Petzoldt, T., and Krems, J. (2015). Learning and development of trust, acceptance and the mental model of ACC. A longitudinal on-road study. *Transp. Res. Part F* 35, 75–84. doi: 10.1016/j.trf.2015.10.005

Bengler, K., Kohlmann, M., and Lange, C. (2012). Assessment of cognitive workload of in-vehicle systems using a visual peripheral and tactile detection task setting. *Work* 41(Suppl. 1), 4919–4923. doi: 10.3233/WOR-2012-0786-4919

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Quigley, K. S., and Lozano, D. (2007). Cardiovascular psychophysiology. *Handb. Psychophysiol.* 3, 182–210. doi: 10.1017/CBO9780511546396.008

Biondi, F. N., Lohani, M., Hopman, R., Mills, S., Cooper, J. M., and Strayer, D. L. (2018). "80 MPH and out-of-the-loop: effects of real-world partially automated driving on driver workload and arousal," in *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, Vol. 62, (Los Angeles, CA: SAGE Publications), 1878–1882. doi: 10.1177/1541931218621427

Broadhurst, P. L. (1959). The interaction of task difficulty and motivation: the Yerkes Dodson Law revived. *Acta Psychol.* 16, 321–338. doi: 10.1016/0001-6918(59)90105-2

Brouwer, A., Snelting, A., Jaswa, M., Flascher, O., Krol, L., and Zander, T. (2017). "Physiological effects of adaptive cruise control behaviour in real driving," in *Proceedings of the 2017 ACM Workshop on An Application-oriented Approach to BCI out of the Laboratory*, Limassol. doi: 10.1145/3038439.3038441

Brown, S. B. R. E, Brosschot, J. F., Versluis, A., Thayer, J. F., and Verkuil, B. (2020) Assessing new methods to optimally detect episodes of non-metabolic heart rate variability reduction as an indicator of psychological stress in everyday life: a thorough evaluation of six methods. *Front. Neurosci.* 14:564123. doi: 10.3389/fnins.2020.564123

Bruyas, M. P., and Dumont, L. (2013). "Sensitivity of Detection Response Task (DRT) to the driving demand and task difficulty," in *Proceedings of the 7th International Driving Symposium on Human Factors in Driver Assessment, Training, and Vehicle Design. Symposium presented at Bolton Landing*, New York, NY, 17–20.

Cacioppo, J. T., and Tassinary, L. G. (1990). Inferring psychological significance from physiological signals. *Am. Psychol.* 45, 16–28. doi: 10.1037/0003-066X.45.1.16

Calvi, A., D'Amico, F., Ciampoli, L. B., and Ferrante, C. (2020). Evaluation of driving performance after a transition from automated to manual control: a

driving simulator study. *Transp. Res. Procedia* 45, 755–762. doi: 10.1016/j.trpro.2020.02.101

Cisler, D., Greenwood, P. M., Roberts, D. M., McKendrick, R., and Baldwin, C. L. (2019). Comparing the relative strengths of EEG and low-cost physiological devices in modeling attention allocation in semiautonomous vehicles. *Front. Hum. Neurosci.* 13:109. doi: 10.3389/fnhum.2019.00109

Cooper, J. M., Castro, S. C., and Strayer, D. L. (2016). "Extending the Detection Response Task to simultaneously measure cognitive and visual task demands," in *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, Vol. 60, Prague, 1962–1966. doi: 10.1177/1541931213601447

Fu, R., Wang, H., and Zhao, W. (2016). Dynamic driver fatigue detection using hidden Markov model in real driving condition. *Expert Syst. Appl.* 63, 397–411. doi: 10.1016/j.eswa.2016.06.042

Gershon, P., Shinar, D., and Ronen, A. (2009). Evaluation of experience-based fatigue countermeasures. *Accid. Anal. Prev.* 41, 969–975. doi: 10.1016/j.aap.2009.05.012

Haouij, N. E., Poggi, J.-M., Sevestre-Ghalila, S., Ghozi, R., and Jaïdane, M. (2018). "AffectiveROAD system and database to assess driver's attention," in *Proceedings of the 33rd Annual ACM Symposium on Applied Computing*, Pau. doi: 10.1145/3167132.3167395

Hebb, D. O. (1955). Drives and the CNS (conceptual nervous system). *Psychol. Rev.* 62, 243–254. doi: 10.1037/h0041823

Heikoop, D. D., de Winter, J. C., van Arem, B., and Stanton, N. A. (2019). Acclimatizing to automation: driver workload and stress during partially automated car following in real traffic. *Transp. Res. Part F* 65, 503–517. doi: 10.1016/j.trf.2019.07.024

Heine, T., Lenis, G., Reichensperger, P., Beran, T., Doessel, O., and Deml, B. (2017). Electrocardiographic features for the measurement of drivers' mental workload. *Appl. Ergon.* 61, 31–43. doi: 10.1016/j.apergo.2016.12.015

International Organization for Standardization [ISO] (2016). *Road Vehicles – Transport Information and Control Systems – Detection Response Task (DRT) for Assessing Attentional Effects of Cognitive Load in Driving (Rep. ISO 17488)*. Geneva: International Organization for Standardization.

Jasper, P., Sibley, C., and Coyne, J. (2016). "Using heart rate variability to assess operator mental workload in a command and control simulation of multiple unmanned aerial vehicles," in *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, Vol. 60, (Los Angeles, CA: SAGE Publications), 1125–1129. doi: 10.1177/1541931213601264

Kahneman, D. (1973). *Attention and Effort*. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Kim, J., Revell, K., Langdon, P., Bradley, M., Politis, I., Thompson, S., et al. (2020). "Drivers' interaction with, and perception toward semi-autonomous vehicles in naturalistic settings," in *Proceedings of the International Conference on Intelligent Human Systems Integration Springer*, Cham, 20–26. doi: 10.1007/978-3-030-39512-4_4

Kruschke, J. K. (2011). Bayesian assessment of null values via parameter estimation and model comparison. *Perspect. Psychol. Sci.* 6, 299–312. doi: 10.1177/1745691611406925

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research–recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lee, H. B., Kim, J. S., Kim, Y. S., Baek, H. J., Ryu, M. S., and Park, K. S. (2007). "The relationship between HRV parameters and stressful driving situation in the real road," in *Proceedings of the 6th International Special Topic Conference on Information Technology Applications in Biomedicine, 2007*, Tokyo. doi: 10.1109/ITAB.2007.4407380

Lee, M. D., and Wagenmakers, E. J. (2014). *Bayesian Cognitive Modeling: A Practical Course*. Cambridge: Cambridge University Press.

Lenneman, J. K., and Backs, R. W. (2009). Cardiac autonomic control during simulated driving with a concurrent verbal working memory task. *Hum. Fact.* 51, 404–418. doi: 10.1177/0018720809337716

Liang, W. C., Yuan, J., Sun, D. C., and Lin, M. H. (2009). Changes in physiological parameters induced by indoor simulated driving: effect of lower body exercise at mid-term break. *Sensors* 9, 6913–6933. doi: 10.3390/s90906913

Lohani, M., Cooper, J. M., Erickson, G., Simmons, T., McDonnell, A., Crabtree, K., et al. (2020). *Driver Arousal and Workload Under Partial Vehicle Automation: A Pilot Study. Proceedings of the Human Factors Ergonomic Society*. Available online at: https://journals.sagepub.com/doi/abs/10.1177/1071181320641471?journalCode=proe (accessed June 1, 2020).

Lohani, M., Payne, B. R., and Strayer, D. L. (2019). A review of psychophysiological measures to assess cognitive states in real-world driving. *Front. Hum. Neurosci.* 13:57. doi: 10.3389/fnhum.2019.00057

Malik, M. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur. Heart J.* 17, 354–381.

Mauss, I. B., and Robinson, M. D. (2010). Measures of emotion: a review. *Cogn. Emot.* 23, 209–237, doi: 10.1080/02699930802204677

McDonnell, A. S., Simmons, T. G., Erickson, G. G., Lohani, M., Cooper, J. M., and Strayer, D. L. (in review). This is your brain on autopilot: neural indices of driver workload and engagement during partial vehicle automation.

Mehler, B., Reimer, B., and Wang, Y. (2011). "A comparison of heart rate and heart rate variability indices in distinguishing single-task driving and driving under secondary cognitive workload," in *Proceedings of the 6th International Driving Symposium on Human Factors in Driver Assessment, Training, and Vehicle Design: Driving Assessment, 2011*, Lake Tahoe, CA, 590–597. doi: 10.17077/drivingassessment.1451

Morey, R. D., Rouder, J. N., and Jamil, T. (2018). *BayesFactor: Computation of Bayes Factors for common designs. R package version 0.9. 12-4.2. Computer software*. Available online at: https://CRAN.R-project.org/package=BayesFactor (accessed June 1, 2020).

Mulder, G. (1985). "Attention, effort and sinus arrhythmia: how far are we," in *Psychophysiology of Cardiovascular Control*, eds J. F. Orlebeke, G. Mulder, and L. P. J. van Doornen (New York, NY: Plenum Press), 407–424.

Mulder, G., and Mulder, L. J. (1981). Information processing and cardiovascular control. *Psychophysiology* 18, 392–402. doi: 10.1111/j.1469-8986.1981.tb02470.x

Noda, A., Miyaji, M., Wakuda, Y., Hara, Y., and Yasuma, F. (2015). Simultaneous measurement of heart rate variability and blinking duration to predict sleep onset and drowsiness in drivers. *J. Sleep Disord. Ther.* 4, 2167–2277. doi: 10.4172/2167-0277.1000213

Nowara, E. M., Marks, T. K., Mansour, H., and Veeraraghavan, A. (2018). "SparsePPG: towards driver monitoring using camera-based vital signs estimation in near-infrared," in *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition Workshops*, Salt Lake City, UT. doi: 10.1109/CVPRW.2018.00174

Paredes, P. E., Ordonez, F., Ju, W., and Landay, J. A. (2018). "Fast & furious: detecting stress with a car steering wheel," in *Proceedings of the 2018 CHI Conference on Human Factors in Computing Systems*, Montreal, QC. doi: 10.1145/3173574.3174239

Peltola, M. A. (2012). Role of editing of R-R intervals in the analysis of heart rate variability. *Front. Physiol.* 23:148. doi: 10.3389/fphys.2012.00148

Piotrowski, Z., and Szypulska, M. (2017). Classification of falling asleep states using HRV analysis. *Biocybern. Biomed. Eng.* 37, 290–301. doi: 10.1016/j.bbe.2017.02.003

Quintana, D. S., and Williams, D. R. (2018). Bayesian alternatives for common null-hypothesis significance tests in psychiatry: a non-technical guide using JASP. *BMC Psychiatry* 18:178. doi: 10.1186/s12888-018-1761-4

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/.

Rastgoo, M. N., Nakisa, B., Rakotonirainy, A., Chandran, V., and Tjondronegoro, D. (2018). A critical review of proactive detection of driver stress levels based on multimodal measurements. *ACM Comput. Surv.* 51, 1–35. doi: 10.1145/3186585

Robbins, T. W., and Everitt, B. J. (1995). "Arousal systems and attention," in *The Cognitive Neurosciences*, ed. M. S. Gazzaniga (Cambridge, MA: The MIT Press), 703–720.

SAE (2016). *Taxonomy and Definitions for Terms Related to Driving Automation Systems for on-Road Motor Vehicles (Surface Vehicle Recommended Practice: Superseding J3016 Jan 2014), SAE International*. Available online at: https://www.sae.org/standards/content/j3016_201806/. (accessed June 1, 2020).

Satpute, A. B., Kragel, P. A., Barrett, L. F., Wager, T. D., and Bianciardi, M. (2019). Deconstructing arousal into wakeful, autonomic and affective varieties. *Neurosci. Lett.* 693, 19–28. doi: 10.1016/j.neulet.2018.01.042

Shaffer, F., McCraty, R., and Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front. Psychol.* 5:1040. doi: 10.3389/fpsyg.2014.01040

Sibi, S., Baiters, S., Mok, B., Steiner, M., and Ju, W. (2017). "Assessing driver cortical activity under varying levels of automation with functional near infrared spectroscopy," in *Proceedings of the 2017 IEEE Intelligent Vehicles Symposium (IV)*, Los Angeles, CA, 1509–1516.

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Solís-Marcos, I., Ahlström, C., and Kircher, K. (2018). Performance of an additional task during Level 2 automated driving: an on-road study comparing drivers with and without experience with partial automation. *Hum. Factors* 60, 778–792. doi: 10.1177/0018720818773636

Stapel, J., Mullakkal-Babu, F. A., and Happee, R. (2019). Automated driving reduces perceived workload, but monitoring causes higher cognitive load than manual driving. *Transp. Res. Part F* 60, 590–605. doi: 10.1016/j.trf.2018.11.006

Stuiver, A., Brookhuis, K. A., de Waard, D., and Mulder, B. (2014). Short- term cardiovascular measures for driver support: increasing sensitivity for detecting changes in mental workload. *Int. J. Psychophysiol.* 92, 35–41. doi: 10.1016/j.ijpsycho.2014.01.010

Sugie, R., Arakawa, T., and Kozuka, K. (2016). Detection of fatigue in long-distance driving by heart rate variability. *Innovat. Comput. Inform. Control Exp. Lett.* 10, 1553–1559.

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

van Gent, P., Farah, H., van Nes, N., and van Arem, B. (2019). Analysing noisy driver physiology real-time using off-the-shelf sensors: heart rate analysis software from the taking the fast lane project. *J. Open Res. Softw.* 7:32. doi: http://doi.org/10.5334/jors.241

Várhelyi, A., Kaufmann, C., Johnsson, C., and Almqvist, S. (2020). Driving with and without automation on the motorway–an observational study. *J. Intell. Transp. Syst.* 1–22.

Walker, F., Boelhouwer, A., Alkim, T., Verwey, W. B., and Martens, M. H. (2018). Changes in trust after driving level 2 automated cars. *J. Adv. Transp.* 2018:1045186. doi: 10.1155/2018/1045186

Walker, F., Wang, J., Martens, M. H., and Verwey, W. B. (2019). Gaze behaviour and electrodermal activity: objective measures of drivers' trust in automated vehicles. *Transp. Res. Part F* 64, 401–412.

Wekselblatt, J. B., and Niell, C. M. (2015). Behavioral state—getting in the zone. *Neuron* 87, 7–9. doi: 10.1016/j.neuron.2015.06.020

Wickens, C. D. (2002). Multiple resources and performance prediction. *Theor. Issues Ergon. Sci.* 3, 159–177.

Wu, Y., Kihara, K., Takeda, Y., Sato, T., Akamatsu, M., and Kitazaki, S. (2019). Effects of scheduled manual driving on drowsiness and response to take over request: a simulator study towards understanding drivers in automated driving. *Accid. Anal. Prev.* 124, 202–209.

Yerkes, R. M., and Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *J. Comp. Neurol. Psychol.* 18, 459–482. doi: 10.1002/cne.920180503

Young, R. A., Hsieh, L., and Seaman, S. (2013). "The tactile detection response task: preliminary validation for measuring the attentional effects of cognitive load," in *Proceedings of the Seventh International Driving Symposium on Human Factors in Driver Assessment, Training and Vehicle Design*, New York, NY, 71–77. doi: 10.17077/drivingassessment.1469

Zhai, J., and Lu, G. (2019). "A study of how drivers' subjective workload and driving performance change under varying levels of automation and critical situations," in *Proceedings of the 19th COTA International Conference of Transportation Professionals*, Nanjing, 344–355.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 29 June 2021
doi: 10.3389/fphys.2021.619722

# Optimizing Autonomic Function Analysis via Heart Rate Variability Associated With Motor Activity of the Human Colon

*M. Khawar Ali[1,2], Lijun Liu[2], Ji-Hong Chen[2] and Jan D. Huizinga[1,2]**

*[1] Faculty of Engineering, School of Biomedical Engineering, McMaster University, Hamilton, ON, Canada, [2] Division of Gastroenterology, Department of Medicine, Faculty of Health Sciences, Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada*

The parameters of heart rate variability (HRV) can non-invasively assess some autonomic activities, and HRV is influenced by many bodily actions. Although parasympathetic activity is the primary driver of colonic propulsive activity, and sympathetic activity a major inhibitor of colonic motility, they are rarely measured and almost play no role in diagnosis of colon motor dysfunction or in standard treatments. Here we set out to optimize HRV analysis of autonomic nervous system changes related to human colon motility. The electrocardiogram and impedance were recorded in synchrony with colonic motor patterns by high-resolution manometry. Respiratory sinus arrhythmia (RSA), root mean square of successive differences of beat-to-beat intervals (RMSSD), the Baevsky Index or Sympathetic Index (SI), and the ratios of SI/RSA and SI/RMSSD were shown to indicate a marked increase in parasympathetic and withdrawal of sympathetic activity during the high-amplitude propagating pressure waves (HAPWs). Strong associations were seen with HAPWs evoked by a meal and rectal bisacodyl indicating a marked increase in parasympathetic and withdrawal of sympathetic activity during the gastrocolic reflex and the defecation reflex. When HAPWs occurred in quick succession, parasympathetic activation (RSA and RMSSD) occurred in a rhythmic fashion. Hence, during propulsive motor patterns, an overall shift in autonomic activity toward increased parasympathetic control was shown to be reflected in HRV. HRV assessment may therefore be valuable in the assessment of autonomic dysfunction related to colonic dysmotility.

**Keywords: high-amplitude propagating pressure waves, RMSSD, RSA, Baevsky's Stress Index, autonomic nervous system, colonic motility**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer, The Ohio State University, United States*

***Reviewed by:***
*Katja Weimer, University of Ulm, Germany*
*DeWayne P. Williams, University of California, Irvine, United States*

***Correspondence:**
*Jan D. Huizinga*
*huizinga@mcmaster.ca*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

***Received:*** *21 October 2020*
***Accepted:*** *24 May 2021*
***Published:*** *29 June 2021*

***Citation:***
*Ali MK, Liu L, Chen J-H and Huizinga JD (2021) Optimizing Autonomic Function Analysis via Heart Rate Variability Associated With Motor Activity of the Human Colon. Front. Physiol. 12:619722. doi: 10.3389/fphys.2021.619722*

## INTRODUCTION

Measurement of autonomic function does not yet play a significant role in colon dysmotility diagnosis, despite the fact that propulsive contractions in the human colon are orchestrated by the parasympathetic nervous system (De Groat and Krier, 1976; Browning and Travagli, 2019). Some studies have linked gastrointestinal activity such as the postprandial state (Lu et al., 1999)

**Abbreviations:** HAPW, High-Amplitude Propagating Pressure Wave; HAPW-SPW, High-Amplitude Propagating Pressure Wave followed by a Simultaneous Pressure Wave; HRV, Heart Rate Variability; RSA, Respiratory Sinus Arrythmia; SI, Baevsky's Stress Index or Sympathetic Index; RMSSD, Root Mean Square of Successive Differences; PEP, Pre-Ejection Period; HF, High Frequency; LF, Low Frequency; SD1, Standard deviation of minor axis of Poincare' Plot; SD2, Standard deviation of major axis of Poincare' Plot.

and gastric hypersensitivity (Ouyang et al., 2020), to high frequency (HF) and low frequency (LF) parameters. Autonomic activity associated with Irritable Bowel Syndrome (IBS) (Bharucha et al., 1993) and chronic intestinal pseudo obstruction (Camilleri et al., 1993) were studied using heart rate interval parameters, heart rate response to deep breathing and other tests. Autonomic function associated with functional dyspepsia was studied using HF power and root mean square of successive differences (RMSSD) (Lorena et al., 2002).

Heart rate can react momentarily to changes in nervous input from the autonomic nervous system to the sinoatrial node, and this property establishes heart rate variability (HRV) (Thayer et al., 2012; Baevsky and Chernikova, 2017; Shaffer and Ginsberg, 2017). Several time and frequency domain analyses and non-linear methods have been developed to analyse HRV. Especially spectral analysis of beat-to-beat intervals, assessing the band power of low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.4 Hz), are used as matrices of the sympathetic and parasympathetic nervous systems (Hayano and Yuda, 2019). The HF band power is considered a measure of parasympathetic nervous system activity, the effect of activity of the final vagal fibers innervating the sinoatrial node, a culmination of vagal innervation that was influenced by a myriad of factors, primarily breathing but also activity from regulatory nuclei such as the nucleus tractus solitarius (NTS) that orchestrate coordination between respiratory, cardiac and gastrointestinal activities to optimize responses to metabolic demands and hence influence the autonomic outflow to the heart (Grossman and Taylor, 2007; Browning and Travagli, 2014; Singh and Jaryal, 2020). Dendritic projections from efferent vagal motor neurons to the colon extent throughout the NTS and intermingle within the various subnuclei so as to co-ordinate homeostatic reflexes across autonomically controlled organs (Browning and Travagli, 2014). The NTS is a key structure for autonomic and neuroendocrine integration (Jean, 1991). The coordination of gastrointestinal, respiratory and cardiac function is dramatically seen in cases of emergency. The afferent information from the airways is first processed at the level of NTS and results in various reflexes that are required for modification of ongoing breathing along with modulation of autonomic output to the cardiovascular and respiratory systems (Singh and Jaryal, 2020). It may be assumed that similar processes are involved in the central control of colon motility where the NTS plays a critical role (Browning and Travagli, 2014).

Effects of organ activities on HRV are difficult to predict and sometimes counterintuitive (La Rovere et al., 2003). The major motor pattern of the human colon, the high-amplitude propagating pressure wave is associated with the autonomic nervous system in two ways. It is orchestrated by the parasympathetic and enteric nervous system and its occurrence, due to increased intraluminal pressure and distention of the colon, will activate stretch sensitive neurons. The role of the autonomic nervous in orchestrating colonic motility is exemplified by the sacral defection reflex (Bharucha and Brookes, 2018) that starts with rectal sensation, which activates the sacral sensory nerves. Then information is signaled into the sacral parasympathetic nucleus (also called the sacral defecation center) from where information travels into the brain stem and frontal cortex to either prevent or initiate a defecation. A bowel movement may be produced via activation of sacral parasympathetic nerves and via the enteric nervous system (Browning and Travagli, 2014; Furness et al., 2014; Brookes et al., 2016; Bharucha and Brookes, 2018). The primary driver of the HAPWs is the parasympathetic nervous system (Devroede and Lamarche, 1974; De Groat and Krier, 1978; Callaghan et al., 2018). Resection of the parasympathetic pelvic splanchnic nerves causes loss of the defecation reflex (Devroede and Lamarche, 1974). HAPWs are not observed in ex vivo preparations of the human colon (Dinning et al., 2016). In the cat, HAPWs and HAPW-SPWs were identified in vivo and shown to be associated with firing of parasympathetic efferents (De Groat and Krier, 1978). Stimulation of sacral extrinsic nerves has also been shown to be a treatment for constipation (Leblanc et al., 2015). Interestingly, propulsive motor patterns can be evoked by injection of a ghrelin agonist in the sacral spinal cord (Shimizu et al., 2006) or by stimulation of surgically placed electrodes in the S2 region of the spinal cord (Devroede et al., 2012).

This study was designed to evaluate which of the myriad of HRV parameters best reflect autonomic nervous system activity using an established supine to standing protocol, and autonomic tone and reactivity associated with the high-amplitude pressure wave that is associated with human colon transit and defecation. We included HF and LF power, to directly compare RSA and HF power for statistical analysis, and to compare the disputed LF power as a measure of sympathetic activity with the Baevsky Index. We also separated the analysis by intervention, so that we could assess shifts in autonomic activity during HAPWs in response to a meal (the gastrocolonic reflex), and in response to rectal bisacodyl (the sacral autonomic (defecation) reflex, and in response to distention. We chose a combination of high amplitude propagating pressure waves (HAPWs) and high amplitude propagating pressure waves followed by simultaneous pressure waves (HAPW-SPWs) to incorporate all individual HAPWs in the statistical analysis, *excluding* in this analysis bisacodyl-induced multiple HAPWs since they sometimes are accompanied by pain and changes in breathing pattern. For bisacodyl-induced multiple HAPW activity we devised a new method for continuous assessment of HRV parameters. To study shifts in autonomic balance we propose new ratios of sympathetic over parasympathetic parameters.

# MATERIALS AND METHODS

## Participants

Eleven healthy volunteers (7 males, 4 females, age 30 ± 10 years) without any current or prior history of cardiovascular or gastrointestinal disease and not on any medications affecting cardiac or gastrointestinal function were recruited by local advertisement (wall posters) for this study. Each participant was paid 600 CA$ to complete this study. The study was carried out at McMaster University with ethics approval from the Hamilton Integrated Research Ethics Board, and written consent from all participants.

## Heart Rate and Impedance Measurements

The electrocardiogram (ECG) was recorded using seven electrodes on the subject's torso. Three electrodes formed a modified Lead II configuration for ECG recording. Four electrodes were used in a standard tetrapolar electrode configuration for impedance recording, where two electrodes supplied a constant current source, and two electrodes registered the changes in the transfer impedance (reflecting changes in activity of the sympathetic nervous system). ECG and impedance were recorded using a MindWare impedance cardio GSC monitor with a sampling frequency of 500 Hz. (MindWare Technologies Ltd., Gahanna, OH, United States) and MindWare BioLab Recording Software. MindWare HRV 3.1 was used for artifact correction of the ECG signal, to generate beat-to-beat intervals (RR intervals) and for the calculation of RSA, RMSSD, HF and LF band powers. PEP was generated by Mindware Cardiac Impedance software (MindWare Technologies Ltd., Gahanna, OH, United States). MATLAB codes were generated to calculate SD1 and SD2 (Poincare plot) as well as the sympathetic Index (SI) using the RR interval signal. The breathing frequency was generated by the Mindware impedance analysis software.

## HRV Related to Posture Change

To test general autonomic reactivity using a standard method, heart rate and HRV changes of all participants were measured related to posture change. The participants refrained from smoking, caffeine intake and heavy eating for 2 h prior to the testing. During the test, they were accommodated in a quiet room with normal lighting and room temperature. After resting in supine position for a minimum 10 min, the ECG and impedance were recorded for 6 min in the supine position, 6 min in sitting position and immediately upon standing for 6 min. The HRV parameters tested are shown in **Table 1**. We calculated the Baevsky's Stress Index (SI) (Baevsky and Chernikova, 2017) according to the formula

$$SI = \frac{AM_o \times 100\%}{2M_o \times M_x DM_n}$$

where the mode ($M_o$) is the most frequent RR interval expressed in seconds. The amplitude of mode ($AM_o$) was calculated, using a 50 ms bin width, as the number of the RR intervals in the bin containing the $M_o$, expressed as a percentage of the total number of intervals measured. The variability is reflected in $M_xDM_n$ as the difference between longest ($M_x$) and shortest ($M_n$) RR interval values, expressed in seconds. The SI is expressed as $s^{-2}$.

## HRV Related to Colonic Motor Patterns

Raw data were obtained from a study that was reported on previously (Milkova et al., 2020; Yuan et al., 2020). High-Resolution Colonic Manometry was performed using an 84-sensor water perfused catheter that detected luminal pressures at 1 cm intervals from the proximal colon to the anal sphincter. The catheter was custom-made by Mui Scientific (Mississauga, ON, Canada) and the acquisition hardware was made by Medical Measurement Systems (Laborie, Toronto, ON, Canada). The sampling frequency of the system is 10 Hz. After the catheter was placed inside the colon with the assistance of colonoscopy, a 6–8 h high-resolution colonic manometry (HRCM) procedure was executed. All participants underwent synchronized HRCM, ECG, and impedance recording during 90 min of baseline, followed by 20 min of proximal balloon distention, 20 min of rectal balloon distention using a standard anorectal manometry balloon assembly, 90 min following intake of a meal, consisting of organic yogurt fortified by organic milk fat to make it 1,000 kcal (Mapleton Organic, ON, Canada), and 45 min after administration of rectal bisacodyl. Participants were supine during all recordings except during the actual intake of the meal.

The manometric analysis was carried out in ImageJ and MATLAB. All High-Amplitude Propagating Pressure Waves (HAPWs) with or without an associated SPW, occurring as single isolated events (Chen et al., 2017) were included in the present study; the motor pattern needed to have a 2 min quiet period before and after the motor pattern. All analysis for the present study was based only on raw data from our studies. Autonomic reactivity to HAPWs was identified by comparing the 2 min period prior to the occurrence of an HAPW, during the occurrence of an HAPW, and the first 2 min immediately after the HAPW. The HRV signal was divided into segments of 1 min and the HRV parameters were calculated for each individual

**TABLE 1 |** Autonomic reactivity associated with posture change.

| | Supine Mean ± SEM | Supine Mean ± SEM | *p*-value | (*t*, *df*) (*t*-test)/ *rs* (Wilcoxon) |
|---|---|---|---|---|
| RSA [ln(ms)] | 6.76 ± 0.28 | 5.80 ± 0.32 | ***0.0006 | *t* = 4.958, *df* = 10 |
| RMSSD (ms) | 57.90 ± 3.65 | 28.49 ± 3.65 | ***0.001 | *rs* = 0.7671 |
| SD1 (ms) | 58.77 ± 6.85 | 29.53 ± 2.64 | **0.0012 | *t* = 4.459, *df* = 10 |
| SD2 (ms) | 102.94 ± 12.52 | 82.99 ± 4.77 | 0.1434 | *t* = 1.588, *df* = 10 |
| HF Power ($ms^2$) | 1552.88 ± 537.25 | 498.55 ± 120.61 | **0.0020 | *rs* = 0.7455 |
| LF Power ($ms^2$) | 1064.53 ± 417.59 | 1122.36 ± 240.33 | 0.5771 | *rs* = 0.1182 |
| PEP (ms) | 121.64 ± 4.94 | 122.75 ± 6.40 | 0.8772 | *t* = 0.1585, *df* = 10 |
| SI ($s^{-2}$) | 32.85 ± 6.96 | 50.73 ± 5.72 | *0.0322 | *rs* = 0.4455 |
| LF/HF Ratio | 0.69 ± 0.13 | 3.19 ± 0.64 | **0.0049 | *rs* = −0.02727 |
| SD2/SD1 | 1.77 ± 0.07 | 2.97 ± 0.21 | ***0.0004 | *t* = 5.196, *df* = 10 |
| SI/RSA | 5.20 ± 1.21 | 9.33 ± 1.30 | *0.0244 | *rs* = 0.5727 |
| SI/RMSSD | 0.80 ± 0.22 | 2.38 ± 0.47 | **0.0020 | *rs* = 0.5982 |
| HR (bpm) | 63.78 ± 2.68 | 80.50 ± 3.24 | *** <0.0001 | *t* = 9.854, *df* = 10 |

*Number of subjects N = 11; t-value and df are reported when the t-test was applied while the rs (Spearman) value is reported where the non-parametric Wilcoxon signed rank test was applied. Abbreviations in this all and other tables: RSA, Respiratory Sinus Arrythmia; SI, Baevsky's Stress Index or Sympathetic Index; RMSSD, Root Mean Square of Successive Differences; PEP, Pre-Ejection Period; HF, High Frequency; LF, Low Frequency; SD1, Standard deviation of minor axis of Poincare' Plot; SD2, Standard deviation of major axis of Poincare'x Plot; HR, heart rate.*

segment. Even if the HAPW lasted 50 sec, the whole segment of 1 min was taken into account. In case of before and after, where the time period taken into account was 2 min, the data was analyzed for each minute separately (using a 1 min window) and the mean of the results of the two segments was taken to represent the HRV parameter. RSA, RMSSD, HF power and SD1 were calculated as measures of parasympathetic activity and LF power, PEP and SI were calculated as measures of sympathetic activity. LF/HF, SD2/SD1 and SI/RSA and SI/RMSSD ratios were also calculated for each phase.

## Analysis of HRV in Association With Motor Complexes

Autonomic activity related to motor complexes, more than one HAPW as a cluster, was assessed graphically by generating time matched images of the motor complexes in HRCM with the frequency domain HF band (the RSA band) images of the HRV data. The process of generating the HF power (RSA band power) image started by importing the ECG and impedance signal into ImageJ using the Cardio Images plugins (Parsons, 2019). In the Cardio Images plugin, the peak detection and correction of the ECG signal was carried out by a Pan-Tomkins algorithm as well as by a Neural Networks model generated and trained in TensorFlow, followed by manually checking and editing the wrongly detected/edited R peaks. The tachogram of RR intervals was plotted as a raster image using a sampling frequency of 10 Hz, image width of 5 s with cubic interpolation in Intervals plugin. The Frequency Win Plugin was used to calculate FFT spectra of the tachogram raster image using window length of 60 s and intervals of 10 s. The power spectra are collated into an image with time on the y-axis and frequency on the x-axis with pixel intensity as amplitude (ms). Similarly, the HRCM data was converted into an image using the Event Series plugin in ImageJ. Both the images were then imported, and time matched in MATLAB as shown in **Figure 1**. A Win frequency plugin generated the HRV spectrogram from 0 to 5 Hz, to study the RSA/HF band only; the lower frequency band (0–0.14 Hz.) as well as the frequency band above 1Hz was removed in MATLAB, and the spectrogram with the frequency band of 0.14–1 Hz. was plotted as an aligned image with the HRCM image as shown in **Figure 1B**. Similarly, the raster image of RR intervals was imported into Matlab and was used to calculate RMSSD and SI, which were also plotted as aligned images with the HRCM **Figures 1C,D**.

## Statistical Analysis

### Supine to Standing

To evaluate HRV related to posture change, all the HRV parameters were analyzed independently. Each HRV parameter was calculated for supine and standing position for all the participants ($n$ = 11) and tested for normal distribution using the Shapiro-Wilk Normality test. If the data for both supine and standing was normally distributed, the comparison was carried out using the paired $t$-test. The Wilcoxon Matched Pair Signed Rank test was used in case one or both of the supine and standing HRV parameter data was not distributed normally. The change in each HRV parameter was considered significant between supine and standing, if the calculated $p$-value was less than 0.05.

### High-Resolution Colonic Manometry (HRCM)

All HAPWs ($n$ = 65) that had a 2 min period before and after without major motor patterns, in order to obtain a "baseline" and "recovery" period, from all the participants were investigated. For each HRV parameter, the results from all HAPWs were averaged for each subject and were presented as one reading with three data points (Before-During-After). These averaged results from all the participants were used for further analysis. Initially, the data was tested for normal distribution using the Shapiro-Wilk Normality test. If the data was normally distributed, the parametric test ANOVA followed by Bonferroni Multiple Comparison test was used for comparison. While the non-parametric Friedman test followed by Dunn's Multiple Comparison test was used for data sets that were not normally distributed. The $p$-value was calculated for before-to-during [$p$-value (B-D)] and during-to-after [$p$-value (D-A)]. A difference was considered significant when $p < 0.05$. The $t$-values and degrees of freedoms are reported with parametric tests while the $z$-value is reported with non-parametric tests.

In addition, the HAPW's were grouped based on the HRCM condition with 12 HAPW's observed during baseline, 16 during meal, 14 during prucalopride, 5 during proximal balloon distension, 7 during distal balloon distension and 11 during bisacodyl. The same statistical procedures as mentioned above were applied to each group separately to identify the effect of the stimulus conditions on the association of autonomic nervous system with HAPW's.

# RESULTS

## Autonomic Reactivity Associated With Posture Change

The parasympathetic parameters RSA, RMSSD, SD1, and HF power all decreased from supine to standing consistent with a decrease in parasympathetic reactivity. The sympathetic parameter SI showed a significant increase from supine to standing. PEP did not show any significant change. The shift from parasympathetic to sympathetic going from supine to standing was reflected in the change in LF/HF ratio, SD2/SD1 ratio, SI/RSA as well as the SI/RMSSD ratio. The posture change resulted in an increase in heart rate. SD2 and LF power did not change, likely a reflection of the fact that these parameters are associated with both sympathetic and parasympathetic changes (**Table 1**).

## Autonomic Reactivity Associated With HAPWs

A significant increase in RSA indicated activation of the parasympathetic nervous system during the motor activity as compared to the period before the motor pattern and the change recovered within 2 min (**Table 2**). An increase in RSA during the HAPWs was seen in all subjects, average 9.3%, with recovery afterward. Similarly, an increase in RMSSD was seen in all subjects except one. There was an average increase of 24.6% in

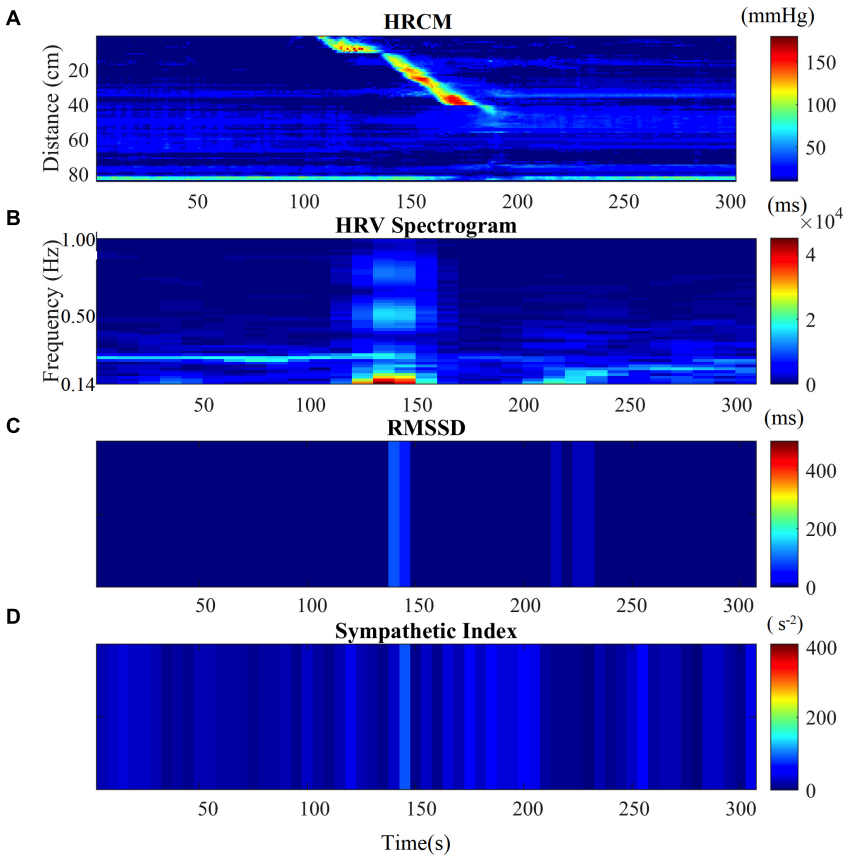



**FIGURE 1 |** HRV parameters associated with a single HAPW. **(A)** 2-min before, during, and 2-min after HAPW recorded by HRCM. **(B)** HF power/RSA band of HRV signal time matched with HRCM recording. **(C)** RMSSD time matched with HRCM. **(D)** SI time matched with HRCM. Distance at 0 cm is positioned at the proximal colon, distance at 80 cm is just proximal to the anal sphincter.

**TABLE 2 |** Autonomic nervous system modulation in association with all individual HAPWs and HAPW-SPWs combined.

| | Before Mean ± SEM | During Mean ± SEM | After Mean ± SEM | *p*-value (B-D) (*t, df*) or (*z*-value) | *p*-value (D-A) (*t, df*) or (*z*-value) |
|---|---|---|---|---|---|
| RSA [ln(ms)] | 6.38 ± 0.27 | 6.90 ± 0.26 | 6.46 ± 0.24 | ***0.0182 (3.420, 8)** | ***0.0220 (3.290, 8)** |
| RMSSD (ms) | 53.24 ± 6.73 | 66.35 ± 10.35 | 52.03 ± 8.34 | 0.1609 (1.862, 8) | 0.1608 (1.973, 8) |
| SD1 (ms) | 45.19 ± 4.66 | 56.44 ± 6.93 | 40.93 ± 4.42 | 0.1201 (2.189, 8) | 0.0593 (2.641, 8) |
| SD2 (ms) | 100.02 ± 7.64 | 126.93 ± 12.76 | 79.44 ± 0.18 | ***0.0069 (4.094, 8)** | ***0.0166 (3.481, 8)** |
| HF Power ($ms^2$) | 1675.88 ± 715.99 | 1594.33 ± 425.33 | 1074.31 ± 319.33 | 0.1979 (1.650) | 0.1187 (1.886) |
| LF Power ($ms^2$) | 1477.46 ± 241.43 | 1223.29 ± 200.60 | 588.49 ± 143.83 | 0.8796 (0.8128, 8) | ***0.0128 (3.661, 8)** |
| PEP (ms) | 115.04 ± 3.02 | 114.91 ± 4.14 | 118.06 ± 7.25 | >0.9999 (0.053, 7) | 0.4309 (1.362, 7) |
| SI ($s^{-2}$) | 94.9 ± 29.1 | 51.92 ± 18.43 | 88.81 ± 25.62 | ***0.0190 (2.593)** | ****0.0044 (3.064)** |
| LF/HF Ratio | 2.75 ± 0.75 | 1.21 ± 0.24 | 0.88 ± 0.20 | 0.4772 (1.179) | 0.1187 (1.886) |
| SD2/SD1 | 2.62 ± 0.19 | 2.53 ± 0.23 | 2.15 ± 0.18 | >0.9999 (0.00) | 0.1542 (1.768) |
| SI/RSA | 18.76 ± 6.92 | 8.69 ± 3.69 | 16.13 ± 5.27 | ****0.0094 (2.828)** | *****0.0008 (3.536)** |
| SI/RMSSD | 5.53 ± 2.60 | 1.97 ± 1.24 | 3.52 ± 1.72 | ****0.0094 (2.828)** | *****0.0008 (3.536)** |
| HR (bpm) | 69.39 ± 3.98 | 66.73 ± 3.79 | 64.33 ± 3.64 | 0.1434 (2.075, 8) | 0.4711 (1.283, 8) |

*The number of subjects, N = 9; the number of HAPW's, n = 65; t-value and df are reported for the parametric test (ANOVA) and z-value is reported in case of the non-parametric Friedman test. *P ≤ 0.05; **P ≤ 0.01, ***P ≤ 0.001.*

the RMSSD during the HAPW (**Table 2**). Due to one outlier, the change in RMSSD did not reach statistical significance. Similarly, SD1 also increased numerically in all volunteers but one, and did not reach statistical significance (**Table 2**).

Although RSA showed a significant increase, the HF power, derived from the same data set as the RSA, did not show a significant change (**Table 2**). RSA is the natural log (*ln*) of HF power and taking a natural log will remove the effect of large

outliers. Indeed, when we removed 4 out of 65 values from the HF power data set that showed more than 3 SD units off the mean value, the HF power changed from 993.07 ± 300.47 before the motor pattern to 1769.64 ± 546.76 ($p = 0.0485$) and recovered to 1135.88 ± 403.85 ($p = 0.0485$); the increase in RMSSD during the HAPW and its decrease afterward, also became significant.

The change in sympathetic index (SI) indicated a decrease in sympathetic activity during the motor patterns that recovered within 2 min (**Table 2**). The PEP did not show significant changes (**Table 2**).

The SI/RSA decreased 42% during an HAPW and recovered within 2 min, consistent with activity shifting toward parasympathetic activity during the motor activity. Similarly, SI/RMSSD showed a 64.4% decrease. Both the LF/HF and SD2/SD1 ratios did not change significantly with motor activity. The heart rate did not show any significant change with motor activity (**Table 2**).

Since RSA is sensitive to respiratory rate changes and to respiratory tidal volume changes, the breathing frequency and volume were calculated before during and after all HAPWs. The breathing frequencies before and during all HAPWs, were 15.9 ± 0.4 and 15.4 ± 0.5 breaths/min ($P = 0.225$), and 15.20 ± 0.47 ($p > 0.9999$) after the HAPW. The values for volume were 0.0123 ± 0.0113 and 0.0131 ± 0.0008 $V^2$ ($P > 0.999$), respectively, and it was 0.008 ± 0.007 $V^2$ ($p = 0.7420$) after the HAPW. Hence, no significant change in breathing frequency was observed in response to an HAPW.

Autonomic activity associated with HAPWs may arise from the activity that initiates the HAPW and from potential mechanoreceptors activated by the actual HAPW. Although rectal bisacodyl almost always evoked HAPWs in the present cohort of healthy subjects, in one subject, two low amplitude simultaneous pressure waves were associated with an increase in RSA from 4.83 to 5.88 *ln(ms)* with a concomitant decrease in SI from 159 to 84 $s^{-2}$; consistent with the notion that the initiating autonomic activity is seen by HRV and that the change may not solely dependent on the strong HAPW evoking distention.

## Autonomic Nervous System Associations With HAPW's in the Different Conditions

Activity of autonomic nervous system activity during an HAPW may be different in different conditions, hence we assessed HRV parameter changes separately under each condition: baseline, meal, prucalopride, proximal balloon distension, distal balloon distension and bisacodyl, The dramatic shift in autonomic balance toward a dominant parasympathetic activity that was described above was observed during the HAPWs that were evoked by a meal (**Table 3A**) and by rectal bisacodyl (**Table 3B**) as reflected by RSA, RMSSD, SI and SI/RSA and SI/ RMSSD.

During baseline, the mean values of all the HRV parameters during HAPW changed in the expected direction (5.03% increase in RSA, 6.53% increase is RMSSD, 24.79% increase in SD1, 38.68% increase in HF power, 30.44% decrease in SI), but the changes did not reach statistical significance (**Table 3C**).

The 90 min period after oral prucalopride, where we hypothesize that prucalopride stimulates the gastric mucosa to evoke HAPWs as a gastrocolic reflex, both RSA and RMSSD increased significantly, and SI decreased significantly during the HAPW's. and recovery afterward in both RMSSD and SI was also significant. A significant shift in autonomic balance toward parasympathetic activity was indicated by a decrease in SI/RMSSD (**Table 3D**).

The periods of balloon distention had low n numbers, nevertheless, distal balloon distension was accompanied by a significant increase in RSA and recovery after the HAPW (**Table 3E**), but changes in response to proximal balloon distention did not reach significance (**Table 3F**).

## Autonomic Reactivity Associated With Motor Complexes

Motor complexes are defined here as more than one HAPW and/or HAPW-SPW that occurred close together such that they could not be analyzed separately. In order to assess HRV during the motor complexes, a continuous assessment procedure was developed as outlined in the methods section. The major finding was that motor complexes were associated with an increase in HF power that was not continuous but rhythmic. The average duration of RSA reactivity, measured at 0.14–0.5 Hz, was 50 ± 10 s and the frequency of occurrence was 0.8 ± 0.2 cycles/min which was similar to the HAPW frequency within motor complexes (**Figure 2**). However, with long HAPWs, more than one RSA band occurred, giving the RSA activity a distinct rhythmic appearance (**Figure 3**). 37 out of a total 40 motor complexes studied, had RSA bands associated with them. Although there was complete synchronization of individual HAPWs and bursts of RSA activity, with motor complexes ($n = 34$), rhythmic RSA activity sometimes ($n = 6$) continued after the HAPW to slowly die out. Sometimes ($n = 3$), the RSA activity started prior to the measured HAPW, but the HAPW likely originated earlier at a more proximal site, beyond the reach of the catheter. RMSSD also increased during the HAPWs and motor complexes. There was complete synchronization between RSA and RMSSD. SI changes were observed as more or less reciprocal to the RMSSD and RSA bands (**Figures 1**, **2**). During all 90 min baseline periods, when HAPWs are rare, there was never rhythmic HF activity although very low amplitude HF activity was continuously observed (**Figure 4**).

# DISCUSSION

## Assessment of Sympathetic Activity During Posture Change

In the assessment of sympathetic increase in the supine to standing protocol, the Baevsky Stress Index or Sympathetic Index increased 52%, whereas SD2, PEP and the LF power did not show significant changes. In order to maintain a near constant blood pressure, in response to the postural changes from supine to standing when blood is pooled in the legs and blood pressure decreases, the baroreceptor reflex increases sympathetic activity

**TABLE 3A |** HRV parameters associated with HAPWs in response to the meal ($n$ = 16).

| | Before ± SEM | During ± SEM | After ± SEM | $p$-value (B-D) ($t$, $df$) or ($z$-value) | $p$-value (D-A) ($t$, $df$) or ($z$-value) |
|---|---|---|---|---|---|
| RSA [ln (ms)] | 6.22 ± 0.24 | 6.72 ± 0.20 | 6.21 ± 0.17 | ***0.0473 (2.54, 14)** | ****0.0013 (4.47, 13)** |
| RMSSD (ms) | 48.15 ± 10.45 | 47.93 ± 6.90 | 38.35 ± 5.13 | ***0.0352 (2.37)** | ****0.0038 (3.10)** |
| SD1 (ms) | 40.24 ± 6.68 | 46.34 ± 4.85 | 35.53 ± 4.69 | 0.0569 (2.19) | ****0.002 (3.29)** |
| SD2 (ms) | 94.45 ± 15.96 | 112.38 ± 10.387 | 79.72 ± 9.3107 | ***0.0352 (2.37)** | ***0.0212** (2.56) |
| HF Power ($ms^2$) | 1930.41 ± 1100.6 | 1199.00 ± 295.64 | 641.37 ± 112.73 | 0.1358 (1.83) | ****0.0038 (3.10)** |
| LF Power ($ms^2$) | 1239.22 ± 405 | 1296.89 ± 355.38 | 667.15 ± 135.33 | 0.4025 (1.28) | ****0.0212 (2.56)** |
| PEP (ms) | 120.91 ± 2.29 | 123.27 ± 2.36 | 124.00 ± 1.72 | 0.2575 (1.58, 13) | 0.9107 (0.11, 13) |
| SI ($s^{-2}$) | 77.34 ± 10.17 | 55.30 ± 7.01 | 89.57 ± 12.22 | ***0.0467 (2.27)** | ****0.0092 (2.84)** |
| LF/HF Ratio | 2.06 ± 0.84 | 1.26 ± 0.27 | 1.43 ± 0.37 | >0.9999 (0.36) | 0.9304 (0.73) |
| SD2/SD1 | 2.67 ± 0.60 | 2.40 ± 0.22 | 2.63 ± 0.34 | 0.4025 (1.79) | >0.9999 (0.36) |
| SI/RSA | 11.60 ± 1.82 | 8.12 ± 1.27 | 13.87 ± 2.03 | ***0.0123 (2.78)** | ****0.002 (3.29)** |
| SI/RMSSD | 2.33 ± 0.44 | 1.48 ± 0.32 | 2.69 ± 0.46 | ***0.0123 (2.74)** | ****0.002 (3.29)** |
| HR (bpm) | 71.50 ± 1.96 | 70.33 ± 1.80 | 70.35 ± 1.80 | >0.9999 (0.18) | >0.9999 (0.09) |

*t-value and df are reported for parametric tests (ANOVA) and the z-value is reported with non-parametric Friedman tests. *P ≤ 0.05; **P ≤ 0.01.*

**TABLE 3B |** HRV parameters associated with HAPWs in response to rectal bisacodyl ($n$ = 11).

| | Before ± SEM | During ± SEM | After ± SEM | $p$-value (B-D) ($t$, $df$) or ($z$-value) | $p$-value (D-A) ($t$, $df$) or ($z$-value) |
|---|---|---|---|---|---|
| RSA [ln (ms)] | 5.67 ± 0.57 | 6.13 ± 0.48 | 5.68 ± 0.47 | ***0.0407 (2.35, 10)** | 0.2529 (1.23, 8) |
| RMSSD (ms) | 32.95 ± 7.45 | 46.00 ± 9.52 | 33.79 ± 7.90 | *****0.0007 (3.58)** | ***0.0278 (2.46)** |
| SD1 (ms) | 26.87 ± 4.38 | 36.90 ± 6.70 | 22.48 ± 4.22 | ***0.0268 (2.99, 10)** | **0.0582 (2.21, 8)** |
| SD2 (ms) | 75.42 ± 9.70 | 78.24 ± 7.89 | 62.71 ± 8.77 | 0.2896 (1.12, 10) | 0.1159 (2.19, 8) |
| HF Power ($ms^2$) | 694.90 ± 226.5 | 1037.75 ± 296.52 | 544.23 ± 142.21 | 0.3594 (1.34) | 0.0883 (2.01) |
| LF Power ($ms^2$) | 1181.99 ± 409.5 | 1079.44 ± 231.31 | 465.79 ± 108.27 | 0.7422 (0.98) | ***0.0278 (2.46)** |
| PEP (ms) | 108.00 ± 3.25 | 109.23 ± 3.19 | 111.11 ± 3.00 | 0.5032 (1.09, 13) | 0.5032 (1.09, 9) |
| SI ($s^{-2}$) | 222.29 ± 75.10 | 146.39 ± 58.42 | 223.09 ± 72.76 | ***0.0278 (2.46)** | ****0.0073 (2.91)** |
| LF/HF Ratio | 2.57 ± 0.49 | 2.21 ± 0.80 | 1.36 ± 0.42 | 0.5271 (1.12) | 0.5271 (1.12) |
| SD2/SD1 | 2.95 ± 0.31 | 2.44 ± 0.22 | 2.68 ± 0.43 | 0.2544 (1.59, 13) | 0.6402 (0.48, 9) |
| SI/RSA | 54.98 ± 21.95 | 29.75 ± 13.02 | 45.09 ± 15.53 | ***0.0146 (2.68)** | ****0.0016 (3.35)** |
| SI/RMSSD | 23.32 ± 7.98 | 11.53 ± 4.61 | 18.85 ± 6.58 | ****0.0094 (2.83)** | ****0.0094 (2.83)** |
| HR (bpm) | 86.22 ± 5.18 | 84.00 ± 5.50 | 82.98 ± 4.93 | 0.0883 (2.01) | >0.9999 (0.34) |

*t-value and df are reported for parametric tests (ANOVA) and the z-value is reported with non-parametric Friedman tests. *P ≤ 0.05; **P ≤ 0.01, ***P ≤ 0.001.*

**TABLE 3C |** HRV parameters associated with HAPWs during baseline ($n$ = 12).

| | Before ± SEM | During ± SEM | After ± SEM | $p$-value (B-D) (t, df) or ($z$-value) | $p$-value (D-A) ($t$, $df$) or ($z$-value) |
|---|---|---|---|---|---|
| RSA [ln (ms)] | 7.01 ± 0.30 | 7.37 ± 0.33 | 6.81 ± 0.33 | 0.6419 (1.25, 21) | 0.0695 (1.91, 21) |
| RMSSD (ms) | 72.23 ± 9.83 | 76.94 ± 11.77 | 60.62 ± 9.37 | > 0.9999 (0.43) | 0.066 (2.12) |
| SD1 (ms) | 64.65 ± 7.83 | 80.68 ± 8.91 | 66.18 ± 7.31 | 0.0562 (2.31, 11) | ****0.0086 (3.67, 10)** |
| SD2 (ms) | 111.94 ± 8.06 | 152.09 ± 10.06 | 110.47 ± 9.33 | ***0.0136 (2.93, 11)** | ****0.001 (5.06, 10)** |
| HF Power ($ms^2$) | 1964.75 ± 519.63 | 2724.68 ± 1146.1 | 1766.25 ± 629.52 | > 0.9999 (0.21) | 0.5728 (1.06) |
| LF Power ($ms^2$) | 1649.09 ± 397.7 | 1629.77 ± 369.21 | 970.19 ± 402.49 | > 0.9999 (0.61) | > 0.9999 (0.61) |
| PEP (ms) | 120.50 ± 2.41 | 125.33 ± 2.11 | 125.45 ± 2.26 | 0.1181 (2.09, 11) | 0.9486 (0.07, 10) |
| SI ($s^{-2}$) | 34.00 ± 5.89 | 23.65 ± 3.41 | 57.88 ± 12.46 | 0.3316 (1.39) | ****0.004 (3.09)** |
| LF/HF Ratio | 2.04 ± 0.77 | 1.08 ± 0.30 | 1.04 ± 0.23 | 0.7845 (0.85) | > 0.9999 (0.21) |
| SD2/SD1 | 2.04 ± 0.25 | 2.11 ± 0.23 | 1.83 ± 0.19 | 0.7648 (0.31, 11) | 0.2662 (1.59, 10) |
| SI/RSA | 4.99 ± 0.94 | 3.35 ± 0.52 | 9.27 ± 2.25 | 0.4017 (1.28) | ****0.0028 (3.20)** |
| SI/RMSSD | 0.63 ± 0.16 | 0.39 ± 0.08 | 1.39 ± 0.41 | 0.2712 (1.49) | *****0.0006 (3.62)** |
| HR (bpm) | 56.92 ± 2.18 | 54.42 ± 1.93 | 54.36 ± 1.50 | 0.0897 (2.25, 11) | 0.9206 (0.10, 10) |

*t-value and df are reported for parametric tests (ANOVA) and the z-value is reported with non-parametric Friedman tests. *P ≤ 0.05; **P ≤ 0.01, ***P ≤ 0.001.*

**TABLE 3D |** HRV parameters associated with HAPWs in response to prucalopride ($n$ = 14).

| | Before ± SEM | During ± SEM | After ± SEM | $p$-value (B-D) (t, df) or ($z$-value) | $p$-value (D-A) (t, df) or ($z$-value) |
|---|---|---|---|---|---|
| RSA [ln (ms)] | 6.17 ± 0.23 | 6.80 ± 0.28 | 6.42 ± 0.35 | ***0.0216 (2.55)** | 0.2333 (1.57) |
| RMSSD (ms) | 43.30 ± 6.16 | 55.97 ± 7.42 | 53.95 ± 0.35 | ***0.0467 (2.27)** | ***0.0467 (2.27)** |
| SD1 (ms) | 35.68 ± 5.253 | 45.74 ± 6.24 | 41.2817 ± 7.23 | 0.1025 (2.12, 13) | 0.2165 (1.30, 13) |
| SD2 (ms) | 76.897925 ± 5.93 | 93.16 ± 9.52 | 82.4542 ± 10.48 | 0.0731 (2.32, 13) | 0.0731 (1.98, 13) |
| HF Power ($ms^2$) | 694.95 ± 205.84 | 1327.93 ± 351.56 | 1304.46 ± 472.90 | 0.0997 (1.96) | 0.6536 (0.98) |
| LF Power ($ms^2$) | 595.53 ± 119.8 | 911.97 ± 274.02 | 607.13 ± 242.66 | > 0.9999 (0.39) | 0.5615 (1.08) |
| PEP (ms) | 110.67 ± 3.74 | 113.33 ± 3.62 | 115.17 ± 3.60 | 0.23 (1.65, 13) | 0.2692 (1.15, 13) |
| SI ($s^{-2}$) | 91.06 ± 12.60 | 65.34 ± 11.87 | 87.27 ± 19.55 | ***0.0465 (2.56, 13)** | ***0.0465 (2.34, 13)** |
| LF/HF Ratio | 1.20 ± 0.23 | 0.75 ± 0.10 | 0.64 ± 0.16 | 0.2333 (1.57) | > 0.9999 (0.39) |
| SD2/SD1 | 2.478 ± 0.27 | 2.275 ± 0.26 | 2.31167 ± 0.32 | 0.8994 (0.76) | > 0.9999 (0.38) |
| SI/RSA | 15.17 ± 2.15 | 10.43 ± 2.17 | 15.00 ± 3.62 | 0.2611 (1.51) | 0.1176 (1.89) |
| SI/RMSSD | 2.94 ± 0.45 | 1.65 ± 0.37 | 3.63 ± 1.09 | ***0.0467 (2.27)** | ***0.0092 (2.84)** |
| HR (bpm) | 71.75 ± 4.19 | 70.33 ± 4.01 | 70.07 ± 3.64 | > 0.9999 (0.38) | > 0.9999 (0.19) |

*t-value and df are reported for parametric tests (ANOVA) and the z-value is reported with non-parametric Friedman tests. *P ≤ 0.05.*

**TABLE 3E |** HRV parameters associated with HAPWs in response to distal balloon distention ($n$ = 7).

| | Before ± SEM | During ± SEM | After ± SEM | $p$-value (B-D) ($t$, $df$) or ($z$-value) | $p$-value (D-A) ($t$, $df$) or ($z$-value) |
|---|---|---|---|---|---|
| RSA [ln (ms)] | 6.56 ± 0.41 | 7.31 ± 0.20 | 6.25 ± 0.21 | ***0.0138 (4.42, 5)** | ***0.0008 (10.88, 4)** |
| RMSSD (ms) | 51.73 ± 8.29 | 61.19 ± 8.43 | 43.51 ± 3.59 | 0.7593 (1.85, 5) | 0.4863 (1.35, 4) |
| SD1 (ms) | 52.82 ± 10.39 | 49.69 ± 5.95 | 42.03 ± 4.57 | 0.7649 (0.31, 6) | 0.7351 (0.77, 4) |
| SD2 (ms) | 105.08 ± 11.97 | 120.76 ± 13.77 | 90.732 ± 11.20 | 0.2277 (1.58) | 0.4118 (1.26) |
| HF Power ($ms^2$) | 1449.08 ± 561.1 | 1844.86 ± 344.43 | 631.55 ± 162.59 | > 0.9999 (0.53, 5) | ***0.0493 (4.20, 3)** |
| LF Power ($ms^2$) | 842.96 ± 438.2 | 1574.24 ± 370.03 | 520.67 ± 212 | 0.3146 (1.41) | 0.1542 (1.77) |
| PEP (ms) | 123.43 ± 2.09 | 126.00 ± 2.68 | 127.60 ± 1.91 | 0.6296 (0.92, 6) | 0.638 (0.51, 4) |
| SI ($s^{-2}$) | 48.39 ± 10.42 | 43.67 ± 7.95 | 61.04 ± 12.76 | 0.7077 (0.39, 6) | 0.6823 (0.86, 4) |
| LF/HF Ratio | 7.92 ± 2.02 | 5.88 ± 0.94 | 10.06 ± 2.35 | 0.5777 (1.06) | 0.5777 (1.06) |
| SD2/SD1 | 2.594 ± 0.47 | 3.31 ± 1.15 | 2.21 ± 0.28 | > 0.9999 (0.32) | > 0.9999 (0.63) |
| SI/RSA | 7.92 ± 2.02 | 5.88 ± 0.94 | 10.06 ± 2.35 | 0.4536 (0.88, 6) | 0.4536 (1.31, 4) |
| SI/RMSSD | 1.30 ± 0.43 | 0.79 ± 0.13 | 1.52 ± 0.40 | 0.5194 (1.24, 6) | 0.5168 (1.32, 4) |
| HR (bpm) | 63.29 ± 1.70 | 64.14 ± 2.06 | 62.31 ± 1.07 | 0.6954 (0.81, 6) | 0.6954 (0.76.4) |

*t-value and df are reported for parametric tests (ANOVA) and the z-value is reported with non-parametric Friedman tests. *P ≤ 0.05.*

**TABLE 3F |** HRV parameters associated with HAPWs in response to proximal balloon distention ($n$ = 5).

| | Before ± SEM | During ± SEM | After ± SEM | $p$-value (B-D) ($t$, $df$) or ($z$-value) | $p$-value (D-A) ($t$, $df$) or ($z$-value) |
|---|---|---|---|---|---|
| RSA [ln (ms)] | 6.63 ± 0.36 | 6.75 ± 0.18 | 7.10 ± 0.31 | > 0.9999 (0.33, 4) | 0.6283 (1.18, 4) |
| RMSSD (ms) | 46.44 ± 0.36 | 43.99 ± 5.85 | 48.88 ± 3.65 | > 0.9999 (0.45, 5) | > 0.9999 (0.64, 4) |
| SD1 (ms) | 39.03 ± 2.91 | 40.57 ± 7.04 | 46.17 ± 4.60 | 0.7593 (0.27, 5) | 0.4863 (0.66, 4) |
| SD2 (ms) | 87.99 ± 14.16 | 85.74 ± 9.28 | 100.57 ± 11.71 | > 0.9999 (0.32) | > 0.9999 (0.32) |
| HF Power ($ms^2$) | 805.16 ± 293 | 818.90 ± 134.34 | 1119.5 ± 444.78 | > 0.9999 (0.05, 5) | > 0.9999 (0.60, 4) |
| LF Power ($ms^2$) | 1347.52 ± 529.7 | 601.13 ± 212.58 | 879.83 ± 444.92 | 0.6856 (0.95) | > 0.9999 (0.00) |
| PEP (ms) | 118.33 ± 3.38 | 111.33 ± 6.35 | 118.40 ± 3.68 | 0.2225 (1.88, 5) | 0.2225 (1.87, 4) |
| SI ($s^{-2}$) | 61.36 ± 7.67 | 67.27 ± 15.99 | 57.17 ± 10.36 | 0.9121 (0.40, 5) | 0.9121 (0.35, 4) |
| LF/HF Ratio | 10.20 ± 1.87 | 10.19 ± 2.38 | 8.24 ± 1.60 | 0.3095 (1.42) | > 0.9999 (0.47) |
| SD2/SD1 | 2.28 ± 0.32 | 2.34 ± 0.31 | 2.39 ± 0.49 | 0.993 (0.11, 5) | 0.993 (0.076, 4) |
| SI/RSA | 10.20 ± 1.87 | 10.19 ± 2.38 | 8.24 ± 1.60 | 0.9942 (0.01, 5) | 0.8978 (0.44, 4) |
| SI/RMSSD | 1.16 ± 0.21 | 1.61 ± 0.57 | 1.23 ± 0.25 | 0.7325 (1.02, 4) | > 0.9999 (0.45, 4) |
| HR (bpm) | 65.00 ± 2.75 | 65.50 ± 3.45 | 64.58 ± 3.77 | 0.8425 (0.31, 5) | 0.8425 (0.56, 4) |

*t-value and df are reported for parametric tests (ANOVA) and the z-value is reported with non-parametric Friedman tests.*

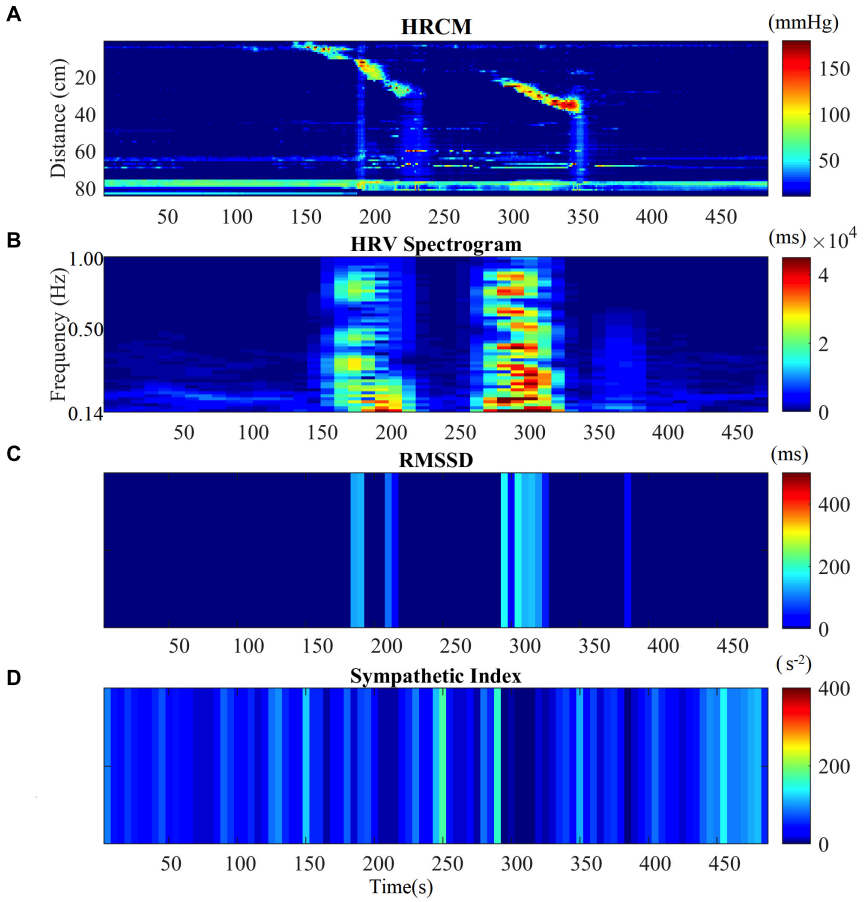



**FIGURE 2 |** HRV parameters associated with two consecutive HAPWs. **(A)** Before-During and After a Motor Complex. **(B)** Time matched HF Power/RSA band of the HRV signal before-during and after MC. **(C)** RMSSD time matched with HRCM. **(D)** SI time matched with HRCM.

to increase heart rate. Baroreceptor action potentials are relayed to the nucleus tractus solitarius, which uses action potential frequency as a measure of blood pressure. The end-result of baroreceptor de-activation is excitation of the sympathetic nervous system and inactivation of the parasympathetic nervous system. Houtveen et al. (2005) recorded PEP at different breathing frequencies during supine, sitting and standing; PEP increased from supine to standing hence the sympathetic activity appeared to decrease. This was also observed by Cacioppo et al. (1994). PEP is a measure of ventricular contractility, influenced by beta-adrenergic receptor mediated ventricular sympathetic innervation (Van Lien et al., 2015). Nitroprusside causes vasodilation, baroreceptor unloading and a reflex increase in sympathetic tone, which was associated with a significant decrease in PEP (Schächinger et al., 2001). Our study indicates that the sympathetic activity that is increased upon standing is not reflected in the PEP value.

Sympathetic pathways within the body form a vast network; only those sympathetic activities that interact with, or directly or indirectly take part in sympathetic regulation of heart rate will be seen in HRV. For example, muscle sympathetic nerve activity measured at the peroneal nerve induces vasoconstriction and is modulated by the baroreflex but it represents regional sympathetic neural activity, it does not equal sympathetic discharge directed to the heart (Schächinger et al., 2001).

Both LF power and SD2 did not significantly increase with posture change in the present study. Many studies continue to presume that LF power, especially if adjusted for HF power, total power, or respiration, provides an index of cardiac sympathetic "tone" and that the ratio of LF:HF power indicates "sympathovagal balance," but strong evidence has been presented that LF power neither reflects cardiac sympathetic tone at supine nor in response to standing (Goldstein et al., 2011). There is also no evidence that the LFa (low frequency area) (Nguyen et al., 2020) is specific for sympathetic activity (Goldstein et al., 2011; Rahman et al., 2011). Rahman et al. (2018) showed that SD2 as well as the ratio of SD1 and SD2 are not related to cardiac sympathetic activity.

Baevsky et al. developed an index of regulation strain, or stress index, or, relevant to our study, a "Sympathetic Index" which illustrates the sympathetic or central regulation activity (Baevsky and Chernikova, 2017). The activation of sympathetic regulation results in the stabilization of the heart rhythm which causes a decrease in variation of RR intervals and an increase in the

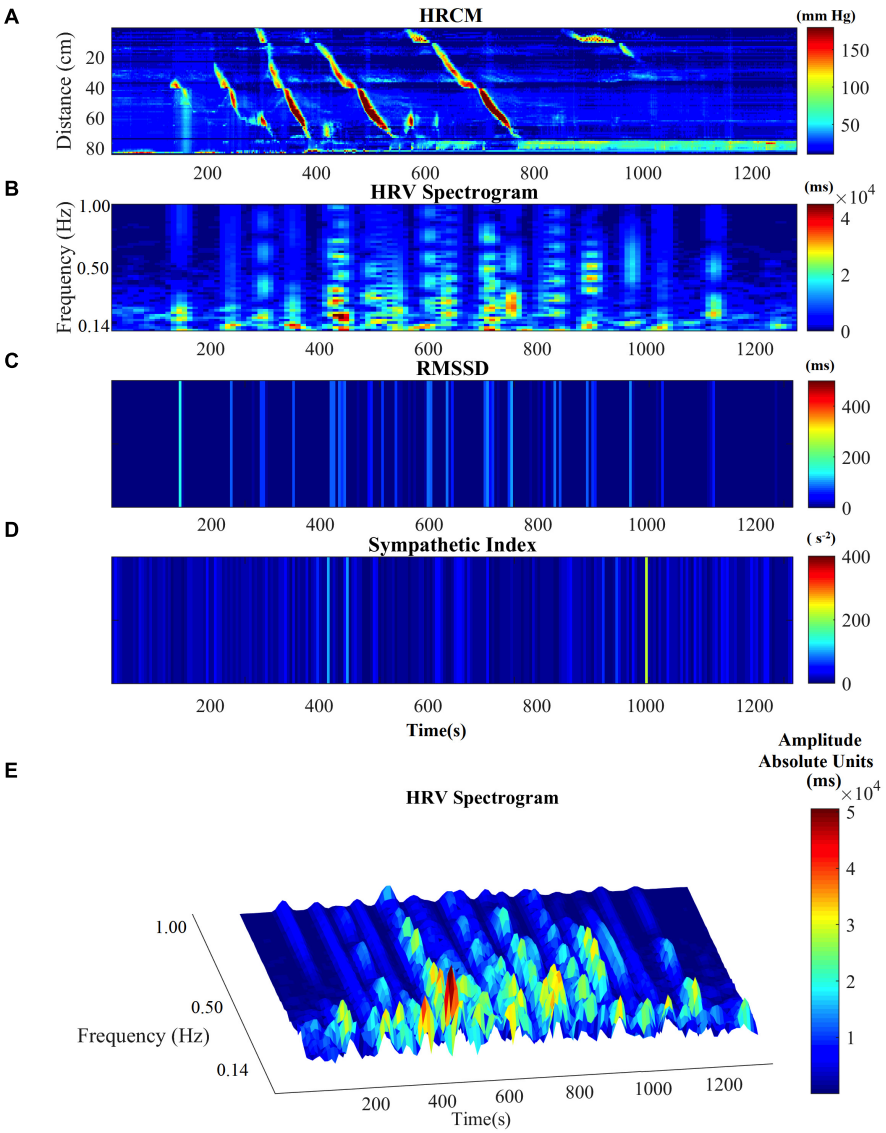



**FIGURE 3 |** HRV parameters associated with a complex of multiple HAPWs (a motor complex). **(A)** HRCM recording with a motor complex containing long overlapping HAPWs. **(B)** HF power/RSA band of HRV signal. **(C)** RMSSD. **(D)** SI. **(E)** HF/RSA power band in 3D.

number of intervals with similar duration. The histogram of RR intervals becomes narrower and increases in height. Although the SI is not yet widely applied for sympathetic measurement, it is used by commercially available HRV data analysis software as one of the measures of sympathetic activity (Kubios, 2020); they use the square root of SI to minimize the effect of outliers. The marked change in SI due to posture change in the present study suggests it to be a sensitive marker for orthostatic sympathetic change.

## Sympathetic Activity and the Colonic Propulsive Motor Patterns

The HAPWs, the most significant propulsive motor pattern of the human colon, were accompanied by a significant decrease in SI, hence a decrease in sympathetic activity. During motor complexes, SI was always showing some value above zero, which indicates that there was continuous sympathetic activity which was inhibited during HAPWs. We infer that withdrawal of sympathetic activity is part of the autonomic reflexes that initiate the HAPW, e.g., in response to a meal or rectal bisacodyl. Could stretch receptors be activated during the HAPW? Viscerofugal neurons, connecting to the sympathetic prevertebral ganglia allow the colon to fill, and when the circular muscle of the colon wall contracts to empty the segment, the mechanoreceptors are unloaded and synaptic input decreases (Szurszewski and Miller, 2006). Hence the marked reduction in SI observed in the present study is consistent with a withdrawal of sympathetic activity, allowing the HAPW to proceed.

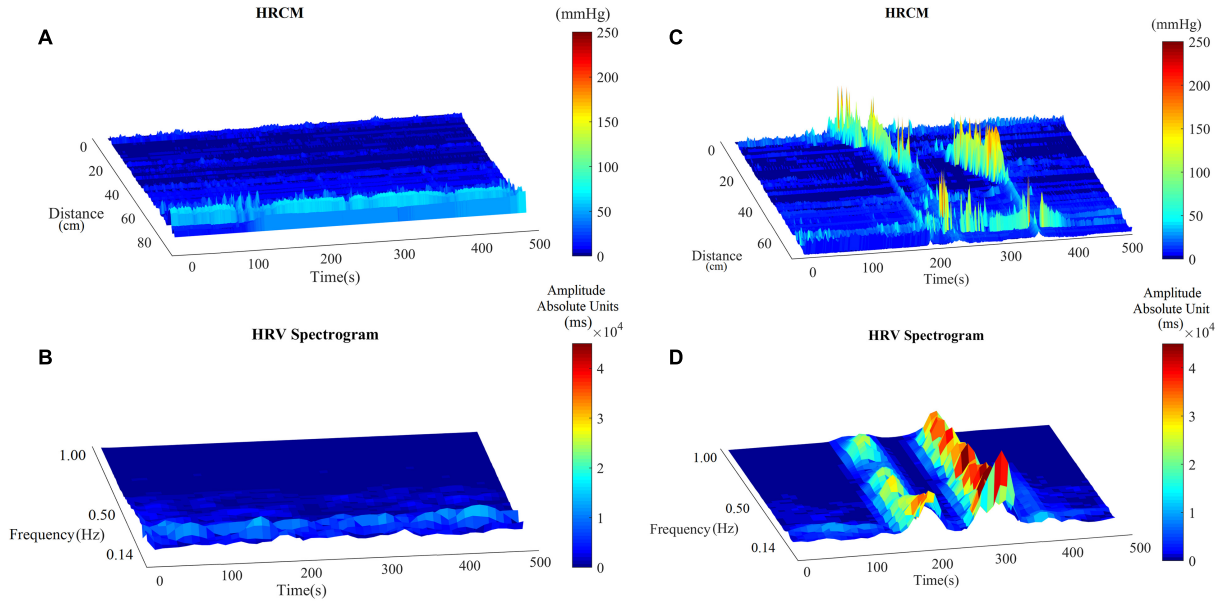



**FIGURE 4 |** The RSA band in baseline and with HAPWs. **(A)** Baseline HRCM recording without any colonic motor pattern. **(B)** HF power/RSA band during the baseline. **(C)** HRCM recording with Motor Complex. **(D)** HF power/RSA band during the Motor Complex.

## Parasympathetic Activity and the Colonic Propulsive Motor Patterns

All HRV parameters studied that are associated with parasympathetic activity, were decreased in response to posture change from supine to standing reflecting the well-known decrease in parasympathetic activity to prevent orthostatic hypotension.

Individual HAPWs, were associated with a significant increase in RSA. Many of these HAPWs occurred during baseline or in the aftermath of taking a meal where the HAPWs are not felt and do not cause a sensation and are not associated with evoked body movements, discomfort or changes in breathing patterns. We suggest that this may reflect the activity in the parasympathetic nervous system associated with the initiation of the HAPW. HAPWs occur most often after a meal as a result of the gastro-colonic reflex, or in response to rectal stimulation where they are the result of a sacral defecation reflex. The sacral defecation reflex involves the sacral defecation center (the parasympathetic nucleus), Barrington's nucleus and the NTS (Taché and Million, 2015). Barrington's nucleus and the NTS are also involved in cardiac homeostasis (Gasparini et al., 2020), and in this way, the neuronal traffic in association with HAPWs can influence heart rate, similar to the influence of breathing on the heart rate: "breathing at different rates within the 9–24 bpm range, which changes HF power, does not change mean heart rate" (Shaffer and Ginsberg, 2017). The fact that a significant change occurred in RSA in association with the motor patterns without a change in heart rate is consistent with the hypothesis that the origin of the parasympathetic activity is the neural activity associated with gut activity and not cardiac activity. The fact that the heart rate does not change suggests that the vagal tone, the mean vagal efferent effects to the sinus node, does not change (Grossman and Taylor, 2007).

Vagal afferent neurons are likely activated by colonic motor patterns and their dendritic projections extent throughout the NTS and intermingle within the subnuclei providing a potential means to coordinate respiratory and cardiac autonomic activities (Browning and Travagli, 2014). Sensory nerves in the pelvic plexus will also convey colonic information to the spinal cord (Smith-Edwards et al., 2019). Hence neuronal traffic originating for an HAPW may also influence HRV. Research is ongoing to distinguish efferent and afferent neuronal traffic associated with HAPWs and to explore their role in HRV changes and diagnostic value. It is also possible that HAPWs induce colonic blood pressure changes that might influence HRV (Semba and Fujii, 1970).

When HAPWs occur in quick succession within motor complexes, overlapping in time, RSA was markedly increased. Strong motor patterns induced by bisacodyl sometimes results in discomfort and increased breathing frequency; increased breathing frequency results in decreased RSA but this was not found, likely superseded by processes that increased RSA. Importantly, there was never continuous RSA activity even when HAPWs were continuously present. RSA increases occurred in bursts that were not synchronous with individual HAPWs within the burst, and they continued for several minutes and then diminished gradually (**Figure 3**). The HF "rhythmicity" suggests that there is a refractory period in the parasympathetic neural activity. In some instances, the RSA bands started prior to the HAPW's, however, in those cases the HAPWs were seen in the most proximal sensor hence the true beginning of the HAPW was not captured by the catheter. Since contractions are ongoing, the rhythmicity of the parasympathetic activity suggests that it arises

from activity orchestrating the HAPWs and not from distention, but this remains to be investigated.

## HRV Parameters Associated With Autonomic Reflexes

When the HRV parameters associated with HAPWs were analyzed within each intervention separately, strong associations between HAPWs and sympathetic decrease and parasympathetic increase were observed in response to a meal, which reflect the gastrocolic reflex, in response to rectal bisacodyl that reflect the sacral autonomic (defecation) reflex and in a rapid response to oral prucalopride which we hypothesize is due to a gastrocolic reflex mediated by stimulation of gastric enterochromaffin cells and subsequent activation of vagal sensory nerves. In a subset of patients with chronic constipation, absence of the autonomic reflexes is associated with high sympathetic activity (Chen and Liu, unpublished observations).

## RSA vs. RMSSD and HF Power

The RMSSD increased one to one with the increase in RSA associated with HAPWs and within the motor complexes, confirming the marked association between parasympathetic activity and human colon propulsive motor patterns. The association between RMSSD and single HAPWs occurred despite the fact that the recording period of 1 min during the HAPW was too short for an ideal assessment as argued by Baek et al. (2015).

The RSA is the natural log of the HF power; the HF power did not show a significant change likely because with a few HAPWs (4/65), the HF power was more than 3 standard deviations from the mean values. If we took these values out and assessed 61 out of 65 motor patters, the HF power showed a significant increase going from baseline to motor pattern and back to recovery.

## The Significance of Changes in Breathing Frequency

The HF band, also known as the respiratory band, is associated with variation in heart rate due to respiration. The HF band has been set to range from 0.15 to 0.4 Hz. which corresponds to the respiration frequency of 9–24 breaths/min, the normal frequency range in adults. The heart rate increases during inhalation due to inhibition of vagal outflow and decreases during exhalation due to the restoration of vagal outflow by release of acetylcholine (Gasparini et al., 2020). If the breathing rate is outside the range of 9–24 breaths/min, then the calculated HF power will be due to noise or harmonics of lower frequency bands and the respiration frequency power will not be included in the HF power. If the respiration frequency is lower than 9 breaths/min, as is the case with slow, deep breathing, the most prominent component of the respiration frequency power will lie in the LF band, and without adjusting the HF band, it will indicate low HF power and high LF power and lead to wrong interpretations of HF power. Similarly, during exercise, a breathing frequency of over 24 breaths/min, the prominent component of parasympathetic power will be out of the range and will wrongly represent low parasympathetic power. If during experimental conditions the breathing frequency goes outside the 9–24 breaths/min range, then the HF power band can be adjusted based on the respiration frequency. This was used in a recent study by Nguyen et al. where the HF or parasympathetic band was replaced by "RFa" (respiratory frequency area; Colombo et al., 2015; Nguyen et al., 2020). In the present study the subjects were lying in a relaxed supine position throughout the recording and were not asked to breathe deeply, they were only asked to change position from supine to sitting for a period of 15 min while eating. The respiration rate was within the range of 9–24 breaths/min almost all of the time. In our study, any incident for which the respiration frequency was out of the range of 9–24 breaths/min was rejected during analysis; hence, there appears to be no benefit in using RFa under our experimental conditions.

## The New Parameters SI/RSA and SI/RMSSD

The two branches of the autonomic nervous system can work reciprocally but they can also work independently. Hence a sympathetic over parasympathetic ratio may not reflect an autonomic "balance" and cannot be used as a singular parameter of autonomic balance. Furthermore, LF and SD2 are not considered good parameters for sympathetic activity, making the LF/HF and SD2/SD1 ratios less useful. These ratios in our study behaved significantly different from SI/RSA and SI/RMSSD. Hence, we calculated SI/RSA and SI/RMSSD *in conjunction with* the SI, RSA and RMSSD values as *additional* parameters to evaluate changes in autonomic activity. The SI/RSA and the SI/RMSSD decreased markedly with the HAPWs confirming a shift to parasympathetic activity.

In conclusion, we show that HAPWs are associated with measurable changes in HRV parameters reflecting parasympathetic and sympathetic activity. Most of the single HAPWs reported here were not noticed by the subjects, they occurred without discomfort or change in respiration pattern. Under normal conditions, they would just be part of everyday movement of content into anal direction without urge to defecate. These motor patterns were not associated with a change in heart rate, suggesting a physiological correlation between the HAPW, the gastrocolic reflex, the sacral defecation reflex, and the autonomic parameters RSA, RMSSD and SI. Our inference is that these motor patterns and reflexes are directed by autonomic activity reflected in HRV, hence RSA, RMSSD, SI, SI/RSA, and SI/RMSSD may develop as biomarkers of autonomic (dys)regulation of colonic motility.

# DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

# ETHICS STATEMENT

The studies involving human participants were reviewed and approved the by Hamilton Integrated Research Ethics Board. The

patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MKA analyzed all the data, incorporated the Baevsky's Stress Index, and wrote the first draft of the manuscript. LL assisted with data analysis and manuscript writing. J-HC directed and performed all volunteer HRCM studies and discussed manuscript writing. JDH oversaw the autonomic nervous system analysis and manuscript writing. All authors approved the manuscript.

## FUNDING

This study was supported by the Natural Sciences and Engineering Research Council (NSERC) Grant 386877 to JDH. MKA was supported by a fellowship from the Farncombe Family Digestive Health Research Institute and NSERC.

## ACKNOWLEDGMENTS

We gratefully acknowledge that Dr. Sean Parsons provided all ImageJ plug-ins. An abstract of this work was presented at the 2021 Canadian Digestive Diseases Week.

## REFERENCES

Baek, H. J., Cho, C. H., Cho, J., and Woo, J. M. (2015). Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability. *Telemed. J. E Health* 21, 404–414. doi: 10.1089/tmj.2014.0104

Baevsky, R. M., and Chernikova, A. G. (2017). Heart rate variability analysis: physiological foundations and main methods. *Cardiometry* 66–67. doi: 10.12710/cardiometry.2017.10.6676

Bharucha, A. E., and Brookes, S. J. H. (2018). "Neurophysiologic mechanisms of human large intestinal motility," in *Physiology of the Gastrointestinal Tract*, ed H. Said, (New York, NY: Elsevier), 517–564. doi: 10.1016/b978-0-12-809954-4.00023-2

Bharucha, A. E., Camilleri, M., Low, P. A., and Zinsmeister, A. R. (1993). Autonomic dysfunction in gastrointestinal motility disorders. *Gut* 34, 397–401. doi: 10.1136/gut.34.3.397

Brookes, S., Chen, N., Humenick, A., Spencer, N. J., and Costa, M. (2016). Extrinsic sensory innervation of the gut: structure and function. *Adv. Exp. Med. Biol.* 891, 63–69. doi: 10.1007/978-3-319-27592-5_7

Browning, K. N., and Travagli, R. A. (2014). Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. *Compr. Physiol.* 4, 1339–1368. doi: 10.1002/cphy.c130055

Browning, K. N., and Travagli, R. A. (2019). Central control of gastrointestinal motility. *Curr. Opin. Endocrinol. Diabetes Obes.* 26, 11–16.

Cacioppo, J. T., Berntson, G. G., Binkley, P. F., Quigley, K. S., Uchino, B. N., and Fieldstone, A. (1994). Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. *Psychophysiology* 31, 586–598. doi: 10.1111/j.1469-8986.1994.tb02351.x

Callaghan, B., Furness, J. B., and Pustovit, R. V. (2018). Neural pathways for colorectal control, relevance to spinal cord injury and treatment: a narrative review. *Spinal Cord* 56, 199–205. doi: 10.1038/s41393-017-0026-2

Camilleri, M., Balm, R. K., and Low, P. A. (1993). Autonomic dysfunction in patients with chronic intestinal pseudo-obstruction. *Clin. Auton. Res.* 3, 95–100. doi: 10.1007/bf01818993

Chen, J.-H., Yu, Y., Yang, Z., Yu, W.-Z., Chen, W. L., Kim, M. J. M., et al. (2017). Intraluminal pressure patterns in the human colon assessed by high-resolution manometry. *Sci. Rep.* 7:41436. doi: 10.1038/srep41436

Colombo, J., Arora, R., DePace, N. L., and Vinik, A. I. (2015). *Clinical Autonomic Dysfunction. Measurement, Indications, Therapies, and Outcomes*. Heidelberg: Springer.

De Groat, W. C., and Krier, J. (1976). An electrophysiological study of the sacral parasympathetic pathway to the colon of the cat. *J. Physiol.* 260, 425–445. doi: 10.1113/jphysiol.1976.sp011523

De Groat, W. C., and Krier, J. (1978). The sacral parasympathetic reflex pathway regulating colonic motility and defaecation in the cat. *J. Physiol.* 276, 481–500. doi: 10.1113/jphysiol.1978.sp012248

Devroede, G., Giese, C., Wexner, S. D., Mellgren, A., Coller, J. A., Madoff, R. D., et al. (2012). Quality of life is markedly improved in patients with fecal incontinence after sacral nerve stimulation. *Female Pelvic Med. Reconstr. Surg.* 18, 103–112. doi: 10.1097/spv.0b013e3182486e60

Devroede, G., and Lamarche, J. (1974). Functional importance of extrinsic parasympathetic innervation to the distal colon and rectum in man. *Gastroenterology* 66, 273–280. doi: 10.1016/s0016-5085(74)80114-9

Dinning, P. G., Sia, T. C., Kumar, R., Mohd Rosli, R., Kyloh, M., Wattchow, D. A., et al. (2016). High-resolution colonic motility recordings in vivo compared with ex vivo recordings after colectomy, in patients with slow transit constipation. *Neurogastroenterol. Motil.* 28, 1824–1835. doi: 10.1111/nmo.12884

Furness, J. B., Callaghan, B. P., and Rivera, L. R. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv. Exp. Med. Biol.* 817, 39–71. doi: 10.1007/978-1-4939-0897-4_3

Gasparini, S., Howland, J. M., Thatcher, A. J., and Geerling, J. C. (2020). Central afferents to the nucleus of the solitary tract in rats and mice. *J. Comp. Neurol.* 528, 2708–2728.

Goldstein, D. S., Bentho, O., Park, M., and Sharabi, Y. (2011). Low−frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Grossman, P., and Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsycho.2005.11.014

Hayano, J., and Yuda, E. (2019). Pitfalls of assessment of autonomic function by heart rate variability. *J. Physiol. Anthropol.* 38:3.

Houtveen, J. H., Groot, P. F., and Geus, E. J. (2005). Effects of variation in posture and respiration on RSA and pre-ejection period. *Psychophysiology* 42, 713–719. doi: 10.1111/j.1469-8986.2005.00363.x

Jean, A. (1991). [The nucleus tractus solitarius: neuroanatomic, neurochemical and functional aspects]. *Arch. Int. Physiol. Biochim. Biophys.* 99, A3–A52.

Kubios (2020). Available online at: https://www.kubios.com/about-hrv (accessed August 28, 2020).

La Rovere, M. T., Pinna, G. D., Maestri, R., Mortara, A., Capomolla, S., Febo, O., et al. (2003). Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation* 107, 565–570. doi: 10.1161/01.cir.0000047275.25795.17

Leblanc, D., McFadden, N., Lebel, M., and Devroede, G. (2015). Fecal continence can be restored by sacral neurostimulation after traumatic unilateral pudendal neuropathy: a case report. *Int. J. Colorectal Dis.* 30, 569–570. doi: 10.1007/s00384-014-2019-3

Lorena, S. L., Figueiredo, M. J., Almeida, J. R., and Mesquita, M. A. (2002). Autonomic function in patients with functional dyspepsia assessed by 24-hour heart rate variability. *Dig. Dis. Sci.* 47, 27–31.

Lu, C. L., Zou, X., Orr, W. C., and Chen, J. D. (1999). Postprandial changes of sympathovagal balance measured by heart rate variability. *Dig. Dis. Sci.* 44, 857–861.

Milkova, N., Parsons, S. P., Ratcliffe, E., Huizinga, J. D., and Chen, J.-H. (2020). On the nature of high-amplitude propagating pressure waves in the human colon. *Am. J. Physiol. Gastrointest. Liver Physiol.* 318, G646–G660. doi: 10.1152/ajpgi.00386.2019

Nguyen, L., Wilson, L. A., Miriel, L., Pasricha, P. J., Kuo, B., Hasler, W. L., et al. (2020). Autonomic function in gastroparesis and chronic unexplained nausea

and vomiting: relationship with etiology, gastric emptying, and symptom severity. *Neurogastroenterol. Motil.* 32:e13810.

Ouyang, X., Li, S., Zhou, J., and Chen, J. D. (2020). Electroacupuncture ameliorates gastric hypersensitivity via adrenergic pathway in a rat model of functional dyspepsia. *Neuromodulation* 23, 1137–1143. doi: 10.1111/ner.13154

Parsons, S. (2019) Available online at: http://scepticalphysiologist.com.html (accessed August 28, 2020).

Rahman, F., Pechnik, S., Gross, D., Sewell, L., and Goldstein, D. S. (2011). Low frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Clin. Auton. Res.* 21, 133–141. doi: 10.1007/s10286-010-0098-y

Rahman, S., Habel, M., and Contrada, R. J. (2018). Poincaré plot indices as measures of sympathetic cardiac regulation: responses to psychological stress and associations with pre-ejection period. *Int. J. Psychophysiol.* 133, 79–90. doi: 10.1016/j.ijpsycho.2018.08.005

Schächinger, H., Weinbacher, M., Kiss, A., Ritz, R., and Langewitz, W. (2001). Cardiovascular indices of peripheral and central sympathetic activation. *Psychosom. Med.* 63, 788–796. doi: 10.1097/00006842-200109000-00012

Semba, T., and Fujii, Y. (1970). Relationship between venous flow and colonic peristalsis. *Jpn. J. Physiol.* 20, 408–416. doi: 10.2170/jjphysiol.20.408

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Shimizu, Y., Chang, E. C., Shafton, A. D., Ferens, D. M., Sanger, G. J., Witherington, J., et al. (2006). Evidence that stimulation of ghrelin receptors in the spinal cord initiates propulsive activity in the colon of the rat. *J. Physiol.* 576, 329–338. doi: 10.1113/jphysiol.2006.116160

Singh, A., and Jaryal, A. K. (2020). "Neurophysiology of Respiratory System," in *Brain and Lung Crosstalk*, eds H. Prabhakar and C. Mahajan (Singapore: Springer), 1–38. doi: 10.1007/978-981-15-2345-8_1

Smith-Edwards, K. M., Najjar, S. A., Edwards, B. S., Howard, M. J., Albers, K. M., and Davis, B. M. (2019). Extrinsic primary afferent neurons link visceral pain to colon motility through a spinal reflex in mice. *Gastroenterology* 157, 522–536.e2.

Szurszewski, J., and Miller, S. M. (2006). "Physiology of prevertebral sympathetic ganglia," in *Physiology of the Gastrointestinal Tract*, ed. L. R. Johnson (San Diego, CA: Academic Press ), 603–627. doi: 10.1016/b978-012088394-3/50025-8

Taché, Y., and Million, M. (2015). Role of corticotropin-releasing factor signaling in stress-related alterations of colonic motility and hyperalgesia. *J. Neurogastroenterol. Motil.* 21, 8–24.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Van Lien, R., Neijts, M., Willemsen, G., and de Geus, E. J. (2015). Ambulatory measurement of the ECG T-wave amplitude. *Psychophysiology* 52, 225–237. doi: 10.1111/psyp.12300

Yuan, Y., Ali, M. K., Mathewson, K. J., Sharma, K., Faiyaz, M., Tan, W., et al. (2020). Associations between colonic motor patterns and autonomic nervous system activity assessed by high-resolution manometry and concurrent heart rate variability. *Front. Neurosci.* 13:1447. doi: 10.3389/fnins.2019.01447

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

SYSTEMATIC REVIEW
published: 09 July 2021
doi: 10.3389/fphys.2021.611609

# The Heart in the Mind: A Systematic Review and Meta-Analysis of the Association Between Theory of Mind and Cardiac Vagal Tone

*Marta Zammuto[1], Cristina Ottaviani[2,3], Fiorenzo Laghi[1] and Antonia Lonigro[4]**

*[1] Department of Social and Developmetal Psychology, Sapienza University of Rome, Rome, Italy, [2] Department of Psychology, Sapienza University of Rome, Rome, Italy, [3] Functional Neuroimaging Lab, IRCCS Fondazione Santa Lucia, Rome, Italy, [4] Department of Human Sciences, European University of Rome, Rome, Italy*

Theory of mind (ToM) is the human ability to infer the mental states of others in order to understand their behaviors and plan own actions. In the past decades, accumulating evidence has shown that heart rate variability (HRV), an index of parasympathetic control of the heart, is linked to behavioral regulation, social competence, and social cognition abilities, all implicated—to some extent—in ToM. This study aims to systematically review and meta-analyze the available studies, investigating the relation between ToM and HRV in typically developing people. Six studies were eligible for the meta-analysis, yielding a significant association between HRV and ToM of a small-to-medium effect size ($g$ = 0.44). This result was not influenced by publication bias. Due to the small number of studies eligible for the meta-analysis, it was not possible to test for the effect of categorical moderators. The moderating role of sex and quality of the studies was examined by meta-regression analysis. Moderation analysis did not yield any significant effect; however, at a descriptive level, studies yielding the largest effect size were characterized by the use of high frequency-HRV assessment at rest and the Reading the Mind in the Eyes Test to evaluate ToM abilities. The results preliminarily suggest that tonic HRV might be used as an indicator of the ability to understand the content of mind of others.

**Keywords: heart rate variability, vagal tone, parasympathetic, theory of mind, social cognition, meta-analysis, systematic review**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University, United States*

***Reviewed by:***
*Lauro Vianna,*
*University of Brasilia, Brazil*
*Andrew Kemp,*
*Swansea University, United Kingdom*

***Correspondence:**
*Antonia Lonigro*
*antonia.lonigro@unier.it*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

***Received:*** *29 September 2020*
***Accepted:*** *14 June 2021*
***Published:*** *09 July 2021*

***Citation:***
*Zammuto M, Ottaviani C, Laghi F and Lonigro A (2021) The Heart in the Mind: A Systematic Review and Meta-Analysis of the Association Between Theory of Mind and Cardiac Vagal Tone. Front. Physiol. 12:611609. doi: 10.3389/fphys.2021.611609*

## INTRODUCTION

### Theory of Mind and Social Behavior

Theory of mind (ToM) is a component of social cognition (Arioli et al., 2018) and it denotes the ability to infer the inner states of others, such as doubts, purposes, intentions, beliefs, and thoughts (Frith and Frith, 2003; Flavell, 2004). Since 1978, when Premack and Woodruff (1978) introduced the expression of ToM in their pioneering study with primates, the interest toward this ability has largely grown, allowing us to recognize its great impact on social life. During social exchanges, people must constantly impute mental states of their interlocutors in order to correctly attribute a meaning to their behaviors.

As a whole, good ToM abilities facilitate the engagement in social behavior. The majority of studies documented a positive relation between ToM and social relationships (for a review, see Hughes and Leekam, 2004), peer popularity (for a meta-analysis, see Slaughter et al., 2015), prosocial behavior (for a meta-analysis, see Imuta et al., 2016), and social adjustment (Capage and Watson, 2001; Banerjee and Watling, 2005). However, other investigations—especially those regarding bullying, machiavellianism, and conduct disorders (Astington, 2003; Lonigro et al., 2014)—pointed out a "dark side" of ToM, revealing that the relation between ToM and prosocial conduct is far from straightforward and simple. High levels of ToM abilities at times may lead to negative outcomes, as demonstrated by Hughes et al. (1998) in their study. The authors found that controls outperformed children with conduct disorders on an affective perspective taking task but no differences emerged between the groups on a deception task. Evidence on the maleficent use of ToM has led Astington to postulate that "although false-belief understanding is necessary for some social behaviors, it is never sufficient to guarantee the performance of such behavior" (Astington, 2003, p. 33). In other words, some people may decide to use ToM for prosocial purposes, namely, nice ToM, but others may use it for antisocial purposes, such as threatening, manipulating, or bullying (nasty ToM; Ronald et al., 2005; McEwen et al., 2007).

At present, it is not completely clear why children with the same ToM abilities may engage in different social behaviors. Ronald et al. (2005) argued that the social use of ToM abilities is difficult to understand because the ToM tasks that are traditionally used (e.g., false-belief understanding) are neutral with respect to the nature of the social behaviors involved. In fact, in daily life, few situations requiring ToM are neutral. In this regard, while some researchers have directed their attention to the role of moral disengagement (Ettekal and Ladd, 2020), others have focused on empathy, in particular, affective or emotional empathy pointing to its significant impact on social behavior (e.g., Lonigro et al., 2014). ToM and empathy are fundamental constructs for successful social interaction and are often considered interchangeable. ToM refers to the ability to infer the cognitive (e.g., thoughts and beliefs) and affective contents (e.g., desires and emotions) of unobservable mental states. Such ability requires the representation of a propositional attitude, namely, meta-representation. Cognitive empathy is commonly conceptualized as the ability to reason about the emotions of others, whereas emotional empathy concerns engaging in a congruent and immediate response with such an emotional state. Thus, affective empathy encompasses empathetic concern, sympathy, or compassion for the emotions of others (Schurz et al., 2021). Dvash and Shamay-Tsoory (2014) have proposed a model in which ToM and cognitive empathy overlap, involving the same neural networks, while affective empathy represents a different system (Tholen et al., 2020). Notably, the development of mentalizing abilities follows a distinct trajectory from that of affective empathy (Sebastian et al., 2012; Slaughter et al., 2015). In this study, we adopted the same approach used by previous meta-analytic studies on ToM among typically developing people (Slaughter et al., 2015; Imuta et al., 2016), thus excluding affective empathy.

## The Polyvagal Theory and the Social Engagement System

From the early 2000s, the field of behavioral medicine has identified several physiological correlates of different aspects of social cognition. For example, within the framework of the polyvagal theory (Porges, 2003), parasympathetic influences on emotional and socio-cognitive processes have been deeply investigated with the ultimate aim to explain social behavioral development (Porges et al., 1996; Porges, 2001). This theory considers cardiac vagal tone as a behavior regulator responding to external and internal environmental cues. In detail, Porges theorized that two vagal circuits regulate affiliation and social behavior, i.e., a more archaic branch called *dorsal vagal*, which regulates visceral and homeostatic functions, and a more recent branch composed of the myelinated vagal fibers (*ventrovagal*) that form the neural substrate of the social engagement system (Porges, 2007).

An indirect, well-validated measure of vagal modulation of the heart is heart rate variability (HRV; Kuo et al., 2005), a measure of beat-to-beat variations in heart rate over time. Empirical evidence has shown that higher resting HRV is linked to behavioral regulation (e.g., Calkins, 1997), social behavior (e.g., Stifter and Corey, 2001), emotional regulation adaptive strategies (e.g., Cole et al., 1996; Santucci et al., 2008), and prosocial traits (e.g., Miller et al., 2017) in developmental years. As a result, HRV is likewise linked to prosociality (e.g., Kogan et al., 2014), cooperative behavior (e.g., Beffara et al., 2016a), compassion (Di Bello et al., 2020 for a meta-analysis), emotional recognition (e.g., Quintana et al., 2012), memory (e.g., Mattarozzi et al., 2019), empathy (e.g., Lischke et al., 2018), and social connectedness (e.g., Kok and Fredrickson, 2010) in adulthood. Only a few studies, however, investigated phasic HRV responses during tasks assessing ToM-related abilities, although this would represent an important measure of momentary physiological concomitants of such abilities (Park et al., 2014).

## Theory of Mind and HRV: The Present Study

In the past decade, researchers investigated whether inter-individual differences in vagally mediated HRV would be associated with inter-individual differences in mindreading ability. As a matter of fact, autonomic dysfunctions have been found in atypical development syndromes, namely, autism spectrum disorder (ASD) (Benevides and Lane, 2015; Saghir et al., 2017) and schizophrenia (Jáuregui et al., 2011), which are characterized by social cognition difficulties. In one of the few experimental studies, Colzato et al. (2017) observed that transcutaneous vagus nerve stimulation improves ToM, measured with the Reading the Mind in the Eyes Test, during which participants must infer what a person is thinking or feeling only by looking

at photographs of the eye region of the face, with the possibility to choose the correct answer among four words (Baron-Cohen et al., 2001a).

An association between better social cognition abilities and higher HRV, however, has not always been found (e.g., see Hamilton et al., 2014). This may be due to the fact that "social cognition" is a comprehensive term that includes different abilities, including ToM (Baars and Gage, 2013), which are probably differently associated with vagal modulation of the heart.

Adopting the same approach used by previous meta-analyses on ToM (Slaughter et al., 2015; Imuta et al., 2016), this study focused on tasks that evaluate the ability to understand the mental states of others (e.g., intentions, desires, thoughts, and knowledge). Thus, this study aimed to systematically review and meta-analyze the available studies examining the relation between (tonic and phasic) HRV and ToM in typically developing people. The moderating role of sex was examined, due to sex differences in autonomic function, with females showing greater high-frequency HRV (HF-HRV) (for a meta-analysis, see Koenig and Thayer, 2016). In contrast, sex differences in ToM appear controversial. While in some studies males and females had the same performance on ToM tasks (Di Tella et al., 2020), in other studies females had better performance compared with males during preschool and elementary school years (Charman et al., 2002; Walker, 2005; Calero et al., 2013), adolescence (Gabriel et al., 2021), and adulthood (Wacker et al., 2017).

# METHODS

## Search Strategy

A systematic analysis of the international literature was carried out by searching articles on PubMed, PsycINFO, and Web of Science databases. The last search was conducted on January 22, 2020. The results were restricted to "from the 1980s," considering the year in which ToM conceptualization appeared in the literature for the first time (Wimmer and Perner, 1983). The keywords used in the literature search were as follows: "heart rate," "autonomic nervous system," "heart rate variability," "root mean square successive difference," "respiratory sinus arrhythmia," "vagal," "vagus," "sympathetic," "psychophys*," "parasympathetic" (see Ottaviani et al., 2016) in combination with each of the following key words, using the Boolean connector and, "theory of mind," "ToM," "mentalizing," "mindreading," "mind understanding," "social understanding," "mental representations," "mental states," "false belief," "perspective taking," and "social cognition" (see Imuta et al., 2016).

## Eligibility Criteria

In agreement with the above-mentioned definition provided for ToM, we included studies in which ToM was evaluated with first-order and/or second-order false belief tasks, perspective taking tasks, and other tasks related to the ability to infer the mental states of others. The studies had to include HRV assessment in typically developing participants. The reports written in English and Italian were considered. Single case and review studies were excluded.

## Selection and Coding of Primary Studies

The review was conducted according to the PRISMA statement methodology (Moher et al., 2009; Crocetti, 2016). Once the 1,772 duplicates were removed, the remaining 2,587 records were independently screened by the first (MZ) and the last (AL) authors. Inter-coder agreement was very high at the abstract stage (% agreement = 99.23%; Cohen's kappa = 0.84). When the independent coders examined the full text of the 74 articles identified as eligible, the agreement between the coders remained high (% agreement = 97.26%; Cohen's kappa = 0.77). Disagreements were resolved through discussion. **Figure 1** illustrates the reasons for excluding 65 studies: 9 studies were included in the qualitative synthesis and 6 of them were included in the quantitative analysis.

The data from the studies of interest were extracted and inserted in a structured dataset sheet. According to the PICOS procedure (Liberati et al., 2009), names of authors, year of publication, number of participants,% of females, mean age, the country where the study was conducted, precautions taken before the HRV assessment, HRV parameters, assessment duration, ToM task(s), quality of the study, and main results were extracted. All the authors reviewed the summary table of the included studies. Only papers (published or sent by the authors) that provided the data needed to compute the correlation between measures of ToM and HRV were included in the meta-analysis. Zero-order correlations were preferred to compute effect sizes. The quality of selected studies was assessed with a modified version of the Newcastle–Ottawa Scale (Wells et al., 2011) previously adopted in a meta-analysis on psychophysiological measures (e.g., Ottaviani et al., 2016). The scores are comprised between 0 and 9, and a higher score is indicative of a higher quality of the study.

## Strategy of Analysis

Effect sizes were computed with the meta-analytic ProMeta 3 software (IDo Statistics-Internovi, Italy) using the random-effects model. Hedges' $g$ was preferred over Cohen's $d$ due to the small number of studies included in the meta-analysis. Heterogeneity across studies was estimated with the $Q$ statistic and the $I^2$ index. Sex and the quality of studies were tested as possible moderators using the meta-regression analysis. Publication bias was estimated by using the Egger's linear regression method and Kendall's tau.

# RESULTS

The flowchart in **Figure 1** reports the study selection process and illustrates the results in each step. Unpublished studies were not included in the meta-analysis because (i) dissertations did not include correlational data; (ii) the results found in conference papers were the same as reported later in published articles

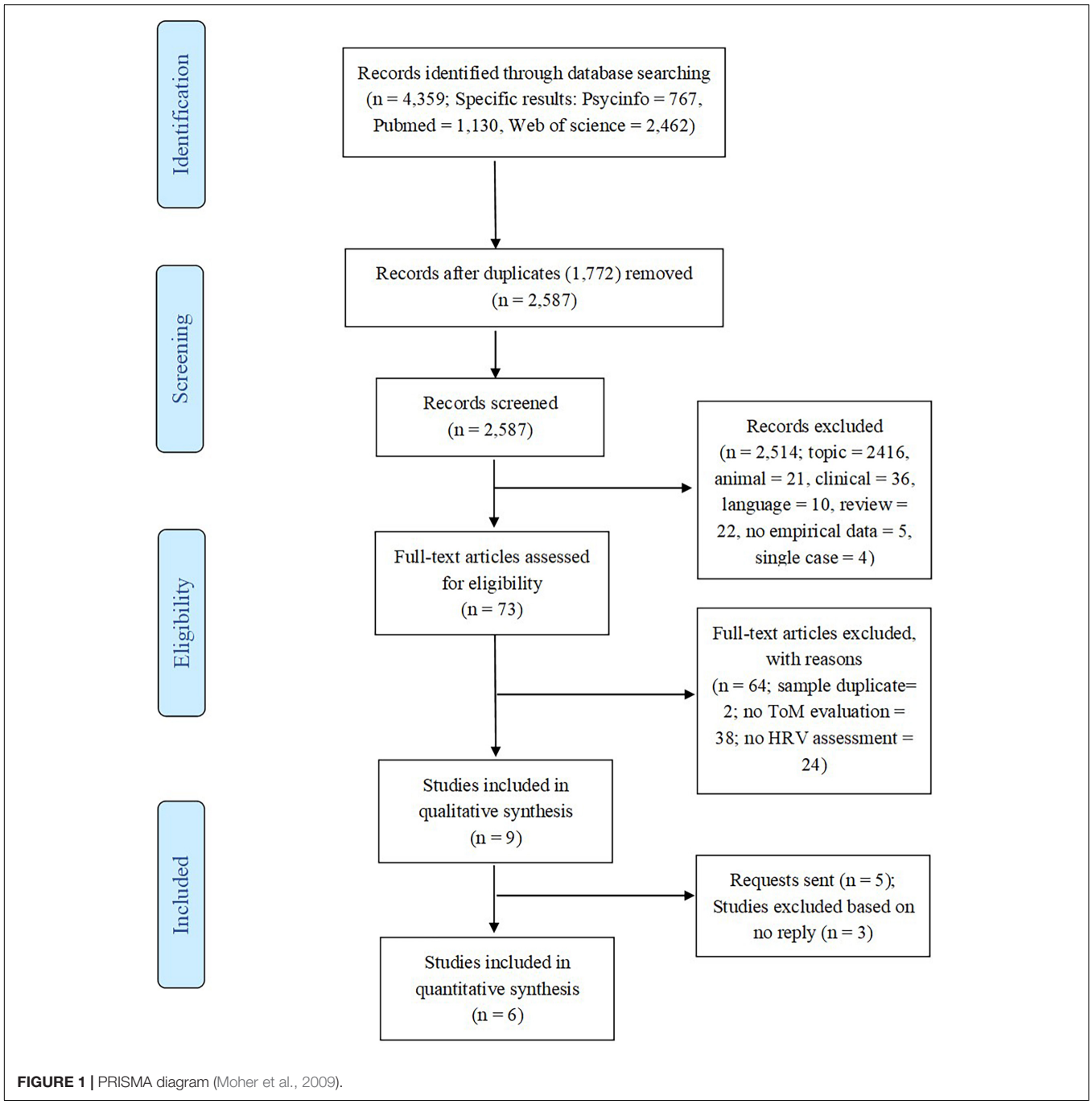



**FIGURE 1 |** PRISMA diagram (Moher et al., 2009).

(coded as "sample duplicate" in **Figure 1**); and (iii) the authors did not respond to data requests.

## Study Selection and Characteristics of Included Findings

Nine articles were included in the qualitative systematic review (see **Table 1**). All study samples were composed of adults, except for one that involved children (Kushki et al., 2014).

Two studies (Cugnata et al., 2018; Deuter et al., 2018) were not included in the meta-analysis because of multilevel analyses. At present, the way to correctly extract the effect sizes from these types of analyses continues to be controversial (Selya et al., 2012). In the case of clinical studies (Jáuregui et al., 2011; Kushki et al., 2014; Iorfino et al., 2016; Varas-Díaz et al., 2017), we focused only on data related to the control group.

## Theory of Mind Evaluation

In studies linking ToM with HRV, the Reading the Mind in the Eyes Test (RMET; Baron-Cohen et al., 1997a,b; Baron-Cohen et al., 2001a,b) is mostly used. This visual test, which

**TABLE 1 |** Summary of the studies included in the systematic review and in the meta-analysis.

| Study | Sample | *N* | Age M (SD) | Females | Country | HRV measure | Precautions | Assessment | ToM task | Relation between HRV and ToM performance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. Cugnata et al. (2018) | TD | 91 | 26.78 (18–52) | 48.35% | Italy | RMMSD | n.s. | RS | RMET | Effect of item difficulty and physiological activation ($p$ = n.s.) |
| 2. Deuter et al. (2018)* | TD | 90 | 23.5 (3.5) | 50% | Germany | RMMSD | No medications | RS | MET | Cognitive empathy and HRV ($r$ = −0.05, $p$ = 0.65; sent by the authors) |
| 3. Iorfino et al. (2016)* | CG | 25 | 23.96 (2.19) | 0% | Australia | HF-HRV | No alcohol and illicit substance 24 h prior to testing, and no smoking, food, and drinking 3 h before testing | RS and Task | RMET split version | HF at baseline and RMET score during no facial cooling ($p$ = n.s.) |
| 4. Jáuregui et al. (2011)* | CG | 19 | 27.6 (6) | 42.11% | Argentina | HF-HRV | Abstain from smoking for 2 h, no caffeinated beverages for at least 6 h, and no strenuous physical exercise for 24 h | RS and Task | RMET and Faux Pas Test (Stone et al., 1998) | Performance on the Faux Pas Test and HF-HRV during the test ($r$ = 0.436, $p < 0.05$) |
| 5. Kushki et al. (2014) | CG | 34 | 12.5 (2.9) | 44.1% | Canada | RSA | No medications and no premature birth | RS and Task | RMET-C | In response to the RMET-C, marginally larger decrease in RSA in the ASD group and larger RSA increase during recovery from the RMET-C ($p$ = n.s.) |
| 6. Lischke et al. (2017)* | TD | 37 | 23.03 (3.56) | 50% | Germany | HF-HRV | No medications and oral contraceptives | RS | RMET | Partial correlations# between HF-HRV and correctly identified positive ($r$ = 0.379, $p$ = 0.009)* and negative ($r$ = −0.084; $p$ = 0.305) states |
| 7. Okruszek et al. (2017) | TD | 25 | 35.9 (12.5) | 48% | United Kingdom | RMMSD | No psychotropic medications | During daily activities | Hinting Task (Corcoran et al., 1995) | Performance and HRV parameters during daily life (8-h max recording period) ($p$ = n.s.) |
| 8. Quintana et al. (2012)* | TD | 65 | 20.91 (6.16) | 53.8% | Australia | HF-HRV | No caffeine, nicotine, and alcohol on the testing day. Not current use of antidepressants | RS (after Task) | RMET | HF-HRV at rest (adjusted for covariates) and RMET scores weighted for difficulty ($r$ = 0.290, $p < 0.05$) |
| 9. Varas-Díaz et al. (2017)* | CG | 18 | 28.83 (3.36) | 50% | Chile | RMMSD, HF-HRV | Refrain from coffee and medication 24 h before | RS and Task | RMET | % of RMET correct answers and (a) resting RMSSD ($r$ = 0.381, $p$ = 0.119)* and HF ($r$ = 0.221, $p$ = 0.379) and (b) RMSSD ($r$ = 0.477, $p$ = 0.045) and HF ($r$ = 0.470, $p$ = 0.049) during the task (sent by the authors) |

**Studies/analysis included in the meta-analysis.*

*TD, typical development; CG, control group; M, mean; SD, standard deviation; n.s., not specified; RSA, respiratory sinus arrhythmia; RMSSD, root mean square of successive differences; HF-HRV, high frequency-heart rate variability; RMET, Reading the Mind in the Eyes Test (Baron-Cohen et al., 2001a); RMET-C, The Reading the Mind in the Eyes Test-Child version (Baron-Cohen et al., 2001b); MET, Multifaceted Empathy Test (Dziobek et al., 2008); Faux Pas Test (Stone et al., 1998); Hinting Task (Corcoran et al., 1995); RS, Resting State; r, Pearson correlation coefficient; p, significance value.*

*#Controlling for sex, age, and psychopathology.*

is traditionally used to measure ToM abilities, consists of 36 pictures of the eye region showing different emotions and mental states. Participants must infer the correct emotional/mental state in the image, choosing one of four words presented in the picture. The RMET scores are generally computed by summing the correct answers. Specifically, seven of the included studies (Jáuregui et al., 2011; Quintana et al., 2012; Kushki et al., 2014; Iorfino et al., 2016; Lischke et al., 2017; Varas-Díaz et al., 2017; Cugnata et al., 2018) used the RMET to evaluate ToM abilities as a component of social cognition. Among these, two studies conceptualized the RMET as an emotional recognition task, not referring to ToM abilities (Varas-Díaz et al., 2017; Cugnata et al., 2018). One of these studies (Kushki et al., 2014) examined a developmental sample and therefore used the child version of the test[1] (Baron-Cohen et al., 2001b). All the authors calculated the scores considering the total of the correct answers except for one (Lischke et al., 2017) that computed the difference between the percentage of correctly identified positive relative to neutral states and the percentage of correctly identified negative relative to neutral states. Jáuregui et al. (2011) also used other tests besides the RMET, i.e., the Faux Pas Test[2] (Stone et al., 1998; Baron-Cohen et al., 1999), the Baron-Cohen Faces Test[3] (Baron-Cohen et al., 1997b), and the Happè ToM Story Test[4] (Happé et al., 1999). The authors, however, reported only results on the relation among HRV, the Faux Pas Test, and the RMET (Jáuregui et al., 2011).

Deuter et al. (2018) administered a PC-assisted short-version of the Multifaceted Empathy Test (MET; Dziobek et al., 2008) to assess cognitive empathy intended as a social cognition measure. The participants were required to infer the mental state of the person in the photo and were asked to indicate the correct emotion from a list of four. The test consists of 30 picture stimuli with people in emotionally charged situations, and in this case (Deuter et al., 2018), the images were presented in 3 blocks of 10 picture stimuli to detect the cognitive components of empathy. The accuracy in the detection of emotional states is usually considered an index of "cognitive empathy" (Blair, 2008), conceptualized as the ability to create a theory about the mental state of others and take their perspective (Perry and Shamay-Tsoory, 2013).

Okruszek et al. (2017) used the Hinting Task (Corcoran et al., 1995) consisting of 10 short vignettes that are read out to the participants and left in front of the participants. The vignettes end with one of the characters giving a hint to the other character. The participant is asked what the character really meant with his/her assertion. An appropriate inference scores two points. If no inference is drawn, a second more obvious hint is added, and the participant is asked to infer the intention again. A correct response at this stage is given a score of 1, an incorrect response is given a score of 0, and the next item is introduced.

Notably, tests that involve faces stimuli (i.e., the Faces Test and the RMET) brought about phasic decreases in total HRV, while the Happè ToM Stories Test produced modest and significant increases in the SD of normal-to-normal intervals (Jáuregui et al., 2011). When the study carried out in children was considered, an increase in respiratory sinus arrhythmia (RSA) during the task and a decrease during recovery after the task were observed (Kushki et al., 2014).

Studies that did not use traditional ToM tasks (Okruszek et al., 2017; Deuter et al., 2018) failed to report an association between HRV and performance on (1) the MET (Dziobek et al., 2008), which requires inferring mental states from pictures with people in emotionally charged situations (Deuter et al., 2018), not presenting neutral background as in the RMET and (2) the Hinting Task (Corcoran et al., 1995), which is both a visual and a verbal ToM task, and it also involves other comprehension abilities (Okruszek et al., 2017).

To summarize, four out of the seven studies that used the RMET found positive and significant associations between HRV and ToM performance (Quintana et al., 2012; Lischke et al., 2017; Varas-Díaz et al., 2017; Cugnata et al., 2018), although different HRV indices were considered.

## Characteristics of HRV Assessment: Measures, Timing, and Precautions

Except for Kushki et al. (2014), who used RSA, the most commonly used index of HRV was HF-HRV (Jáuregui et al., 2011; Quintana et al., 2012; Iorfino et al., 2016; Lischke et al., 2017; Varas-Díaz et al., 2017). Okruszek et al. (2017); Cugnata et al. (2018), and Deuter et al. (2018) analyzed a time-domain component, namely, the root mean square of successive differences (RMSSD). Both indices are measures of vagally mediated HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

In most studies, the authors recorded physiological activity for 5 min with a minimum of 4 and a maximum of 10 min in

[1]Children were presented with a set of 28 photographs (selected from the adult version of the task) depicting the eye region of the face and were asked to choose the word that best described what the person was thinking or feeling from a total of 4 items (only 1 correct). Performance was measured as the number of correct responses (Baron-Cohen et al., 2001b).

[2]The test contained 20 stories, half of which including a social faux pas. The stories were presented one at a time. The text of each story was printed on a single page and posed in front of the participant. The researcher read the story aloud and then asked a series of questions about it. The authors (Jáuregui et al., 2011) quantified correct detections of unintentional Faux Pas (i.e., hits), correct rejections (i.e., detection of non-Faux Pas stories), and control questions designed to assess the comprehension of the story (Stone et al., 1998; Baron-Cohen et al., 1999).

[3]This task consisted of 20 photographs of the frontal face of an actress displaying different facial expressions, photographed under controlled and standardized conditions: 10 photographs displaying emotions such as happiness (repeated 2 times), sadness, anger (repeated 2 times), sorrow, surprise (repeated 2 times), disgust, and distress, and 10 photographs showing complex mental states, such as scheming, guilt, thoughtfulness, admiration, quizzically, flirtatiousness, boredom, interest (repeated 2 times), and arrogance. Each picture had two labels, giving participants a choice between two emotions (one correct and the other incorrect). The authors (Jáuregui et al., 2011) selected the label pairs out of a group of 20 emotions and arranged them pseudo-randomly. The participants were asked to identify the label that most appropriately described the "thinking or feeling" mental state of an actress (Baron-Cohen et al., 1997b).

[4]The verbal test consisted of 16 short stories, half of which invoking ToM. The ToM stories involved the interaction between people and contained double bluff, mistakes, persuasion, or white lies, with two examples of each type presented. Each ToM story was followed by a question testing the ability of participant to make inferences about mental states of others, usually the intentions of actors (Happé et al., 1999).

laboratory studies. Only one ambulatory study (Okruszek et al., 2017) assessed HRV during 8 h for 6 days of activities, including socializing moments.

Regarding the time of the day in which HRV was assessed, Jáuregui et al. (2011) and Quintana et al. (2012) recorded parasympathetic activity in the morning, while Varas-Díaz et al. (2017) assessed it from 3 to 6 p.m. and Iorfino et al. (2016) from 12 a.m. to 7 p.m.

Neurological, psychiatric, or psychological disorders, acute or persistent neurological or cardiovascular disease, and disability were excluded in all of the examined studies. Additional exclusion criteria were to refrain from medication in general (Kushki et al., 2014; Varas-Díaz et al., 2017; Deuter et al., 2018), oral contraceptives (Lischke et al., 2017), antidepressants (Quintana et al., 2012), psychotropic medication (Okruszek et al., 2017), antiparkinsonian anticholinergic agents, beta-blockers, and angiotensin-converting enzyme inhibitors (Jáuregui et al., 2011). Most of the authors asked participants to refrain from caffeinated beverages, food, and smoking before the testing session, from a minimum of 2 h (Jáuregui et al., 2011) to a maximum of 24 h (Varas-Díaz et al., 2017), and not to engage in physical activities (Jáuregui et al., 2011). Some authors also had alcohol consumption (Quintana et al., 2012; Iorfino et al., 2016) and illicit substances (Iorfino et al., 2016) as exclusionary criteria (see **Table 1** for further methodological details).

Resting (i.e., tonic) HRV was mostly assessed, sometimes failing to show a significant association with performance on ToM tasks (Iorfino et al., 2016; Okruszek et al., 2017; Deuter et al., 2018). It has to be noted, however, that when time trajectories of phasic HRV in response to the task was examined, positive association between the performance on the RMET and RMSSD (Cugnata et al., 2018) and between performance on a verbal ToM test (Faux Pas Test) and HF-HRV (Jáuregui et al., 2011) were reported, respectively.

## Meta-Analysis on the Association Between HRV and ToM

Six of the nine studies included in the qualitative review were included in the meta-analysis as (i) they included correlational data on HRV and ToM (Jáuregui et al., 2011; Quintana et al., 2012; Iorfino et al., 2016; Lischke et al., 2017) or (ii) such data were provided by the authors on request (Varas-Díaz et al., 2017; Deuter et al., 2018)[5].

Analysis of the six studies (i.e., 255 participants; 119 females) showed a significant association between HRV and ToM performance [$g$ = 0.44, 95% CI (0.07, 0.82), and $p$ = 0.021], revealing a small-to-medium effect size[6]. **Figure 2** shows the forest plot. No significant heterogeneity was detected $Q$ (5) = 8.90, $p$ = 0.113; $I^2$ = 43.82. Egger's test (intercept = 2.51, $t$ = 1.92, and $p$ = 0.127) and Kendall's tau ($Z$ = 0.56 and $p$ = 0.573) did not detect the presence of publication bias.

Meta-regression analysis was computed considering sex as a continuous variable (i.e., % of women). The meta-regression analysis did not show a statistically significant role of sex in moderating the association between resting HRV and performance on ToM tasks ($p$ = 0.712). The quality of studies did not emerge as a significant moderator ($p$ = 0.988).

# DISCUSSION

## Summary of Main Findings

The aim of the present systematic review and meta-analysis was to ascertain the relation between performance on tasks aimed at assessing the ability to infer mental states of others, namely ToM, and resting HRV. The effect size computed on the six studies with available data (Jáuregui et al., 2011; Quintana et al., 2012; Iorfino et al., 2016; Lischke et al., 2017; Varas-Díaz et al., 2017; Deuter et al., 2018) revealed a significant association of a small-to-medium size between tonic HRV and performance on ToM tasks. Neither sex nor the quality of the study moderated the relation between these two variables, likely due to the paucity of studies.

When considered individually, only two of the included studies were characterized by statistically significant results (Quintana et al., 2012; Lischke et al., 2017). In both studies, ToM was evaluated with the RMET, and HF-HRV was assessed as a measure of vagally mediated HRV. Lischke et al. (2017) evidenced a positive correlation between the identification of positive (but not negative) mental states of others and HF-HRV at rest. These findings are in line with the notion that individuals with high resting HF-HRV are more successful in initiating and maintaining social relationships than the individuals with low resting HF-HRV (e.g., Shahrestani et al., 2015).

A significant association between resting HRV and ToM performance also emerged when the RMET items were weighted for difficulty and HF-HRV values were adjusted for covariates, such as sex, body mass index, smoking habits, physical activity, and levels of depression, anxiety, and stress (Quintana et al., 2012). It has been reported that the RMET captures ToM deficits shown by people with ASD more than other tests do (Peterson and Slaughter, 2009) because it requires not only the identification of primary emotions from facial expression but also the recognition of mental states from a specific portion of the face. In fact, the understanding of inner states is more complex than the recognition of primary emotions, which has been more deeply investigated in relation to the vagal modulation of the heart (Bal et al., 2010; Park et al., 2012; Quintana et al., 2012; Beffara et al., 2016b; Lischke et al., 2017).

Two other studies that used the RMET failed to find a significant association with HRV (Iorfino et al., 2016; Varas-Díaz et al., 2017). In comparison with the previously examined studies,

[5]Varas-Díaz et al. (2017) provided HRV and ToM values, and we calculated the Pearson's correlation between RMSSD and HF at baseline and during the task and ToM score (% of correct answers). The results are reported in **Table 1**. In the meta-analysis, resting RMSSD was used, because all of the included studies used HRV values at rest with the exception of the study by Jáuregui et al. (2011).

[6]The effect size was considered small, medium or large if equal to 0.20, 0.50, and 0.80, respectively (Cohen, 1988).

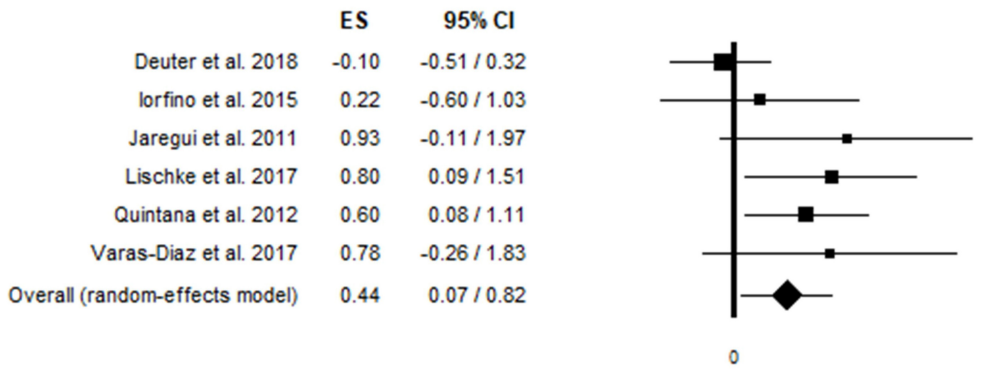



**FIGURE 2 |** Forest plot for meta-analysis on the association between HRV values and ToM performance.

Iorfino et al. (2016) used the split version of the RMET, and Varas-Díaz et al. (2017) assessed vagally mediated HRV with time-domain measures (i.e., RMSSD). Other studies that obtained non-significant findings used the Faux Pas Test (Jáuregui et al., 2011) or the MET (Deuter et al., 2018).

In summary, the use of HF-HRV at rest and the RMET appear to best capture the relation between the vagally mediated HRV and ToM. Notably, when HRV and ToM were examined in children with ASD and controls (Baron-Cohen et al., 2001b), an atypical parasympathetic modulation in the ASD group during the RMET, but not at rest, was emerged (Kushki et al., 2014).

## Limitations and Future Directions

The main limitation of this study is the paucity of studies included in the meta-analysis; thus, caution is required when interpreting the results. For this reason, the impact of categorical moderators could not be considered.

Second, the current meta-analysis was not preregistered, and we are aware that protocol registration is a highly desirable practice that allows avoiding research duplications or overlaps, ensuring a careful study plan and research implementation promotion (Shamseer et al., 2015). However, Xu et al. (2019) compared meta-analyses with and without protocol, demonstrating that protocol registration was associated with better quality of reporting but not with improved methodological quality.

Third, the polyvagal theory, which together with the neurovisceral integration model (Thayer and Lane, 2000, 2009; Thayer et al., 2009) provided the theoretical background for this study, has been criticized for the weak empirical evidence on its phylogenetic basis. More specifically, Berntson et al. (2007) have argued that the smart vagus is already present not only in mammals but also across vertebrate species (e.g., cartilaginous fish). Moreover, these authors have pointed out the difficulty to clearly distinguish the dorsal motor nucleus and nucleus ambiguous contributions to human behavior, suggesting that the association between specific behavioral patterns (e.g., adaptive behavior, mobilization, and immobilization response) and the myelinated or unmyelinated vagus nerve appears to be misleading (Berntson et al., 2007).

Meanwhile, the results from several meta-analytic works suggested that vagal control is positively associated with social functioning (Shahrestani et al., 2014, 2015) and positive effect (Di Bello et al., 2020), as well as negatively correlated with psychopathological symptoms and conditions (e.g., Chalmers et al., 2014; Koenig et al., 2016; Ottaviani et al., 2016; Koch et al., 2019). More recently, Marmerstein et al. (2021) have questioned the link between vagal modulation and HRV, showing a lack of association between HRV measures (e.g., RMSSD, HF-HRV, and LF-HRV) and tonic vagal activity assessed in the left cervical vagus and with a respiratory vagal difference in rats with and without anesthesia. Conversely, other researchers have asserted that HF-HRV and other time-domain metrics (e.g., RMSSD) constitute a good measure of vagal activity in humans, as suggested by a seminal preclinical study on this topic (Ter Horst and Postema, 1997). Such opposite views represent a challenge for future research.

The neurovisceral integration model (Thayer and Lane, 2000, 2009; Thayer et al., 2009) provides an important description of the network that includes neural, visceral, and cardiac components, in regulating affective and social processes. Considering the recent formulation of the model (Smith et al., 2017), it is important to evaluate vagal modulation both at rest and when social cognition reasoning is engaged. In fact, based on the different levels of communication between the cortex system, neural afferents, and cardiac circuits, which are hierarchically organized, complex cognitive and emotive functions can be carried on only if basic processes are satisfied. Thus, we can view such integration capacity only during specific tasks that require the engagement of more complex networks. Resting HRV is generally mostly used as an index of physical and psychological wellbeing (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). On the other hand, phasic fluctuations in HRV may reflect effortful emotional regulation during ToM tasks.

Finally, it seems that frontal lobe contributions to ToM might be important for representing mental states, whereas the parietal lobe role might be more specifically involved in reasoning about beliefs (Sabbagh, 2013). In future research, it would be useful to

adopt measures of implicit ToM, such as the "Triangles Playing Tricks" (Heider and Simmel, 1944; Abell et al., 2000), which requires the understanding of the mental state of others using social intuition.

## CONCLUSION

The prevalence of studies included adult samples, used visual ToM tasks, and considered RMSSD and HF-HRV as measures of vagally mediated HRV. Given the variety of tasks used, replication is warranted using more consistent experimental methods. The assessment of vagally mediated HRV both at rest and during the ToM tasks is also warranted as it provides different and complementary information. Finally, longitudinal and experimental studies on the association between the ability to infer the mental states of others and vagally mediated HRV are needed, since conclusions on directionality cannot be drawn based on the existing data.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

MZ, CO, FL, and AL conceived the idea. MZ and AL performed the systematic research. AL encouraged and supervised MZ's work. All authors discussed the results and contributed to the final manuscript.

## REFERENCES


Abell, F., Happe, F., and Frith, U. (2000). Do triangles play tricks? Attribution of mental states to animated shapes in normal and abnormal development. *Cogn. Dev.* 15, 1–16. doi: 10.1016/S0885-2014(00)00014-9

Arioli, M., Crespi, C., and Canessa, N. (2018). Social cognition through the lens of cognitive and clinical neuroscience. *BioMed Res. Int.* 2018:4283427. doi: 10.1155/2018/4283427

Astington, J. W. (2003). "Sometimes necessary, never sufficient: false-belief understanding and social competence," in *Macquarie Monographs in Cognitive Science. Individual Differences in Theory of Mind: Implications for Typical and Atypical Development*, eds B. Repacholi and V. Slaughter (Hove: Psychology Press), 13–38.

Baars, B., and Gage, N. M. (2013). "Social cognition," in *Fundamentals of Cognitive Neuroscience: A Beginner's Guide*, eds N. M. Cage and B. J. Baars (Cambridge, MA: Academic Press), 357–382.

Bal, E., Harden, E., Lamb, D., Van Hecke, A. V., Denver, J. W., and Porges, S. W. (2010). Emotion recognition in children with autism spectrum disorders: relations to eye gaze and autonomic state. *J. Autism Dev. Disord.* 40, 358–370. doi: 10.1007/s10803-009-0884-3

Banerjee, R., and Watling, D. (2005). Children's understanding of faux pas: associations with peer relations. *Hellenic J. Psychol.* 2, 27–45.

Baron-Cohen, S., O'riordan, M., Stone, V., Jones, R., and Plaisted, K. (1999). Recognition of faux pas by normally developing children and children with Asperger syndrome or high-functioning autism. *J. Autism Dev. Disord.* 29, 407–418. doi: 10.1023/A:1023035012436

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., and Plumb, I. (2001a). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J. Child Psychol. Psychiatry Allied Disciplines* 42, 241–251. doi: 10.1017/S0021963001006643

Baron-Cohen, S., Wheelwright, S., Spong, A., Scahill, V., and Lawson, J. (2001b). Are intuitive physics and intuitive psychology independent? A test with children with Asperger Syndrome. *J. Dev. Learn. Disord.* 5, 47–78.

Baron-Cohen, S., Jolliffe, T., Mortimore, C., and Robertson, M. (1997a). Another advanced test of theory of mind: evidence from very high functioning adults with autism or Asperger syndrome. *J. Child Psychol. Psychiatry* 38, 813–822. doi: 10.1111/j.1469-7610.1997.tb01599.x

Baron-Cohen, S., Wheelwright, S., and Jolliffe, A. T. (1997b). Is there a" language of the eyes"? Evidence from normal adults, and adults with autism or Asperger syndrome. *Vis. Cogn.* 4, 311–331. doi: 10.1080/713756761

Beffara, B., Bret, A. G., Vermeulen, N., and Mermillod, M. (2016a). Resting high frequency heart rate variability selectively predicts cooperative behavior. *Physiol. Behav.* 164, 417–428. doi: 10.1016/j.physbeh.2016.06.011

Beffara, B., Vermeulen, N., and Mermillod, M. (2016b). Resting high frequency heart rate variability is not associated with the recognition of emotional facial expressions in healthy human adults. *bioRxiv* [Preprint]. doi: 10.1101/077784

Benevides, T. W., and Lane, S. J. (2015). A review of cardiac autonomic measures: considerations for examination of physiological response in children with autism spectrum disorder. *J. Autism Dev. Disord.* 45, 560–575. doi: 10.1007/s10803-013-1971-z

Berntson, G. G., Cacioppo, J. T., and Grossman, P. (2007). Whither vagal tone. *Biol. Psychol.* 74, 295–300. doi: 10.1016/j.biopsycho.2006.08.006

Blair, R. J. R. (2008). Fine cuts of empathy and the amygdala: dissociable deficits in psychopathy and autism. *Q. J. Exp. Psychol.* 61, 157–170. doi: 10.1080/17470210701508855

Calero, C. I., Salles, A., Semelman, M., and Sigman, M. (2013). Age and gender dependent development of Theory of Mind in 6- to 8-years old children. *Front. Hum. Neurosci.* 7:281. doi: 10.3389/fnhum.2013.00281

Calkins, S. D. (1997). Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. *Dev. Psychobiol.* 31, 125–135. doi: 10.1002/(sici)1098-2302(199709)31:2<125::aid-dev5>3.0.co;2-m

Capage, L., and Watson, A. C. (2001). Individual differences in theory of mind, aggressive behavior, and social skills in young children. *Early Educ. Dev.* 12, 613–628. doi: 10.1207/s15566935eed1204_7

Chalmers, J. A., Quintana, D. S., Abbott, M. J., and Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Front. Psychiatry* 5:80. doi: 10.3389/fpsyt.2014.00080

Charman, T., Ruffman, T., and Clements, W. (2002). Is there a gender difference in false belief development? *Soc. Dev.* 11, 1–10. doi: 10.1111/1467-9507.00183

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*, 2nd Edn. Mahwah, NJ: Lawrence Erlbaum Associates. doi: 10.1111/1467-9507.00183

Cole, P. M., Zahn-Waxler, C., Fox, N. A., Usher, B. A., and Welsh, J. D. (1996). Individual differences in emotion regulation and behavior problems in preschool children. *J. Abnorm. Psychol.* 105, 518–529. doi: 10.1037/0021-843X.105.4.518

Colzato, L. S., Sellaro, R., and Beste, C. (2017). Darwin revisited: the vagus nerve is a causal element in controlling recognition of other's emotions. *Cortex* 92, 95–102. doi: 10.1016/j.cortex.2017.03.017

Corcoran, R., Mercer, G., and Frith, C. D. (1995). Schizophrenia, symptomatology and social inference: investigating "theory of mind" in people with schizophrenia. *Schizophr. Res.* 17, 5–13. doi: 10.1016/0920-9964(95)00024-G

Crocetti, E. (2016). Systematic reviews with meta-analysis: why, when, and how? *Emerg. Adulthood* 4, 3–18. doi: 10.1177/2167696815617076

Cugnata, F., Martoni, R. M., Ferrario, M., Di Serio, C., and Brombin, C. (2018). Modeling physiological responses induced by an emotion recognition task using latent class mixed models. *PLoS One* 13:e0207123. doi: 10.1371/journal.pone.0207123

Deuter, C. E., Nowacki, J., Wingenfeld, K., Kuehl, L. K., Finke, J. B., Dziobek, I., et al. (2018). The role of physiological arousal for self-reported emotional empathy. *Auton. Neurosci.* 214, 9–14. doi: 10.1016/j.autneu.2018.07.002

Di Bello, M., Carnevali, L., Petrocchi, N., Thayer, J., Gilbert, P., and Ottaviani, C. (2020). The compassionate vagus: a meta-analysis on the connection between compassion and vagally-mediated heart rate variability. *Neurosci. Biobehav. Rev.* 116, 21–30. doi: 10.1016/j.neubiorev.2020.06.016

Di Tella, M., Miti, F., Ardito, R. B., and Adenzato, M. (2020). Social cognition and sex: are men and women really different? *Personal. Individ. Differ.* 162:110045. doi: 10.1016/j.paid.2020.110045

Dvash, J., and Shamay-Tsoory, S. G. (2014). Theory of mind and empathy as multidimensional constructs: neurological foundations. *Top. Lang. Disord.* 34, 282–295. doi: 10.1097/TLD.0000000000000040

Dziobek, I., Rogers, K., Fleck, S., Bahnemann, M., Heekeren, H. R., Wolf, O. T., et al. (2008). Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the Multifaceted Empathy Test (MET). *J. Autism Dev. Disord.* 38, 464–473. doi: 10.1007/s10803-007-0486-x

Ettekal, I., and Ladd, G. W. (2020). Development of aggressive-victims from childhood through adolescence: associations with emotion dysregulation, withdrawn behaviors, moral disengagement, peer rejection, and friendships. *Dev. Psychopathol.* 32, 271–291. doi: 10.1017/S0954579419000063

Flavell, J. H. (2004). Theory-of-mind development: retrospect and prospect. *Merrill-Palmer Q.* 50, 274–290. doi: 10.1353/mpq.2004.0018

Frith, U., and Frith, C. D. (2003). Development and neurophysiology of mentalizing. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 358, 459–473. doi: 10.1098/rstb.2002.1218

Gabriel, E. T., Oberger, R., Schmoeger, M., Deckert, M., Vockh, S., Auff, E., et al. (2021). Cognitive and affective Theory of Mind in adolescence: developmental aspects and associated neuropsychological variables. *Psychol. Res.* 85, 533–553. doi: 10.1007/s00426-019-01263-6

Hamilton, H. K., Sun, J. C., Green, M. F., Kee, K. S., Lee, J., Sergi, M., et al. (2014). Social cognition and functional outcome in schizophrenia: the moderating role of cardiac vagal tone. *J. Abnorm. Psychol.* 123, 764–770. doi: 10.1037/a0037813

Happé, F., Brownell, H., and Winner, E. (1999). Acquired theory of mind'impairments following stroke. *Cognition* 70, 211–240. doi: 10.1016/S0010-0277(99)00005-0

Heider, F., and Simmel, M. (1944). An experimental study of apparent behavior. *Am. J. Psychol.* 57, 243–259. doi: 10.2307/1416950

Hughes, C., Dunn, J., and White, A. (1998). Trick or treat? Uneven understanding of mind and emotion and executive dysfunction in "hard–to–manage preschoolers. *J. Child Psychol. Psychiatry* 39, 981–994. doi: 10.1111/1469-7610.00401

Hughes, C., and Leekam, S. (2004). What are the links between theory of mind and social relations? Review, reflections and new directions for studies of typical and atypical development. *Soc. Dev.* 13, 590–619. doi: 10.1111/j.1467-9507.2004.00285.x

Imuta, K., Henry, J. D., Slaughter, V., Selcuk, B., and Ruffman, T. (2016). Theory of mind and prosocial behavior in childhood: a meta-analytic review. *Dev. Psychol.* 52, 1192–1205. doi: 10.1037/dev0000140

Iorfino, F., Alvares, G. A., Guastella, A. J., and Quintana, D. S. (2016). Cold face test-induced increases in heart rate variability are abolished by engagement in a social cognition task. *J. Psychophysiol.* 30, 38–46. doi: 10.1027/0269-8803/a000152

Jáuregui, O. I., Costanzo, E. Y., de Achával, D., Villarreal, M. F., Chu, E., Mora, M. C., et al. (2011). Autonomic nervous system activation during social cognition tasks in patients with schizophrenia and their unaffected relatives. *Cogn. Behav. Neurol.* 24, 194–203. doi: 10.1097/WNN.0b013e31824007e9

Koch, C., Wilhelm, M., Salzmann, S., Rief, W., and Euteneuer, F. (2019). A meta-analysis of heart rate variability in major depression. *Psychol. Med.* 49, 1948–1957. doi: 10.1017/S0033291719001351

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Koenig, J., Williams, D. P., Kemp, A. H., and Thayer, J. F. (2016). Vagally mediated heart rate variability in headache patients—a systematic review and meta-analysis. *Cephalalgia* 36, 265–278. doi: 10.1177/0333102415583989

Kogan, A., Oveis, C., Carr, E. W., Gruber, J., Mauss, I. B., Shallcross, A., et al. (2014). Vagal activity is quadratically related to prosocial traits, prosocial emotions, and observer perceptions of prosociality. *J. Personal. Soc. Psychol.* 107, 1051–1063. doi: 10.1037/a0037509

Kok, B. E., and Fredrickson, B. L. (2010). Upward spirals of the heart: autonomic flexibility, as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biol. Psychol.* 85, 432–436. doi: 10.1016/j.biopsycho.2010.09.005

Kuo, T. B., Lai, C. J., Huang, Y. T., and Yang, C. C. (2005). Regression analysis between heart rate variability and baroreflex-related vagus nerve activity in rats. *J. Cardiovasc. Electrophysiol.* 16, 864–869. doi: 10.1111/j.1540-8167.2005.40656.x

Kushki, A., Brian, J., Dupuis, A., and Anagnostou, E. (2014). Functional autonomic nervous system profile in children with autism spectrum disorder. *Mol. Autism* 5:39. doi: 10.1186/2040-2392-5-39

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J. Clin. Epidemiol.* 62, e1–e34. doi: 10.1016/j.jclinepi.2009.06.006

Lischke, A., Lemke, D., Neubert, J., Hamm, A. O., and Lotze, M. (2017). Inter-individual differences in heart rate variability are associated with inter-individual differences in mind-reading. *Sci. Rep.* 7:11557. doi: 10.1038/s41598-017-11290-1

Lischke, A., Pahnke, R., Mau-Moeller, A., Behrens, M., Grabe, H. J., Freyberger, H. J., et al. (2018). Inter- individual differences in heart rate variability are associated with inter-individual differences in empathy and alexithymia. *Front. Psychol.* 9:229. doi: 10.3389/fpsyg.2018.00229

Lonigro, A., Laghi, F., Baiocco, R., and Baumgartner, E. (2014). Mind reading skills and empathy: evidence for nice and nasty ToM behaviours in school-aged children. *J. Child Fam. Stud.* 23, 581–590. doi: 10.1007/s10826-013-9722-5

Marmerstein, J. T., McCallum, G. A., and Durand, D. M. (2021). Direct measurement of vagal tone in rats does not show correlation to HRV. *Sci. Rep.* 11:1210. doi: 10.1038/s41598-020-79808-8

Mattarozzi, K., Colonnello, V., Thayer, J. F., and Ottaviani, C. (2019). Trusting your heart: long-term memory for bad and good people is influenced by resting vagal tone. *Conscious. Cogn.* 75:102810. doi: 10.1016/j.concog.2019.102810

McEwen, F., Happé, F., Bolton, P., Rijsdijk, F., Ronald, A., Dworzynski, K., et al. (2007). Origins of individual differences in imitation: links with language, pretend play, and socially insightful behaviour in two–year–old twins. *Child Dev.* 78, 474–492. doi: 10.1111/j.1467-8624.2007.01010.x

Miller, J. G., Kahle, S., and Hastings, P. D. (2017). Moderate baseline vagal tone predicts greater prosociality in children. *Dev. Psychol.* 53, 274–289. doi: 10.1037/dev0000238

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann. Int. Med.* 151, 264–269. doi: 10.1093/ptj/89.9.873

Okruszek, Ł, Dolan, K., Lawrence, M., and Cella, M. (2017). The beat of social cognition: exploring the role of heart rate variability as marker of mentalizing abilities. *Soc. Neurosci.* 12, 489–493. doi: 10.1080/17470919.2016.1244113

Ottaviani, C., Thayer, J. F., Verkuil, B., Lonigro, A., Medea, B., Couyoumdjian, A., et al. (2016). Physiological concomitants of perseverative cognition: a systematic review and meta-analysis. *Psychol. Bull.* 142, 231–251. doi: 10.1037/bul0000036

Park, G., Van Bavel, J. J., Vasey, M. W., Egan, E. J., and Thayer, J. F. (2012). From the heart to the mind's eye: cardiac vagal tone is related to visual perception of fearful faces at high spatial frequency. *Biol. Psychol.* 90, 171–178. doi: 10.1016/j.biopsycho.2012.02.012

Park, G., Vasey, M. W., Van Bavel, J. J., and Thayer, J. F. (2014). When tonic cardiac vagal tone predicts changes in phasic vagal tone: the role of fear and perceptual load. *Psychophysiology* 51, 419–426. doi: 10.1111/psyp.12186

Perry, A., and Shamay-Tsoory, S. (2013). "Understanding emotional and cognitive empathy: a neuropsychological perspective," in *Understanding Other Minds: Perspectives From Developmental Social Neuroscience*, eds S. Baron-Cohen, H. Tager-Flusberg, and M. V. Lombardo (Oxford: Oxford University Press), 178–194. doi: 10.1093/acprof:oso/9780199692972.003.0011

Peterson, C. C., and Slaughter, V. (2009). Theory of mind (ToM) in children with autism or typical development: links between eye-reading and false belief understanding. *Res. Autism Spectr. Disord.* 3, 462–473. doi: 10.1016/j.rasd.2008.09.007

Porges, S. W. (2001). The polyvagal theory: phylogenetic substrates of a social nervous system. *Int. J. Psychophysiol.* 42, 123–146. doi: 10.1016/S0167-8760(01)00162-3

Porges, S. W. (2003). The polyvagal theory: phylogenetic contributions to social behavior. *Physiol. Behav.* 79, 503–513. doi: 10.1016/S0031-9384(03)00156-2

Porges, S. W. (2007). The polyvagal perspective. *Biol. Psychol.* 74, 116–143. doi: 10.1016/j.biopsycho.2006.06.009

Porges, S. W., Doussard-Roosevelt, J. A., Portales, A. L., and Greenspan, S. I. (1996). Infant regulation of the vagal "brake" predicts child behavior problems: a psychobiological model of social behavior. *Dev. Psychobiol.* 29, 697–712. doi: 10.1002/(SICI)1098-2302(199612)29:8<697::AID-DEV5>3.0.CO;2-O

Premack, D., and Woodruff, G. (1978). Does the chimpanzee have a theory of mind? *Behav. Brain Sci.* 1, 515–526. doi: 10.1017/S0140525X00076512

Quintana, D. S., Guastella, A. J., Outhred, T., Hickie, I. B., and Kemp, A. H. (2012). Heart rate variability is associated with emotion recognition: direct evidence for a relationship between the autonomic nervous system and social cognition. *Int. J. Psychophysiol.* 86, 168–172. doi: 10.1016/j.ijpsycho.2012.08.012

Ronald, A., Happé, F., Hughes, C., and Plomin, R. (2005). Nice and nasty theory of mind in preschool children: nature and nurture. *Soc. Dev.* 14, 664–684. doi: 10.1111/j.1467-9507.2005.00323.x

Sabbagh, M. A. (2013). *Brain Electrophysiological Studies of Theory of Mind. Understanding Other Minds: Perspectives From Developmental Social Neuroscience*. New York, NY: Oxford University Press.

Saghir, H., Dupuis, A., Chau, T., and Kushki, A. (2017). Atypical autonomic nervous system complexity accompanies social cognition task performance in ASD. *Res. Autism Spectr. Disord.* 39, 54–62. doi: 10.1016/j.rasd.2017.04.004

Santucci, A. K., Silk, J. S., Shaw, D. S., Gentzler, A., Fox, N. A., and Kovacs, M. (2008). Vagal tone and temperament as predictors of emotion regulation strategies in young children. *Dev. Psychobiol.* 50, 205–216. doi: 10.1002/dev.20283

Schurz, M., Radua, J., Tholen, M. G., Maliske, L., Margulies, D. S., Mars, R. B., et al. (2021). Toward a hierarchical model of social cognition: a neuroimaging meta-analysis and integrative review of empathy and theory of mind. *Psychol. Bull.* 147, 293–327. doi: 10.1037/bul0000303

Sebastian, C. L., Fontaine, N. M., Bird, G., Blakemore, S. J., Brito, S. A., McCrory, E. J., et al. (2012). Neural processing associated with cognitive and affective Theory of Mind in adolescents and adults. *Soc. Cogn. Affect. Neurosci.* 7, 53–63. doi: 10.1093/scan/nsr023

Selya, A. S., Rose, J. S., Dierker, L. C., Hedeker, D., and Mermelstein, R. J. (2012). A practical guide to calculating Cohen's f(2), a measure of local effect size, from PROC MIXED. *Front. Psychol.* 3:111. doi: 10.3389/fpsyg.2012.00111

Shahrestani, S., Stewart, E. M., Quintana, D. S., Hickie, I. B., and Guastella, A. J. (2014). Heart rate variability during social interactions in children with and without psychopathology: a meta-analysis. *J. Child Psychol. Psychiatry Allied Disciplines* 55, 981–989. doi: 10.1111/jcpp.12226

Shahrestani, S., Stewart, E. M., Quintana, D. S., Hickie, I. B., and Guastella, A. J. (2015). Heart rate variability during adolescent and adult social interactions: a meta-analysis. *Biol. Psychol.* 105, 43–50. doi: 10.1016/j.biopsycho.2014.12.012

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 350:g7647. doi: 10.1136/bmj.g7647

Slaughter, V., Imuta, K., Peterson, C. C., and Henry, J. D. (2015). Meta-analysis of theory of mind and peer popularity in the preschool and early school years. *Child Dev.* 86, 1159–1174. doi: 10.1111/cdev.12372

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Stifter, C. A., and Corey, J. M. (2001). Vagal regulation and observed social behavior in infancy. *Soc. Dev.* 10, 189–201. doi: 10.1111/1467-9507.00158

Stone, V. E., Baron-Cohen, S., and Knight, R. T. (1998). Frontal lobe contributions to theory of mind. *J. Cogn. Neurosci.* 10, 640–656. doi: 10.1162/089892998562942

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Ter Horst, G. J., and Postema, F. (1997). Forebrain parasympathetic control of heart activity: retrograde transneuronal viral labeling in rats. *Am. J. Physiol.* 273, H2926–H2930. doi: 10.1152/ajpheart.1997.273.6.H2926

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart–brain connection: Further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Tholen, M. G., Trautwein, F. M., Böckler, A., Singer, T., and Kanske, P. (2020). Functional magnetic resonance imaging (fMRI) item analysis of empathy and theory of mind. *Hum. Brain Mapp.* 41, 2611–2628. doi: 10.1002/hbm.24966

Varas-Díaz, G., Brunetti, E. P., Rivera-Lillo, G., and Maldonado, P. E. (2017). Patients with chronic spinal cord injury exhibit reduced autonomic modulation during an emotion recognition task. *Front. Hum. Neurosci.* 11:59. doi: 10.3389/fnhum.2017.00059

Wacker, R., Bölte, S., and Dziobek, I. (2017). Women know better what other women think and feel: gender effects on mindreading across the adult life Span. *Front. Psychol.* 8:1324. doi: 10.3389/fpsyg.2017.01324

Walker, S. (2005). Gender differences in the relationship between young children's peer-related social competence and individual differences in theory of mind. *J. Genet. Psychol.* 166, 297–312. doi: 10.3200/GNTP.166.3.297-312

Wells, G. A., Shea, B., O'Connell, D., Peterson, J., Welch, V., Losos, M., et al. (2011). *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses*. Ontario: Ottawa Hospital Research Institute.

Wimmer, H., and Perner, J. (1983). Beliefs about beliefs: representation and constraining function of wrong beliefs in young children's understanding of deception. *Cognition* 13, 103–128. doi: 10.1016/0010-0277(83)90004-5

Xu, C., Cheng, L. L., Liu, Y., Jia, P.-L., Gao, M.-Y., and Zhang, C. (2019). Protocol registration or development may benefit the design, conduct and reporting of dose-response meta-analysis: empirical evidence from a literature survey. *BMC Med. Res. Methodol.* 19:78. doi: 10.1186/s12874-019-0715-y

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Sports and Active Living

CASE REPORT
published: 03 September 2021
doi: 10.3389/fspor.2021.612782

# Utilizing Heart Rate Variability for Coaching Athletes During and After Viral Infection: A Case Report in an Elite Endurance Athlete

*Laura Hottenrott*[1]*, *Thomas Gronwald*[2], *Kuno Hottenrott*[3], *Thimo Wiewelhove*[1] and *Alexander Ferrauti*[1]

[1] Department of Training and Exercise Science, Faculty of Sports Science, Ruhr University Bochum, Bochum, Germany, [2] Department of Performance, Neuroscience, Therapy and Health, Faculty of Health Sciences, Medical School Hamburg, Hamburg, Germany, [3] Institute of Sports Science, Department of Training Science and Sports Medicine, Martin-Luther-University Halle-Wittenberg, Halle, Germany

**Background:** Viral diseases have different individual progressions and can lead to considerable risks/long-term consequences. Therefore, it is not suitable to give general recommendations on a time off from training for athletes. This case report aims to investigate the relevance of detecting heart rate (HR) and HR variability (HRV) during an orthostatic test (OT) to monitor the progression and recovery process during and after a viral disease in an elite endurance athlete.

**Methods:** A 30-year-old elite marathon runner contracted a viral infection (upper respiratory tract infection) 4 weeks after a marathon race. RR intervals in HR time series in supine and standing positions were monitored daily in the morning. Analyzed parameters included HR, the time-domain HRV parameter root mean square of successive difference (RMSSD), peak HR (HRpeak) in a standing position, and the time to HR peak (tHRpeak).

**Results:** During the 6-day viral infection period, HR increased significantly by an average of 11 bpm in the supine position and by 22 bpm in the standing position. In addition, the RMSSD decreased from 20.8 to 4.2 ms, the HRpeak decreased by 13 bpm, and the tHRpeak increased by 18 s in the standing position significantly. There were no significant changes in the pre-viral infection RMSSD values in the supine position. The viral infection led to a significant change in HR and HRV parameters. The cardiac autonomic system reacted more sensitively in the standing position compared to the supine position after a viral infection in the present case study.

**Conclusion:** These data have provided supportive rationale as to why the OT with a change from supine to standing body position and the detection of different indicators based on HR and a vagal driven time-domain HRV parameter (RMSSD) is likely to be useful to detect viral diseases early on when implemented in daily routine. Given the case study nature of the findings, future research has to be conducted to investigate whether the use of the OT might be able to offer an innovative, non-invasive, and time-efficient possibility to detect and evaluate the health status of (elite endurance) athletes.

**Keywords: orthostatic test, athlete monitoring, heart rate variability, viral infection, return to sport, endurance sport, cardiac autonomic control, marathon**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Fábio Yuzo Nakamura, Instituto Universitário da Maia (ISMAI), Portugal*
*Ross Sherman, Grand Valley State University, United States*

***Correspondence:**
*Laura Hottenrott*
*laura.hottenrott@rub.de*

***Specialty section:***
*This article was submitted to Elite Sports and Performance Enhancement, a section of the journal Frontiers in Sports and Active Living*

***Received:*** *30 September 2020*
***Accepted:*** *28 June 2021*
***Published:*** *03 September 2021*

***Citation:***
*Hottenrott L, Gronwald T, Hottenrott K, Wiewelhove T and Ferrauti A (2021) Utilizing Heart Rate Variability for Coaching Athletes During and After Viral Infection: A Case Report in an Elite Endurance Athlete. Front. Sports Act. Living 3:612782. doi: 10.3389/fspor.2021.612782*

## INTRODUCTION

With the pandemic spread of the novel coronavirus (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19; World Health Organization, 2020), healthcare professionals and coaches are faced with an increasing number of athletes seeking advice on when and how to restart training after recovery from viral diseases. This is challenging, as practical and evidence-based recommendations for a return to sports after infectious episodes are limited and heterogeneous. Preliminary approaches regarding COVID-19 provide symptom-based decision schemes to make recommendations for additional assessment and intervention measures (Nieß et al., 2020; Schnellhorn et al., 2020). It is the aim of this case report to investigate the relevance of detecting heart rate (HR) and HR variability (HRV) during an orthostatic test (OT) to monitor the progression and recovery process during and after a viral disease in an elite endurance athlete.

Due to the negative effects on health status and performance capacity, viral infections in sports must be detected early and physical exercise is not recommended in the case of a viral infection (Roberts, 1986). Especially, myocarditis is a significant cause of sudden cardiac death and sudden cardiac arrest (SCD/SCA) in athletes (Harmon et al., 2016) with case series reporting myocarditis as a potential source of SCD/SCA in up to 8% (Halle et al., 2020). In this regard, it is important to find very early indicators and sensitive markers (ideally before the onset of symptoms) that are practicable and can map the course of viral disease and recovery for detailed control diagnostics. According to Friman and Wesslén (2000), fever (>38°C or 0.5–1° higher than usual) and an increased resting heart rate (>10 bpm higher than normal) are contraindications for physical training. Therefore, deciding on the right time to restart training is essential (Dores and Cardim, 2020). Long breaks in physical training are critical for performance development in elite athletes; too short breaks in the case of viral infection can cause a relapse and incomplete curing of an infection can lead to severe health risks (Verwoert et al., 2020).

The scientific community is working on the topic of how to optimize the time to return to sports after a viral infection and which diagnostic indicators could support decision making (Dores and Cardim, 2020; Nieß et al., 2020; Schnellhorn et al., 2020; Verwoert et al., 2020). Viral infections affect the cardiovascular system and lead to an increased resting heart rate (HR) in the presence of fever (Karjalainen and Viitasalo, 1986). Acute upper respiratory tract infections (URTIs), including influenza, respiratory syncytial virus, and bacterial pneumonias, are well-recognized triggers for cardiovascular diseases (Cowan et al., 2018). The emergence of SARS-CoV-2 has rapidly grown into a pandemic and practical parameters for daily health monitoring are necessary. A large proportion of affected patients have been reported to have underlying cardiovascular diseases, and myocardial infarctions were noted to occur after an infection (Madjid et al., 2020). A recent case study on cardiovascular changes occurring during an infection with COVID-19 shows that heart rate variability (HRV) decreases but HR does not increase at rest (Buchhorn et al., 2020). Thus, it is not sufficient to only examine resting HR, but further parameters are required for a differential analysis.

For athletes and coaches, it is essential to perform detailed health monitoring in conjunction with performance monitoring to ensure a very early detection of a viral infection and to guarantee a safe return to training (Hagen et al., 2020). For the control of physical load, athletes often use HR and HRV measurements to individualize the training load and to detect symptoms of overtraining at an early stage (Uusitalo et al., 2000; Buchheit, 2014; Plews et al., 2014; Hottenrott and Hoos, 2017). To the best of our knowledge, no study has examined HR and HRV measurements in elite athletes to monitor health status during and after a viral infection and to individualize return to training.

Cardiac vagal control reflects the activity of the vagus nerve regulating cardiac functioning and can be inferred via HRV measurements (Laborde et al., 2018; Schneider et al., 2018). Most often, HRV analysis is derived from resting data in a single supine or sitting position. However, some studies show that RR measurements in a single resting position are not sufficient to detect training-induced fatigue in athletes (Buchheit, 2014; Plews et al., 2014; Bellenger et al., 2016a,b). A passive head-up tilt test in supine and upright positions results in specific changes in the spectral characteristics of HRV as a result of reduced vagal and enhanced sympathetic outflow and gives valuable indications that a change of body position can lead to additional information about regulation patterns of the cardiac autonomic system (Tulppo et al., 2001). An active switching from the supine to the upright position imposes stress by gravitational pooling of the blood in the splanchnic venous reservoir and leg veins (Stewart et al., 2006). Consequently, the autonomic nervous system is required to maintain the hemodynamic to avoid cerebral hypoperfusion. From supine to standing, HR increases (RR intervals become shorter) and high frequency power (parasympathetic) is depressed compared to supine, whereas low frequency power (partially sympathetic) increases (Aubert et al., 2003).

An orthostatic test (OT) provides a practical method for the detection of overload and overtraining in athletes and can be used to assess the autonomic nervous system's response to physical exercise and training (Le Meur et al., 2013; Buchheit, 2014; Plews et al., 2014; Schneider et al., 2019; Barrero et al., 2020). In addition, changes in body position can provoke specific responses in HR dynamics and could therefore provide more specific information about autonomic nervous system regulation patterns (Tulppo et al., 2001). During orthostatic tolerance assessment, HRV patterns in both supine and standing positions are affected by the different involvement of cardiopulmonary receptors, i.e., cardiac preload, and hence, tuned changes in plasma volume and/or peripheral vasomotor tone. Among other factors, these parameters likely support changes in autonomic patterns and HRV also during different training loads and phases (Schmitt et al., 2015). For precise analysis, monitoring in athletes should not be limited to the measurement of cardiac autonomic function in just one body position but should consider assessing the response in two different body positions (e.g., supine and standing) (Buchheit, 2014; Schmitt et al., 2015; Ravé and Fortrat, 2016; Hottenrott and Hoos, 2017; Hottenrott et al., 2019).

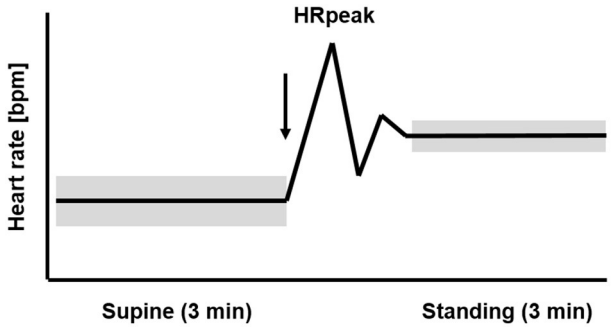



**FIGURE 1 |** Temporal course of the heart rate (HR) and heart rate variability (HRV) during 3 min supine position followed by 3 min standing position (orthostatic test, including HRpeak) in healthy athletes with uncompromised performance. The arrow marks the change in body position from supine to standing up. The widths of the bars represent the higher HRV in the supine than the standing position (mod. Hottenrott and Hoos, 2017).

**Figure 1** displays the temporal course of the heart rate in healthy athletes with uncompromised performance during the OT. The HR is low in the supine position and rises rapidly during active standing-up. In the supine position, the efferent vagal activity often calculated with the parameter root mean square of successive differences (RMSDD) is much higher than in the standing position (Bellenger et al., 2016a). After active standing-up, the HR will reach the first peak (HRpeak) after 15–20 s (Flachenecker, 2000; Rooke and Sparks, 2003). The rate of heart rate increase from rest to exercise or supine to standing is another parameter providing information about the cardiac autonomic system to detect training-induced fatigue (Nelson et al., 2014; Bellenger et al., 2016a,c). Thereafter, a counter-regulation with a rapid decrease of the HR occurs, before the HR rises again and stabilizes in the standing position. When there is high circulation stability, HR in the standing position remains higher than the HR in the supine position. The average HR in the standing position of healthy and non-fatigued athletes is on average 10–20 bpm higher than the HR at rest in the supine position (Aubert et al., 2003; Hottenrott and Hoos, 2017). Standing also induces a three- to four-fold decrease of vagal HRV indices compared to the supine position (Aubert et al., 2003; Hottenrott and Hoos, 2017).

Viral diseases (e.g., influenza, Epstein-Barr virus, coronavirus) have different individual progressions (Polak et al., 2020) and can lead to considerable risks/long-term consequences. Therefore, it is not suitable to give general recommendations on time off from training for athletes. The severity of the disease and the course of the recovery process can be very individual. In athletes, viral diseases are usually mild and are often not taken seriously, although considerable risks/long-term consequences can nevertheless occur if training is continued during the infection (Roberts, 1986). Regarding daily monitoring with the OT, it is expected that a viral infection with the presence of fever will lead to an increase in supine resting heart rate and affect the overall cardiac autonomic system (Karjalainen and Viitasalo, 1986; Polak et al., 2020).

It is the aim of the present case report to display how the OT, which is based on the detection of RR intervals in heart rate time series in supine and standing positions, can monitor the progression and recovery process before, during, and after a viral disease in a high-performance endurance athlete providing immediate day-to-day feedback about autonomic nervous system recovery status.

# METHODS

## Participant

The participant is a 30-year-old male elite marathon runner with a personal best time in the marathon of 2:18 h. He has competed in the sport of running since he was 13 years. He has a resting HR of 47 bpm, a maximal HR of 188 bpm, and a $VO_2$max of 71 ml/kg/min and receives medical clearance in a sports medical check-up annually. The athlete's training is monitored routinely throughout the entire year and he performs an OT daily (**Figure 1**). The participant contracted an upper respiratory tract infection (URTI, diagnosed by a physician) 4 weeks after running a marathon race in 2:21 h. He had intense flu symptoms for 6 days and fever between 38.5° and 39.3°C for 3 days on days 13, 14, and 15; he did not take any fever-lowering medication. Clinical diagnostics were not accompanied by laboratory diagnostics.

The participant provided informed consent in accordance with the institutional review board and the guidelines of the Helsinki World Medical Association Declaration.

## Measurements

Ten days before (pre), during, and 10 days after (post) the viral infection, a daily (after a night's sleep) orthostatic test (OT) and a position change test with 3 min in the supine and 3 min in the standing position were performed (Bourdillon et al., 2017; Hottenrott and Hoos, 2017). The recordings were taken in the supine position without a given breathing rhythm after the morning visit to the restroom. The OT was performed with continuous beat-to-beat recording of the heart rate (RR measurement with V800 and H10 sensor, Polar Electro GmbH, Finland, Gilgen-Ammann et al., 2019). The calculated parameters of the OT included HR and the vagal time-domain HRV parameter RMSSD in supine and standing positions as well as the peak HR (HRpeak) and time to HR peak (tHRpeak) in the standing position. Daily the athlete recorded his training and recorded information about his state of health, especially about symptoms of illness.

## Data Analysis and Statistics

The calculation of the RR data from the OT was performed with the Kubios HRV Premium Software (Version 3.4.1, Tarvainen et al., 2014). The RMSSD was calculated for 2 min in the supine and 2 min in standing position. The first of the 3 min in the supine position was not used for data analysis but served for physiological stabilization (Bourdillon et al., 2017; Hottenrott and Hoos, 2017). The smallest worthwhile change (SWC) in standing RMSSD from baseline was deemed as 0.5 of the individual baseline coefficient variation (CV) (Plews et al., 2013; Buchheit, 2014) from the baseline during average training load

at sea level in a healthy state over 3 month prior to the viral infection (as a fixed reference point). Differences in HR, RMSSD, HRpeak, and tHRpeak between the measure points (days) were evaluated. Statistical analysis was performed with the software SPSS 25.0 (IBM Statistics, USA) for Windows. Prior to the analysis of the differences between 10 days before, during, and 10 days after the upper respiratory tract infection (URTI), Gaussian distribution of the data was verified by the Shapiro–Wilk test. A single factor repeated measures ANOVA was used to test whether there were statistical differences between the mean values of the respective parameters over the three measurement points (pre, URTI, post). For statistical analysis, ANOVA with *post-hoc* multiple comparisons and Bonferroni correction was applied. The sphericity was determined in advance using the Mauchly test. The data are presented as mean ± standard deviation. The level of significance was set at 0.05 ($p < 0.05$).

## RESULTS

The athlete maintained his average training load of 14–21 km of easy to moderate running per day on days 1–11. He had 1 day of rest on day 9. The athlete was absent from training on day 12–20 and restarted training on day 21 with an easy 10 km run (aerobic) followed by 2 days of easy running training of 8–12 km, on day 24, he completed 20 km of easy running, and on day 25, he did no running but did 3 h of biking. The ANOVA analysis shows significant main time effects for HR supine [$F_{(2.10)} = 21.913$, $p < 0.001$, partial $\eta^2 = 0.814$], HR standing [$F_{(2.10)} = 47.253$, $p < 0.001$, partial $\eta^2 = 0.904$], HRpeak [$F_{(2.10)} = 22.586$, $p < 0.001$, partial $\eta^2 = 0.819$], tHRpeak [$F_{(2.10)} = 38.261$, $p < 0.001$, partial $\eta^2 = 0.819$], RMSSD supine [$F_{(2.10)} = 5.625$, $p = 0.023$, partial $\eta^2 = 0.529$], and RMSSD standing [$F_{(2.10)} = 21.761$, $p < 0.001$, partial $\eta^2 = 0.813$]. *Post-hoc* comparisons showed that during the 6-day viral infection period, all values, except the RMSSD value in the supine position, changed significantly from the previous 10-day pre-measurement (**Table 1**). The HR increased by an average of 11 bpm in the supine position and by 22 bpm in the standing position, the HRpeak by 13 bpm, and the tHRpeak increased by 18 s. The RMSSD in the standing position decreased from 20.8 ms to 4.2 ms and showed the largest changes over the course of the viral infection days (**Table 1**; **Figure 2**).

The 10-day post-measurements differed significantly in all parameters on average from the 6-day viral infection period. The HR in the supine position decreased by 14 bpm, the HR in the standing position by 19 bpm, and the HRpeak by 18 bpm. The RMSSD values increased by 20 ms (supine) and 17 ms (standing), and the HR ascended faster again. The 10-day post RMSSD values in the supine and standing positions and the tHRpeak did not differ from the 10-day pre-measurements (**Figure 2**).

The graph in **Figure 2** displays that the RMSSD values were already decreasing days before the viral infection symptoms were felt. Training was then stopped when the viral infection symptoms (sore throat) appeared (day 12). Upon relief of the viral infection symptoms, RMSSD levels immediately increased. The athlete restarted his training (day 21) after the RMSSD values had approximately reached the initial values before the illness.

**Figure 3** shows the HR curves from OT for the 6-day viral infection period and a typical curve before and after the viral infection period. An increase in HR values in supine and standing positions as well as 2 days with extremely slow tHRpeaks (52 s and 59 s) were apparent, and counter-regulation was missing. On the days of the slow tHRpeak (days 4 and 5 of vthe iral infection), the HRpeak reached the highest values (122 bpm and 111 bpm).

## DISCUSSION

The present case study is the first to show the interaction between a viral infection and the daily monitoring of the cardiac autonomic control in an elite athlete. The case study is particularly unique due to the fact that the data are ecologically valid (not lab-based), and obtained from an elite endurance athlete.

The main finding is that a viral infection had a direct influence on HR and HRV. An increase in HR was accompanied by a decrease in RMSSD in the OT in the standing position. It seems that cardiac vagal activity is suppressed in the presence of a viral infection, which is also the case in patients with fever (Lin et al., 2006; Carter et al., 2014). An increase in resting HR and a decrease in RMSSD suggests a suppression of parasympathetic activity (Buchheit, 2014; Laborde et al., 2018). The kinetics of HR from supine to standing was significantly different in the HR increase (tHRpeak) from a typical course in a healthy condition. With the onset of the viral infection symptoms, there was a slower increase, which was particularly pronounced after 3 days of the 6-day period. The state of research according to changes in cardiac autonomic regulation during viral infection

**TABLE 1 |** Values (mean ± SD) of HR, RMSSD, HRpeak, and tHRpeak (time from HR in the supine position until HRpeak in the standing position) in the orthostatic test (OT) 10 days before, during, and 10 days after the upper respiratory tract infection (URTI) from a 30-year-old elite marathon runner.

| Position | Parameter | Pre<br>days 1–10 | URTI<br>days 11–16 | Post<br>days 17–26 | Pre-URTI<br>*p*-value | URTI-post<br>*p*-value | Pre-post<br>*p*-value |
|---|---|---|---|---|---|---|---|
| 2 min supine | HR [bpm] | 55.5 ± 2.8 | 66.0 ± 7.2 | 52.1 ± 3.2 | 0.013 | 0.013 | 0.070 |
| | RMSSD [ms] | 18.0 ± 9.2 | 11.5 ± 7.6 | 31.4 ± 20.3 | 0.595 | 0.005 | 0.65 |
| 2 min standing | HR [bpm] | 86.0 ± 4.6 | 107.7 ± 9.2 | 79.0 ± 6.0 | 0.001 | 0.002 | 0.178 |
| | RMSSD [ms] | 20.8 ± 5.7 | 4.2 ± 2.8 | 21.0 ± 7.9 | 0.002 | 0.041 | 0.307 |
| Supine-standing | HRpeak [bpm] | 93.4 ± 4.5 | 106.3 ± 9.4 | 88.4 ± 4.7 | 0.012 | 0.010 | 0.184 |
| | tHRpeak [s] | 16.8 ± 2.2 | 35.0 ± 16.2 | 18.9 ± 3.5 | 0.003 | 0.008 | 0.124 |

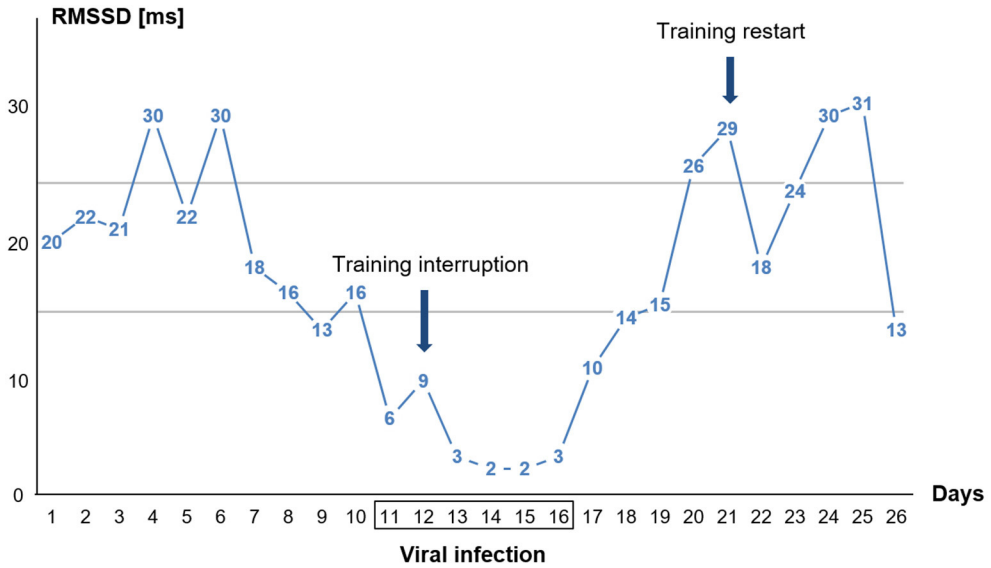



**FIGURE 2 |** Single day values for time-domain heart rate variability (HRV) parameter RMSSD in the standing position of the orthostatic test (OT) before, during, and after the viral infection. The athlete had fever on days 13, 14, and 15. The two horizontal lines indicate the area of the individual smallest worthwhile change (SWC) in standing RMSSD during baseline (see section Methods).

strengthen the evidence that specific measures of HRV are valid indicators of cardiac autonomic responsiveness (Malik et al., 2019). Bellenger et al. (2016c) found a slower HR acceleration at the onset of exercise in athletes suffering from exercise-induced fatigue (overload training). A reduced performance capacity of the athletes and altered cardiac autonomic control due to fatigue may also apply to a viral infection. In the context of viral diseases, HR increase in regard to a change in body position has not been investigated so far. A small number of studies conducted with endurance athletes demonstrated a decrease in HR acceleration following overload training, indicating it may be a potential indicator of training-induced fatigue (Nelson et al., 2014; Bellenger et al., 2016a).

The second main result is that HR and HRV values changed more substantially in the standing position than in the supine position during viral infection. This was particularly pronounced in the RMSSD values, which decreased from 20 ms before to 4 ms during viral infection. The cardiac autonomic system reacted more sensitively in the standing position compared to the supine position in the present data. The RMSSD changed significantly from pre to viral infection values in the standing position only (**Table 1**). The analysis of the single day values for the RMSSD in the standing position shows that the athlete stopped training when the flu symptoms appeared. However, according to the HRV analysis, a training suspension 2–3 days earlier might have been preferable to avoid weakening the immune system further (**Figure 2**). The return to training was reasonably chosen after the HRV values had returned to the initial level and were elevated for several days. The further fluctuations of the post-values of the 6-day viral infection period are related to the training process. Moderate aerobic training (low-intensity training) leads to better values in RMSSD compared to pre-values due to positive effects on vagal activity (increases in parasympathetic activity) (Stanley et al., 2013). The reason for the strong decrease in RMSSD values on day 26 could be the more intensive training the day before (training phases with increased time spent at high intensity suppress parasympathetic activity) (Buchheit and Gindre, 2006; Plews et al., 2014; Schneider et al., 2019). This might indicate that the performance capacity and health status level for high training stimuli was not yet given.

## LIMITATIONS

We chose morning resting HRV recordings due to the practicality of the measurement and with a focus on the vagal HRV parameter RMSSD because this parameter provides valid results in longitudinal analysis without a given breathing rate in the context of training and health status, which cannot be guaranteed using frequency-domain analysis of HRV (e.g., low frequency and high frequency power) without a given breathing control (Nakamura et al., 2015).

Furthermore, it could be shown that RMSSD determined in a short period of already 1 min (calculation of 1 min after 1 min stabilization period) is sensitive to training-induced changes in athletes, and can be used to track cardiac autonomic adaptations (Nakamura et al., 2015). In regard to OT, Schäfer et al. (2015) found no differences using 2 min intervals compared to 4 min intervals in both supine and standing positions for monitoring training and recovery processes. Therefore, the chosen procedure of a 3-min recording time in supine and standing positions with analyzed intervals of 2 min respectively should provide representative values of HR and RMSSD.

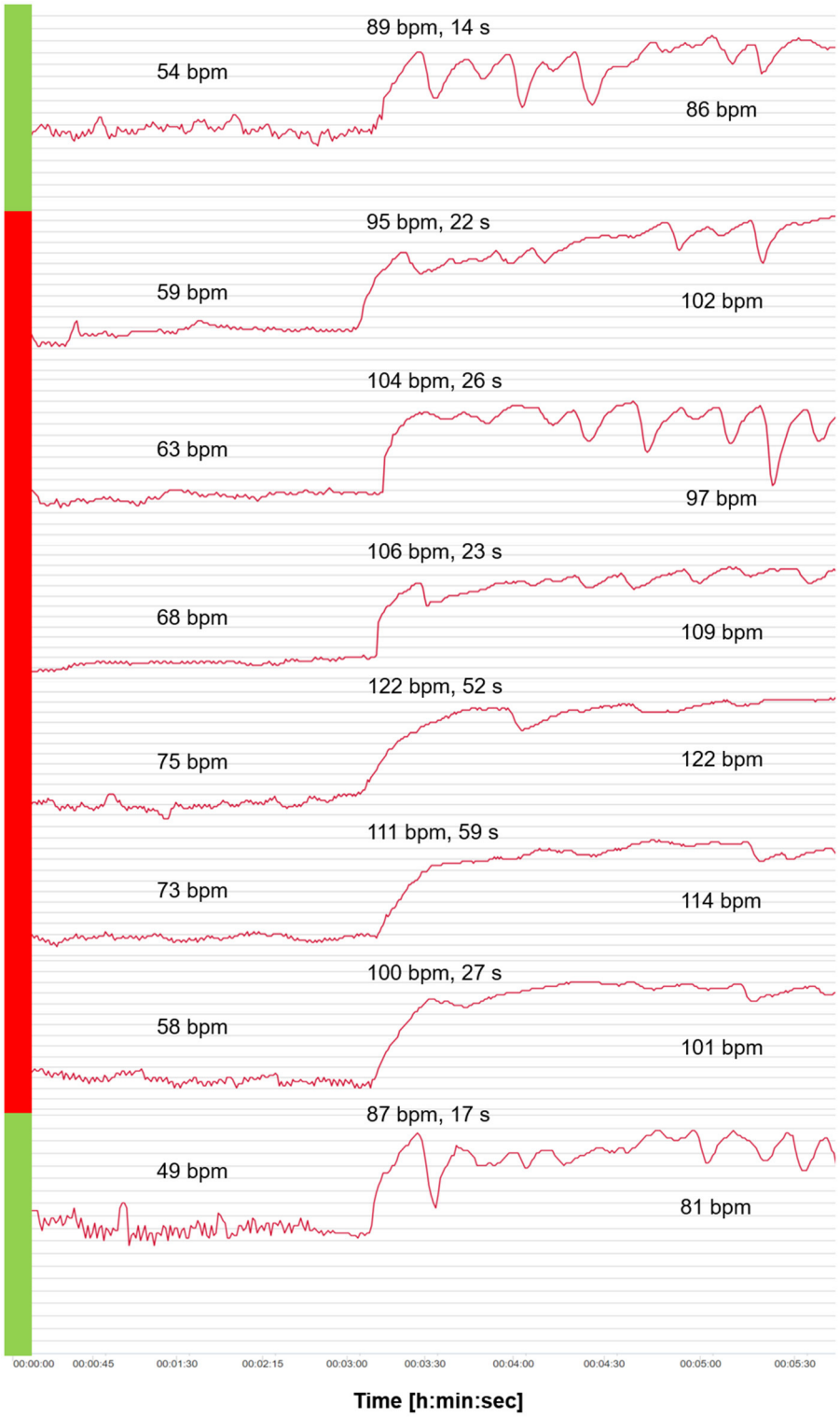



**FIGURE 3 |** Heart rate (HR) curves from the orthostatic test (OT) for the 6-day viral infection period (red) and a curve before and after the viral infection period (green). Mean values of HR analyzed after 2 min in the supine position, and HRpeak, tHRpeak, and mean values of HR analyzed after 2 min in the standing position.

## CONCLUSION

The findings of this case report have some implications for sports practitioners and coaches looking to both ensure the health of their athletes, and for using HRV as a tool to monitor training process and the return to sport after a viral infection. For endurance athletes, a control by means of resting HR in one body position does not seem to be sufficient. Accordingly, the data have provided supportive rationale as to why the OT with a change from supine to standing body position and the detection of different indicators based on HR and a vagal driven time-domain HRV parameter (RMSSD) is likely to be useful to detect viral diseases early on when implemented in a daily routine. Given the case study nature of the findings, future research has to be conducted to investigate whether the use of the OT might be able to offer an innovative, non-invasive, and time-efficient possibility to detect and evaluate the health status of (elite endurance) athletes.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

LH: conceptualization, methodology, investigation, writing—original draft preparation, and project administration. LH, KH, and TG: validation. LH and KH: data curation and visualization. LH, KH, TG, TW, and AF: writing—review and editing. AF: supervision. All authors have read and agreed to the published version of the manuscript.

## FUNDING

We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-Universität Bochum.

## REFERENCES

Aubert, A. E., Seps, B., and Beckers, F. (2003). Heart rate variability in athletes. *Sports Med.* 33, 889–919. doi: 10.2165/00007256-200333120-00003

Barrero, A., Le Cunuder, A., Carrault, G., Carr,é, F., Schnell, F., and Le Douairon Lahaye, S. (2020). Modeling stress-recovery status through heart rate changes along a cycling grand tour. *Front. Neurosci.* 14:576308. doi: 10.3389/fnins.2020.576308

Bellenger, C. R., Fuller, J. T., Thomson, R. L., Davison, K., Robertson, E. Y., and Buckley, J. D. (2016a). Monitoring athletic training status through autonomic heart rate regulation: a systematic review and meta-analysis. *Sports Med*. 46, 1461–1486. doi: 10.1007/s40279-016-0484-2

Bellenger, C. R., Karavirta, L., Thomson, R. L., Robertson, E. Y., Davison, K., and Buckley, J. D. (2016b). Contextualizing parasympathetic hyperactivity in functionally overreached athletes with perceptions of training tolerance. *Int. J. Sport Physiol. Perform.* 11, 685–692. doi: 10.1123/ijspp.2015-0495

Bellenger, C. R., Thomson, R. L., Howe, P. R., Karavirta, L., and Buckley, J. D. (2016c). Monitoring athletic training status using the maximal rate of heart rate increase. *J. Sci Med. Sport* 19, 590–595. doi: 10.1016/j.jsams.2015.07.006

Bourdillon, N., Schmitt, L., Yazdani, S., Vesin, J. M., and Millet, G. P. (2017). Minimal window duration for accurate HRV recording in athletes. *Front. Neurosci.* 11:456. doi: 10.3389/fnins.2017.00456

Buchheit, M. (2014). Monitoring training status with HR measures: do all roads lead to Rome? *Front Physiol.* 5:73. doi: 10.3389/fphys.2014.00073

Buchheit, M., and Gindre, C. (2006). Cardiac parasympathetic regulation: respective associations with cardiorespiratory fitness and training load. *Am. J. Physiol. Heart Circ. Physiol.* 291, 451–458. doi: 10.1152/ajpheart.00008.2006

Buchhorn, R., Baumann, C., and Willaschek, C. (2020). Heart rate variability in a patient with coronavirus disease 2019. *Int. Cardiovasc. Forum J.* 20:2020050209. doi: 10.20944/preprints202005.0209.v1

Carter, I. I. I., R., Hinojosa-Laborde, C., and Convertino, V. A. (2014). Heart rate variability in patients being treated for dengue viral infection: new insights from mathematical correction of heart rate. *Front. Physiol.* 5:46. doi: 10.3389/fphys.2014.00046

Cowan, L. T., Lutsey, P. L., Pankow, J. S., Matsushita, K., Ishigami, J., and Lakshminarayan, K. (2018). Inpatient and outpatient infection as a trigger of cardiovascular disease: the ARIC study. *J. Am. Heart Assoc.* 7:e009683. doi: 10.1161/JAHA.118.009683

Dores, H., and Cardim, N. (2020). Return to play after COVID-19: a sport cardiologist's view. *Br. J. Sports Med.* 54, 1132–1133. doi: 10.1136/bjsports-2020-102482

Flachenecker, P. (2000). *Klinische Standarduntersuchungen autonomer Funktionen-Parasympathikusfunktionen. Jörg J: Autonome Diagnostik und Schlafpolygraphie in Klinik und Praxis*. Darmstadt: Steinkopff.

Friman, G., and Wesslén, L. (2000). Infections and exercise in high-performance athletes. *Immunol. Cell Biol.* 78, 510–522. doi: 10.1111/j.1440-1711.2000.t01-12-.x

Gilgen-Ammann, R., Schweizer, T., and Wyss, T. (2019). RR interval signal quality of a heart rate monitor and an ECG Holter at rest and during exercise. *Eur. J. Appl. Physiol.* 119, 1525–1532. doi: 10.1007/s00421-019-04142-5

Hagen, J., Stone, J. D., Hornsby, W. G., Stephenson, M., Mangine, R., Joseph, M., et al. (2020). COVID-19 surveillance and competition in sport: utilizing sport science to protect athletes and staff during and after the pandemic. *J. Funct. Morphol. Kinesiol.* 5:69. doi: 10.3390/jfmk5030069

Halle, M., Binzenhofer, L., Mahrholdt, H., Schindler, J. M., Esefeld, K., and Tschope, C. (2020). Myocarditis in athletes: a clinical perspective. *Eur. J. Prev. Cardiol.* 28, 1050–1057. doi: 10.1177/2047487320909670

Harmon, K. G., Asif, I. M., Maleszewski, J. J., Owens, D. S., Prutkin, J. M., Salerno, J. C., et al. (2016). Incidence and etiology of sudden cardiac arrest and death in high school athletes in the United States. *Mayo Clin. Proc.* 91, 1493–1502. doi: 10.1016/j.mayocp.2016.07.021

Hottenrott, K., and Hoos, O. (2017). "Heart rate variability analysis in exercise physiology," in *ECG Time Series Variability Analysis: Engineering and Medicine*, eds H. F. Jelinek, D. J. Cornforth, and A. H. Khandoker (Boca Raton, FL: CRC Press), 249–279.

Hottenrott, L., Hottenrott, K., and Ketelhut, S. (2019). Commentary: vagal tank theory: the three rs of cardiac vagal control functioning–resting, reactivity, and recovery. *Front. Neurosci.* 13:1300. doi: 10.3389/fnins.2019.01300

Karjalainen, J., and Viitasalo, M. (1986). Fever and cardiac rhythm. *Arch. Intern. Med.* 146, 1169–1171. doi: 10.1001/archinte.1986.00360180179026

Laborde, S., Mosley, E., and Mertgen, A. (2018). A unifying conceptual framework of factors associated to cardiac vagal control. *Heliyon* 4:e01002. doi: 10.1016/j.heliyon.2018.e01002

Le Meur, Y., Pichon, A., Schaal, K., Schmitt, L., Louis, J., Gueneron, J., et al. (2013). Evidence of parasympathetic hyperactivity in functionally overreached athletes. *Med. Sci. Sports Exerc*. 45, 2061–2071. doi: 10.1249/MSS.0b013e3182980125

Lin, M. T., Wang, J. K., Lu, F. L., Wu, E. T., Yeh, S. J., Lee, W. L., et al. (2006). Heart rate variability monitoring in the detection of central nervous system complications in children with enterovirus infection. *J. Crit. Care* 21, 280–286. doi: 10.1016/j.jcrc.2006.02.005

Madjid, M., Safavi-Naeini, P., Solomon, S. D., and Vardeny, O. (2020). Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol.* 5, 831–840. doi: 10.1001/jamacardio.2020.1286

Malik, M., Hnatkova, K., Huikuri, H. V., Lombardi, F., Schmidt, G., and Zabel, M. (2019). CrossTalk proposal: heart rate variability is a valid measure of cardiac autonomic responsiveness. *J. Physiol.* 597:2595. doi: 10.1113/JP277500

Nakamura, F. Y., Flatt, A. A., Pereira, L. A., Ramirez-Campillo, R., Loturco, I., and Esco, M. R. (2015). Ultra-short-term heart rate variability is sensitive to training effects in team sports players. *J. Sci. Med. Sport.* 14:602.

Nelson, M. J., Thomson, R. L., Rogers, D. K., et al. (2014). Maximal rate of increase in heart rate during the rest-exercise transition tracks reductions in exercise performance when training load is increased. *J. Sci. Med. Sport.* 17, 129–133. doi: 10.1016/j.jsams.2013.02.016

Nieß, A. M., Bloch, W., Friedmann-Bette, B., Grim, C., Halle, M., Hirschmüller, A., et al. (2020). Position stand: return to sport in the current coronavirus pandemic. *Ger. J. Sport Med*. 71, 1–4. doi: 10.5960/dzsm.2020.437

Plews, D. J., Laursen, P. B., Kilding, A. E., and Buchheit, M. (2014). Heart-rate variability and training-intensity distribution in elite rowers. *Int. J. Sports Physiol. Perform.* 9, 1026–1032. doi: 10.1123/ijspp.2013-0497

Plews, D. J., Laursen, P. B., Stanley, J., Kilding, A. E., and Buchheit, M. (2013). Training adaptation and heart rate variability in elite endurance athletes: opening the door to effective monitoring. *Sports Med.* 43, 773–781. doi: 10.1007/s40279-013-0071-8

Polak, S. B., Van Gool, I. C., Cohen, D., Jan, H., and van Paassen, J. (2020). A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. *Modern Pathol.* 33, 2128–2138. doi: 10.1038/s41379-020-0603-3

Ravé, G., and Fortrat, J. O. (2016). Heart rate variability in the standing position reflects training adaptation in professional soccer players. *Eur. J. Appl. Physiol.* 116, 1575–1582. doi: 10.1007/s00421-016-3416-9

Roberts, J. A. (1986). Viral illnesses and sports performance. *Sports Med.* 3, 296–303. doi: 10.2165/00007256-198603040-00006

Rooke, T. W., and Sparks, H. V. (2003). "Control mechanisms in circulatory function," in *George AT*: *Medical physiology*, ed A. R. Rodney (Philadelphia, PA: Lippincott Williams and Wilkins), 290–308.

Schäfer, D., Olstad, B. H., and Wilhelm, M. (2015). Can heart rate variability segment length during orthostatic test be reduced to 2 min?: 227 Board# 78 May 27, 1100 AM-1230 PM. *Med. Sci. Sports Exerc.* 47:48. doi: 10.1249/01.mss.0000476531.84848.dd

Schmitt, L., Regnard, J., and Millet, G. P. (2015). Monitoring fatigue status with HRV measures in elite athletes: an avenue beyond RMSSD? *Front. Physiol.* 6:343. doi: 10.3389/fphys.2015.00343

Schneider, C., Hanakam, F., Wiewelhove, T., Döweling, A., Kellmann, M., Meyer, T., et al. (2018). Heart rate monitoring in team sports—a conceptual framework for contextualizing heart rate measures for training and recovery prescription. *Front. Physiol.* 9:639. doi: 10.3389/fphys.2018.00639

Schneider, C., Wiewelhove, T., Raeder, C., Flatt, A. A., Hoos, O., Hottenrott, L., et al. (2019). Heart rate variability monitoring during strength and high-intensity interval training overload microcycles. *Front. Physiol.* 10:582. doi: 10.3389/fphys.2019.00582

Schnellhorn, P., Klingel, K., and Burgstahler, C. (2020). Return to sports after COVID-19 infection. Do we have to worry about myocarditis? *Eur. Heart J.* 41, 4382–4384. doi: 10.1093/eurheartj/ehaa448

Stanley, J., Peake, J. M., and Buchheit, M. (2013). Cardiac parasympathetic reactivation following exercise: implications for training prescription. *Sports Med.* 43, 1259–1277. doi: 10.1007/s40279-013-0083-4

Stewart, J. M., Medow, M. S., Glover, J. L., and Montgomery, L. D. (2006). Persistent splanchnic hyperemia during upright tilt in postural tachycardia syndrome. *Am. J. Physiol. Heart Circ. Physiol.* 290, H665–H673. doi: 10.1152/ajpheart.00784.2005

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Meth. Prog. Bio.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Tulppo, M. P., Hughson, R. L., Mäkikallio, T. H., Airaksinen, K. J., Seppänen, T., and Huikuri, H. V. (2001). Effects of exercise and passive head-up tilt on fractal and complexity properties of heart rate dynamics. *Am. J. Physiol. Heart Circ. Physiol.* 280, H1081–H1087. doi: 10.1152/ajpheart.2001.280.3.H1081

Uusitalo, A. L., Uusitalo, A. J., and Rusko, H. K. (2000). Heart rate and blood pressure variability during heavy training and overtraining in the female athlete. *Int. J. Sports Med.* 21, 45–53. doi: 10.1055/s-2000-8853

Verwoert, G. C., de Vries, S. T., Bijsterveld, N., Willems, A. R., vd Borgh, R., Jongman, J. K., et al. (2020). Return to sports after COVID-19: a position paper from the Dutch Sports Cardiology Section of the Netherlands Society of Cardiology. *Neth. Heart J.* 28, 391–395. doi: 10.1007/s12471-020-01469-z

World Health Organization (2020). *Coronavirus Disease (COVID-19) Pandemic*. Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed September 25, 2020).

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 07 September 2021
doi: 10.3389/fnins.2021.612712

# Morality of the Heart: Heart Rate Variability and Moral Rule Adherence in Men

*Alexander Lischke[1,2]*, Matthias Weippert[3], Anett Mau-Moeller[3] and Rike Pahnke[3]**

*[1] Department of Psychology, Medical School Hamburg, Hamburg, Germany, [2] Department of Psychology, University of Greifswald, Greifswald, Germany, [3] Department of Sport Science, University of Rostock, Rostock, Germany*

Moral rules are a cornerstone of many societies. Most moral rules are concerned with the welfare of other individuals, reflecting individuals' innate aversion against harming other individuals. Harming others is associated with aversive experiences, implying that individuals who are sensitive to the aversiveness of these experiences are more likely to follow moral rules than individuals who are insensitive to the aversiveness of these experiences. Individuals' sensitivity for aversive experiences depends on individuals' ability to integrate the underlying neural and physiological processes: Individuals who are more efficient in integrating these processes are more sensitive to the aversiveness that is associated with moral rule violations than individuals who are less efficient in integrating these processes. Individuals who differ in their ability to integrate these processes may, thus, also differ in their inclination to follow moral rules. We tested this assumption in a sample of healthy individuals (67 males) who completed measures of moral rule adherence and integration abilities. Moral rule adherence was assessed with self-report measure and integration abilities were assessed with a resting state measure of heart rate variability (HRV), which reflects prefrontal–(para-)limbic engagement during the integration of physical and neural processes. We found a positive association between individuals' HRV and individuals' moral rule adherence, implying that individuals with efficient integration abilities were more inclined to follow moral rules than individuals with inefficient integration abilities. Our findings support the assumption that individuals with different integration abilities also differ in moral rule adherence, presumably because of differences in aversiveness sensitivity.

**Keywords: moral reasoning, moral decision making, idealism, harm avoidance, vagal tone**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer, The Ohio State University, United States*

***Reviewed by:***
*DeWayne P. Williams, University of California, Irvine, United States*
*Gewnhi Park, Hope College, United States*

****Correspondence:***
*Alexander Lischke*
*alexander.lischke@medicalschool-hamburg.de*
*Rike Pahnke*
*rike.pahnke@gmx.de*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *30 September 2020*
***Accepted:*** *25 June 2021*
***Published:*** *07 September 2021*

***Citation:***
*Lischke A, Weippert M, Mau-Moeller A and Pahnke R (2021) Morality of the Heart: Heart Rate Variability and Moral Rule Adherence in Men. Front. Neurosci. 15:612712. doi: 10.3389/fnins.2021.612712*

## INTRODUCTION

Across industrialized and non-industrialized societies, individuals share a common set of moral rules. Most moral rules are concerned with the welfare of others (Haidt and Joseph, 2004), which may be a reflection of individuals' innate aversion against harming others (Hamlin et al., 2007, 2010). Regardless whether individuals imagine or perform harmful actions, they experience aversive emotional reactions that are accompanied by corresponding processes on the neural and physiological level (Cushman et al., 2012; Decety et al., 2012). The aversiveness of these experiences depends on individuals' ability to integrate the corresponding neural and physiological processes (Critchley, 2005). Individuals who are efficient in integrating these processes are more

sensitive to the aversiveness of harmful actions than individuals who are inefficient in integrating these processes (Cushman et al., 2012; Decety et al., 2012). As a consequence, individuals with efficient integration abilities are more motivated to avoid aversive experiences that are associated with moral rule violations than individuals with inefficient integration abilities (Decety and Cowell, 2014), implying more moral rule adherence in individuals with efficient than inefficient integration abilities.

Individuals' integration abilities can be differentiated on the basis of individuals' heart rate variability (HRV), which serves as a proxy for the interplay of prefrontal and (para-)limbic brain regions during the integration of neural and physiological processes (Smith et al., 2017). Individuals with higher HRV are generally more efficient in integrating these processes than individuals with lower HRV, implying that individuals with higher HRV are also more efficient in integrating neural and physiological processes that are associated with aversive experiences. These differences in integration abilities may render individuals with higher HRV more sensitive to the aversiveness of others' harm than individuals with lower HRV. Individuals with higher HRV are, in fact, more responsive to others' needs and more concerned with others' welfare than individuals with lower HRV (Kogan et al., 2014; Stellar et al., 2015; Lischke et al., 2018a), presumably because individuals with higher HRV are more empathetic and less alexithymic than individuals with lower HRV (Lischke et al., 2017, Lischke et al., 2018b). Due to these differences in aversiveness sensitivity, individuals with higher HRV may be more inclined to follow moral rules than individuals with lower HRV.

Following this notion, we performed an exploratory study where we investigated the association between HRV and moral rule adherence in a sample of healthy individuals. In the absence of previous studies on HRV and moral rule adherence, we felt obliged to provide a concise description rather than a complex explanation of the association between HRV and moral rule adherence. We, thus, tested whether HRV was associated with moral rule adherence and refrained from testing whether this association would be moderated or mediated by aversiveness sensitivity. This allowed us to analyze the association between HRV and moral rule adherence in a clear and simple manner, thereby avoiding issues arising from the use of more complex analyses (e.g., overfitting in structural equation models).

As we were interested to investigate the association between HRV and moral rule adherence in a clear and simple manner, we made arrangements to reduce the complexity of the study design. Given that male and female individuals differ in moral rule adherence and HRV (Abhishekh et al., 2013; Friesdorf et al., 2015), we only included male individuals in our investigation. We, thus, did not have to control for sex- or menstrual cycle-related differences in moral rule adherence and HRV in our analyses. To reduce the number of possible analyses to a minimum, we used a limited set of measures for the assessment of moral rule adherence and HRV. HRV was assessed with a resting state measure of high-frequency HRV (HF-HRV). HF-HRV measures the integration of neurophysiological processes that are associated with an empathic reaction to others' harm (Kogan et al., 2014; Stellar et al., 2015; Lischke et al., 2018b), implying that this may also be the case during violations of moral rules that are concerned with others' welfare. Moral rule adherence was assessed with a self-report measure that differentiated between moral idealism and moral relativism (Forsyth, 1980). Whereas moral idealism is characterized by strict rule following that precludes the violation of moral rules, moral relativism is characterized by flexible rule following that allows the violation of moral rules (Forsyth, 1980). Given that moral idealism reflects moral rule following to a greater extent than moral relativism, we expected individuals' HRV to be associated with individuals' moral idealism rather than with individuals' moral relativism.

# MATERIALS AND METHODS

## Participants

According to an *a priori* power analysis with G*Power[1] , we had to investigate a minimum of 67 individuals to be able to detect medium- to large-sized associations between HRV and moral rule adherence in a series of dimensional and categorical analyses [$1 - \beta = 0.80$, $\alpha = 0.05$, $f^2 = 0.20$, and $f = 0.35$]. In order to be included in the study, individuals had to be males with an age range of 18 to 35 years. Individuals who were in psychotherapeutic or psychopharmacological treatment were excluded from the study. Inclusion and exclusion of individuals was determined on the basis of an in-house interview that assessed individuals' demographic (age and sex), anthropometric (height and weight), and health (physical activity, psychotherapeutic treatment, and psychopharmacological treatment) characteristics. Of the 67 individuals who had been recruited for the study, 3 individuals had to be excluded because they were in psychotherapeutic treatment. The final sample, thus, comprised 64 instead of 67 individuals (see **Table 1**). However, all individuals had provided written informed consent to the study protocol that was approved by the ethics committee of the University of Rostock and that was carried out in accordance with the Declaration of Helsinki.

## Procedure

We followed an established procedure that has been described in more detail elsewhere (Lischke et al., 2017, Lischke et al., 2018a,b). At the beginning of the experimental session, individuals were asked to use the bathroom to control for the effects of bladder filling and gastric distension on individuals' HRV. Thereafter, individuals were seated in a comfortable chair and prepared for a 5-min lasting resting state heart rate (HR) recording. Individuals' HR was recorded with a polar watch (RS800, Polar Electro, Oy, Kempele, Finland) that allowed an accurate assessment of consecutive changes in heartbeats. The consecutive changes in heartbeats were later used for the determination of individuals' HRV. During the HR recording, individuals were instructed to sit still, to breathe spontaneously, and to keep their eyes open. After the HR recording, individuals had to complete a self-report

[1]http://www.gpower.hhu.de

**TABLE 1 |** Sample characteristics.

| | *M* | SEM |
|---|---|---|
| Age (years) | 23.91 | 0.49 |
| Body mass index (kg/m$^2$) | 23.76 | 0.28 |
| Activity (h/week) | 7.66 | 0.49 |
| Moral relativism (EPQ-IDE) | 5.98 | 0.11 |
| Moral idealism (EPQ-REL) | 5.68 | 0.14 |
| Heart rate variability (HF-HRV, ms$^2$)[a] | 2.73 | 0.05 |
| Heart rate variability (RMSSD, ms)[a] | 1.59 | 0.02 |

*EPQ-REL, Ethical Position Questionnaire—Moral relativism (Forsyth, 1980); EPQ-IDE, Ethical Position Questionnaire—Moral idealism (Forsyth, 1980); HF-HRV, (log-transformed) high-frequency heart rate variability (Shaffer and Ginsberg, 2017); and RMSSD, (log-transformed) root mean square of successive differences between consecutive heart beats (Shaffer and Ginsberg, 2017).*
[a] *Data were missing for one participant due to a recording error.*

measure of moral rule adherence (Ethical Position Questionnaire, EPQ; Forsyth, 1980). At the end of the experimental session, individuals were debriefed and dismissed.

## Heart Rate Variability

Kubios HRV 2.2 (Tarvainen et al., 2014) was used to determine individuals' HRV on the basis of the HR recordings. The HR recordings were detrended (smoothn priors: $\lambda = 500$) and, if necessary, artifact corrected (adaptive filtering: cubic spine interpolation) before they were subjected to a spectral analysis (Fast Fourier Transformation) and a time domain analysis. The spectral analysis was used to determine the HRV measure of interest: HF-HRV (0.15–0.4 Hz). HF-HRV was the HRV measure of interest because HF-HRV tracks the integration of neurophysiological processes that are associated with an empathic reaction to others' harm (Kogan et al., 2014; Stellar et al., 2015; Lischke et al., 2018b), indicating that HF-HRV reflects aversive reactions to violations of others' welfare (see **Supplementary Material 1**). The time domain analysis was used for the determination of a HRV measure that tracks similar processes as HF-HRV (Shaffer and Ginsberg, 2017): the root mean square of successive differences between consecutive heart beats (RMSSD). This HRV measure was used to determine whether possible associations between HF-HRV and moral rule adherence would generalize across different HRV measures.

## Moral Rule Adherence

The EPQ (Forsyth, 1980) was used to determine individuals' moral rule adherence in terms of moral idealism and moral relativism. The self-report measure comprises 20 items, 10 items that assess moral idealism [$\alpha = 0.64$] and 10 items that assess moral relativism [$\alpha = 0.73$]. Whereas moral idealism refers to moral rule adherence in terms of strict rule following that precludes the violation of moral rules in all circumstances, moral relativism refers to moral rule adherence in terms of flexible rule following that allows the violation of moral rules in some circumstances. Given that moral rule adherence is motivated by the concern about others' welfare (Haidt and Joseph, 2004), it is not surprising that moral idealism (i.e., strict rule following) rather than moral relativism (i.e., flexible rule following) is associated with aversive reactions to violations of others' welfare (see **Supplementary Material 2**).

## Statistical Analysis

In line with recent recommendations (Laborde et al., 2017), dimensional and categorical analyses were performed to investigate the association between HRV and moral rule adherence in the sample of individuals. Combining dimensional and categorical analyses allowed a cross-validation of the respective findings, thereby providing a robustness check for any conclusions that were based on the findings of a particular analysis. For the dimensional analysis, hierarchical regression analyses were run to investigate whether HRV was associated with moral rule adherence among all individuals. For the categorical analyses, analyses of covariance (ANCOVAs) were run to investigate whether moral rule adherence differed between individuals who had been assigned to a high and low HRV group on the basis of a median-split. For both types of analyses, HRV was log-transformed (log 10) to account for deviations from normality. Age, body mass index, and physical activity were under statistical control in these analyses because these characteristics may affect the association between individuals' HRV and individuals' moral rule adherence (De Meersman, 1993; Abhishekh et al., 2013; Koenig et al., 2014). To facilitate the interpretation of the analyses (Cohen, 1992; Cumming, 2014), significance values ($p$) and effect size measures ($\eta^2$, $B$, $R^2$, and $\Delta R^2$) were determined. All analyses were performed with SPSS 24 (SPSS Inc., Chicago, IL, United States).

# RESULTS

## Association Between Moral Relativism (EPQ-REL) and Heart Rate Variability (HF-HRV)

A hierarchical regression analysis was run to investigate the association between HF-HRV and moral relativism among all individuals. Entering individuals' age, body mass index, and physical activity in a first step into the regression model did not explain any variance in individuals' moral relativism [$R^2 = 0.02$, $F(3, 59) = 0.49$, and $p = 0.693$; see **Table 2**]. Age, body mass index, and physical activity were not associated with moral relativism [all $B \leq |0.05|$, all $t(59) \leq |1.20|$, and all $p \geq 0.233$; see **Table 2**]. Entering individuals' HF-HRV in a second step into the regression model also explained no variance in moral relativism [$\Delta R^2 = 0.01$, $\Delta F(1, 58) = 0.38$, and $p = 0.538$; see **Table 2**]. HF-HRV was, similar to age, body mass index, and physical activity [all $B \leq |0.04|$, all $t(58) \leq |1.07|$, and all $p \geq 0.287$; see **Table 2**], not associated with moral relativism [$B = 0.24$, $t(58) = 0.62$, and $p = 0.538$; see **Table 2** and **Figure 1**]. A subsequent ANCOVA revealed that moral relativism was equally pronounced among individuals with higher and lower HF-HRV [$F(1,58) = 0.31$, $p = 0.581$, and $\eta^2 = 0.005$; see **Figure 2**], thereby confirming the absence of an association between individuals' HF-HRV and individuals' moral relativism. Repeating the analyses with RMSSD instead of HF-HRV revealed exactly the same findings

**TABLE 2 |** Association of moral idealism (EPQ-IDE) or moral relativism (EPQ-REL) with heart rate variability (HF-HRV).

| | Moral idealism (EPQ-IDE) | | | | | Moral relativism (EPQ-REL) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | ***B*** | **SE B** | ***t*** | ***p*** | **Model 2** | ***B*** | **SE B** | ***t*** | ***p*** |
| ***Step 1*** | | | | | ***Step 1*** | | | | |
| Age (years) | −0.01 | 0.04 | −0.34 | 0.738 | Age (years) | 0.05 | 0.04 | 1.20 | 0.233 |
| Body mass index (kg/m$^2$) | 0.00 | 0.05 | −0.07 | 0.947 | Body mass index (kg/m$^2$) | −0.04 | 0.08 | −0.54 | 0.595 |
| Activity (h/week) | −0.01 | 0.03 | −0.39 | 0.699 | Activity (h/week) | 0.01 | 0.04 | 0.29 | 0.776 |
| *Step 2* | | | | | *Step 2* | | | | |
| Age (years) | −0.02 | 0.04 | −0.70 | 0.486 | Age (years) | 0.04 | 0.04 | 1.07 | 0.287 |
| Body mass index (kg/m$^2$) | 0.03 | 0.05 | 0.45 | 0.655 | Body mass index (kg/m$^2$) | −0.03 | 0.08 | −0.37 | 0.717 |
| Activity (h/week) | −0.03 | 0.03 | −0.95 | 0.347 | Activity (h/week) | 0.00 | 0.04 | 0.11 | 0.913 |
| Heart rate variability (HF-HRV, ms$^2$)[a] | 0.66 | 0.33 | 2.12 | 0.039* | Heart rate variability (HF-HRV, ms$^2$)[a] | 0.24 | 0.44 | 0.62 | 0.538 |

*Model 1: step 1: $R^2 = 0.01$, $F(3, 59) = 0.10$, $p = 0.959$, step 2: $\Delta R^2 = 0.07$, $\Delta F(1, 58) = 4.48$, $p = 0.039$*; model 2: step 1: $R^2 = 0.02$, $F(3, 59) = 0.49$, $p = 0.693$, step 2: $\Delta R^2 = 0.01$, $\Delta F(1, 58) = 0.38$, $p = 0.538$.*

*EPQ-REL, Ethical Position Questionnaire—Moral relativism (Forsyth, 1980); EPQ-IDE, Ethical Position Questionnaire—Moral idealism (Forsyth, 1980); and HF-HRV, (log transformed) high-frequency heart rate variability (Shaffer and Ginsberg, 2017).*

[a] *Data were missing for one participant due to a recording error.*

* $p \leq 0.05$.

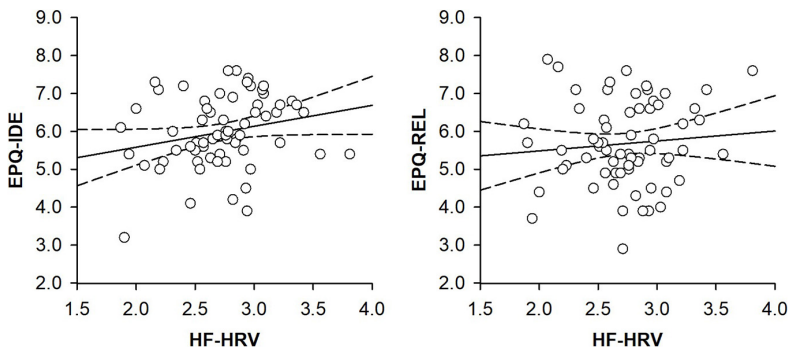



**FIGURE 1 |** Scatter plots with lines of best fit and 95% confidence intervals demonstrating associations between (log-transformed) heart rate variability (HF-HRV) and moral idealism (EPQ-IDE) or moral relativism (EPQ-REL) among all individuals.

(see **Supplementary Material 3**), indicating that the findings generalize across different HRV measures (HF-HRV, RMSSD).

## Association Between Moral Idealism (EPQ-IDE) and Heart Rate Variability (HF-HRV)

Another hierarchical regression analysis was run to investigate the association between HF-HRV and moral idealism among all individuals. Entering individuals' age, body mass index, and physical activity in a first step into the regression model explained no variance in individuals' moral idealism [$R^2 = 0.01$, $F(3, 59) = 0.10$, and $p = 0.959$; see **Table 2**]. Age, body mass index and physical activity were not associated with moral idealism [all $B \leq |-0.01|$, all $t(59) \leq |-0.39|$, and all $p \geq 0.699$; see **Table 2**]. Entering individuals' HF-HRV in a second step into the regression model explained 7% of the variance in individuals' moral idealism [$\Delta R^2 = 0.07$, $\Delta F(1, 58) = 4.48$, and $p = 0.039$; see **Table 2**]. Whereas age, body mass index, and physical activity remained to be unassociated with moral idealism [all $B \leq |-0.03|$, all $t(58) \leq |-0.95|$, and all $p \geq 0.347$; see **Table 2**], HF-HRV turned out to be associated with moral idealism [$B = 0.66$, $t(58) = 2.12$, and $p = 0.039$; see **Table 2** and **Figure 1**]. A subsequent ANCOVA showed that moral idealism was more pronounced among individuals with higher than lower HF-HRV [$F(1,58) = 5.11$, $p = 0.028$, and $\eta^2 = 0.081$, see **Figure 2**], thereby confirming the existence of a positive association between individuals' HF-HRV and individuals' moral idealism. The positive association between individuals' HF-HRV and individuals' moral idealism remained unchanged when controlling for individuals' moral realism in the analyses [$\Delta R^2 = 0.07$, $\Delta F(1, 57) = 4.16$, $p = 0.046$; $B = 0.65$, $t(57) = 2.04$, and $p = 0.046$; see **Table 3**]. Repeating the analyses with RMSSD instead of HF-HRV revealed similar findings (see **Supplementary Material 4**), indicating that the findings generalize across different HRV measures (HF-HRV, RMSSD).

## DISCUSSION

In the present study, we investigated whether individuals' ability to integrate neural and physiological processes was associated

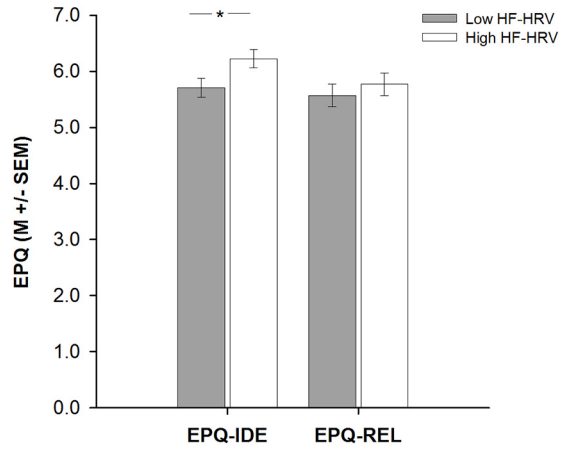



**FIGURE 2 |** Bar plots demonstrating differences in moral idealism (EPQ-IDE) or moral relativism (EPQ-REL) between individuals with lower and higher heart rate variability (HF-HRV). Bars represent M ± SEM. *$p \leq 0.05$.

**TABLE 3 |** Association of moral idealism (EPQ-IDE) with heart rate variability (HF-HRV) under control of moral realism (EPQ-REL).

| | Moral Idealism (EPQ-IDE) | | | |
|---|---|---|---|---|
| **Model 1** | ***B*** | **SE B** | ***t*** | ***p*** |
| *Step 1* | | | | |
| Age (years) | −0.02 | 0.04 | −0.47 | 0.640 |
| Body mass index (kg/m$^2$) | 0.00 | 0.06 | 0.00 | 0.997 |
| Activity (h/week) | −0.01 | 0.03 | −0.42 | 0.675 |
| Moral relativism (EPQ-REL) | 0.10 | 0.11 | 0.90 | 0.373 |
| *Step 2* | | | | |
| Age (years) | −0.03 | 0.04 | −0.80 | 0.428 |
| Body mass index (kg/m$^2$) | 0.03 | 0.06 | 0.48 | 0.631 |
| Activity (h/week) | −0.03 | 0.04 | −0.96 | 0.343 |
| Moral relativism (EPQ-REL) | 0.08 | 0.11 | 0.75 | 0.454 |
| Heart rate variability (HF-HRV, ms$^2$)[a] | 0.65 | 0.35 | 2.04 | 0.046* |

*Note. Model 1: step 1: $R^2 = 0.02$, $F(3, 58) = 0.28$, $p = 0.892$, step 2: $\Delta R^2 = 0.07$, $\Delta F(1, 57) = 4.16$, $p = 0.046$*.*
*EPQ-REL, Ethical Position Questionnaire—Moral relativism (Forsyth, 1980); EPQ-IDE, Ethical Position Questionnaire—Moral idealism (Forsyth, 1980); and HF-HRV, (log-transformed) high-frequency heart rate variability (Shaffer and Ginsberg, 2017).*
[a] *Data were missing for one participant due to a recording error.*
*$p \leq 0.05$.

with individuals' tendency to follow moral rules. Individuals' integration abilities were determined on the basis of a HRV measure that served as a proxy for the interplay of prefrontal and (para-)limbic brain regions during the regulation of neural and physiological processes (Smith et al., 2017). Individuals' tendency to follow moral rules was assessed with a self-report measure that differentiated between moral idealism and moral relativism (Forsyth, 1980). Applying these measures to a sample of healthy individuals, we found an association between HRV and moral idealism but no association between HRV and moral relativism: moral idealism was more pronounced among individuals with higher than lower HRV, whereas moral relativism was equally pronounced among individuals with higher and lower HRV. These findings emerged in a series of complementary analyses, which helped to ascertain the robustness of the observed associations. To understand these associations, we have to consider that moral idealism and moral relativism refer to distinct but overlapping aspects of moral rule following. Whereas moral idealism refers to strict rule following that precludes the violation of moral rules in all circumstances, moral relativism refers to flexible rule following that allows the violation of moral rules in some circumstances. We considered the conceptual overlap of moral idealism and moral relativism in our analyses and still found an association between moral idealism and HRV. Our findings, thus, show that individuals with higher HRV follow moral rules to a greater extent (i.e., in all circumstances) than individuals with lower HRV. Given that differences in HRV reflect differences in neurophysiological integration (Smith et al., 2017), our findings support the assumption that individuals with higher integration abilities show more moral rule adherence than individuals with lower integration abilities.

Whether individuals with efficient and inefficient integration abilities follow moral rules may depend on their sensitivity for aversive experiences that are associated with real or imagined violations of moral rules (Cushman et al., 2012; Decety et al., 2012). Individuals whose psychological traits render them aversiveness sensitive, like, for example, empathetic individuals (Conway and Gawronski, 2013; Reynolds and Conway, 2018), are more inclined to follow moral rules than individuals whose psychological traits render them aversiveness insensitive, like, for example, alexithymic individuals (Koven, 2011; Patil and Silani, 2014). These differences in moral rule adherence are even more pronounced in individuals who show abnormal representations of these psychological traits, like, for, example, autistic or psychopathic individuals (Koenigs et al., 2012; Patil et al., 2016). However, individuals who differ in empathy, alexithymia, autism, or psychopathy also seem to differ in their ability to integrate neural and physiological processes as suggested by the respective differences in individuals' HRV (Hansen et al., 2007; Kuiper et al., 2017; Lischke et al., 2018b). It may, thus, be possible that differences in individuals' integration abilities contribute to differences in individuals' aversiveness sensitivity that lead to differences in individuals' moral rule adherence. Considering that individuals' HRV reflect differences in individuals' integration abilities (Smith et al., 2017), we assume that individuals with lower HRV showed more moral rule adherence than individuals with higher HRV because of differences in individuals' aversiveness sensitivity for moral rule violations.

Individuals with efficient integration abilities are more successful in engaging prefrontal and (para-)limbic brain regions for the regulation of neural and physiological processes than individuals with inefficient integration abilities (Critchley, 2005), indicating that differences in prefrontal–(para-)limbic engagement may account for differences in aversiveness sensitivity between individuals with efficient and inefficient integration abilities. However, differences in

prefrontal–(para-)limbic engagement may account not only for differences in individuals' aversiveness sensitivity but also for differences in individuals' moral rule adherence because naturally occurring or experimentally induced alterations in these brain regions impair individuals' integration abilities as well as individuals' moral rule adherence (Mendez and Shapira, 2009; Moretto et al., 2010; Tassy et al., 2012). Individuals with abnormal representations of empathy, alexithymia, autism, and psychopathy also show alterations in prefrontal and (para-)limbic brain regions that are associated with impairments in aversiveness sensitivity and moral rule adherence (Silani et al., 2008; Decety et al., 2013), indicating that overlapping networks of prefrontal and (para-)limbic brain regions are implicated in the integration of neural and physiological processes that are relevant for the experience of aversiveness in the context of moral rule violations (Decety and Cowell, 2014). The interplay of prefrontal and (para-)limbic brain regions can be assessed on the basis of individuals' HRV (Smith et al., 2017), suggesting that differences in individuals' HRV reflect differences in individuals' integration abilities that are due to differences in prefrontal–(para-)limbic engagement. We, thus, assume that individuals with higher HRV showed more moral rule adherence than individuals with lower HRV because individuals with higher HRV were more efficient in engaging prefrontal and (para-)limbic brain regions for the integration of neurophysiological processes that account for the aversiveness of moral rule violations than individuals with lower HRV.

Although these assumptions appear to be plausible, we think that the assumptions have to be validated in further studies that follow a less exploratory approach than the present study. These studies should investigate the association between individuals' HRV and individuals' moral rule adherence with more complex measures and in more diverse samples than the present study. The present study investigated this association in a homogenous sample of male individuals, leaving open whether similar associations would emerge in heterogeneous samples that include male and female individuals. Given that female individuals have a higher HRV and a higher moral rule adherence than male individuals (Abhishekh et al., 2013; Friesdorf et al., 2015), the association between HRV and moral rule adherence may be more pronounced among female than male individuals. Future studies that include male and female individuals may help to determine whether this is the case. These studies should also include male and female individuals with a more diverse background in their investigation to determine whether the proposed associations between individuals' HRV and individuals' moral rule adherence generalize across different populations (e.g., including individuals with different ages or ethnicities). The present study explored this association with a combination of physiological and self-report measures. Although these measures allowed us to describe the association between HRV and moral rule adherence in a concise manner, they did not allow us to provide a complex explanation of this association. Neural, physiological, and behavioral measures that may have helped to provide such an explanation were not employed (e.g., task-based measures of moral rule adherence and aversiveness sensitivity and imaging-based measures of neurophysiological integration). We were, thus, unable to probe the psychological and neurobiological mechanisms underlying the association between HRV and moral rule adherence (e.g., testing the mediating or moderating role of aversiveness sensitivity). These mechanisms may involve the neurophysiological integration of aversive reactions to violations of others' welfare, but whether this is in fact the case remains to be determined. Studies that combine neural, physiological, and behavioral measures in their investigation may be more successful in elucidating the psychological and neurobiological mechanisms of HRV and moral rule adherence than the present study (e.g., combining task-based measures of moral rule adherence and aversiveness sensitivity with imaging-based measures of neurophysiological integration). Studies that manipulate these mechanisms with appropriate methods may help to make causal inferences about the association between HRV and moral rule adherence (e.g., increasing or decreasing HRV with brain stimulation techniques), thereby providing first insights into intervention programs for individuals who have difficulties in moral rule following (e.g., HRV biofeedback training for individuals with psychopathy). We hope that our exploratory study opened an avenue for these types of studies.

To sum up, we found a positive association between HRV and moral rule adherence in a sample of healthy individuals. Individuals with higher HRV showed more moral rule adherence than individuals with lower HRV. We assume that the differences in individuals' moral rule adherence were due to differences in individuals' aversiveness sensitivity for moral rule violations that were determined by differences in individuals' prefrontal–(para-)limbic engagement during the integration of neurophysiological processes. As we based these assumptions on the findings of previous studies (Decety and Cowell, 2014; Smith et al., 2017), we encourage researchers to validate these assumptions in further studies. Together, these studies will provide important insights into the psychological and neurobiological mechanisms underlying the association between individuals' HRV and individuals' moral rule adherence. Whether these studies will help to develop treatment interventions for individuals who have difficulties in moral rule adherences has to be seen in the future.

## DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of ethical restrictions. Requests to access the datasets should be directed to AL.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Rostock. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

AL and RP designed the study, analyzed the data, and wrote the manuscript. AL, AM-M, and MW collected the data. AM-M and MW contributed to writing, reviewing, and editing of the manuscript. All authors approved the final version of the manuscript.

## FUNDING

Funding for this study was provided by a grant from the German Research Foundation to AL (DFG; LI 2517/2-1). The funding source had no further role in study design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

## ACKNOWLEDGMENTS

The authors would like to thank Stephan Lau for valuable comments on an earlier version of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.612712/full#supplementary-material

## REFERENCES

Abhishekh, H. A., Nisarga, P., Kisan, R., Meghana, A., Chandran, S., Trichur, R., et al. (2013). Influence of age and gender on autonomic regulation of heart. *J. Clin. Monit. Comput.* 27, 259–264. doi: 10.1007/s10877-012-9424-3

Cohen, J. (1992). A power primer. *Psychol. Bull.* 112, 155–159.

Conway, P., and Gawronski, B. (2013). Deontological and utilitarian inclinations in moral decision making: a process dissociation approach. *J. Pers. Soc. Psychol.* 104, 216–235. doi: 10.1037/a0031021

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *J. Comp. Neurol.* 493, 154–166. doi: 10.1002/cne.20749

Cumming, G. (2014). The new statistics: why and how. *Psychol. Sci.* 25, 7–29. doi: 10.1177/0956797613504966

Cushman, F., Gray, K., Gaffey, A., and Mendes, W. B. (2012). Simulating murder: the aversion to harmful action. *Emotion* 12, 2–7. doi: 10.1037/a0025071

De Meersman, R. E. (1993). Heart rate variability and aerobic fitness. *Am. Heart J.* 125, 726–731. doi: 10.1016/0002-8703(93)90164-5

Decety, J., Chen, C., Harenski, C., and Kiehl, K. A. (2013). An fMRI study of affective perspective taking in individuals with psychopathy: imagining another in pain does not evoke empathy. *Front. Hum. Neurosci.* 7:489. doi: 10.3389/fnhum.2013.00489

Decety, J., and Cowell, J. M. (2014). The complex relation between morality and empathy. *Trends Cogn. Sci.* 18, 337–339. doi: 10.1016/j.tics.2014.04.008

Decety, J., Michalska, K. J., and Kinzler, K. D. (2012). The contribution of emotion and cognition to moral sensitivity: a neurodevelopmental study. *Cereb. Cortex* 22, 209–220. doi: 10.1093/cercor/bhr111

Forsyth, D. R. (1980). A taxonomy of ethical ideologies. *J. Pers. Soc. Psychol.* 39:175. doi: 10.1037/0022-3514.39.1.175

Friesdorf, R., Conway, P., and Gawronski, B. (2015). Gender differences in responses to moral dilemmas: a process dissociation analysis. *Pers. Soc. Psychol. Bull.* 41, 696–713. doi: 10.1177/0146167215575731

Haidt, J., and Joseph, C. (2004). Intuitive ethics: how innately prepared intuitions generate culturally variable virtues. *Daedalus* 133, 55–66. doi: 10.1162/0011526042365555

Hamlin, J. K., Wynn, K., and Bloom, P. (2007). Social evaluation by preverbal infants. *Nature* 450, 557–559. doi: 10.1038/nature06288

Hamlin, J. K., Wynn, K., and Bloom, P. (2010). Three-month-olds show a negativity bias in their social evaluations. *Dev. Sci.* 13, 923–929. doi: 10.1111/j.1467-7687.2010.00951.x

Hansen, A. L., Johnsen, B. H., Thornton, D., Waage, L., and Thayer, J. F. (2007). Facets of psychopathy, heart rate variability and cognitive function. *J. Pers. Disord.* 21, 568–582. doi: 10.1521/pedi.2007.21.5.568

Koenig, J., Jarczok, M. N., Warth, M., Ellis, R. J., Bach, C., Hillecke, T. K., et al. (2014). Body mass index is related to autonomic nervous system activity as measured by heart rate variability-a replication using short term measurements. *J. Nutr. Health Aging* 18, 300–302. doi: 10.1007/s12603-014-0022-6

Koenigs, M., Kruepke, M., Zeier, J., and Newman, J. P. (2012). Utilitarian moral judgment in psychopathy. *Soc. Cogn. Affect. Neurosci.* 7, 708–714. doi: 10.1093/scan/nsr048

Kogan, A., Oveis, C., Carr, E. W., Gruber, J., Mauss, I. B., Shallcross, A., et al. (2014). Vagal activity is quadratically related to prosocial traits, prosocial emotions, and observer perceptions of prosociality. *J. Pers. Soc. Psychol.* 107, 1051–1063. doi: 10.1037/a0037509

Koven, N. S. (2011). Specificity of meta-emotion effects on moral decision-making. *Emotion* 11, 1255–1261. doi: 10.1037/a0025616

Kuiper, M. W. M., Verhoeven, E. W. M., and Geurts, H. M. (2017). Heart rate variability predicts inhibitory control in adults with autism spectrum disorders. *Biol. Psychol.* 128, 141–152. doi: 10.1016/j.biopsycho.2017.07.006

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research - Recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lischke, A., Lemke, D., Neubert, J., Hamm, A. O., and Lotze, M. (2017). Inter-individual differences in heart rate variability are associated with inter-individual differences in mind-reading. *Sci. Rep.* 7:11557. doi: 10.1038/s41598-017-11290-1

Lischke, A., Mau-Moeller, A., Jacksteit, R., Pahnke, R., Hamm, A. O., and Weippert, M. (2018a). Heart rate variability is associated with social value orientation in males but not females. *Sci. Rep.* 8:7336. doi: 10.1038/s41598-018-25739-4

Lischke, A., Pahnke, R., Mau-Moeller, A., Behrens, M., Grabe, H. J., Freyberger, H. J., et al. (2018b). Inter-individual differences in heart rate variability are associated with inter-individual differences in empathy and alexithymia. *Front. Psychol.* 9:229. doi: 10.3389/fpsyg.2018.00229

Mendez, M. F., and Shapira, J. S. (2009). Altered emotional morality in frontotemporal dementia. *Cogn. Neuropsychiatry* 14, 165–179. doi: 10.1080/13546800902924122

Moretto, G., Ladavas, E., Mattioli, F., and di Pellegrino, G. (2010). A psychophysiological investigation of moral judgment after ventromedial prefrontal damage. *J. Cogn. Neurosci.* 22, 1888–1899. doi: 10.1162/jocn.2009.21367

Patil, I., Melsbach, J., Hennig-Fast, K., and Silani, G. (2016). Divergent roles of autistic and alexithymic traits in utilitarian moral judgments in adults with autism. *Sci. Rep.* 6:23637. doi: 10.1038/srep23637

Patil, I., and Silani, G. (2014). Reduced empathic concern leads to utilitarian moral judgments in trait alexithymia. *Front. Psychol.* 5:501. doi: 10.3389/fpsyg.2014.00501

Reynolds, C. J., and Conway, P. (2018). Not just bad actions: affective concern for bad outcomes contributes to moral condemnation of harm in moral dilemmas. *Emotion* 18, 1009–1023. doi: 10.1037/emo0000413

Shaffer, F., and Ginsberg, J. P. (2017). An overview of heart rate variability metrics and norms. *Front. Public Health* 5:258. doi: 10.3389/fpubh.2017.00258

Silani, G., Bird, G., Brindley, R., Singer, T., Frith, C., and Frith, U. (2008). Levels of emotional awareness and autism: an fMRI study. *Soc. Neurosci.* 3, 97–112. doi: 10.1080/17470910701577020

Smith, R., Thayer, J. F., Khalsa, S. S., and Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296. doi: 10.1016/j.neubiorev.2017.02.003

Stellar, J. E., Cohen, A., Oveis, C., and Keltner, D. (2015). Affective and physiological responses to the suffering of others: compassion and vagal activity. *J. Pers. Soc. Psychol.* 108, 572–585. doi: 10.1037/pspi0000010

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV–heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Tassy, S., Oullier, O., Duclos, Y., Coulon, O., Mancini, J., Deruelle, C., et al. (2012). Disrupting the right prefrontal cortex alters moral judgement. *Soc. Cogn. Affect. Neurosci.* 7, 282–288. doi: 10.1093/scan/nsr008

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 10 September 2021
doi: 10.3389/fphys.2021.678428

# Association of Short-Term Heart Rate Variability With Breast Tumor Stage

*Shuang Wu[1], Man Chen[1], Jingfeng Wang[2,3], Bo Shi[2,3]* and Yufu Zhou[1]**

[1]*Department of Radiation Oncology, First Affiliated Hospital, Bengbu Medical College, Bengbu, China,* [2]*School of Medical Imaging, Bengbu Medical College, Bengbu, China,* [3]*Anhui Key Laboratory of Computational Medicine and Intelligent Health, Bengbu Medical College, Bengbu, China*

Cardiac autonomic modulation, assessed by heart rate variability (HRV), is associated with tumor pathogenesis and development as well as invasion and metastasis. This study aimed to examine this association in breast cancer (BC) patients. A total of 133 patients (average age 49.2 years) with BC or benign breast tumors were divided into three groups: benign group, early-stage group, and advanced-stage group. About 5-min resting ECG was collected for the analysis of linear and nonlinear HRV parameters. Multiple logistic regression models were performed to test the independent contribution of HRV to breast tumor stage. The advanced-stage group had significantly reduced HRV compared to the benign and early-stage groups. In particular, for each 1-SD increase in SD2, SD of normal-to-normal intervals, very-low frequency, total power, and low frequency, the odds of having advanced staging decreased by 69.3, 64.3, 58.3, 53.3, and 65.9%, respectively. These associations were independent of age, body mass index, mean heart rate (HR), and respiratory rate (RR). These findings suggest an association between HRV and breast tumor stage, and HRV parameters may help construct an effective early diagnostic and clinical prognostic model.

**Keywords: autonomic modulation, breast tumors, heart rate variability, nonlinear dynamics, tumor-node-metastasis stages**

OPEN ACCESS

***Edited by:***
*Julian F. Thayer,*
*The Ohio State University,*
*United States*

***Reviewed by:***
*Yori Gidron,*
*UMR9193 Laboratoires Sciences Cognitives et Sciences Affectives (SCALab), France*
*Raquel Bailón,*
*University of Zaragoza, Spain*

****Correspondence:***
*Yufu Zhou*
*byyfyzyf@163.com*
*Bo Shi*
*shibo@bbmc.edu.cn*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Physiology*

***Received:*** *09 March 2021*
***Accepted:*** *18 August 2021*
***Published:*** *10 September 2021*

***Citation:***
*Wu S, Chen M, Wang J, Shi B and Zhou Y (2021) Association of Short-Term Heart Rate Variability With Breast Tumor Stage. Front. Physiol. 12:678428. doi: 10.3389/fphys.2021.678428*

## INTRODUCTION

Breast cancer (BC) is the most common cancer among women worldwide. In 2020, approximately 2.26 million new cases of BC were diagnosed. BC represents approximately 11.7% of all cancers and is the fifth leading cause of cancer deaths worldwide (680,000 deaths; Sung et al., 2021).

The vagus nerve, a major part of the parasympathetic nerve system, regulates the development and progression of cancer (De Couck and Gidron, 2013). Increased vagal nerve activity has a profound inhibitory effect on oxidative stress, DNA cell damage, inflammation, and sympathetic nervous system overreaction (Maki et al., 2006; Tsutsumi et al., 2007; Tracey, 2009; De Couck et al., 2012). Studies have demonstrated the bidirectional relationship between cancer and decreased vagal nerve activity (De Couck et al., 2018). Therefore, study of the vagus nerve could provide valuable prognostic information and guide therapy in breast cancer.

Heart rate variability (HRV) is a promising biomarker that can be used to evaluate autonomic nervous system function and it may be associated with vagal nerve function (Vanderlei et al., 2009; Karvinen et al., 2013). One group showed the clinical significance of HRV in patients with BC (Arab et al., 2016). However, few studies have explored the association of HRV with tumor-node-metastasis (TNM) in patients with BC.

In a previous study, patients with BC had lower the SD of all normal-to-normal intervals (SDNN) and the root mean square of successive interval differences (RMSSD) than women without BC, no matter how long after the surgery (Palma et al., 2016). In addition, Arab et al. (2018) found that SDNN and RMSSD negatively correlated with BC staging after analyzing time and frequency domains of HRV in patients. In patients with advanced-stage BC, low SDNN might be correlated with a poor prognosis. Kim et al. (2010) reported that an SDNN value of <21.3 ms in brain metastasis predicted poor survival, while Wang et al. (2013) found that an SDNN value of <10 ms in terminal-stage cancer predicted poor survival. These studies suggested a potential role of time-domain parameters of HRV as a prognostic factor. However, further research is warranted to clarify the potential role of time-domain parameters in the survival of patients with advanced-stage BC.

Previous studies indicated that higher resting high-frequency power (HF) was strongly associated with longer overall survival in patients with recurrent or metastatic BC (Giese-Davis et al., 2015). Chiang et al. (2010) found that the survival time of patients with terminal hepatocellular carcinoma was significantly related to HF. These studies suggested that, as a long-term predictor of survivors, HF may offer early estimation of clinical prognosis for cancer patients. More importantly, the results of these studies also indicated that HF strongly positively correlated with prognosis, particularly in patients with advanced-stage cancer. The vagal nerve activity might be of more importance in advanced stages.

Therefore, this study aimed to compare short-term HRV in the tumor stage of patients with BC. It was hypothesized that HRV in patients with advanced cancer would be lower than that in patients with early-stage disease. The analysis confirmed the aforementioned hypothesis and had clinical implications: HRV might be a potentially feasible tool in clinic to evaluate the prognosis of BC. More importantly, clinicians could ascertain patients at risk for disease progression through the long-term monitoring of HRV.

# MATERIALS AND METHODS

## Participants and Procedures

This study followed the regulations of the National Research Ethics Committee and obtained the approval of the Clinical Medical Research Ethics Committee of the First Affiliated Hospital of Bengbu Medical College (Bengbu, Anhui, China; registration number: 2019KY031). In this study, women diagnosed with breast tumors were selected by the pathological examination from 2019 to 2020. All participants volunteered for this study and provided informed consent.

The function of the vagal nervous system was assessed by analyzing HRV using an ECG recorder (HeaLink-R211B; HeaLink Ltd., Bengbu, China). The sampling rate of the ECG signal was 400 Hz. ECG data were collected at room temperature (23 ± 1°C) and always 3 days prior to radiotherapy/operation. Participants were explained the ECG collection procedure and were asked to assume the supine position and keep quiet during ECG examination. The ECG acquisition time using V5-lead was 5 min.

The following conditions that are known to alter HRV were used as exclusion criteria: (1) diabetes mellitus; (2) heart diseases; (3) use of anti-arrhythmic drugs or beta-blockers; (4) pacemaker; (5) poor ECG quality; (6) ectopic beats (>10% of all beats); and (7) chemotherapy or surgery in the 3 weeks before the examination. Therefore, our study analyzed the data of 133 participants.

## HRV Analysis

The ECG R peaks were extracted using an algorithm based on the Pan–Tompkins algorithm (Pan and Tompkins, 1985). The technical and physiological artifacts within R-R intervals (RRI) were corrected by applying an automatic artefact correction algorithm. Subsequent HRV indices for both linear (time and frequency domain) and nonlinear methods were calculated.

Commonly used time-domain indices include SDNN and RMSSD. SDNN, a total variability index, represents the involvement of all cyclic components. RMSSD reflects parasympathetic activity (Camm et al., 1996; Vanderlei et al., 2009).

The RRI time series was converted into power spectral analysis to analyze the frequency domain, and the power spectral density was obtained using the Fast Fourier Transform algorithm. Prior to frequency-domain analysis, the RRI time series was evenly resampled at 4 Hz using cubic spline interpolation. Frequency-domain parameters included total power (TP, 0–0.4 Hz), high-frequency power (HF, 0.15–0.4 Hz), low-frequency power (LF, 0.04–0.15 Hz), very-low frequency power (VLF, 0–0.04 Hz), and the ratio of LF to HF (LF/HF). LF and HF parameters were expressed in normalized units: normalized HF [HF n.u. = HF/(TP – VLF)] and normalized LF [LF n.u. = LF/(TP – VLF); Montano et al., 1994; Camm et al., 1996; Vanderlei et al., 2009].

The LF corresponds to the co-regulation of sympathetic and vagal nerve tones, HF indicates the vagal nerve tone, and the LF/HF reflects interactions of both sympathetic nervous system and parasympathetic nervous system, but they are limited to the case where the respiratory frequency is in the HF band (Hernando et al., 2016; Varon et al., 2019). The difference in respiratory rate (RR) will lead to the analysis of HRV in the standard frequency band cannot accurately estimate the activity of autonomic nervous system. For example, when the RR is higher than the upper limit of the HF band, vagus activity may be underestimated. In contrast, when the RR is within the LF band, sympathetic activity is overestimated and vagus activity is underestimated. Therefore, respiratory influences need to be separated in order to better estimate the activity of sympathetic and vagus nerves (Varon et al., 2019). An estimate of RR was calculated using an ECG-derived respiration approach

(Moody et al., 1985). It is important to check whether RR is below 0.15 Hz or higher than 0.4 Hz in all the enrolled subjects, in order to trust in the interpretation of LF and HF related indices.

Each RRI time series included eight nonlinear HRV indices, including approximate entropy (ApEn), sample entropy (SampEn), Poincare plot: SD1, SD2, and SD2/SD1, detrended fluctuation analysis (DFA): α1 and α2, and correlation dimension (CD). The estimated ApEn and SampEn depended on three parameters: the embedding dimension $m$, the tolerance value $r$, and the data length $N$. The parameters are set as $m = 2$ and $r = 0.2\sigma$, where $\sigma$ was the SD of each realization (Camm et al., 1996; Vanderlei et al., 2009; Voss et al., 2009; de Godoy, 2016).

All the above processing steps were performed using the Kubios HRV Premium software (version 3.1.0, Kubios Oy, Kuopio, Finland).[1]

## Breast Tumor Groups

Patients with breast tumors were divided into three groups: benign group, early-stage group, and advanced-stage group. Patients with benign breast tumors (i.e., benign epithelial proliferations, intraductal papilloma, phyllodes tumor, breast hyperplasia, and fibroadenomas) were selected as the controls. According to the National Comprehensive Cancer Network Clinical Practice Guidelines TNM staging version 3.2020 (Gradishar et al., 2020), the remaining participants were divided into early-stage and advanced-stage groups. The early-stage group consisted of T1–2, N0–1, and M0, and T3N0M0 cancers, while the advanced-stage group consisted of T0–4, any N, and M0–1 cancers. The non-advanced-stage group included controls and early-stage patients.

## Statistical Analysis

Descriptive statistical data were expressed as mean (SD), median (Q1, Q3), or percentage. The Shapiro–Wilk test was used to test the normality of HRV indices. A chi-square test was used to analyze the difference between the two cancer groups. Dependent variable analyses for linear and nonlinear HRV parameters were separately conducted using parametric and nonparametric tests. One-way ANOVA was used to calculate normal data and Fisher's least significant difference (LSD) was used for multiple comparisons between groups. The Kruskal–Wallis test was used to analyze non-normal data. Finally, separate multiple logistic regression models were performed with breast tumor stage as an outcome and with each significant HRV parameter set as a predictor while adjusted for age, body mass index (BMI), mean heart rate (HR), and RR. SPSS Statistics 25.0 (IBM Corp., Chicago, Illinois, United States) was used, and a value of $p < 0.05$ was considered statistically significant.

# RESULTS

**Table 1** presents the demographics and HRV indices of patients with breast tumors. In TNM staging, the early-stage group mainly comprised patients with stages T1–2, N0–1, and M0 cancers, while the advanced-stage group commonly comprised patients with stages T1–3, N2–3, and M0 cancers. Twelve patients in the advanced-stage group had distant metastases to the brain, bones, and lungs. Among patients with stages I–IV, the early-stage group mostly comprised stages Ia, IIa, and IIb; however, the advanced-stage group mostly comprised stage IIIa, IIIc, and IV. Invasive carcinoma with no special type is mostly frequent in patients with BC. The early-stage group had a more noninvasive type of BC compared to the advanced-stage group. The results of the molecular typing revealed no significant differences between the groups (**Table 2**).

**TABLE 1** | Demographics and heart rate variability (HRV) of breast tumor patients.

| Variables | Values |
|---|---|
| *N* (Female) | 133 |
| Age (years) | 49.2 (10.5) |
| BMI (kg/m$^2$) | 24.5 (3.6) |
| Mean HR (bpm) | 79.8 (11.5) |
| RR (Hz) | 0.31 (0.05) |
| SDNN (ms) | 28.8 (11.5) |
| RMSSD (ms) | 17.1 (10.3, 24.1) |
| VLF (ms$^2$) | 359 (167, 573) |
| LF (ms$^2$) | 116 (59, 260) |
| HF (ms$^2$) | 122 (55, 255) |
| TP (ms$^2$) | 640 (344, 1,072) |
| LF n.u. (%) | 53.1 (18.7) |
| HF n.u. (%) | 47.1 (18.7) |
| LF/HF | 1.102 (0.599, 2.114) |
| SD1 (ms) | 12.1 (7.3, 16.9) |
| SD2 (ms) | 38.1 (14.8) |
| SD2/SD1 | 3.392 (1.270) |
| ApEn | 1.126 (0.091) |
| SampEn | 1.441 (0.268) |
| $\alpha_1$ | 1.068 (0.263) |
| $\alpha_2$ | 1.070 (0.188) |
| CD | 0.494 (0.228, 0.974) |

*ApEn, approximate entropy; BMI, body mass index; bpm, beats per minute; CD, correlation dimension; RR, respiration rate; HR, heart rate; HF, high-frequency power; LF, low-frequency power; LF/HF, ratio of low-frequency power to high-frequency power; N, number of individuals; RMSSD, root mean square of successive interval differences; SampEn, sample entropy; SD, standard deviation; SDNN, SD of all normal-to-normal intervals; TP, total power; and VLF, very low frequency. Values are expressed as the number of patients, mean (SD), or median (Q1, Q3).*

Differences in SDNN ($p < 0.001$), RMSSD ($p = 0.006$), VLF ($p < 0.001$), LF ($p < 0.001$), HF ($p = 0.005$), TP ($p < 0.001$), SD1 ($p = 0.007$), SD2 ($p < 0.001$), and CD ($p < 0.001$) between groups were shown using one-way ANOVA and Kruskal–Wallis test. Furthermore, SDNN, RMSSD, VLF, LF, HF, TP, SD1, SD2, and CD were significantly decreased in the advanced-stage group compared to the corresponding values in the benign and early-stage groups. However, differences in HRV indices between the benign and early-stage groups were not significantly different. Moreover, there were no statistically significant differences in the LF n.u, HF n.u, LF/HF, SD2/SD1, ApEn, SampEn, $\alpha_1$, and $\alpha_2$ among the groups (**Table 3**).

With SDNN = 20 ms as the cutoff value, the subjects were divided into two subgroups: a low-SDNN subgroup (SDNN < 20 ms; $n = 31$) and a high-SDNN subgroup

[1]https://www.kubios.com

**TABLE 2 |** Clinical characteristics of breast cancer (BC) patients.

| | Early stage (*N* = 50) (%) | Advanced stage (*N* = 40) (%) | *p* |
|---|---|---|---|
| **TNM staging** | | | |
| T – primary tumor in greatest dimension | | | |
| T1 (≤20 mm) | 20 (40) | 11 (27.5) | **0.002** |
| T2 (>20 mm and ≤ 50) | 28 (56) | 15 (37.5) | |
| T3 (>50 mm) | 2 (4) | 9 (22.5) | |
| T4 (any size with extension to the chest wall and/or to the skin – ulceration or skin nodules) | 0 (0) | 5 (12.5) | |
| N – regional lymph nodes metastases | | | |
| N0 (none) | 35 (70) | 0 (0) | **<0.001** |
| N1 | 15 (30) | 5 (12.5) | |
| N2 | 0 (0) | 16 (40) | |
| N3 | 0 (0) | 19 (47.5) | |
| M – distant metastases | | | |
| M0 (no clinical or radiographic evidence) | 50 (100) | 28 (70) | **<0.001** |
| M1 (with distant detectable metastases) | 0 (0) | 12 (30) | |
| **I–IV staging** | | | |
| Ia – T1, N0, M0 | 16 (32) | 0 (0) | **<0.001** |
| Ib – T0–1, N1 micrometastases, M0 | 0 (0) | 0 (0) | |
| IIa – T0–1, N1, M0 or T2, N0, M0 | 21 (42) | 0 (0) | |
| IIb – T2, N1, M0 or T3, N0, M0 | 13 (26) | 0 (0) | |
| IIIa – T0–2, N2, M0 or T3, N1–2, M0 | 0 (0) | 13 (32.5) | |
| IIIb – T4, N0–2, M0 | 0 (0) | 3 (7.5) | |
| IIIc – T0–4, N3, M0 | 0 (0) | 12 (30) | |
| IV – T0–4, N0–3, M1 | 0 (0) | 12 (30) | |
| Type of breast cancer | | | |
| Noninvasive type | 11 (22) | 3 (7.5) | 0.169 |
| Invasive (no special type) | 38 (76) | 36 (90) | |
| Invasive (special type) | 1 (2) | 1 (2.5) | |
| Molecular typing | | | |
| Luminal A | 12 (24) | 10 (25) | 0.794 |
| Luminal B | 19 (38) | 14 (35) | |
| Her-2 overexpressing | 10 (20) | 11 (27.5) | |
| Triple negative | 9 (18) | 5 (12.5) | |

*Values are expressed as the number of individuals (percentages). Bold values of p indicate statistical significance (p < 0.05). Luminal A: ER/PR (+), HER2 (–), and ki-67 <14%. Luminal B: ER/PR (+), HER2 (–), and ki-67 ≥14%; ER/PR/HER2 (+), any level of ki-67. Her-2-overexpressing: ER/PR (–) and HER2 (+). Triple negative: ER/PR/HER2 (–).*

**TABLE 3 |** Comparison of HRV parameters among the benign, early-stage, and advanced-stage groups.

| Variables | Benign (*N* = 43) | Early stage (*N* = 50) | Advanced stage (*N* = 40) | *p* |
|---|---|---|---|---|
| Mean HR (bpm) | 78.9 ± 11.7 | 77.9 ± 10.5 | 83.0 ± 12.2 | 0.099 |
| RR (Hz) | 0.30 ± 0.04 | 0.31 ± 0.05 | 0.31 ± 0.06 | 0.489 |
| SDNN (ms) | 32.7 ± 9.3 | 29.3 ± 11.6 | 23.1 ± 11.6*,† | **<0.001** |
| RMSSD (ms) | 19.2 (13.8, 27.4) | 18.6 (12.2, 27.8) | 11.2 (8.0, 18.0)*,† | **0.006** |
| VLF ($ms^2$) | 443 (259, 674) | 410 (177, 604) | 199 (103, 355)*,† | **<0.001** |
| LF ($ms^2$) | 155 (102, 297) | 154 (59, 295) | 66 (37, 142)*,† | **<0.001** |
| HF ($ms^2$) | 151 (78, 300) | 151 (55, 270) | 60 (23, 136)*,† | **0.005** |
| TP ($ms^2$) | 903 (485, 1,147) | 781 (429, 1,190) | 351 (187, 619)*,† | **<0.001** |
| LF n.u. (%) | 52.7 ± 18.6 | 54.4 ± 18.9 | 52.0 ± 18.9 | 0.821 |
| HF n.u. (%) | 47.2 ± 18.5 | 46.4 ± 19.1 | 48.0 ± 18.9 | 0.927 |
| LF/HF | 1.214 (0.682, 2.030) | 1.401 (0.579, 2.213) | 0.952 (0.597, 2.165) | 0.852 |
| SD1 (ms) | 13.6 (9.8, 17.3) | 13.1 (8.7, 19.7) | 8.0 (5.7, 12.8)*,† | **0.007** |
| SD2 (ms) | 43.8 ± 12.7 | 39.6 ± 14.8 | 30.2 ± 13.8*,† | **<0.001** |
| SD2/SD1 | 3.444 ± 1.237 | 3.348 ± 1.358 | 3.391 ± 1.220 | 0.937 |
| ApEn | 1.10 ± 0.10 | 1.13 ± 0.10 | 1.15 ± 0.10 | 0.055 |
| SampEn | 1.40 ± 0.27 | 1.47 ± 0.27 | 1.46 ± 0.26 | 0.411 |
| $\alpha_1$ | 1.11 ± 0.26 | 1.07 ± 0.27 | 1.02 ± 0.25 | 0.355 |
| $\alpha_2$ | 1.05 ± 0.19 | 1.07 ± 0.18 | 1.09 ± 0.19 | 0.557 |
| CD | 0.73 (0.39, 1.05) | 0.56 (0.31, 1.69) | 0.20 (0.08, 0.57)*,† | **<0.001** |

*ApEn, approximate entropy; bpm, beats per minute; CD, correlation dimension; HF, high-frequency power; HR, heart rate; LF, low-frequency power; LF/HF, ratio of low-frequency power to high-frequency power; RMSSD, root mean square of successive interval differences; RR, respiration rate; SampEn, sample entropy; SD, standard deviation; SDNN, standard deviation of all normal-to-normal intervals; TP, total power; and VLF, very low frequency. Values are expressed as mean (SD) with normal distribution or median (Q1, Q3) without normal distribution. Bold values of p indicate statistical significance (value of p < 0.05).*
**p < 0.05 advanced-stage group vs. benign group.*
*†p < 0.05 advanced-stage group vs. early-stage group.*

(SDNN > 20 ms; $n = 102$). There was no statistically significant difference in age between the non-advanced and advanced groups (low-SDNN subgroup, $p = 0.311$; high-SDNN subgroup, $p = 0.218$; **Figure 1**).

To test the independent contribution of HRV to breast tumor stage, we performed multiple logistic regression models after adjusting for age, BMI, mean HR, and RR (**Table 4**). The associations of tumor stage with SD2, SDNN, VLF, TP, and LF were significant in logistic regression analysis. Specifically, for each 1-SD increase in SD2, SDNN, VLF, TP, and LF, the odds of having advanced staging decreased by 69.3% [odds ratio (OR): 0.307, 95% CI: (0.155, 0.606)], 64.3% [OR: 0.357, 95% CI: (0.183, 0.694)], 58.3% [OR: 0.417, 95% CI: (0.216, 0.807)], 53.3% [OR: 0.467, 95% CI: (0.239, 0.910)], and 65.9% [OR: 0.341, 95% CI: (0.130, 0.898)], respectively. The associations of tumor stage with CD, RMSSD, SD1, and HF were not significant in the logistic regression models.

## DISCUSSION

This study aimed to compare the HRV of patients with breast tumors (benign tumors, early-stage BC, and advanced-stage BC) and evaluate the feasibility of HRV as a tool for the early diagnosis and prognosis of BC patients. Our results revealed that patients with advanced-stage BC had lower HRV than those with benign tumors and early-stage BC. However, no statistically significant difference was observed in the HRV indices between the benign and early-stage groups. After adjusting for age, BMI, Mean HR, and RR, our results showed that SD2, SDNN, VLF, TP, and LF were associated with tumor stage.

The vagus nerve also called the wandering nerve, works *via* many neurotransmitters and plays an important role in multiple systems, such as cardiovascular, neuroendocrine, and

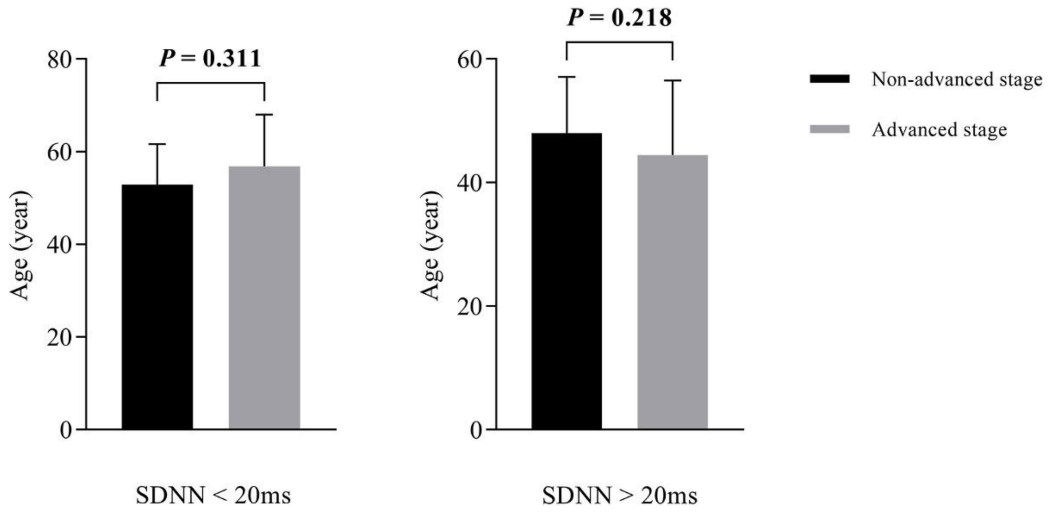



**FIGURE 1** | Subgroup analysis of age between non-advanced and advanced groups.

**TABLE 4** | Results from Logistic regression models (adjusted for age, BMI, Mean HR, and RR).

| Variables | OR | 95% CI for OR | | *p* |
|---|---|---|---|---|
| | | Lower | Upper | |
| SD2 (ms) | 0.307 | 0.155 | 0.606 | **0.001** |
| SDNN (ms) | 0.357 | 0.183 | 0.694 | **0.002** |
| VLF ($ms^2$) | 0.417 | 0.216 | 0.807 | **0.009** |
| TP ($ms^2$) | 0.467 | 0.239 | 0.910 | **0.025** |
| LF ($ms^2$) | 0.341 | 0.130 | 0.898 | **0.029** |
| CD | 0.599 | 0.326 | 1.099 | 0.098 |
| RMSSD (ms) | 0.848 | 0.492 | 1.462 | 0.553 |
| SD1 (ms) | 0.869 | 0.505 | 1.495 | 0.612 |
| HF ($ms^2$) | 1.000 | 0.632 | 1.583 | 1.000 |

*BMI, body mass index; CI, confidence interval; CD, correlation dimension; HF, high-frequency power; HR, heart rate; LF, low-frequency power; OR, odds ratio; RMSSD, root mean square of successive interval differences; RR, respiration rate; SD, standard deviation; SDNN, standard deviation of all normal-to-normal intervals; TP, total power; and VLF, very low frequency. Bold values of p indicate statistical significance (value of $p < 0.05$).*

immunological (Tracey, 2009). Studies have showed that the vagal nerve system transmits a variety of signals to the brain in order to restore the body to a steady state (Tracey, 2009; Ohira et al., 2013). Moreover, published preliminary studies have also demonstrated that vagal nerve tone is vital in the prognosis of cancer. A higher vagal nerve tone may protect cancer patients by reducing inflammation (De Couck et al., 2016). Measuring the HRV is a noninvasive approach of measuring vagal nerve activity (Vanderlei et al., 2009). HRV is known to be associated with vagal nerve tone ($r = 0.88$; Kuo et al., 2005). In recent years, various linear and nonlinear methods have been applied to analyze the time series of heartbeat cardiac intervals, reflecting the physiological and pathological information contained in the HRV signal from different angles.

Few studies have explored the association of HRV with TNM in patients with breast cancer. In one study, Mouton et al. (2012) examined the data of 72 patients with colorectal cancer and found that the baseline HRV could predict the carcinoembryonic antigen levels at 12 months. Moreover, they found that SDNN < 20 ms was associated with significantly higher CEA at 12 months. Arab et al. (2016) showed the clinical significance of HRV in patients with breast cancer.

Several studies indicated that higher resting HF was strongly associated with longer overall survival in patients with recurrent or metastatic BC (Giese-Davis et al., 2015). Chiang et al. (2010) found that the survival time of patients with terminal hepatocellular carcinoma was significantly related to HF. More importantly, the results of these studies also indicated that HF strongly positively correlated with prognosis, particularly in patients with advanced-stage cancer. However, despite the interesting results of our study, we did not find any statistically significant differences in the HF among the three cancer stage groups. The difference in mean HR and RR may cause HF to not accurately estimate the cardiac autonomic regulation activity. In our study, the difference was not statistically significant in mean HR and RR between the groups, and the RR of all the enrolled subjects were in the HF band. Therefore, the interpretation of spectral HRV indices as autonomic cardiac regulation markers in our study was more accurate and reliable. The findings of the present study could be verified through a prospective study performed over a longer follow-up period.

Similar to the above results, the median HF at baseline was 122 (IQR, 55, 255) $ms^2$ in our study, and the median HF values in benign, early-stage, and advanced-stage BC were 151 (78, 300), 151 (55, 270), and 60 (23, 136), respectively, indicating that a higher HF may be associated with benign or early-stage BC, and consequently a better survival rate. However, unlike Mouton et al. (2012), we used a cutoff of SDNN = 20 ms, and found that the SDNN showed a significant inverse association with tumor stage. A lower SDNN was found to be associated with more advanced-stage BC.

The interpretation of LF/HF is controversial. Malliani et al. (1991) showed that the LF/HF could reflect the balance between the vagus nerve and the sympathetic nerve. But LF/HF has been largely criticized as a marker of sympatho-vagal balance (Billman, 2011, 2013). First, LF power is not a pure index of sympathetic nerve, it may also be affected by vagus nerve and other unspecified factors. Second, sympathetic and vagus nerve can be simultaneously active, and their interaction is complex and nonlinear. Third, respiratory parameters and mechanical factors will also cause uncertainty in the contribution of sympathetic and vagus nerve to LF/HF. Finally, HR can affect LF/HF independently of cardiac autonomic nerve activity (Billman, 2013). In our study, no statistically significant difference was observed in LF/HF among the three cancer stage groups. This could be because of the complex physiological basis of LF/HF and the other unidentified factors.

Linear methods cannot be used to describe properly the complex nonlinear behavior, which is predominant in human systems. Therefore, it is necessary to search the novel indexes to reflect the correlation and the complexity characteristics of the HRV signal. The characteristic of HRV nonlinear analysis can better express the irregularity, complexity, and other dynamic characteristics of heartbeat fluctuations. Across various studies in the field of cardiovascular disorders, nonlinear dynamical HRV analysis is significantly superior to linear time-domain and frequency-domain methods (Mäkikallio et al., 2001; Stein et al., 2005; Voss et al., 2009; de Godoy, 2016). Some preliminary studies also explored the correlation between cancer and several nonlinear heartbeat dynamics measurements. Bettermann et al. (2001) showed that the variability, complexity, or rhythmicity of HRV in patients with BC was lower than that in patients with diabetes and age-matched healthy women. In particular, while comparing patients with and without BC metastasis, patients with metastasis had lower ApEn compared with those without metastasis. Shi et al. (2019) explored the perturbations of HRV nonlinear dynamical patterns to predict the increase in the severity of gastric cancer and found that nonlinear HRV parameters were the markers of autonomic nervous function to tumor progression.

Although, analysis of HRV by methods based on nonlinear dynamics do not reflect vagal or sympathetic regulation, we found significant correlations between time- and frequency domain indices and some of the nonlinear HRV parameters in patients with BC. For example, SampEn, $\alpha_1$, and CD correlated with RMSSD [SampEn ($r = 0.546$, $p < 0.001$), $\alpha_1$ ($r = -0.564$, $p < 0.001$), and CD ($r = 0.800$, $p < 0.001$)], and also correlated with HF [SampEn ($r = 0.521$, $p < 0.001$), $\alpha_1$ ($r = -0.530$, $p < 0.001$), and CD ($r = 0.792$, $p < 0.001$)]. This illustrates that the nonlinear parameters also contain the component of time-frequency domain index. The nonlinear analysis method is still in the preliminary exploration stage, and the exact physiological and pathological background has not been fully clarified. The findings of the current study might provide new evidence on the role of nonlinear HRV in cancer. Further studies are needed to clarify the correlation of nonlinear HRV as a long-term predictor of survival.

This study had some limitations. First, its cross-sectional study design was a major notable limitation. The correlation between HRV parameters and outcomes could not be inferred. Second, a comprehensive understanding of the connections of nonlinear HRV with BC prognosis is currently lacking. Third, more background variables, such as physical activity, stress levels, use of medications, and other relevant medical variables, could not be included. To address these limitations, studies with larger sample sizes, more detailed background variables, and a prospective design should be conducted to clarify the correlation of linear and nonlinear HRV parameters with BC prognosis.

## CONCLUSION

This novel study investigated linear and nonlinear HRV parameters in breast tumor groups. It found that the HRV was related to BC staging, indicating a correlation between tumor and HRV. The results of our study showed that patients with advanced-stage BC had lower HRV and might have a poor prognosis, and demonstrated that nonlinear HRV parameters might predict tumor staging in patients with breast tumors. Nonlinear approaches are of great significance in coping well with the nonstationary and nonlinear nature of heartbeat fluctuations. It is suggested that the combined measurement of linear and nonlinear HRV parameters may benefit future investigations. Researchers should identify a comprehensive biomarker for predicting BC prognosis by leveraging existing linear methods and nonlinear indicators. In addition to evaluating tumor stage, vagal nerve activity should be considered to estimate the prognosis of a cancer patient. Vagal nerve activity can be easily assessed and has the potential to provide healthcare professionals with incremental information based on the treatment plans. Future research should investigate the therapeutic potential of vagal nerve activation in cancer treatments through different supportive therapies such as relaxation, exercise interventions, and Traditional Chinese Medicine treatments (Niederer et al., 2013).

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

This study followed the regulations of the National Research Ethics Committee and obtained the approval of the Clinical Medical Research Ethics Committee of the First Affiliated Hospital of Bengbu Medical College, China. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

BS: conceptualization, resource allocation, and review and editing of the manuscript. SW and MC: data collection, interpretation of the results, and manuscript preparation. JW: data analysis. YZ: supervision and resource allocation. All authors contributed to the article and approved the submitted version.

## FUNDING

This study was funded by the "512" Outstanding Talents Fostering Project of Bengbu Medical College under Grant BY51201312, the Scientific Research Innovation Project of Bengbu Medical College under Grant BYKC201905, and the Students Scientific Research Innovation Project of Bengbu Medical College under Grant byycx20103.

## REFERENCES

Arab, C., Dias, D. P. M., Barbosa, R. T. A., Carvalho, T. D., Valenti, V. E., Crocetta, T. B., et al. (2016). Heart rate variability measure in breast cancer patients and survivors: a systematic review. *Psychoneuroendocrinology* 68, 57–68. doi: 10.1016/j.psyneuen.2016.02.018

Arab, C., Vanderlei, L. C. M., da Silva Paiva, L., Fulghum, K. L., Fristachi, C. E., Nazario, A. C. P., et al. (2018). Cardiac autonomic modulation impairments in advanced breast cancer patients. *Clin. Res. Cardiol.* 107, 924–936. doi: 10.1007/s00392-018-1264-9

Bettermann, H., Kröz, M., Girke, M., and Heckmann, C. (2001). Heart rate dynamics and cardiorespiratory coordination in diabetic and breast cancer patients. *Clin. Physiol.* 21, 411–420. doi: 10.1046/j.1365-2281.2001.00342.x

Billman, G. E. (2011). Heart rate variability–a historical perspective. *Front. Physiol.* 2:86. doi: 10.3389/fphys.2011.00086

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26. doi: 10.3389/fphys.2013.00026

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., et al. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European society of cardiology and the north American society of pacing and electrophysiology. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Chiang, J. K., Koo, M., Kuo, T. B. J., and Fu, C. H. (2010). Association between cardiovascular autonomic functions and time to death in patients with terminal hepatocellular carcinoma. *J. Pain Symptom Manag.* 39, 673–679. doi: 10.1016/j.jpainsymman.2009.09.014

De Couck, M., Caers, R., Spiegel, D., and Gidron, Y. (2018). The role of the vagus nerve in cancer prognosis: a systematic and a comprehensive review. *J. Oncol.* 2018, 1–11. doi: 10.1155/2018/1236787

De Couck, M., and Gidron, Y. (2013). Norms of vagal nerve activity, indexed by heart rate variability, in cancer patients. *Cancer Epidemiol.* 37, 737–741. doi: 10.1016/j.canep.2013.04.016

De Couck, M., Maréchal, R., Moorthamers, S., Laethem, J.-L. V., and Gidron, Y. (2016). Vagal nerve activity predicts overall survival in metastatic pancreatic cancer, mediated by inflammation. *Cancer Epidemiol.* 40, 47–51. doi: 10.1016/j.canep.2015.11.007

De Couck, M., Mravec, B., and Gidron, Y. (2012). You may need the vagus nerve to understand pathophysiology and to treat diseases. *Clin. Sci.* 122, 323–328. doi: 10.1042/cs20110299

de Godoy, M. F. (2016). Nonlinear analysis of heart rate variability: a comprehensive review. *J. Cardiol. Ther.* 3, 528–533. doi: 10.17554/j.issn.2309-6861.2016.03.101-4

Giese-Davis, J., Wilhelm, F. H., Tamagawa, R., Palesh, O., Neri, E., Taylor, C. B., et al. (2015). Higher vagal activity as related to survival in patients with advanced breast cancer. *Psychosom. Med.* 77, 346–355. doi: 10.1097/PSY.0000000000000167

Gradishar, W. J., Anderson, B. O., Abraham, J., Aft, R., Agnese, D., Allison, K. H., et al. (2020). Breast cancer, version 3.2020, NCCN clinical practice guidelines in oncology. *J. Natl. Compr. Cancer Netw.* 18, 452–478. doi: 10.6004/jnccn.2020.0016

Hernando, A., Lazaro, J., Gil, E., Arza, A., Garzon, J. M., Lopez-Anton, R., et al. (2016). Inclusion of respiratory frequency information in heart rate variability analysis for stress assessment. *IEEE J. Biomed. Health Inform.* 20, 1016–1025. doi: 10.1109/JBHI.2016.2553578

Karvinen, K. H., Murray, N. P., Arastu, H., and Allison, R. R. (2013). Stress reactivity, health behaviors, and compliance to medical care in breast cancer survivors. *Oncol. Nurs. Forum* 40, 149–156. doi: 10.1188/13.ONF.149-156

Kim, D. H., Kim, J. A., Choi, Y. S., Kim, S. H., Lee, J. Y., and Kim, Y. E. (2010). Heart rate variability and length of survival in hospice cancer patients. *J. Korean Med. Sci.* 25, 1140–1145. doi: 10.3346/jkms.2010.25.8.1140

Kuo, T. B., Lai, C. J., Huang, Y. T., and Yang, C. C. (2005). Regression analysis between heart rate variability and baroreflex-related vagus nerve activity in rats. *J. Cardiovasc. Electrophysiol.* 16, 864–869. doi: 10.1111/j.1540-8167.2005.40656.x

Maki, A., Kono, H., Gupta, M., Asakawa, M., Suzuki, T., Matsuda, M., et al. (2006). Predictive power of biomarkers of oxidative stress and inflammation in patients with hepatitis c virus-associated hepatocellular carcinoma. *Ann. Surg. Oncol.* 14, 1182–1190. doi: 10.1245/s10434-006-9049-1

Mäkikallio, T. H., Huikuri, H. V., Mäkikallio, A., Sourander, L. B., Mitrani, R. D., Castellanos, A., et al. (2001). Prediction of sudden cardiac death by fractal analysis of heart rate variability in elderly subjects. *J. Am. Coll. Cardiol.* 37, 1395–1402. doi: 10.1016/s0735-1097(01)01171-8

Malliani, A., Pagani, M., Lombardi, F., and Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84, 482–492. doi: 10.1161/01.CIR.84.2.482

Montano, N., Ruscone, T. G., Porta, A., Lombardi, F., Pagani, M., and Malliani, A. (1994). Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation* 90, 1826–1831. doi: 10.1161/01.CIR.90.4.1826

Moody, G., Mark, R., Zoccola, A., and Mantero, S. (1985). Derivation of respiratory signals from multi-lead ECGs. *Comput. Cardiol.* 12, 113–116.

Mouton, C., Ronson, A., Razavi, D., Delhaye, F., Kupper, N., Paesmans, M., et al. (2012). The relationship between heart rate variability and time-course of carcinoembryonic antigen in colorectal cancer. *Auton. Neurosci.* 166, 96–99. doi: 10.1016/j.autneu.2011.10.002

Niederer, D., Vogt, L., Thiel, C., Schmidt, K., Bernhörster, M., Lungwitz, A., et al. (2013). Exercise effects on HRV in cancer patients. *Int. J. Sports Med.* 34, 68–73. doi: 10.1055/s-0032-1314816

Ohira, H., Matsunaga, M., Osumi, T., Fukuyama, S., Shinoda, J., Yamada, J., et al. (2013). Vagal nerve activity as a moderator of brain–immune relationships. *J. Neuroimmunol.* 260, 28–36. doi: 10.1016/j.jneuroim.2013.04.011

Palma, M. R., Vanderlei, L. C. M., Ribeiro, F. E., Mantovani, A. M., Christofaro, D. G. D., and Fregonesi, C. E. P. T. (2016). The relationship between post-operative time and cardiac autonomic modulation in breast cancer survivors. *Int. J. Cardiol.* 224, 360–365. doi: 10.1016/j.ijcard.2016.09.053

Pan, J., and Tompkins, W. J. (1985). A real-time QRS detection algorithm. *IEEE Trans. Biomed. Eng.* 32, 230–236. doi: 10.1109/TBME.1985.325532

Shi, B., Wang, L., Yan, C., Chen, D., Liu, M., and Li, P. (2019). Nonlinear heart rate variability biomarkers for gastric cancer severity: a pilot study. *Sci. Rep.* 9:13833. doi: 10.1038/s41598-019-50358-y

Stein, P. K., Domitrovich, P. P., Huikuri, H. V., and Kleiger, R. E. (2005). Traditional and nonlinear heart rate variability are each independently associated with mortality after myocardial infarction. *J. Cardiovasc. Electrophysiol.* 16, 13–20. doi: 10.1046/j.1540-8167.2005.04358.x

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71, 209–249. doi: 10.3322/caac.21660

Tracey, K. J. (2009). Reflex control of immunity. *Nat. Rev. Immunol.* 9, 418–428. doi: 10.1038/nri2566

Tsutsumi, T., Ide, T., Yamato, M., Kudou, W., Andou, M., Hirooka, Y., et al. (2007). Modulation of the myocardial redox state by vagal nerve stimulation after experimental myocardial infarction. *Cardiovasc. Res.* 77, 713–721. doi: 10.1093/cvr/cvm092

Vanderlei, L. C. M., Pastre, C. M., Hoshi, R. A., Carvalho, T. D., and Godoy, M. F. (2009). Basic notions of heart rate variability and its clinical applicability. *Rev. Bras. Cir. Cardiovasc.* 24, 205–217. doi: 10.1590/S0102-76382009000200018

Varon, C., Lazaro, J., Bolea, J., Hernando, A., Aguilo, J., Gil, E., et al. (2019). Unconstrained estimation of HRV indices after removing respiratory influences from heart rate. *IEEE J. Biomed. Health Inform.* 23, 2386–2397. doi: 10.1109/JBHI.2018.2884644

Voss, A., Schulz, S., Schroeder, R., Baumert, M., and Caminal, P. (2009). Methods derived from nonlinear dynamics for analysing heart rate variability. *Philos. Trans. A Math. Phys. Eng. Sci.* 367, 277–296. doi: 10.1098/rsta.2008.0232

Wang, Y. M., Wu, H. T., Huang, E. Y., Kou, Y. R., and Hseu, S. S. (2013). Heart rate variability is associated with survival in patients with brain metastasis: a preliminary report. *Biomed. Res. Int.* 2013, 1–6. doi: 10.1155/2013/503421

**Conflict of Interest:** A direct family member of BS owns stock in HeaLink Ltd., Bengbu, China.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neuroscience

BRIEF RESEARCH REPORT
published: 05 October 2021
doi: 10.3389/fnins.2021.615363

# Personal Resources and Organizational Outcomes: Sex as a Moderator of the Complex Relationships Between Self-Esteem, Heart Rate Variability, and Work-Related Exhaustion

*Evelina De Longis[1]*, Cristina Ottaviani[1,2] and Guido Alessandri[1]*

[1] Department of Psychology, Sapienza University of Rome, Rome, Italy, [2] IRCCS Santa Lucia Foundation, Rome, Italy

Global self-esteem represents a protective personal resource lowering the risk of psychological distress. Research conducted in the work setting has confirmed the psychosocial benefits of high self-esteem. However, research linking self-esteem to neurobiological adaptability appears quite scarce. In this study, we propose a theoretical model in which self-esteem predicts work-related exhaustion indirectly, through the mediation of heart rate variability (HRV) and negative affect at work. Moreover, we explore the relationship between self-esteem and HRV. From one side, one would expect a positive link between self-esteem and HRV, signaling higher autonomic adaptability. However, recent studies have shown that in women, such associations become more complex, with even reversed patterns as compared with that in men. Thus, we included sex as a moderator of the relationship between HRV and self-esteem. The model was tested on a sample of 110 individuals working in the relational professions (54% males; $M_{age}$ = 42.6, SD = 13.73), observed for an entire workday. Results confirmed the protective role of self-esteem against the experience of negative affect and (indirectly) work-related exhaustion. Symptoms of exhaustion at work were also negatively predicted by HRV, and both HRV and negative affect acted as mediators of the relationship between self-esteem and work-related exhaustion. Notably, sex differences emerged in the association between global self-esteem and cardiac vagal tone at work: in women, self-esteem was negatively related to HRV, which in turn led to higher work-related exhaustion, whereas in men, no evidence of this indirect effect appeared. Burnout prevention programs should not ignore important sex differences in how individuals respond to work-related stress.

**Keywords: self-esteem (SE), HRV (heart rate variability), exhaustion, negative affect, sex**

OPEN ACCESS

***Edited by:***
*Sylvain Laborde, German Sport University Cologne, Germany*

***Reviewed by:***
*Sara Lindeberg, Lund University, Sweden*
*Maximilian Schmaußer, German Sport University Cologne, Germany*

****Correspondence:***
*Evelina De Longis*
*Evelina.delongis@uniroma1.it*

***Specialty section:***
*This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience*

***Received:*** *11 December 2020*
***Accepted:*** *07 September 2021*
***Published:*** *05 October 2021*

***Citation:***
*De Longis E, Ottaviani C and Alessandri G (2021) Personal Resources and Organizational Outcomes: Sex as a Moderator of the Complex Relationships Between Self-Esteem, Heart Rate Variability, and Work-Related Exhaustion. Front. Neurosci. 15:615363. doi: 10.3389/fnins.2021.615363*

## INTRODUCTION

Exhaustion is the core component of job burnout syndrome, and it refers to a feeling of depletion of emotional, physical, and cognitive resources (Hobfoll and Shirom, 2000; Maslach et al., 2001). A large body of research documents that exhaustion often results in a number of negative outcomes both for employees and organizations (e.g., Bakker et al., 2014; Bakker and Costa, 2014). Exhaustion, in fact, is associated

with psychological and physical health problems, such as cardiovascular diseases, musculoskeletal disorders, pain experiences, depressive and insomnia symptoms, and mental disorders (for a review, see Bakker et al., 2014; Salvagioni et al., 2017). As for its occupational consequences, exhaustion is related to job dissatisfaction, overload, absenteeism, presenteeism, and reduced job performance (both at the individual and at the team level; see Bakker et al., 2014; Salvagioni et al., 2017). Given its significant impact on individuals' health and well-being, researchers have widely investigated exhaustion's antecedents in order to better understand and prevent this phenomenon. In this regard, a large body of literature has highlighted the role of individual differences in the development of exhaustion symptoms (for a meta-analysis, see Alarcon et al., 2009). Among these, consistent evidence points to the protective role played by self-esteem (e.g., Hobfoll and Freedy, 1993; Janssen et al., 1999; Alarcon et al., 2009; Alessandri et al., 2017), defined as a global subjective judgment of personal worth and self-acceptance (Rosenberg, 1965; Marsh and O'Mara, 2008). Individuals with high self-esteem are usually more satisfied with their life and more optimistic, have a clearer self-concept, are more likely to experience positive emotions, and have a high sense of mastery (Lyubomirsky et al., 2006). In addition, they are less vulnerable to anxiety and depression (Lyubomirsky et al., 2006). In the workplace, self-esteem proved to be a valuable resource, as it was found to be associated with career success, better working conditions and outcomes, better quality of relationships with colleagues, and low levels of exhaustion (e.g., Janssen et al., 1999; Kammeyer-Mueller et al., 2008; Kuster et al., 2013; Orth and Robins, 2013; Perinelli et al., 2021).

Importantly, under the theoretical framework of conservation of resources (COR) theory (Hobfoll, 1989), self-esteem has been conceptualized as a key personal resource that may help individuals to cope with stressful working conditions, as it affects how they react to an actual resource loss or to the threat of a loss. The main tenet of COR theory is that individuals strive to obtain, retain, and foster their resources (Hobfoll, 1989). It follows that stress occurs when individuals lose resources, are threatened with resource loss, or fail to gain resources after an investment (Hobfoll, 1989). Under this theoretical framework, high global self-esteem can be viewed as a "reserve" of self-worth and confidence that influences individuals' ability to cope with a (threat of) resource loss (Grandey and Cropanzano, 1999; Hobfoll, 2010; Alessandri et al., 2017). However, despite the evidence of a negative association between self-esteem and work-related exhaustion, the process underlying this relationship has not yet been fully clarified (e.g., Janssen et al., 1999). At the same time, while the benefits of self-esteem for work adjustment are well acknowledged, relatively few studies have explored its physiological correlates (Martens et al., 2008, 2010; Schwerdtfeger and Scheel, 2012), especially in the work setting.

Some evidence is available of the relationship between self-esteem and biological measures. For example, Pruessner et al. (2005) showed that self-esteem is associated with cortisol responses to a psychosocial stress task as well as with hippocampal volume, supporting the notion that perceiving a situation as more stressful, and therefore activating the hypothalamic–pituitary–adrenal (HPA) axis, might have an effect on specific brain structures via the neurotoxic effects of cortisol. Self-esteem has also been found to modulate neural responses to evaluative feedback in the ventral anterior cingulate cortex/medial prefrontal cortex (mPFC) (Somerville et al., 2010), and it has been linked to alpha asymmetry at the precuneus, which plays a key role in self-referential thought (Alessandri et al., 2015). Of interest, a connection between self-esteem and cardiac vagal tone has been proposed (e.g., Martens et al., 2008; Schwerdtfeger and Scheel, 2012). Specifically, some evidence is available for a positive association of self-esteem with heart rate (HR) variability (HRV; e.g., Martens et al., 2008, 2010; Schwerdtfeger and Scheel, 2012), a measure of parasympathetic modulation of the heart and measured as the variability in time between successive heart beats (Task Force, 1996). For instance, Martens et al. (2008, 2010), moving from the assumption that high self-esteem and high HRV represent resources that buffer threat responses, conducted four studies on state-dependent changes in self-esteem and their association with resting HRV. These authors found that positive self-esteem relevant feedback (i.e., bogus personality feedback) was related to higher HRV compared with negative feedback, even when controlling for negative and positive mood (Martens et al., 2010, Study 1). Furthermore, the authors found that a positive intelligence feedback was associated with an increase in HRV relative to a negative intelligence feedback (Martens et al., 2010, Study 2) and that overall levels of self-esteem over 2 weeks were positively correlated with resting HRV assessed in the laboratory (Martens et al., 2010, Studies 3 and 4). Relatedly, O'Donnell et al. (2008) found that greater global self-esteem was associated with lower HRV during a speech task. Of special interest for the present study, Schwerdtfeger and Scheel (2012), using ecological momentary assessment among college students, found evidence for a positive association between self-esteem and HRV in men, while such relationship was negative, although not significant, in women. The authors also reported a negative relationship between self-esteem and negative affect, which was considerably stronger in women than in men (Schwerdtfeger and Scheel, 2012).

Taken together, these findings are in line with the neurovisceral integration model (Thayer and Lane, 2000, 2009), which suggests that HRV can be used as an index of physiological, emotional, cognitive, and behavioral processes involved in self-regulation and adaptability. In the work setting, evidence is accumulating on the negative relationship between HRV and work-related exhaustion (Lennartsson et al., 2016; Kanthak et al., 2017; De Longis et al., 2020), as well as negative emotions at work (Pieper et al., 2007; Uusitalo et al., 2011; De Longis et al., 2020).

On the basis of these findings and the theoretical arguments derived from COR theory (Hobfoll, 1989; Orth and Robins, 2013; Burić et al., 2019), the present study aims to extend previous research by examining the contribution of HRV to the health-protective effect of self-esteem in the workplace. To this end, we used the experience sampling method combined with HRV assessment to test a comprehensive model (see **Figure 1**), which takes into account the role of both physiological and

psychological variables in the association between self-esteem and work-related exhaustion. Furthermore, in light of recent evidence of substantial sex differences in vagal activity, we also tested the moderating effect of sex in the relationship between self-esteem and HRV (e.g., Snieder et al., 2007; Koenig and Thayer, 2016; Jarczok et al., 2018). More in details, in line with previous studies (e.g., Janssen et al., 1999; Alarcon et al., 2009; Orth and Robins, 2013) and with COR theory tenets, we expect a negative relationship between workers' self-esteem and work-related exhaustion, and we expect HRV and negative affect to act as mediators of this relationship. Self-esteem, in fact, has been found to represent a good proxy measure for well-being (e.g., Diener et al., 1999) and to be related with emotion regulation and emotional experiences (e.g., Nezlek, 2005; Lightsey et al., 2006; Nezlek and Kuppens, 2008). In addition, it seems reasonable to expect that self-esteem, as a coping-enhancing factor, may be related to lower levels of negative emotions and high HRV (e.g., Hughes, 2007). Stated differently, two parallel processes are expected to underlie the relationship between self-esteem and work-related exhaustion. On the one hand, from a psychological point of view, negative affect may act as a mediator of the relationship between self-esteem and work-related exhaustion: those high in self-esteem, and being less prone to experience negative emotions at work, may report lower exhaustion. On the other hand, from a physiological point of view, HRV is also postulated to mediate the negative relationship between self-esteem and work-related exhaustion: high self-esteem, by increasing cardiac vagal tone at work, may lead to reduced exhaustion symptoms. In light of the well-documented sex differences in both the neural control of HRV, with higher HRV in women compared with man (e.g., Koenig and Thayer, 2016; Tobaldini et al., 2020) and in self-esteem (Kling et al., 1999; Zuckerman et al., 2016), we also aimed at exploring the interactive effect of sex and self-esteem on HRV at work. To our knowledge, only one previous study examined the role of sex in the association between self-esteem and HRV. In the study conducted by Schwerdtfeger and Scheel (2012), self-esteem fluctuations were significantly positively associated with HRV for men and were negatively, but not significantly associated with HRV for women, suggesting a sex-specific relationship between self-esteem and HRV.

We tested our model with a sample of workers observed for an entire working day, during working hours. Several studies, indeed, showed that the psychosocial work environment influences HRV (for a review, see Jarczok et al., 2013). Finally, following methodological guidelines (e.g., Quintana et al., 2016), in testing our model, we controlled for body mass index (BMI), age, medications, caffeine, posture, physical effort, and nicotine consumption.

# MATERIALS AND METHODS

## Participants

Our sample consisted of 110 individuals working in the relational professions. Most of them (54.2%) were males, with a mean age of 42.6 years (*SD* = 13.73). They had an average BMI of 24.02 (*SD* = 3.63); 27.7% of participants were smokers, and 77.5% of participants did not take any medication that could affect HRV. A diagnosis of heart disease was an exclusionary criterion. In terms of employment sectors, 24.8% of participants worked in the sales sector, 10.3% of participants worked in the health sector, 8.5% of participants worked in the education sector, 10.8% were entrepreneurs, 9.8% of participants were managers, and the remaining 35.8% were employees in various fields. The mean job tenure was 14.5 years (*SD* = 11).

## Procedure

Participants were recruited mainly through online advertisement, but also via word of mouth. A week before the experience sampling procedure, socio-demographic information and global self-esteem were assessed in an initial online survey. All participants also provided informed consent, as well as their work schedule for the day of the study, indicating their start and end times and breaks at work. Then, in the selected day of the following week, participants were prompted semi-randomly (via a tone signal on their smartphone) six times during working hours. The time interval between two assessments varied depending on the length of the workday (e.g., for a 6-h workday, prompts occurred semi-randomly about every 60 min). Depending on the length of the workday, participants had 10 or 20 min to respond to the initial question (e.g., for a 6-h workday, participants had 10 min to fill in a questionnaire; for an 8-h workday, participants had 20 min to fill in a questionnaire).

## Measures

### Self-Esteem

To measure global self-esteem, we used the Rosenberg Self-Esteem Scale (RSE; Rosenberg, 1965). A sample item is "I feel that I have a number of good qualities." Each of the 10 items was scored on a 4-point scale ranging from 1 = "Strongly disagree" to 4 = "Strongly agree." Alpha coefficient was 0.77.

### Heart Rate

HR was monitored using Bodyguard 2 (Firstbeat) HR monitors, which have been extensively used for HR recording and provide reliable measures of beat-to-beat intervals (e.g., Porto and Junqueira, 2009). Participants were instructed to wear the HR monitor for 24 consecutive hours on the selected day (to include the entire work shift and one night of sleep). As a first step, each of the six surveys entries was labeled in the cardiac data. Then, we divided the 24-h raw beat-to-beat intervals in several blocks, one for each interval between two prompts. We removed from the analysis any break from work (e.g., lunch break). To assess vagally mediated HRV, we computed the root mean square of successive beat-to-beat interval differences (RMSSD), the recommended parameter for field studies, as it reflects vagal regulation of HR being less affected by breathing (Task Force, 1996; Penttilä et al., 2001; Laborde et al., 2017). HRV analyses, outlier, and artifact detection were performed using Kubios HRV software (Tarvainen et al., 2014).

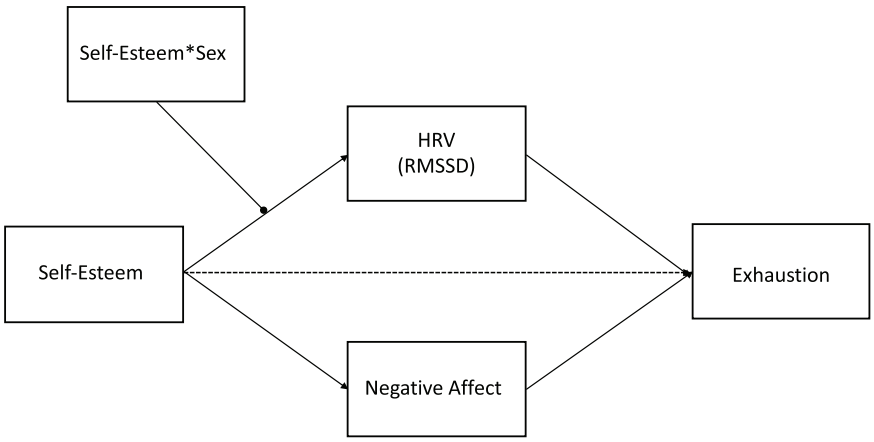



**FIGURE 1 |** Conceptual representation of the hypothesized moderated mediation model. Self-esteem*Sex = interaction term between self-esteem and sex.

### Negative Affect

To measure negative affect, we asked participants to report their current levels of negative emotions. In each survey, participants indicated the extent to which they were currently feeling each of nine negative emotions (sad, angry, anxious, ashamed, frustrated, irritable, guilty, restless, and disgusted) by moving a slider anchored with the numbers 0 and 100. The selected affect words were drawn from the study by Thompson et al. (2012) and De Longis et al. (2020). The between-person reliability, calculated by running mixed models and following the procedure indicated by Bonito et al. (2012), was 0.40, while the within-person reliability was 0.86. The within-person value can be considered substantial (Shrout, 1998). In the present study, an aggregated value of negative affect, representing the observed mean level of negative affect observed across the six prompts that occurred during the workday, was used.

### Exhaustion

At the end of the workday, work-related exhaustion was assessed by using two items drawn from the Maslach Burnout Inventory—General Survey (MBI-GS; Maslach et al., 1996; i.e., "I feel emotionally drained from my work" and "I feel exhausted by my work"). The items were scored on a 5-point scale (1 = very little or not at all; 5 = extremely). Alpha coefficient was 0.82.

## Statistical Analysis

Our model was tested within the framework of Bayesian structural equation modeling (BSEM), using Mplus 8 (Muthén and Muthén, 2018). BSEM fit was assessed according to the following criteria: the posterior predictive *p*-value and the associated 95% credibility interval (Muthén and Asparouhov, 2012). Credibility intervals are the Bayesian analogous of frequentist confidence intervals, from which they differ as they are based on the percentiles computed on the whole distribution of the posterior estimates. In addition, the derivation of these intervals does not rely on large-sample theory and does not assume that the distribution is normal. A good model fit is expected to show a posterior predictive *p*-value of approximately 0.5 and a symmetric 95% credibility interval (CI 95%) centered around zero. All parameter estimates with an associated posterior *p*-value value below 0.05 were considered statistically significant ($p < 0.05$).

# RESULTS

## Descriptive Statistics and Correlations

Descriptive statistics and correlations among the main study variables are reported in **Table 1**. Except for HRV, all variables were significantly correlated in the expected direction. In the overall sample, moderate correlations were found among work-related exhaustion and negative affect, as well as negative affect and self-esteem. HRV was negatively and significantly related only to work-related exhaustion.

**Table 1** also presents descriptive statistics and correlations stratified by sex. Interestingly, self-esteem was negatively correlated with HRV in women, whereas there was no observed correlation in men. Exhaustion was negatively and significantly associated with HRV only in men. The negative correlation between self-esteem and exhaustion was statistically significant only in men.

## Model Testing

The hypothesized model fitted the data well, as indicated by a posterior predictive *p*-value of 0.19 and a symmetric 95% posterior predictive interval ranging from −13.315 to 33.197. This model is shown in **Figure 2**. As it can be seen, the relation between self-esteem and HRV was qualified by a significant interaction between self-esteem and sex. HRV, on the other hand, significantly and negatively predicted work-related exhaustion. At the same time, self-esteem significantly and negatively predicted negative affect, and negative affect significantly and positively predicted work-related exhaustion. Thus, both HRV and negative affect acted as mediators of the relationship between self-esteem and work-related exhaustion. Hence, as a next step, simple slopes at one standard deviation above and below the

**TABLE 1 |** Means, standard deviations (SD), and correlations among variables.

| **Overall sample** | | **Mean** | ***SD*** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|---|---|
| | 1. Self-esteem | 3.28 | 0.43 | 1 | – | – | – |
| | 2. Negative affect | 8.44 | 10.73 | −0.33** | 1 | | – |
| | 3. lnHRV | 1.46 | 0.27 | −0.14 | −0.07 | 1 | – |
| | 4. Exhaustion | 1.76 | 0.88 | −0.31** | −0.39** | −0.19* | 1 |
| | 5. Sex | – | – | 0.13 | −0.18* | 0.03 | 0.00 |
| **Stratified by sex** | | **Mean$_w$ (SD$_w$)** | **Mean$_m$ (SD$_m$)** | **1** | **2** | **3** | **4** |
| | 1. Self-esteem | 1.78 (0.47) | 1.67 (0.38) | 1 | −0.25* | 0.00 | −0.36** |
| | 2. Negative affect | 10.59 (12.47) | 6.70 (8.81) | −0.37** | 1 | −0.18 | 0.39** |
| | 3. lnHRV | 1.45 (0.25) | 1.47 (0.28) | −0.31* | 0.05 | 1 | −0.26** |
| | 4. Exhaustion | 1.76 (0.81) | 1.77 (0.94) | −0.26 | 0.43** | −0.09 | 1 |

*Means for daily hassles and exhaustion were aggregated within and across days. Correlations for women are presented below the diagonal; correlations for men are presented above the diagonal. lnHRV = natural logarithm of heart rate variability; w = women; m = men. *p < 0.01. **p < 0.05.*

mean of the scores on self-esteem were computed. The observed relationship between self-esteem and HRV as a function of sex is represented in **Figure 3**. Results from simple slope analysis indicated that the relationship between self-esteem and HRV was not significant for men ($B$ = 0.024, $p$ = 0.39), while it was significant and negative for women ($B$ = −0.162, $p$ = 0.019). Accordingly, women with low self-esteem were characterized by higher HRV, as compared with women with high self-esteem. The latter, on the contrary, were characterized by lower HRV.

The finding of a conjoint prediction (1) of HRV by the interaction term between self-esteem and sex and (2) of exhaustion by HRV suggested that the indirect association between self-esteem and exhaustion might be moderated by sex. In accordance, we found that the indirect effect of self-esteem on exhaustion via HRV was significant and positive only for women (0.12, $p$ = 0.02) but was not significant for men (−0.02, $p$ = 0.39). Likewise, we found a significant indirect effect of self-esteem on exhaustion symptoms through negative affect (−0.28, $p$ = 0.002).

Finally, among covariates, only age was significantly and negatively associated with HRV (−0.01, $p < 0.001$).

## DISCUSSION

The aim of this study was to investigate two possible pathways through which global self-esteem, one of the most popular individual differences constructs in psychology (Donnellan et al., 2011), may protect workers from developing exhaustion symptoms. Despite that self-esteem has been acknowledged as a key personal resource capable of influencing how individuals react to challenging circumstances (Hobfoll, 1989; Janssen et al., 1999), the mechanisms through which it may operate need further investigation. Studies investigating general self-esteem and its association with neurobiological adaptability are relatively scarce and typically involved samples of college students, leaving the relationship between self-esteem and HRV unexplored in the organizational domain.

A first finding of this study is that global self-esteem is associated with lower negative affect at work, thus indirectly protecting workers from exhaustion symptoms. This result is consistent with COR theory conceptualization of self-esteem as a personal resource that dampens threat responses, as it is associated with a feeling of security and confidence that offsets the impact of job stressors and their consequences (e.g., Hobfoll, 1989; Hobfoll and Freedy, 1993). Furthermore, the negative association between self-esteem and work-related exhaustion is consistent with previous research, suggesting an effect of self-esteem on work outcomes (Kuster et al., 2013). The mediating role of negative affect, on the other hand, broadens our understanding of the mechanisms through which an individual difference (i.e., self-esteem) may protect workers from chronic stress conditions. This pattern of results suggests that the reduced perceived vulnerability associated with self-esteem may affect individuals' affective reactions at work, possibly because it leads to a less threatening interpretation of potentially stressful work events, or because it supports the use of efficient coping strategies.

Symptoms of exhaustion at work were also significantly and negatively predicted by HRV. This result fits well with a plethora of results highlighting the prognostic value of HRV as an indicator of future negative psychological outcomes, for example, daily instability of positive affect (e.g., Koval et al., 2013) or the development of depressive symptoms over time (e.g., Carnevali et al., 2018). The neurovisceral integration model (Thayer and Lane, 2000, 2009) illustrates that this is plausible from a physiological point of view thanks to afferent and efferent vagal pathways, which allow emotional states to affect physiological function and vice versa. According to the model, HRV is a psychophysiological proxy of vagal activity and therefore is a good candidate to reflect good emotion regulation abilities and engagement in context-appropriate behaviors over time.

The second most noteworthy finding, however, concerns the moderating role of sex in the relationship between self-esteem and HRV. Women with high self-esteem, in fact, were characterized by low HRV and, through the mediation of HRV, ended in higher work-related exhaustion than were men. This is not the only paradoxical result that emerged from studies examining HRV in women. Similar to the pattern found between self-esteem and HRV, the association between depressive symptoms and HRV appeared to be positive in female monkeys

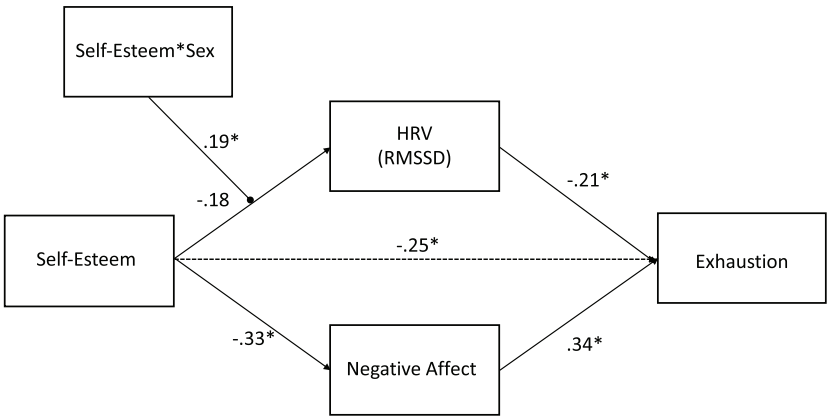



**FIGURE 2 |** The moderated mediation model with parameter estimates. Self-esteem*Sex = interaction term between self-esteem and sex.

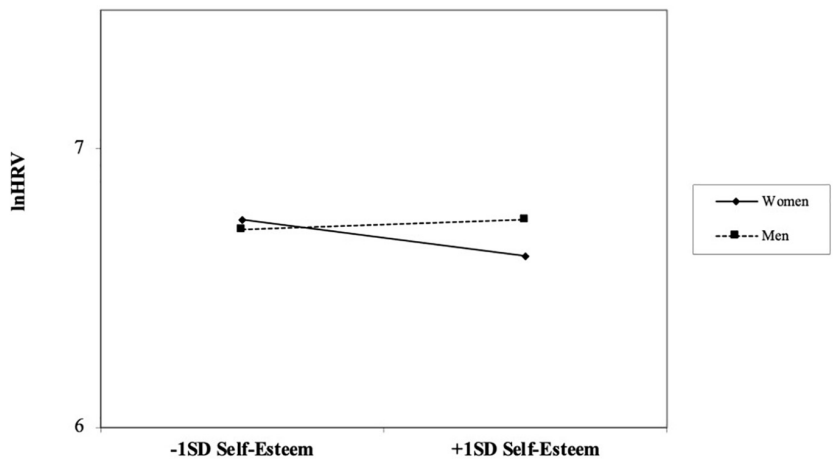



**FIGURE 3 |** Interaction of self-esteem and sex on heart rate variability (HRV).

but not in males (Jarczok et al., 2018). Intriguingly, amygdala activity is negatively correlated with HRV in men but positively correlated in women (Nugent et al., 2011). The amygdala plays a crucial role in adjusting physiological and behavioral responses to stress, for example, exerting strong regulatory influence over the HPA axis and the autonomic nervous system (ANS). When the environment is perceived as safe, amygdala activation is inhibited by the mPFC. Notably, chronic occupational stress is associated with impaired functional connectivity between the amygdala and the mPFC, indicating a reduced capacity to flexibly adjust to the environmental requests (Golkar et al., 2014). A recent review of the literature has reported between-sex differences in the described amygdala–PFC responses to stress (Zhang et al., 2021). Crucially, such differences have been imputed to the role of oxytocin (Bredewold and Veenema, 2018). For example, a psychosocial stressor such as social defeat increases oxytocin production, which in turn regulates output of the vagal dorsal motor nucleus, thereby regulating bodily functions associated with the parasympathetic nervous system, in female mice but not in males (Steinman et al., 2016).

It is therefore clear that there are important sex differences in how individuals respond to stressors. The present study added a further piece in the puzzle showing that the seemingly paradoxical response described above is particularly true for women with lower self-esteem. Importantly, current results replicated in the work setting those of Schwerdtfeger and Scheel (2012), which were obtained by examining a sample of university students. We can speculate that having a higher self-esteem at work comes with a cost for women, as this dispositional characteristic is likely to be associated with an active coping style, while working in the relational professions often requires to suppress and regulate one's emotions (Thayer et al., 2003). Recent evidence points to the "interpersonal roots" of self-esteem by showing the impact of the interpersonal environment at work on perceived self-worth (Perinelli et al., 2021). It is likely that perceived social support may help to explain variations in individual responses to stressors and their well-being at work, as well as results on HRV in women. Of interest, low social support

was found to lead to higher variance in the HRV trajectory, compared with high social support (Baethge et al., 2020). Future studies are certainly needed to clarify the causal mechanisms underlying the role of self-esteem in sex differences in the physiological response to job-related daily stressors.

Overall, findings from this research offer some important contributions to our understanding of the role of self-esteem in workers' adjustment. First, our study encourages further study of the physiological correlates of global self-esteem, as it sheds light on the pathways between self-esteem and health-related outcomes. Despite that the connection between self-esteem and cardiac vagal tone has been proposed to be context dependent (Martens et al., 2008), this is the first study to investigate such relationship in the work setting, where global self-esteem proved to be a valuable resource that protects against work maladjustment (Kuster et al., 2013).

Results from this study come with some limitations. A first limitation is the use of only two items to assess exhaustion. Given the intensive nature of our study design (six assessments during working hours), we tried to reduce the burden on participants as much as possible and considered two representative items to be sufficient. A second possible limitation concerns the temporal frame considered. Future organizational research should consider the use of repeated assessment of HR over multiple days. Future studies should also control for menstrual cycle phase to better investigate the association between psychological and physiological variables in women. A recent meta-analysis supports the notion that hormonal status may greatly affect such associations (Schmalenberger et al., 2019). Ideally, future research should include multiple physiological indicators other than HRV, such as cortisol, which is intimately linked to psychosocial stress. Finally, it would be desirable to test the generalizability of our findings across different populations of workers.

Notwithstanding these limitations, in this study, we found evidence for sex differences in the association between global self-esteem and cardiac vagal tone at work. While self-esteem was negatively related to HRV in women, in men, such relationship was not significant. Furthermore, both HRV and negative affect acted as mediators of the relationship between self-esteem and work-related exhaustion symptoms at the end of the workday, thus offering insights on the physiological and psychological mechanisms of this relationship and on the prevention of work-related stress.

## DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: data from this study are not publicly available as informed consent and ethical approval for public data sharing were not obtained from participants. Requests to access these datasets should be directed to GA and corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Sapienza University of Rome (Prot No. 0000231). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

GA conceptualized this study. ED and GA collected, prepared the data, and performed the data analysis. ED drafted the manuscript. GA and CO commented and revised the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the Sapienza University of Rome Grant No. AR11916B889311C5 to ED, Grant No. RM1181643660189A to CO, and Grants RG11816433CBD8D3 and RM11715C809391B1 to GA.

## REFERENCES

Alarcon, G., Eschleman, K. J., and Bowling, N. A. (2009). Relationships between personality variables and burnout: a meta-analysis. *Work Stress* 23, 244–263. doi: 10.1080/02678370903282600

Alessandri, G., Caprara, G. V., and De Pascalis, V. (2015). Relations among EEG-alpha asymmetry and positivity personality trait. *Brain Cogn.* 97, 10–21. doi: 10.1016/j.bandc.2015.04.003

Alessandri, G., Perinelli, E., De Longis, E., Rosa, V., Theodorou, A., and Borgogni, L. (2017). The costly burden of an inauthentic self: insecure self-esteem predisposes to emotional exhaustion by increasing reactivity to negative events. *Anxiety Stress Coping* 30, 630–646. doi: 10.1080/10615806.2016.1262357

Baethge, A., Vahle-Hinz, T., and Rigotti, T. (2020). Coworker support and its relationship to allostasis during a workday: a diary study on trajectories of heart rate variability during work. *J. Appl. Psychol.* 105, 506–526. doi: 10.1037/apl0000445

Bakker, A. B., and Costa, P. L. (2014). Chronic job burnout and daily functioning: a theoretical analysis. *Burn. Res.* 1, 112–119. doi: 10.1016/j.burn.2014.04.003

Bakker, A. B., Demerouti, E., and Sanz-Vergel, A. I. (2014). Burnout and work engagement: the JD-R approach. *Annu. Rev. Org. Psychol. Org. Behav.* 1, 389–411. doi: 10.1146/annurev-orgpsych-031413-091235

Bonito, J. A., Ruppel, E. K., and Keyton, J. (2012). Reliability estimates for multilevel designs in group research. *Small Group Res.* 43, 443–467. doi: 10.1177/1046496412437614

Bredewold, R., and Veenema, A. H. (2018). Sex differences in the regulation of social and anxiety-related behaviors: insights from vasopressin and oxytocin brain systems. *Curr. Opin. Neurobiol.* 49, 132–140. doi: 10.1016/j.conb.2018.02.011

Burić, I., Slišković, A., and Penezić, Z. (2019). Understanding teacher well-being: a cross-lagged analysis of burnout, negative student-related emotions, psychopathological symptoms, and resilience. *Educ. Psychol.* 39, 1136–1155. doi: 10.1080/01443410.2019.1577952

Carnevali, L., Thayer, J. F., Brosschot, J. F., and Ottaviani, C. (2018). Heart rate variability mediates the link between rumination and depressive symptoms: a longitudinal study. *Int. J. Psychophysiol.* 131, 131–138. doi: 10.1016/j.ijpsycho.2017.11.002

De Longis, E., Alessandri, G., and Ottaviani, C. (2020). Inertia of emotions and inertia of the heart: physiological processes underlying inertia of negative emotions at work. *Int. J. Psychophysiol.* 155, 210–218. doi: 10.1016/j.ijpsycho.2020.06.007

Diener, E., Suh, E. M., Lucas, R. E., and Smith, H. L. (1999). Subjective well-being: three decades of progress. *Psychol. Bull.* 125, 276–302. doi: 10.1037/0033-2909.125.2.276

Donnellan, M. B., Trzesniewski, K. H., and Robins, R. W. (2011). "Self-esteem: enduring issues and controversies," in *The Wiley-Blackwell Handbook of Individual Differences*, eds T. Chamorro-Premuzic, S. von Stumm, and A. Furnham (New York, NY: Wiley-Blackwell), 718–746. doi: 10.1002/9781444343120.ch28

Golkar, A., Johansson, E., Kasahara, M., Osika, W., Perski, A., and Savic, I. (2014). The influence of work-related chronic stress on the regulation of emotion and on functional connectivity in the brain. *PLoS One* 9:e104550. doi: 10.1371/journal.pone.0104550

Grandey, A. A., and Cropanzano, R. (1999). The conservation of resources model applied to work–family conflict and strain. *J. Vocat. Behav.* 54, 350–370. doi: 10.1006/jvbe.1998.1666

Hobfoll, S. E. (1989). Conservation of resources: a new attempt at conceptualizing stress. *Am. Psychol.* 44, 513–524. doi: 10.1037//0003-066x.44.3.513

Hobfoll, S. E. (2010). "Conservation of resources theory: its implication for stress, health, and resilience," in *The Oxford Handbook of Stress, Health, and Coping*, eds S. Folkman and P. E. Nathan (New York, NY: Oxford University Press), 127–147.

Hobfoll, S. E., and Freedy, J. (1993). "Conservation of resources: a general stress theory applied to burnout," in *Professional Burnout: Recent Developments in Theory and Research*, eds W. B. Schaufeli, C. Maslach, and T. Marek (Philadelphia, PA: Taylor & Francis), 115–133. doi: 10.4324/9781315227979-9

Hobfoll, S. E., and Shirom, A. (2000). "Conservation of resources theory: applications to stress and management in the workplace," in *Handbook of Organizational Behavior*, 2nd Edn, ed. R. T. Golembiewski (New York, NY: Marcel Dekker), 57–81.

Hughes, B. M. (2007). Self-esteem, performance feedback, and cardiovascular stress reactivity. *Anxiety Stress Coping* 20, 239–252. doi: 10.1080/10615800701330218

Janssen, P. P., Schaufeli, W. B., and Houkes, I. (1999). Work-related and individual determinants of the three burnout dimensions. *Work Stress* 13, 74–86. doi: 10.1080/026783799296200

Jarczok, M. N., Jarczok, M., Mauss, D., Koenig, J., Li, J., Herr, R. M., et al. (2013). Autonomic nervous system activity and workplace stressors-a systematic review. *Neurosci. Biobehav. Rev.* 37, 1810–1823. doi: 10.1016/j.neubiorev.2013.07.004

Jarczok, M. N., Koenig, J., Shively, C. A., and Thayer, J. F. (2018). Behavioral depression is associated with increased vagally mediated heart rate variability in adult female cynomolgus monkeys (*Macaca fascicularis*). *Int. J. Psychophysiol.* 131, 139–143. doi: 10.1016/j.ijpsycho.2017.11.004

Kammeyer-Mueller, J. D., Judge, T. A., and Piccolo, R. F. (2008). Self-esteem and extrinsic career success: test of a dynamic model. *Appl. Psychol.* 57, 204–224. doi: 10.1111/j.1464-0597.2007.00300.x

Kanthak, M. K., Stalder, T., Hill, L. K., Thayer, J. F., Penz, M., and Kirschbaum, C. (2017). Autonomic dysregulation in burnout and depression: evidence for the central role of exhaustion. *Scand. J. Work Environ. Health* 43, 475–484. doi: 10.5271/sjweh.3647

Kling, K. C., Hyde, J. S., Showers, C. J., and Buswell, B. N. (1999). Gender differences in self-esteem: a meta-analysis. *Psychol. Bull.* 125, 470–500. doi: 10.1037/0033-2909.125.4.470

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Koval, P., Ogrinz, B., Kuppens, P., Van den Bergh, O., Tuerlinckx, F., and Sütterlin, S. (2013). Affective instability in daily life is predicted by resting heart rate variability. *PLoS One* 8:e81536. doi: 10.1371/journal.pone.0081536

Kuster, F., Orth, U., and Meier, L. L. (2013). High self-esteem prospectively predicts better work conditions and outcomes. *Soc. Psychol. Pers. Sci.* 4, 668–675. doi: 10.1177/1948550613479806

Laborde, S., Mosley, E., and Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research–recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* 8:213. doi: 10.3389/fpsyg.2017.00213

Lennartsson, A. K., Jonsdottir, I., and Sjörs, A. (2016). Low heart rate variability in patients with clinical burnout. *Int. J. Psychophysiol.*110, 171–178. doi: 10.1016/j.ijpsycho.2016.08.005

Lightsey, O. R. Jr., Burke, M., Ervin, A., Henderson, D., and Yee, C. (2006). Generalized self-efficacy, self-esteem, and negative affect. *Can. J. Behav. Sci.* 38, 72–80. doi: 10.1037/h0087272

Lyubomirsky, S., Tkach, C., and DiMatteo, M. R. (2006). What are the differences between happiness and self-esteem. *Soc.Ind. Res.* 78, 363–404. doi: 10.1007/s11205-005-0213-y

Marsh, H. W., and O'Mara, A. (2008). Reciprocal effects between academic self-concept, self-esteem, achievement, and attainment over seven adolescent years: unidimensional and multidimensional perspectives of self-concept. *Pers. Soc. Psychol. Bull.* 34, 542–552. doi: 10.1177/0146167207312313

Martens, A., Greenberg, J., and Allen, J. J. (2008). Self-esteem and autonomic physiology: parallels between self-esteem and cardiac vagal tone as buffers of threat. *Pers. Soc. Psychol. Rev.* 12, 370–389. doi: 10.1177/1088868308323224

Martens, A., Greenberg, J., Allen, J. J., Hayes, J., Schimel, J., and Johns, M. (2010). Self-esteem and autonomic physiology: self-esteem levels predict cardiac vagal tone. *J. Res. Pers.* 44, 573–584. doi: 10.1016/j.jrp.2010.07.001

Maslach, C., Jackson, S. E., and Leiter, M. P. (1996). *Maslach Burnout Inventory Manual*, 3rd Edn. Palo Alto, CA: Consulting Psychologists Press.

Maslach, C., Schaufeli, W. B., and Leiter, M. P. (2001). Job burnout. *Annu. Rev. Psychol.* 52, 397–422. doi: 10.1146/annurev.psych.52.1.397

Muthén, B., and Asparouhov, T. (2012). Bayesian SEM: a more flexible representation of substantive theory. *Psychol. Methods* 17, 313–335. doi: 10.1037/a0026802

Muthén, B., and Muthén, L. (2018). *Mplus User's Guide*. Los Angeles, CA: Muthén & Muthén.

Nezlek, J. B. (2005). Distinguishing affective and non-affective reactions to daily events. *J. Pers.* 73, 1539–1568. doi: 10.1111/j.1467-6494.2005.00358.x

Nezlek, J. B., and Kuppens, P. (2008). Regulating positive and negative emotions in daily life. *J. Pers.* 76, 561–580. doi: 10.1111/j.1467-6494.2008.00496.x

Nugent, A. C., Bain, E. E., Thayer, J. F., Sollers, J. J., and Drevets, W. C. (2011). Sex differences in the neural correlates of autonomic arousal: a pilot PET study. *Int. J. Psychophysiol.* 80, 182–191. doi: 10.1016/j.ijpsycho.2011.03.001

O'Donnell, K., Brydon, L., Wright, C. E., and Steptoe, A. (2008). Self-esteem levels and cardiovascular and inflammatory responses to acute stress. *Brain Behav. Immun.* 22, 1241–1247. doi: 10.1016/j.bbi.2008.06.012

Orth, U., and Robins, R. W. (2013). Understanding the link between low self-esteem and depression. *Curr. Dir. Psychol. Sci.* 22, 455–460. doi: 10.1177/0963721413492763

Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol.* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Perinelli, E., Alessandri, G., Cepale, G., and Fraccaroli, F. (2021). The sociometer theory at work: exploring the organizational interpersonal roots of self-esteem. *Appl. Psychol.* doi: 10.1111/apps.12312 *vp,

Pieper, S., Brosschot, J. F., van der Leeden, R., and Thayer, J. F. (2007). Cardiac effects of momentary assessed worry episodes and stressful events. *Psychosom. Med.* 69, 901–909. doi: 10.1097/PSY.0b013e31815a9230

Porto, L. G. G., and Junqueira, L. F. Jr. (2009). Comparison of time-domain short-term heart interval variability analysis using a wrist-worn heart rate monitor and the conventional electrocardiogram. *Pacing Clin. Electrophysiol.* 32, 43–51. doi: 10.1111/j.1540-8159.2009.02175.x

Pruessner, J. C., Baldwin, M. W., Dedovic, K., Renwick, R., Mahani, N. K., Lord, C., et al. (2005). Self-esteem, locus of control, hippocampal volume, and cortisol regulation in young and old adulthood. *Neuroimage* 28, 815–826. doi: 10.1016/j.neuroimage.2005.06.014

Quintana, D. S., Alvares, G. A., and Heathers, J. A. J. (2016). Guidelines for reporting articles on psychiatry and heart rate variability (GRAPH): recommendations to advance research communication. *Transl. Psychiatry* 6, e803–e803. doi: 10.1038/tp.2016.73

Rosenberg, M. (1965). *Society and Adolescent Self-Image*. Princeton, NJ: Princeton University Press.

Salvagioni, D. A. J., Melanda, F. N., Mesas, A. E., González, A. D., Gabani, F. L., and Andrade, S. M. D. (2017). Physical, psychological and occupational consequences of job burnout: a systematic review of prospective studies. *PLoS One* 12:e0185781. doi: 10.1371/journal.pone.0185781

Schmalenberger, K. M., Eisenlohr-Moul, T. A., Würth, L., Schneider, E., Thayer, J. F., Ditzen, B., et al. (2019). A systematic review and meta-analysis of within-person changes in cardiac vagal activity across the menstrual cycle: implications for female health and future studies. *J. Clin. Med.* 8:1946. doi: 10.3390/jcm8111946

Schwerdtfeger, A. R., and Scheel, S. M. (2012). Self-esteem fluctuations and cardiac vagal control in everyday life. *Int. J. Psychophysiol.* 83, 328–335. doi: 10.1016/j.ijpsycho.2011.11.016

Shrout, P. E. (1998). Measurement reliability and agreement in psychiatry. *Stat. Methods Med. Res.* 7, 301–317. doi: 10.1177/096228029800700306

Snieder, H., Van Doornen, L. J., Boomsma, D. I., and Thayer, J. F. (2007). Sex differences and heritability of two indices of heart rate dynamics: a twin study. *Twin Res. Hum. Genet.* 10, 364–372. doi: 10.1375/twin.10.2.364

Somerville, L. H., Kelley, W. M., and Heatherton, T. F. (2010). Self-esteem modulates medial prefrontal cortical responses to evaluative social feedback. *Cereb. Cortex* 20, 3005–3013. doi: 10.1093/cercor/bhq049

Steinman, M. Q., Duque-Wilckens, N., Greenberg, G. D., Hao, R., Campi, K. L., Laredo, S. A., et al. (2016). Sex-specific effects of stress on oxytocin neurons correspond with responses to intranasal oxytocin. *Biol. Psychiatry* 80, 406–414. doi: 10.1016/j.biopsych.2015.10.007

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV – heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Task Force (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use task force of the european society of cardiology and the North American society of pacing and electrophysiology. *Eur. Heart J.* 17, 354–381.

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Dis.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Claude bernard and the heart–brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Rossy, L. A., Ruiz-Padial, E., and Johnsen, B. H. (2003). Gender differences in the relationship between emotional regulation and depressive symptoms. *Cogn. Ther. Res.* 27, 349–364. doi: 10.1023/A:1023922618287

Thompson, R. J., Mata, J., Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Gotlib, I. H. (2012). The everyday emotional experience of adults with major depressive disorder: examining emotional instability, inertia, and reactivity. *J. Abnorm. Psychol.* 121, 819–829. doi: 10.1037/a0027978

Tobaldini, E., Carandina, A., Toschi-Dias, E., Erba, L., Furlan, L., Sgoifo, A., et al. (2020). Depression and cardiovascular autonomic control: a matter of vagus and sex paradox. *Neurosci. Biobehav. Rev.* 116, 154–161. doi: 10.1016/j.neubiorev.2020.06.029

Uusitalo, A., Mets, T., Martinmaki, K., Mauna, S., Kinnunen, U., and Rusko, H. (2011). Heart rate variability related to effort at work. *Appl. Ergonomics* 42, 830–838. doi: 10.1016/j.apergo.2011.01.005

Zhang, W. H., Zhang, J. Y., Holmes, A., and Pan, B. X. (2021). Amygdala circuit substrates for stress adaptation and adversity. *Biol. Psychiatry* 89, 847–856. doi: 10.1016/j.biopsych.2020.12.026

Zuckerman, M., Li, C., and Hall, J. A. (2016). When men and women differ in self-esteem and when they don't: a meta-analysis. *J. Res. Pers.* 64, 34–51. doi: 10.1016/j.jrp.2016.07.007

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# Advantages of publishing in Frontiers

**OPEN ACCESS**
Articles are free to read for greatest visibility and readership

**FAST PUBLICATION**
Around 90 days from submission to decision

**HIGH QUALITY PEER-REVIEW**
Rigorous, collaborative, and constructive peer-review

**TRANSPARENT PEER-REVIEW**
Editors and reviewers acknowledged by name on published articles

**Frontiers**
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

**Visit us:** www.frontiersin.org
**Contact us:** frontiersin.org/about/contact

**REPRODUCIBILITY OF RESEARCH**
Support open data and methods to enhance research reproducibility

**DIGITAL PUBLISHING**
Articles designed for optimal readership across devices

**FOLLOW US**
@frontiersin

**IMPACT METRICS**
Advanced article metrics track visibility across digital media

**EXTENSIVE PROMOTION**
Marketing and promotion of impactful research

**LOOP RESEARCH NETWORK**
Our network increases your article's readership