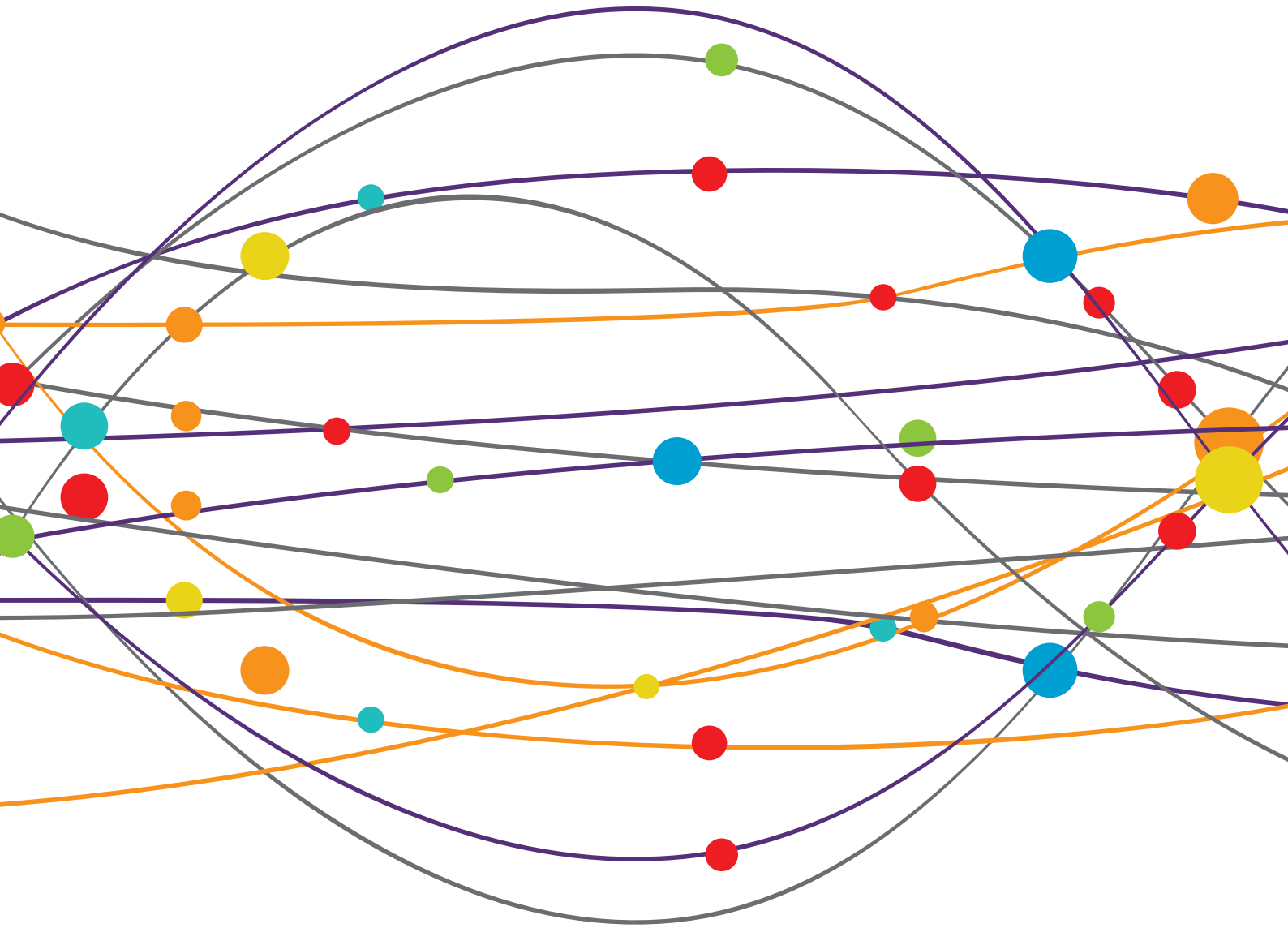
TRANSLATION AND PROCESSING OF LIGHT BY THE NON-IMAGE FORMING VISUAL SYSTEM – CONTEXT, MECHANISMS AND APPLICATIONS

EDITED BY: Fabian-Xosé Fernandez, Shadab Rahman and Manuel Spitschan
PUBLISHED IN: Frontiers in Neurology, Frontiers in Neuroscience and Frontiers in Psychiatry



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-497-1
DOI 10.3389/978-2-88971-497-1

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TRANSLATION AND PROCESSING OF LIGHT BY THE NON-IMAGE FORMING VISUAL SYSTEM – CONTEXT, MECHANISMS AND APPLICATIONS

Topic Editors:
Fabian-Xosé Fernandez, University of Arizona, United States
Shadab Rahman, Harvard Medical School, United States
Manuel Spitschan, University of Oxford, United Kingdom

Citation: Fernandez, F.-X., Rahman, S., Spitschan, M., eds. (2021). Translation and Processing of Light by the Non-Image Forming Visual System – Context, Mechanisms and Applications. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-497-1

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frontiers in Neurology

EDITORIAL
published: 27 August 2021
doi: 10.3389/fneur.2021.727849

Editorial: Translation and Processing of Light by the Non-image Forming Visual System—Context, Mechanisms and Applications

Shadab A. Rahman[1,2]*, *Fabian-Xosé Fernandez*[3,4]* and *Manuel Spitschan*[5,6,7,8]*

[1] Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, MA, United States, [2] Division of Sleep Medicine, Harvard Medical School, Boston, MA, United States, [3] Department of Psychology, University of Arizona, Tucson, AZ, United States, [4] BIO5 and McKnight Brain Research Institutes, University of Arizona, Tucson, AZ, United States, [5] Department of Experimental Psychology, University of Oxford, Oxford, United Kingdom, [6] Nuffield Department of Clinical Neurosciences, Sleep and Circadian Neuroscience Institute (SCNi), University of Oxford, Oxford, United Kingdom, [7] Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Basel, Switzerland, [8] Transfaculty Research Platform Molecular and Cognitive Neurosciences, University of Basel, Basel, Switzerland

Keywords: light, non-visual effects of light, non-imaging-forming pathways, ipRGCs, melanopsin, circadian rhythms, light exposure, intermittent light exposure

Editorial on the Research Topic

Translation and Processing of Light by the Non-image Forming Visual System—Context, Mechanisms and Applications

OPEN ACCESS

Edited and reviewed by:
Yves A. Dauvilliers,
Hôpital Gui De Chauliac, France

***Correspondence:**
Shadab A. Rahman
sarahman@rics.bwh.harvard.edu
Fabian-Xosé Fernandez
fabianf@email.arizona.edu
Manuel Spitschan
manuel.spitschan@psy.ox.ac.uk

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *19 June 2021*
Accepted: *22 July 2021*
Published: *27 August 2021*

Citation:
Rahman SA, Fernandez F-X and Spitschan M (2021) Editorial: Translation and Processing of Light by the Non-image Forming Visual System—Context, Mechanisms and Applications. Front. Neurol. 12:727849. doi: 10.3389/fneur.2021.727849

IT'S ABOUT LIGHT

We have the great pleasure of editing a special issue concerning the sensory integration of visible light across different exposure durations by the non-image forming photobiological system including the central circadian system. Throughout the past several decades, the circadian field has seen extraordinary progress. Sixty years have passed since the seminal Cold Spring Harbor Symposium on Biological Clocks. Forty have passed since the finding that light gates melatonin secretion in humans, while only 20 years separates us from the discovery of intrinsically photosensitive retinal ganglion cells (ipRGCs) and their expression of the short-wavelength photopigment, melanopsin. The field is once again at an exciting watershed, this time created by advances in semiconductor light emission technologies that offer the ability to "tune" multiple characteristics of photic stimulation (e.g., intensity, duration, spectrum, exposure pattern), thereby enabling us to engineer human-centric lighting that is designed for not just optimal vision but also mental and physical health. In observance of this watershed (which is still unfolding), we have drawn together an issue of 16 articles covering a wide range of model organisms and considerations for what constitutes "biologically active" light, how to quantify, measure, and report it, as well as applications of biologically-active exposure patterns that might improve health outcomes. The issue is thus comprised around the following topics.

APPROACHES TO DEFINING LIGHT EXPOSURE

Kicking off the volume with a didactic tutorial, Spitschan introduces the concept of time-varying light (from milliseconds to hours), its properties, and how to describe its ambient/environmental and exposure characteristics during laboratory investigations assessing the impacts of light on sleep and circadian function. Important issues are raised in the specification of these domains,

including: (1) the challenges with calibrating the temporal output of light generators operating on the sub-second timescale, and (2) the possibility of non-linear distortions occurring in light emission across the exposure duration based on input variability (e.g., the output spectra of LEDs can shift by a few nanometers depending on the electrical driver, creating potential artifacts in photoreceptor stimulation). The mini-review ends with a call for more consistency in scientific communication to assure that current reports are "future-proofed" as changes in lighting technology increasingly fuel investigations of non-image forming responses to new and enhanced human centric lighting devices.

Understanding the biological potency of light exposure requires accurate metrology. Schlangen and Price introduce fundamental concepts related to the measurement and characterization of emission sources and of the lighting environment in general, with reference to the recent International Standard CIE S 026/E:2018. This standard recommends grounding predictions about the wake or sleep-promoting effects of light exposure on its activation of each of the five photoreceptor types. The authors close their article by contributing new data illustrating how the melanopic content of light can be derived from everyday light sources, including daylight, and how such assessments, if standardized, can be used to better understand and improve the impact of lighting on human well-being.

Three author teams examine the *bench-to-bedside* extension of laboratory findings on the biological effects of light exposure into everyday application. First, Stefani and Cajochen review current advisories on ambient lighting that are intercalated across various societal frameworks, from academic recommendations to formal "best-practices" guidelines and workplace-industry ordinances. They conclude that in many cases existing standards are based on visual lighting needs for a given occupational setting (e.g., minimum levels of illuminance required for performance or color rendering quality needed to ensure visual comfort) and have yet to fully integrate an understanding of the influence of light exposure on non-visual physiological responses (e.g., minimum levels of melanopic-EDI required for alertness across the workday). Rounding out their perspective, the authors discuss several laboratory experiments suggesting that dynamic lighting mimicking changes in natural daylight can improve circadian and sleep health while maintaining adequate illumination for work-related visual tasks. As such, regulations looking to integrate visual and non-visual lighting considerations can be credibly developed, as embodied in several design guidelines for the built environment already emerging in North America and Europe.

Next, targeting practitioners, Houser and Esposito outline a five-step process for imbuing current scientific knowledge on the non-visual effects of light exposure into (physiologically relevant) human-centric lighting design. These steps include characterizing the lighting application, determining the habitual or desired sleep-wake schedule of the occupants and their sleep needs, and staying informed of building specifications that optimize functional balance between the visual, arousal, and circadian systems. Like Stefani and Cajochen, the authors conclude that architecture lighting (with the proper control systems) can be engineered to deliver biologically potent light during the day and low-potency light at night, all while balancing traditional factors such as color quality, flicker, and visibility.

Soler and Voss discuss principles for the application of emergent lighting technology including LED lighting from an industry perspective. They summarize the influence of important variables such as spectral power distribution and spatial geometry of the built environment that require careful consideration when designing indoor lighting solutions. Incorporating such principles at the design stage has the potential to maximize the ability to create "biologically" brighter days and darker nights. Their piece complements the reviews from Stefani and Cajochen, and from Houser and Esposito providing insights relevant for industry practitioners.

Taking a broad comparative-translational perspective, from rodents to humans, Walbeek et al. review evidence that naturalistic intensities of light at night (nLAN) provide important information that orients mammalian circadian and neuroendocrine physiology. The authors note that the terminology used in the field to date has been wildly inconsistent, with the term "dim light" being used to describe light levels ranging seven orders of magnitude (0.0001 to 500 lux). When properly defined and calibrated to intensities approximating the moon and stars, extant data suggest that nLAN can influence common parameters measured within circadian oscillations (e.g., free-running period and waveform), function as a zeitgeber, and produce direct non-visual effects on melatonin secretion and pupil constriction. While the properties of nLAN have been documented exclusively in rodents, there is a reasonable expectation that they will have translational potential for humans—imparting either analogous effects to those in animal models or more subtle effects on the plasticity of light responses. The authors close their discussion with a consideration of some of the challenges involved with measuring nLAN and the need to possibly rethink the current human-centric lighting recommendation that nights should be *completely* dark.

MODEL SYSTEMS, MECHANISTIC INSIGHTS, AND BENCHMARKING THE EFFECTS OF LIGHT EXPOSURE

Understanding the effects of light exposure on the circadian pacemaker requires understanding the molecular and cellular mechanisms underlying the encoding of stimulus strength and the circuitry that integrates the signal from the retina to the pacemaker. In this issue, four contributions provide unique insights into these mechanisms and pathways, each using a different model system. First, Tabuchi et al. review the signaling biology that connects oscillations of the molecular clock with daily changes in membrane excitability in *Drosophila* timekeeping neurons. At the cross-roads of each is cryptochrome (CRY), a photosensitive molecule in flies that is embedded within the circadian transcriptional–translational machinery and an effector of the potassium ion channel β-subunit redox sensor. In addition to CRY, several other molecular clock constituents have been shown to modulate the neural activity of fly timekeeping neurons via interactions with ligand-gated

and voltage-dependent/independent channels, channel-binding proteins, and subunits of the electrogenic ion pump. The authors suggest that the generalization of this crosstalk motif—between clock gene oscillations and daily changes in neuronal excitability—offers a new paradigm by which to study the effects of light exposure on sleep-wake rhythms, cognition, and neurological disorder.

Hannibal provides an intriguing and in-depth review of the comparative neuroanatomy of the retinohypothalamic tract and its role in mediating non-image forming (NIF) responses to light exposure in mammals. Drawing insights from studies on various genetic models and unique neuroanatomic features (e.g., 90% of retinal ganglion cells in the mole rat express melanopsin compared to 1% of the cell population in most laboratory rodents), Hannibal elucidates the molecular and neuronal pathways underlying NIF responses to light exposure. A spotlight is placed on neurotransmitter systems connecting the front-end of circadian photoreception to the hypothalamus, including those using the neuropeptide PACAP. A perspective is also provided on the harmful effects of light exposure at night on humans and other animals.

Next, Wong and Fernandez zoom out of the picture to discuss how intermittent light exposure through a sequence of millisecond light flashes is encoded as a coherent stimulus by the circadian system. They place the signaling of time at twilight within a theoretical framework that suggests the pivotal role of cones in this process, tying together different ideas from Gestalt theory and bringing them into a non-image forming context, thus generalizing principles of efficient sensory coding from one to the other. To support their "Cone Sentinel" model, the authors provide the first experimental data showing that ipRGC responses to short light flashes are dependent on extrinsic synaptic relays from classic photoreceptor cells and not intrinsic melanopsin expression. These data offer the unique perspective that flash protocols optimizing the stimulus-response characteristics of cone photoreception have the potential to be used clinically to treat sleep/circadian disorders and other medical conditions with an underlying circadian impairment.

While light exposure can be modulated to examine how the circadian system responds to it, it can also be used to disrupt the system—or eliminate it entirely. To do so, Ruby introduces the disruptive phase shift (DPS) protocol, a simple manipulation of ambient lighting that renders animals (Siberian hamsters) arrhythmic without altering their genome, or the tissue and developmental integrity of the brain's clock, the suprachiasmatic nucleus (SCN). The DPS model has the advantage of studying arrhythmia in a "natural" state within the brain. In this model, the SCN circuitry remains normally intact (albeit dysfunctional) and thus can influence other structures to which it projects and receive input from networks returning from those structures. In that regard, Ruby offers an inroad to studying the neurophysiology of circadian dysrhythmia in an animal the way it is most likely to present in a human. DPS hamsters show severe impairments in declarative memory that are associated with fragmentation of electroencephalographic theta oscillations, as well as altered GABAergic and cholinergic signaling in the hippocampus. These phenotypes may point the way toward developing pharmacological treatments for circadian disturbances related to jetlag and those incurred with chronic disease.

Finally, Mure provides a state-of-the-art review on what is currently known about the functional properties of human ipRGCs, an extremely difficult system in which to conduct electrophysiological recordings. The article takes an integrative stance, reviewing transcriptomic and morphological phenotypes that may distinguish various subtypes (GM1, M1-M4) and brain connectivity maps generated via PACAP (pituitary adenylate-cyclase-activating polypeptide) immunohistochemistry. By and large, the light-response characteristics of human ipRGCs are similar to their variants in rodents and primates. In each case, the spectral sensitivity of the cells peaks around ∼460 nm, with kinetic properties of intrinsic responses exhibiting a slow, sustained period of activity during stimulation that continues after light exposure. The author concludes the article by reviewing how altered ipRGC response kinetics or cell loss might provide insight into pathological mechanisms underlying aging-related neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

PRECISION LIGHTING: APPLICATIONS OF TUNABLE LIGHT EXPOSURE

Current lighting technology allows tuning of various stimulus characteristics, including timing, intensity, spectrum, and pattern. Kawasaki et al. investigate the extent to which morning bright light (∼10,000 lux, 30 min per day for 4 weeks) influences metrics related to sleep and non-visual physiological function in patients with glaucoma, an optic neuropathy that results in ipRGC loss. Post-intervention, those exposed to light therapy reported better subjective sleep quality on a Likert scale. The treatment group also demonstrated increased melanopsin-dependent, post-illumination pupil responses that correlated with common measures employed to quantify the robustness of daily ∼24-h oscillations in physical activity assessed using actigraphy. The authors concluded that scheduled bright light exposure may be a cost-effective strategy to improve sleep, circadian entrainment, and general sense of well-being in patients with glaucoma.

Using an overnight (2-day) in-laboratory randomized trial in healthy college-aged young adults, Grant et al. examined whether daytime exposure to LED task lighting with different spectra conferring different melanopic illuminance (melEDI) can affect neurobehavioral performance. Their key finding was that long duration (8 h) light exposure with higher melanopic content was associated with significant improvements in working memory and processing speed (as assessed using a 2-min addition task) and improved procedural learning in a motor sequence task. These data provide preliminary evidence indicating that the incorporation of lighting with higher melEDI in the built environment (i.e., a modification resulting in few changes in perceived lighting) may improve neurobehavioral performance

in the classroom in college-aged young adults with a naturally restricted sleep schedule.

An additional two primary reports from randomized trials elucidate how spectrally tuned light exposure affects the brain and behaviors associated with underlying mood, cognition, and arousal. First, Raikes et al. examined the extent to which daily morning short-wavelength (blue) light therapy over 6 weeks, compared to therapy with longer wavelength (amber) light exposure, changes regional functional connectivity in the brain and ratings of subjective sleepiness in patients recovering from a mild traumatic brain injury (mTBI). At the conclusion of the 6-week treatment, improvements were observed in gray matter volume in the right thalamus and functional connectivity between regions of interest related to attention, somatomotor, and visual networks as well as the default mode network (e.g., those interconnecting the thalamus with the prefrontal and orbitofrontal cortices) in patients receiving blue light exposure as compared to amber light exposure. Furthermore, these morphological and functional connectivity improvements were associated with less daytime sleepiness reported with the Epworth Sleepiness Scale. The authors concluded that blue light therapy might be a non-invasive way of improving sleep and thereby improving functional outcomes after mTBI.

Using a shorter duration (30 min) single-exposure administration of blue and amber light, Alkozei et al. examined changes in indices of functional and directed connectivity between the amygdala and the left dorsolateral prefrontal cortex, and the association of these outcomes with affect. The authors report that individuals receiving blue light exposure had greater bidirectional information flow between these two regions about an hour after the light exposure had stopped. This enhanced connectivity was associated with decreased negative affect. These results suggest that short-interval blue-light therapy can facilitate the engagement of cognitive control strategies needed to regulate arousal/mood and provide some mechanistic insight into why conventional bright light therapy during the winter months might improve depressive symptoms in people with seasonal affective disorder.

Finally, Figueiro and Leggett review the evidence for the beneficial effects of intermittent light exposure in Alzheimer's disease (AD). Two strategies are discussed relating to (1) the delivery of 40-Hz flickering light to promote gamma oscillations and gamma-related clearance of the amyloid beta (Aβ) peptides contributing to plaque formation in the AD brain and (2) the delivery of timed millisecond flashes during sleep to induce phase-resetting responses that may help reinforce entrainment of sleep-wake schedules, thereby also facilitating Aβ clearance (e.g., the recently discovered glymphatic system has been shown to remove Aβ specifically during sleep). Overall, the authors point out that these two strategies are not mutually exclusive when considering how intermittent light stimulation can be parlayed for the treatment of AD-related phenotypes and envision the development of personalized light therapy devices that might one day achieve both.

This volume touches on only a sliver of the developments that are currently permeating the field of circadian photobiology. Nevertheless, we hope that we have been able to capture some of the current "frontier" thinking in editing this issue, thus inching a little beyond the *zeitgeist*.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

F-XF was supported by the Velux Stiftung (Proj. No. 1360).

Conflict of Interest: SAR holds patents for Prevention of Circadian Rhythm Disruption by Using Optical Filters and Improving sleep performance in subject exposed to light at night; SAR owns equity in Melcort Inc.; has provided paid consulting services to Sultan & Knight Limited, Bambu Vault LLC, Lucidity Lighting Inc.; and has received honoraria as an invited speaker and travel funds from Starry Skies Lake Superior, University of Minnesota Medical School, PennWell Corp., and Seoul Semiconductor Co. Ltd. These interests were reviewed and managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies.

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.

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frontiers in Neurology

BRIEF RESEARCH REPORT
published: 15 January 2021
doi: 10.3389/fneur.2020.584479

Can Extra Daytime Light Exposure Improve Well-Being and Sleep? A Pilot Study of Patients With Glaucoma

Aki Kawasaki[1]*, *Morgane Udry*[1], *Mohamad El Wardani*[1,2] and *Mirjam Münch*[3]*

[1] Hôpital Ophtalmique Jules Gonin, Fondation Asile des Aveugles, Department of Biology and Medicine, University of Lausanne, Lausanne, Switzerland, [2] Ophthalmology Department, Calderdale and Huddersfield NHS Foundation, Huddersfield, United Kingdom, [3] Sleep/Wake Research Centre, Massey University, Wellington, New Zealand

Glaucoma damages retinal ganglion cells, including intrinsically photosensitive retinal ganglion cells (ipRGCs). These cells modulate various non-visual physiological and psychological functions which are modulated by light. In patients with glaucoma, we assessed the effect of daily bright light exposure (LE) on several melanopsin-dependent functions, such as the pupil constriction, circadian rest-activity cycles, sleep and subjective well-being including relaxation, alertness and mood. Twenty patients participated in the study (9 women, 11 men, mean age = 67.6 ± 7.5 y). Pupillometry was performed before the LE weeks and repeated on the last day of LE. The post-illumination pupil response (PIPR) was calculated as a proxy for melanopsin-dependent activation. Participants continuously wore an activity monitor and self-assessed sleep quality, well-being and visual comfort for 7 days before and during 4 weeks of daily bright LE (30 min to 10,000 lux polychromatic bright white light). After the LE, there was a significantly greater PIPR and higher subjective sleep quality when compared to the pre-LE week ($p < 0.05$), but no significant changes in 24-h rhythms or sleep parameters. A greater PIPR was correlated with an increase in circadian amplitude and higher inter-daily stability (derived from rest-activity cycles; $p < 0.05$). In a small group of patients with glaucoma, scheduled daily bright light exposure could improve subjective sleep quality. These findings highlight the importance to evaluate and maintain non-visual functions at different levels in patients with progressive loss of ipRGCs.

Keywords: light therapy, pupil, glaucoma, retinal ganglion cells, melanopsin, sleep, mood, circadian

OPEN ACCESS

Edited by:
Shadab Rahman,
Harvard Medical School,
United States

Reviewed by:
Ivy Dreizin,
University of Wisconsin-Madison,
United States
Aleksandra Domagalik,
Jagiellonian University, Poland

***Correspondence:**
Aki Kawasaki
aki.kawasaki@fa2.ch
Mirjam Münch
m.munch@massey.ac.nz

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *17 July 2020*
Accepted: *09 December 2020*
Published: *15 January 2021*

Citation:
Kawasaki A, Udry M, El Wardani M and Münch M (2021) Can Extra Daytime Light Exposure Improve Well-Being and Sleep? A Pilot Study of Patients With Glaucoma. Front. Neurol. 11:584479. doi: 10.3389/fneur.2020.584479

INTRODUCTION

Glaucoma is a common optic neuropathy that results in retinal ganglion cell loss. In patients with advanced glaucoma, loss of intrinsically photosensitive retinal ganglion cells (ipRGCs) has been demonstrated (1). The ipRGCs express melanopsin and synapse centrally to modulate a variety of non-visual physiological functions such as the pupil, circadian rhythms, alertness and sleep (2). Reduced capacity to entrain to light and was first shown in glaucomatous rats (3, 4). Extending those findings to patients with glaucoma,

even those with mild visual dysfunction may demonstrate abnormal light responses including reduced acute alerting effects to light, reduced light-induced suppression of nocturnal melatonin and reduced pupil light reflex to selected light stimuli as these are effects mediated by melanopsin (5–10). Glaucoma patients have also been shown to have a higher prevalence for impaired executive daytime functions, depressive symptoms, impaired mood (11, 12) and anxiety (13) and clinical implications have been discussed [for a reviews see (14–16)].

Additionally, there is evidence that patients with glaucoma have greater daytime sleepiness and/or decreased sleep quality compared to age-matched control subjects (17–19). A large cross-sectional study with more than 6,700 patients reported an association between glaucoma and very long sleep duration (20). Lanzani et al. reported increased wakefulness with lower sleep efficiency at night (21) and similarly Gubin et al. found later bed times and shorter sleep duration in primary open angle glaucoma patients (22) suggesting a higher prevalence for sleep disturbances in patients with glaucoma (13, 23, 24). There is some evidence that obstructive sleep apnea might be a systemic risk factor for glaucoma (25–28), even though not all study reports confirmed this (20, 29).

One factor contributing to the mechanism of impaired sleep in glaucoma may be related to loss of ipRGCs and consequently, a reduction of the melanopsin-mediated light signaling to the suprachiasmatic nuclei (SCN), the principal biological pacemaker in the hypothalamus. Such reduced light input due to glaucoma-related loss of ipRGCs (1) may be further amplified by physiologically occurring age-related ipRGC loss (30), and physiologic changes of circadian rhythm regulation and sleep (31, 32), as glaucoma generally affects elderly persons (30, 33). Since light is the principal external zeitgeber on which the biological circadian clock aligns to the 24-h day–night cycle, the combination of inadequate light exposure during daytime and loss of ipRGCs together may reduce the effectiveness of light as a zeitgeber in patients with glaucoma. The potential consequence is impaired circadian entrainment and adverse influence on well-being, mood, 24-h rest-activity cycles and sleep (34).

We hypothesize that it is possible to enhance the external zeitgeber strength by increasing daytime light exposure in patients with glaucoma. This, in turn, will have beneficial effects, particularly on sleep, 24-h rest-activity cycles, mood and well-being. Because we presume the effect of light is mediated through the melanopsin system, we also hypothesize that melanopsin activity will change and will be detectable in the pupil response (6, 35, 36). Thus, the aim of this study is to assess how scheduled additional bright light exposure during daytime might impact pupil light reflex, sleep, circadian rest-activity cycles and subjective mood and well-being in patients with glaucoma.

METHODS

Participants

Study participants were recruited from patients actively treated for glaucoma at the Hôpital Ophtalmique Jules-Gonin in Lausanne, Switzerland. Patients with the following conditions were excluded: unilateral glaucoma, congenital glaucoma, non-glaucomatous optic neuropathy, diabetes, past or present cancer, sleep apnea, diagnosed mood disorder, recent use of recreational drugs (e.g. cannabis, cocaine), alcohol dependency or pregnancy. Other exclusionary criteria included night shift work within the last three months, current use of sleeping pills or travel across a time zone <1 month before study participation. All participants provided oral and written informed consent and were approved by their treating ophthalmologist to participate in the study. The study was conducted at the Hôpital Ophtalmique Jules Gonin, Lausanne, Switzerland according to the tenets of the Declaration of Helsinki and received authorization from the local ethical review board for human research (Commission d'Ethique de Recherche sur l'être humain de Canton de Vaud, Switzerland no. 2018-01749). All participants were clinically examined at least once by their treating ophthalmologist during or after study participation.

Twenty patients with glaucoma participated in the study. They were 9 women and 11 men, mean age = 67.55 ± 7.45 years, age range 53 to 79 years. The types of glaucoma were primary open angle ($n = 10$), primary angle closure ($n = 8$) and pseudo exfoliative glaucoma ($n = 2$). Eight patients had undergone laser iridoplasty, and seven patients had undergone glaucoma filtration surgery. Ten patients had undergone uncomplicated cataract surgery with intraocular lens replacement. All procedures had been performed at least 6 months prior to study participation. These procedures did not affect the iris sphincter muscle contractibility, as assessed at the slit lamp.

Study Design

From the medical charts, the following ophthalmologic information was extracted for each eye: best-corrected visual acuity, mean defect of the automated visual field, and thickness of the peripapillary retinal nerve fiber layer (RNFL) from the optical coherence tomography (see **Supplementary Table 1**). If the ophthalmologic examination dated more than 6 months before study participation, the participant underwent these tests at the first study session. The study comprised 4 sessions at the Hôpital Ophtalmique Jules Gonin (46.5197° N, 6.6323° E), Switzerland over 5 consecutive weeks and was conducted from January to July 2019.

At session 1, the participant came to the hospital, gave informed written consent and completed six standardized questionnaires: Horne-Ostberg [HO; (37)], Munich Chronotype Questionnaire [MCTQ; (38)], Epworth Sleepiness Scale [ESS; (39)], Pittsburgh Sleep Quality Index [PSQI; (40)], Seasonal Pattern Assessment Questionnaire [SPAQ; (41)] and the Beck Depression Inventory [BDI; (42)]. These questionnaires were used to assess chronotype, daytime sleepiness, habitual sleep quality (during the preceding 4 weeks), seasonal effects and depressive symptoms (see **Supplementary Table 2**). At the end of session 1, the participant received an activity monitor (Actiwatch L, Camntech Cambridge, UK) to be continuously worn on the non-dominant wrist during the next 5 weeks in order to record 24-h rest-activity cycles. The participant was instructed to maintain a daily sleep log (bedtime, wake up time) and note subjective sleep quality (1 = very bad sleep, 10 = excellent sleep)

every morning. Also, the participant made a daily self-assessment of well-being and visual comfort using a visual analog scale (see section Methods).

After 1 week, the participant came again to the hospital (session 2) and underwent pupillometry (see below). At the end of session 2, the participant was given a commercially available table-based light box (EnergyUp / HF3419^{TM} Philips, The Netherlands) for home use. The light source emits a diffuse polychromatic bright white light at 10,000 lux [see light measures according to the CIE Standard, (CIE S 026) in the **Supplementary Table 3**]. The participant was instructed to sit face-forward at 50 cm distance from the light box every morning around the same time for 30 min over the next 4 weeks. No additional change of their usual lifestyle was requested.

After 2 weeks, participants were asked to come to the laboratory for session 3 which served to verify compliance with the light exposure at home and to download rest-activity data of the first 3 weeks. The activity monitor was then returned to participants. After another 2 weeks, participants came to the laboratory for the final session 4 to complete one questionnaire (second PSQI) and to undergo the second pupillometry. Participants also returned the completed subjective assessments, the activity monitor and the light box. An overview of the study design is shown in **Supplementary Figure 1**.

Pupillometry

In this study, pupillometry was used as a quantitative method to assess the functionality of the melanopsin-signaling pathway. The pupillometer used for this study was a monocular device (Neurolight, IDMed, Marseille, France) which presented light stimuli and recorded the pupil of the stimulated eye at a sampling frequency of 67 Hz. The light stimulus was a narrow bandwidth short-wavelength light stimulus (peak wavelength at 470 nm; "blue") having a 1s duration. Two brightness intensities (luminance: 56 cd/m^2 and 170 cd/m^2) were used with intention of activating rod and cone photoreceptors as well as the melanopsin photopigment of ipRGCs, as described in previous studies (36, 43). The pupil test started with 3 s of darkness followed by a blue light stimulus at lower luminance (56 cd/m^2) and then a second blue light stimulus at higher luminance (170 cd/m^2). The inter-stimulus dark interval was 15 s. By convention, the right eye was always tested first. Both eyes were tested under photopic condition (prior adaptation to room light at 150 lux at a vertical direction at eye level for 10 min), and scotopic condition (adaptation to darkness 0 lux for 20 min). The non-tested eye was covered by the participant's hand. Pupils were measured twice, once at session 2, that is, before the start of scheduled daily bright light exposure from the light box and denoted as pre-light exposure (= pre-LE), and again at session 4, that is, after 4 weeks of daily bright light, denoted as post-light exposure (= post-LE).

From the pupillometer device, raw tracings were downloaded with NL Viewer Software (v 1.2, IDMED, Marseille, France). All recorded tracings were visually inspected and artifacts from movement or blinking were removed by linear interpolation in Excel (Microsoft Office, v 7). The following outcome parameters were determined for each pupil recording: baseline pupil size (BL), maximum contraction amplitude (MCA), post-illumination pupil response (PIPR). The BL was calculated as the averaged pupil size during 0.25 s before the first light stimulus. Thereafter, pupil size was normalized by expressing absolute pupil size in mm as a percentage of BL in mm (%). Normalization of pupil size was important because BL of the pupil before the second stimulus was often slightly smaller than BL before the first stimulus. The MCA was identified as the smallest pupil size within 3 s from light stimulus onset and expressed in % as the difference from the baseline pupil size [maximum contraction amplitude = (1 – smallest relative pupil size) × 100%]. The PIPR (in %) was amount of pupil constriction 6 s after the stimulus light was terminated and calculated as: [(1 – relative pupil size at 6 s after light offset) × 100%].

Rest-Activity Recordings for 24-h Rhythms and Sleep

After downloading the rest-activity data (sampling frequency = 1 min), all recordings across 5 weeks were joined to one file per participant and each 24-h epoch was visually inspected. Any 24-h days with more than 3 h of missing data were excluded from further analysis (44). Missing data of <3 h were edited with the mean activity of 24-h using the software Sleep Analysis (v7, Camntech, Cambridge, UK) with an inbuilt algorithm to detect sleep (at medium sensitivity of the device). Using the non-parametric circadian rhythm analysis [NPCRA; (45)] implemented in the software (Sleep Analysis, v7), the following parameters were assessed: intra-daily variability (IV), inter-daily stability (IS), the absolute amplitudes derived from rest-activity oscillations (in arbitrary units) of 24 h (absolute amplitude, AMP) and of the 5 h with least activity (L5) and the 10 h of highest activity (M10) during a 24 h period. The M10 onset typically occurs during daytime and L5 onset typically occurs during nighttime. We also assessed the onset clock time of the 10 consecutive hours of greatest activity (M10on), the onset clock time of 5 consecutive hours of least activity (L5on). The IV evaluates the frequency of transitions between rest and activity per day, which is an indicator of fragmentation of the 24-h rest-activity rhythm. A lower IV score reflects less rest-activity rhythm fragmentation. The IS evaluates the strength of coupling between the rest-activity rhythm (45). A higher IS score is considered as greater invariability between days. The relative amplitude (RA) derives from the difference between M10 minus L5 expressed relative to the 24 h activity, where a higher RA indicates more consolidated high daytime and low nighttime activity.

In addition, bedtime (clock time), get-up time (clock time) were determined semi-manually from the activity recordings (and sleep logs). The sleep analysis tool of the same software (Sleep Analysis, v7) was used to determine the following sleep parameters, derived from rest-activity recordings (sampling frequency = 1 min epochs): time in bed (hours), sleep duration (hours), actual wake time during scheduled sleep (hours), sleep efficiency (sleep duration / time in bed x 100; %), sleep latency (time range between bed time until the first episode of consolidated sleep, as assessed by the sleep software).

Subjective sleep quality was daily assessed for 5 weeks after get-up (on the sleep log) by indicating a number between 1 (very bad sleep) and 10 (excellent sleep) on a paper-based version and individual scores were averaged per week.

Visual Analog Scales for Subjective Well-Being and Visual Comfort

Participants were instructed to complete a paper-based visual analog scale (VAS) for subjective well-being and visual comfort every day around the same clock time in the morning during 5 weeks. For subjective well-being, the VAS consists of a vertical line from 0 and 100 mm and represents the extremes of relaxation, physical comfort, alertness: 0 mm = extremely relaxed/100 mm = extremely tense; 0 mm = physically comfortable/100 mm = physically not at all comfortable; 0 mm = extremely alert/100 mm = extremely sleepy; 0 mm = bad mood/100 mm = very good mood. The participant indicated the current state of these parameters by marking a vertical line on the scale.

For visual comfort, five specific items of lighting were assessed [adapted from the Office Lighting scale (46)] and presented on a VAS between 0 mm and 100 mm: (1) I like the lighting (0 mm)/I do not like the lighting in this room (100 mm); (2) the lighting is pleasant (0 mm)/the lighting is not pleasant at all (100 mm); (3) this room is too bright (0 mm)/this room is too dark (100 mm); (4) there is too much light to read/work properly (0 mm)/there is not enough light to read/work properly (100 mm); and (5) the glare in this room is imperceptible (0 mm)/the glare in this room is intolerable (100 mm). The participant indicated the current state of these items by marking a vertical line on the VAS scale. For analysis, a weekly average was determined from the paper-based daily assessed VAS, for each parameter of well-being (relaxation, physical comfort, alertness, mood) and for five items of visual comfort.

Statistics

For differences between ophthalmological screening measures (left and right eyes) two-tailed *t*-tests were applied. For pupil data, a mixed linear model with the factor WEEK was applied to compare the recording of the pre-LE with the recording after the 4 weeks (= post-LE) with daily scheduled light exposure with AGE, SEX and their interactions also added to the model. For all analyses, AGE was used on dichotomized variables based on median split (median = 69.5 years). For continuous rest-activity and sleep data as well as subjective assessments, all averaged per week, a generalized linear regression model was used with the repeated factor WEEK (i.e., averages of pre-LE week 1 and each of the LE weeks 2-5) and the fixed factors AGE, SEX, and their interactions. *Post-hoc* tests were performed with the Tukey-Kramer, adjusted for multiple comparisons. For tables, averaged values of the pre-LE and the average of LE-weeks 2–5 are shown. For correlations Pearson's correlation was used. The SAS software (v. 9.4.; SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

RESULTS

Participants

There was no significant interocular difference for visual acuity (VA) and for mean deviation (MD) on automated perimetry (2-sided *t*-tests; $p > 0.09$). The VA ranged from 0.3 to 1.5, mean 0.85 ± 0.17 for both eyes (**Supplementary Table 1**). The mean MD for both eyes was 5.41 dB ± 3.17 for both eyes, range −0.9 to 20 dB. On OCT, the peripapillary retinal nerve fiber layer (RNFL) thickness was abnormal or borderline in all but one eye of 19 patients as OCT data from one patient was not available. The descriptive statistics for the questionnaires (MCTQ, ESS, HO, SPAQ, BDI and PSQI) are shown on **Supplementary Table 2**. The post-LE PSQI was not significantly different ($p = 0.67$; generalized linear model) from pre-LE.

Pupillometry

There were small, but statistically significant differences between left and right eyes in scotopic baseline pupil size (**Supplementary Table 4**). Because the right eye was always tested first and reflects the most accurate photopic and scotopic adapted condition, we used the pupil results from right eyes for further analyses. Older participants had a smaller baseline pupil size before the first light stimulus under scotopic conditions (main effect of AGE; $F_{1,20} = 5.49$; $p = 0.03$; mean ± SD older: 4.83 mm ± 0.55 mm; younger participants; 5.38 mm ± 0.84 mm). As expected, the pupil response (MCA and PIPR) to the 1s light stimulus at higher luminance (170 cd/ m^2) was greater (and BL pupil size smaller) than the response to the 1s light stimulus at lower luminance (56 cd/ m^2; **Table 1**; $F_{1,20} > 4.9$; $p < 0.038$) under photopic and scotopic testing conditions.

For all pupil parameters related to the 1s light stimulus at lower luminance (56 cd/ m^2) there was no statistically significant difference between the pre-LE and post-LE (i.e., after 4 weeks of daily scheduled bright light exposure; **Table 1**; $F_{1,20} < 1.5$; $p > 0.24$).

As a main finding of this study, the scotopic post-LE PIPR to the 1s light stimulus at higher luminance (170 cd/ m^2) was, however, significantly greater compared to pre-LE PIPR (**Table 1** and **Figure 1**). The increase in the scotopic post-light PIPR was, on average, 13% and was observed in 14 out of 20 patients (main effect of WEEK; $F_{1,20} = 6.02$, $p = 0.02$, **Supplementary Figure 2**).

24-h Rest-Activity Cycles and Sleep

Results from rest-activity recordings were averaged per week (see also methods; for one participant, the first 2 weeks of rest-activity data with daily bright light exposure were missing, and for one participant the rest-activity data during the last LE week was only available for 2 days). For 24-h rest-activity variables, there was no significant difference between the pre-LE week and the LE weeks values (**Table 2**; $F_{4,60} < 1.8$, $p > 0.17$), except for the RA which dropped in week 5 (0.84 ± 0.09) when compared to week 4 (0.87 ± 0.06; main effect of WEEK; $F_{4,59} = 3.1$, $p = 0.02$). However, there was no difference for RA between the pre-LE week and any single week during the daily light exposure ($p > 0.48$).

The sleep variables did not reveal significant changes between pre-LE and LE weeks for any of the parameters (see means ± SD

TABLE 1 | Mean values (± SD) across patients (for right eyes) for baseline pupil size (BL), maximum contraction amplitude (MCA) and post-illumination pupil response (PIPR).

	Pre-LE week	Post-LE weeks
BL photopic low (mm)	3.50 (0.73)	3.59 (0.72)
BL photopic high (mm) ∧	3.41 (0.73)	3.51 (0.72)
BL scotopic low (mm)	5.07 (0.76)	5.14 (0.77)
BL scotopic high (mm) ∧	4.61 (0.73)	4.64 (0.64)
MCA photopic low (%)	23.86 (8.79)	23.88 (7.97)
MCA photopic high (%) ∧	29.33 (8.56)	29.29 (7.98)
MCA scotopic low (%)	40.74 (6.11)	40.32 (6.37)
MCA scotopic high (%) ∧	46.02 (6.51)	46.40 (6.39)
PIPR photopic low (%)	4.17 (3.33)	3.26 (2.87)
PIPR photopic high (%) ∧	9.48 (5.90)	9.07 (3.92)
PIPR scotopic low (%)	19.14 (8.66)	20.35 (10.15)
PIPR scotopic high (%) ∧,*	25.07 (9.53)	28.71 (10.46)

*BL in mm = 100 %; MCA in % difference to BL and PIPR in % difference to BL. All mean values are shown for recordings before the pre-light exposure (pre-LE) week and the post-light exposure (post-LE), that is, the pupil recording immediately at the end of 4 weeks of scheduled daily bright light exposure. Photopic low = 1s short-wavelength narrow bandwidth light stimulus (= blue light stimulus) at lower luminance (56 cd/m^2) under photopic conditions (i.e., after 10 min of room light adaptation); photopic high = 1s blue light stimulus at higher luminance (170 cd/m^2) under photopic conditions; scotopic low = 1s blue light stimulus at lower luminance (56 cd/m^2) under scotopic conditions (i.e., after 20 min of dark adaptation); scotopic high = 1s blue light stimulus at higher luminance (170 cd/m^2) under scotopic conditions; ∧ = $p < 0.05$, indicate differences between light stimuli used for pupil measures at higher (170 cd/m^2) and lower luminance (56 cd/m^2). * = $p < 0.05$, shows difference between pupil tests pre-light exposure and post-light exposure; n = 20.*

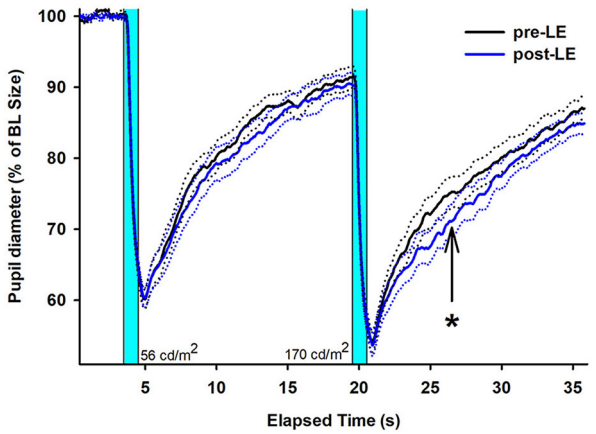

FIGURE 1 | Mean (solid lines) pupil recordings for 20 subjects with glaucoma (right eyes, SEM shown as dotted lines). The pre-LE recordings (taken in week 1) are shown in black; the post-LE recordings (taken immediately after 4 weeks after daily scheduled bright light exposure) are shown in blue (SEM = dotted black and blue lines). LE = light exposure. The two vertical bars indicate when the two blue light stimuli are given (1s duration). The first 1s light stimulus is at lower (56 cd/m^2) and the second 1s light stimulus at higher luminance (170 cd/m^2). The arrow and asterisk designate the PIPR after the second (brighter) light stimulus with a significant difference between pre- and post-LE measures ($p < 0.05$). The data is shown relative to baseline (= 100 %).

for the pre-LE week and averaged LE weeks on **Table 3**). There was a decrease in sleep efficiency from week 3 and 4 to week 5 (main effect of WEEK; $F_{4,62} = 3.3$, $p = 0.02$) from 87.6 ± 5.6 and 87.5 ± 5.8 (weeks 3 and 4) % to 86. 1 ± 6.8 % (week 5). When sleep analysis was performed only between pre-LE and LE week 5, there were no significant differences ($p > 0.36$; **Supplementary Table 5**) for any of the parameters.

TABLE 2 | Mean values (and SD in brackets) for variables from the circadian rest-activity cycles.

Variable	Pre-LE week	LE weeks
IS	0.58 (0.11)	0.59 (0.11)
IV	0.82 (0.28)	0.82 (0.22)
L5	1,434 (759)	1,600 (926)
L5on	24.78 (1.30)	24.92 (1.33)
M10	21,477 (8,100)	21,979 (8,450)
M10on	8.83 (1.63)	8.67 (1.71)
AMP	20,043 (7,935)	20,378 (8,271)
RA ∧	0.87 (0.07)	0.86 (0.08)

IS = inter-daily stability; IV = intra-daily variability; L5 = 5 h with lowest activity; L5 onset = onset time of the 5 h with least activity; M10 = 10 h with highest activity oscillations; M10 onset = onset hours of the 10 h with greatest activity. AMP = absolute amplitude; RA = relative amplitude (see method section for more explanations). Averaged values across participants are shown with SD (in brackets) for the pre – LE week and the LE weeks (i.e., the 4 weeks with scheduled daily bright light; weeks 2–5). ∧ = $p < 0.05$, indicating higher RA during week 4 than 5 (main effect of week; for mean values in weeks 4 and 5 separately see also text); n = 20.

TABLE 3 | Sleep variables derived from rest-activity recordings.

	Pre – LE week	LE weeks
Bedtime (clock time)	23.59 (1.19)	23.67 (1.31)
Sleep onset (clock time)	23.79 (1.18)	23.89 (1.32)
Wake time (clock time)	7.24 (0.99)	7.31 (1.05)
Get-up time (clock time)	7.33 (0.99)	7.39 (1.05)
Time in bed (h)	7.72 (0.74)	7.71 (0.77)
Sleep duration (h)	7.44 (0.73)	7.41 (0.77)
Wake duration (h)	0.68 (0.39)	0.69 (0.34)
Sleep efficiency (%) ∧	87.43 (6.42)	87.14 (5.77)
Sleep latency (h)	0.21 (0.16)	0.22 (0.16)

All values are indicated as means and SD (in brackets) for the pre – light exposure (LE) week and LE weeks (i.e., average of weeks 2–5 when patients were exposed to bright light); n = 20. Bedtimes, sleep onset, wake and get-up times are indicated as clock time (h.mm), and time in bed as well as sleep duration and wakefulness during sleep episodes in hours (h.mm). Sleep efficiency (SE) derived from the ratio of sleep duration / time in bed is expressed as percentage (%). Sleep latency indicates the time range between bedtime and consolidated sleep (indirectly assessed via the inbuilt algorithm of the sleep analysis software). There were no statistically significant differences between pre-LE and LE weeks. ∧ = $p < 0.05$, indicating higher SE during weeks 3 and 4 than 5 (main effect of week; for mean values in weeks 3–5 separately see also text); n = 20.

Subjective Sleep Quality and Well-Being

Subjective sleep quality became significantly better for week 4 of the LE weeks (7.5 ± 1.5 mean ± SD) when compared to the pre-LE week (6.8 ± 1.5; $F_{4,63} = 2.7$, $p = 0.04$; main effect of WEEK). **Table 4a** shows the values for the pre-LE week and the average of all LE weeks. For the time course of subjective sleep quality, see **Figure 2**. The **Supplementary Figure 2** shows individual increases of subjective sleep quality in 15 of 20

TABLE 4a | Subjective assessments for sleep quality [score 1 (very bad) – 10 (excellent)], and results from visual analog scales for relaxation (0 = extremely relaxed, 100 = extremely tense), physical comfort (0 = physically comfortable, 100 = physically not at all comfortable), alertness (0 = extremely alert, 100 = extremely sleepy) and mood (0 = bad mood and 100 = very good mood) on visual analog scales (0–100 mm).

	Pre – LE week	LE weeks
Subjective sleep quality*	6.83 (1.53)	7.25 (1.53)
Relaxation	37.6 (19.0)	36.6 (22.1)
Physical comfort	32.2 (17.7)	34.2 (20.3)
Alertness	35.9 (23.2)	32.7 (19.2)
Mood*	71.3 (16.7)	66.5 (18.2)

*LE = light exposure; all values are indicated as means and SD (in brackets) for the pre – LE week and the light LE weeks (i.e., average of weeks 2–5 when patients were exposed to bright light); n = 20; * = $p < 0.05$, main effect WEEK.*

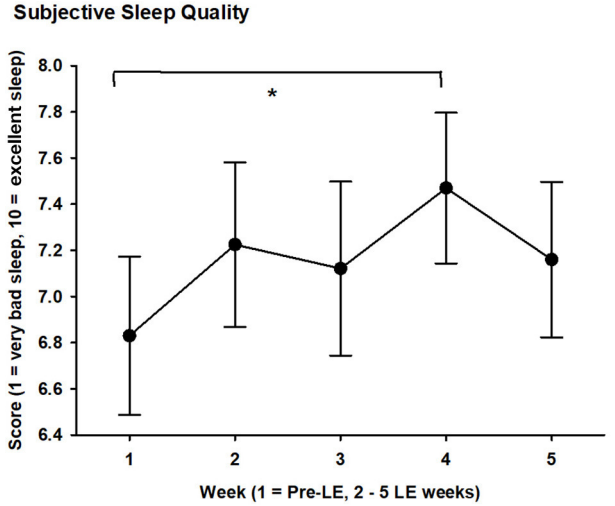

FIGURE 2 | Subjective sleep quality score (1 = very bad sleep, 10 = excellent sleep) averaged per week during 5 weeks for 20 subjects with glaucoma. Mean values (black filled circles) and SEM are shown; LE = light exposure; week 1 = pre-LE week; weeks 2–5 = LE weeks. * = significant differences between weeks 1 and 4 (see text for mean value during week 4; $p < 0.05$).

participants during the LE weeks (subjective sleep quality data for week 3 was missing from one participant).

For relaxation, physical comfort and alertness there was no significant difference between pre-LE and LE weeks values. Mood decreased in the first LE week compared to pre-LE, but was not significantly different from pre-LE for the other 3 weeks (main effect of WEEK, $F_{4,62} = 2.7$, $p = 0.04$).

Visual Comfort

In general, the enhanced daily bright light exposure was well-tolerated by all participants and there were no negative reports from the light exposure such as headache, discomfort glare or eye strain. None of the 5 items of the visual comfort changed between the pre-LE week and the LE weeks (main effect of WEEK; $F_{4,61} < 1.5$, $p > 0.22$). All visual comfort scores were on average in the first half, that is, better comfort (item 1, 2, 5) or neutral (items 3, 4; i.e., in the middle, around 50; **Table 4b**). Visual comfort (and well-being) data from one participant was missing for 2 weeks during light exposure.

TABLE 4b | Subjective daily assessments for visual comfort (five items) on visual analog scales (1 – 100 mm).

	Pre – LE week	LE weeks
I like the light in this room (yes – no)	31.4 (17.0)	30.0 (17.3)
The light is comfortable (yes – no)	30.7 (17.0)	30.1 (17.4)
This room is too bright/too dark	50.7 (13.3)	47.2 (11.5)
There is too much light to read/there is not enough light to read	51.8 (13.8)	48.2 (10.6)
The glare in this room is imperceptible/intolerable	35.4 (16.4)	34.1 (20.5)

LE = light exposure.
All values are shown as mean values and SD (in brackets) for the pre-LE week and LE weeks (i.e., average of weeks 2–5 when patients were exposed to scheduled daily bright light); n = 20.

Correlations

To correlate changes between the pre-LE and the LE week, values of the pre-LE week were subtracted from mean values of 4 LE weeks for the circadian and sleep related parameters as well as the subjective sleep quality and the VAS. Also, the difference between pre-LE and post-LE was used for all pupil parameters from two recordings, and the same was also done for the PSQI. There were significant correlations for increased PIPR (at lower luminance; 56 cd/m^2) during LE weeks with greater inter-daily stability (IS; $R^2 = 0.275$; $p = 0.018$; **Figure 3**, upper graph) and greater relative amplitude of 24-h rest-activity cycles (RA; $R^2 = 0.296$; $p = 0.013$; **Figure 3**, lower graph) during LE-weeks. A statistical trend in the same direction was observed for the higher luminance PIPR (170 cd/m^2) and relative amplitude ($p = 0.07$; $R^2 = 0.17$). An increase in subjective sleep quality during LE was correlated with a lower global PSQI score post LE (a lower PSQI is better), which suggests, that both assessments went in the same (positive) direction ($p = 0.04$; $R^2 = 0.21$; see **Supplementary Figure 3**, upper graph). An increase in subjective mood during the LE weeks was correlated with earlier wake time ($p = 0.03$; $R^2 = 0.23$; see **Supplementary Figure 3**, lower graph).

DISCUSSION

In a small group of patients with glaucoma of heterogeneous types and without severe visual loss, we found that increasing their daily light exposure during daytime by adding a table-based light box in their home had a favorable effect on subjective sleep quality and increased their melanopsin dependent pupil response (PIPR). This effect was observed after only 4 weeks. The scheduled bright light exposure, which was added to habitual light exposure for 30 min daily, did not alter or interfere with the daily activities of the participants. The additional light exposure did not alter timing or duration of habitual sleep nor did it change 24-h rest-activity cycles (both assessed from activity recordings). There was an increase in PIPR at the end of 4 weeks of light exposure and there was a significant correlation between the

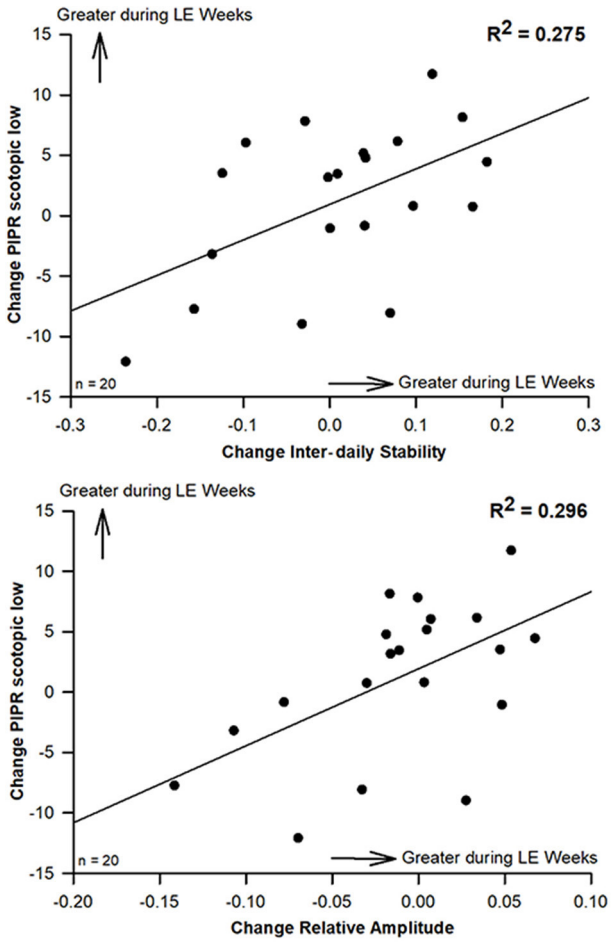

FIGURE 3 | Scatterplots (and regression lines) for significant correlations between changes of the scotopic PIPR (to the lower luminance 1s light stimulus at 56 cd/m^2) and inter-daily stability (IS; upper graph; $R^2 = 0.275$) and with relative amplitude of rest-activity cycles (RA; lower graph; $R^2 = 0.296$; Pearson's correlation; $n = 20$). Positive numbers indicate higher IS and RA as well as greater scotopic PIPR (to the 1s light stimulus at lower luminance; 56 cd/m^2) during LE weeks (compared to pre-LE; $p < 0.05$; $n = 20$.

change in PIPR and the change in 24-h amplitude and inter-daily stability of rest-activity cycles during light exposure.

In line with our hypothesis, we found the melanopsin-mediated pupil response, the PIPR, was greater after 4 weeks of additional daytime light exposure. This change in PIPR suggests that in patients with glaucoma, the melanopsin activity in viable ipRGCs can adapt to different light levels if sustained over a certain period. Why might we think that additional daytime light exposure over 4 weeks is an adaptation response of the melanopsin system? In a previous study, we had demonstrated that the PIPR to a bright narrow-bandwidth short wavelength light modulates with long-term changes in light timing, such as seasonal changes of daylight (36, 47). In the current study, we did not change the light exposure timing, but enhanced brightness of light exposure in the morning over 4 weeks. We found a greater PIPR at the end of 4 weeks without any substantial change in timing of rest-activity, sleep duration or 24-h amplitude as assessed from rest-activity recordings. The significant correlation with higher relative amplitude and inter-daily stability (both derived from rest-activity cycles over 4 weeks) with higher PIPR after LE weeks suggests that there may be a common mode of action conveyed by melanopsin, even though this correlation is not taken as evidence for causality.

We cannot exclude the possibility that the increase in subjective sleep quality is a "placebo" effect or linked to other behavioral factors as it may be the decrease of mood between the pre-LE week and the first week with LE. The absence of change in the objective sleep (or circadian) parameters, assessed from the activity recordings, when compared to the pre-LE week might be taken as support for a placebo effect, especially since we did not ask participants to adhere to a certain sleep-wake schedule. We would argue that this seeming discrepancy in the subjective vs. objective sleep evaluation may be a methodologic one. Given the nature of this field study, we used rest-activity recordings as an indirect objective measure of sleep whereas the studies assessing sleep in a laboratory setting with standardized means, that is, polysomnography, have demonstrated that improved subjective sleep quality implicates objectively improved sleep. These studies found that sleep continuity and rapid eye movement (REM) sleep were correlated with subjective sleep quality (48) and that slow wave sleep ("deep sleep") was the best predictor for subjective sleep quality (49).

Another minor but interesting result of our study was the correlation between changes toward earlier wake times during the LE weeks and better subjective mood when compared to pre-LE. This goes along with well-established evidence for light therapy outcomes for patients with mood disorders. Benedetti and colleagues for example showed, (50) that a combination of light therapy and sleep deprivation resulted in antidepressant effects which were correlated with circadian phase advances of rest-activity acrophases. Even though our patients with glaucoma were not depressed, and we did not determine circadian phase or found a difference in their sleep timing over 4 weeks, the correlation with an earlier wake time and better mood during the LE weeks (compared to pre-LE) points in a similar direction, but will certainly need further consideration to confirm improved non-visual functions in patients with ipRGC loss. We recognize that 10 of 20 patients had an artificial intraocular lens (non-blue-light blocking) which permits greater transmission of the light into the eye compared to the natural lens of the other 10 patients. However, a previous study had shown no correlation between lens transmission of blue light and pupil response (51). The current study assessed the change in pupil response before and after additional daytime light in a within subject-design, thus lens status is not likely to be a confounding variable to our results.

A potential concern regarding use of a light box might be the "blue light hazard." Indeed there is a photochemical risk to the retinal tissues of the eye associated with ocular exposure to bright light sources such as the sun or welding arcs (52). The term "blue light hazard" defines the optical radiation risk for photochemical injury which peaks in the short-wavelength ("blue") part of the optical radiation spectrum around 435 to 440 nm. The International Commission on Illumination (Commission Internationale de l' Eclairage, CIE) published a standard on this [as part of the CIE S 009:2002 "*Photobiological safety of lamps and lamp systems,*" (53)] and states in its position

statement:…*'There is no evidence in humans of any adverse health effects from occasional exposure to optical radiation at the exposure limits.'*…(54). The lamps in our study emitted a broad spectrum white light and exposure followed the manufacturer guidelines. Clinical follow-up of patients within months of study participation did not demonstrate evidence of retinal toxicity.

This study is not a call for the use of light therapy for patients with glaucoma. Rather, the study results lend further support to the notion that patients in whom ipRGCs are damaged or dysfunctional due to glaucoma (or other neuroretinal disorders) may benefit from enhanced daytime light exposure which serves as a more effective zeitgeber. In turn, better distinction of their biological day and night may influence active synchronization of the internal circadian clocks with the external 24-h day (also called entrainment). While more daytime light may seem like obvious good advice, it is not always apparent and in practice. Most persons are indoors during many hours of the day (school, workplace, home) and office lighting is far less bright than natural daylight. Elderly persons tend to remain indoors for a variety of reasons: medical problems such as unsteady gait or poor vision, social issues such as fear of public transport, social isolation or confined living in an institution and personal comfort such as getting cold easily. This study has demonstrated that even a rather short duration of added light exposure (30 min) in a room is beneficial and supports the general advice to elderly persons to sit outdoors or go outside for 30 min each morning.

Thus, while glaucoma patients can never recover the vision lost from damaged retinal ganglion cells, they may be able to maintain a robust day–night cycle and concomitant good circadian entrainment which helps maintain high sleep quality. This will indirectly support good daytime performance and a sense of well-being. All these effects together might reduce vulnerability for other co-morbidities such as depression and disordered sleep. For this reason, further studies examining optimized light (intensity and timing) for glaucoma patients are needed.

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 Commission d'Ethique de Recherche sur l'être humain de Canton de Vaud, Switzerland no. 2018-01749. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AK and MM designed the experiment. MU and ME performed the study. AK, MU, and MM analyzed the data. AK and MM wrote the manuscript. All authors 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/fneur.2020.584479/full#supplementary-material

REFERENCES

1. Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of melanopsin-expressing retinal ganglion cells in severely staged glaucoma patients. *Invest Ophthalmol Vis Sci.* (2016) 57:4661–7. doi: 10.1167/iovs.16-19997
2. Esquiva G, Hannibal J. Melanopsin-expressing retinal ganglion cells in aging and disease. *Histol Histopathol.* (2019) 34:1299–311. doi: 10.14670/HH-18-138
3. Drouyer E, Dkhissi-Benyahya O, Chiquet C, WoldeMussie E, Ruiz G, Wheeler L, et al. Glaucoma alters the circadian timing system. *PLoS ONE.* (2008) 3:e3931. doi: 10.1371/journal.pone.0003931
4. de Zavalía N, Plano SA, Fernandez DC, Lanzani MF, Salido E, Belforte N, et al. Effect of experimental glaucoma on the non-image forming visual system. *J Neurochem.* (2011) 117:904–14. doi: 10.1111/j.1471-4159.2011.07260.x
5. Perez-Rico C, de la Villa P, Arribas-Gomez I, Blanco R. Evaluation of functional integrity of the retinohypothalamic tract in advanced glaucoma using multifocal electroretinography and light-induced melatonin suppression. *Exp Eye Res.* (2010) 91:578–83. doi: 10.1016/j.exer.2010.07.012
6. Kankipati L, Girkin CA, Gamlin PD. The post-illumination pupil response is reduced in glaucoma patients. *Invest Ophthalmol Vis Sci.* (2011) 52:2287–92. doi: 10.1167/iovs.10-6023
7. Münch M, Léon L, Collomb S, Kawasaki A. Comparison of acute non-visual bright light responses in patients with optic nerve disease, glaucoma and healthy controls. *Sci Rep.* (2015) 5:15185. doi: 10.1038/srep15185
8. Ahmadi H, Lund-Andersen H, Kolko M, Bach-Holm D, Alberti M, Ba-Ali S. Melanopsin-mediated pupillary light reflex and sleep quality in patients with normal tension glaucoma. *Acta Ophthalmol.* (2020) 98:65–73. doi: 10.1111/aos.14133
9. Adhikari P, Zele AJ, Thomas R, Feigl B. Quadrant field pupillometry detects melanopsin dysfunction in glaucoma suspects and early glaucoma. *Sci Rep.* (2016) 6:33373. doi: 10.1038/srep33373
10. Rukmini AV, Milea D, Baskaran M, How AC, Perera SA, Aung T, et al. Pupillary responses to high-irradiance blue light correlate with glaucoma severity. *Ophthalmology.* (2015) 122:1777–85. doi: 10.1016/j.ophtha.2015.06.002
11. Yochim BP, Mueller AE, Kane KD, Kahook MY. Prevalence of cognitive impairment, depression, and anxiety symptoms among older adults with glaucoma. *J Glaucoma.* (2012) 21:250–4. doi: 10.1097/IJG.0b013e3182071b7e
12. Ayaki M, Shiba D, Negishi K, Tsubota K. Depressed visual field and mood are associated with sleep disorder in glaucoma patients. *Sci Rep.* (2016) 6:25699. doi: 10.1038/srep25699
13. Agorastos A, Skevas C, Matthaei M, Otte C, Klemm M, Richard G, et al. Depression, anxiety, and disturbed sleep in glaucoma. *J Neuropsychiatry Clin Neurosci.* (2013) 25:205–13. doi: 10.1176/appi.neuropsych.12020030
14. Ksendzovsky A, Pomeraniec IJ, Zaghloul KA, Provencio JJ, Provencio I. Clinical implications of the melanopsin-based non-image-forming visual system. *Neurology.* (2017) 88:1282–90. doi: 10.1212/WNL.0000000000003761
15. Münch M, Kawasaki A. Intrinsically photosensitive retinal ganglion cells: classification, function and clinical implications. *Curr Opin Neurol.* (2013) 26:45–51 doi: 10.1097/WCO.0b013e32835c5e78
16. La Morgia C, Carelli V, Carbonelli M. Melanopsin retinal ganglion cells and pupil: clinical implications for neuro-ophthalmology. *Front Neurol.* (2018) 9:1047. doi: 10.3389/fneur.2018.01047
17. Gracitelli CP, Duque-Chica GL, Moura AL, Roizenblatt M, Nagy BV, Ragot de Melo G, et al. Relationship between daytime sleepiness and intrinsically photosensitive retinal ganglion cells in glaucomatous disease. *J Ophthalmol.* (2016) 2016:5317371. doi: 10.1155/2016/5317371

18. Gracitelli CP, Duque-Chica GL, Roizenblatt M, Moura AL, Nagy BV, Ragot de Melo G, et al. Intrinsically photosensitive retinal ganglion cell activity is associated with decreased sleep quality in patients with glaucoma. *Ophthalmology.* (2015) 122:1139–48. doi: 10.1016/j.ophtha.2015.02.030
19. Chin JY, Toh ZH, Lo YT, Wang HT, Poh EY, Chua CH, et al. Effects of primary glaucoma on sleep quality and daytime sleepiness of patients residing at an equatorial latitude. *Int J Ophthalmol.* (2020) 13:1451–58. doi: 10.18240/ijo.2020.09.18
20. Qiu M, Ramulu PY, Boland MV. Association between sleep parameters and glaucoma in the United States population: National Health and Nutrition Examination Survey. *J Glaucoma.* (2019) 28:97–104. doi: 10.1097/IJG.0000000000001169
21. Lanzani MF, de Zavalía N, Fontana H, Sarmiento MI, Golombek D, Rosenstein RE. Alterations of locomotor activity rhythm and sleep parameters in patients with advanced glaucoma. *Chronobiol Int.* (2012) 29:911–9. doi: 10.3109/07420528.2012.691146
22. Gubin DG, Malishevskaya TN, Astakhov YS, Astakhov SY, Cornelissen G, Kuznetsov VA, et al. Progressive retinal ganglion cell loss in primary open-angle glaucoma is associated with temperature circadian rhythm phase delay and compromised sleep. *Chronobiol Int.* (2019) 36:564–77. doi: 10.1080/07420528.2019.1566741
23. Wang H, Zhang Y, Ding J, Wang N. Changes in the circadian rhythm in patients with primary glaucoma. *PLoS ONE.* (2013) 8:e62841. doi: 10.1371/journal.pone.0062841
24. Guo ZZ, Jiang SM, Zeng LP, Tang L, Li N, Xu ZP, et al. ipRGCs: possible causation accounts for the higher prevalence of sleep disorders in glaucoma patients. *Int J Ophthalmol.* (2017) 10:1163–1167. doi: 10.18240/ijo.2017.07.22
25. Chaitanya A, Pai VH, Mohapatra AK, Ve RS. Glaucoma and its association with obstructive sleep apnea: A narrative review. *Oman J Ophthalmol.* (2016) 9:125–34. doi: 10.4103/0974-620X.192261
26. Aptel F, Khayi H, Pépin JL, Tamisier R, Levy P, Romanet JP, et al. Association of nonarteritic ischemic optic neuropathy with obstructive sleep apnea syndrome: consequences for obstructive sleep apnea screening and treatment. *JAMA Ophthalmol.* (2015) 133:797–804. doi: 10.1001/jamaophthalmol.2015.0893
27. Fraser CL. Obstructive sleep apnea and optic neuropathy: is there a link? *Curr Neurol Neurosci Rep.* (2014) 14:465. doi: 10.1007/s11910-014-0465-5
28. Lin CC, Hu CC, Ho JD, Chiu HW, Lin HC. Obstructive sleep apnea and increased risk of glaucoma: a population-based matched-cohort study. *Ophthalmology.* (2013) 120:1559–64. doi: 10.1016/j.ophtha.2013.01.006
29. Wozniak D, Bourne R, Peretz G, Kean J, Willshire C, Harun S, et al. Obstructive sleep apnea in patients with primary-open angle glaucoma: no role for a screening program. *J Glaucoma.* (2019) 28:668–75. doi: 10.1097/IJG.0000000000001296
30. Esquiva G, Lax P, Pérez-Santonja JJ, García-Fernández JM, Cuenca N. Loss of melanopsin-expressing ganglion cell subtypes and dendritic degeneration in the aging human retina. *Front Aging Neurosci.* (2017) 9:79. doi: 10.3389/fnagi.2017.00079
31. Duffy JF, Zitting KM, Chinoy ED. Aging and circadian rhythms. *Sleep Med Clin.* (2015) 10:423–34. doi: 10.1016/j.jsmc.2015.08.002
32. Cajochen C, Münch M, Knoblauch V, Blatter K, Wirz-Justice A. Age-related changes in the circadian and homeostatic regulation of human sleep. *Chronobiol Int.* (2006) 23:461–74. doi: 10.1080/07420520500545813
33. Turner PL, Mainster MA. Circadian photoreception: ageing and the eye's important role in systemic health. *Br J Ophthalmol.* (2008) 92:1439–44. doi: 10.1136/bjo.2008.141747
34. Wulff K, Foster RG. Insight into the role of photoreception and light intervention for sleep and neuropsychiatric behaviour in the elderly. *Curr Alzheimer Res.* (2017) 14:1022–29. doi: 10.2174/1567205014666170523095231
35. Tsika C, Crippa SV, Kawasaki A. Differential monocular vs. binocular pupil responses from melanopsin-based photoreception in patients with anterior ischemic optic neuropathy. *Sci Rep.* (2015) 5:10780. doi: 10.1038/srep10780
36. Kawasaki A, Wisniewski S, Healey B, Pattyn N, Kunz D, Basner M, et al. Impact of long-term daylight deprivation on retinal light sensitivity, circadian rhythms and sleep during the Antarctic winter. *Sci Rep.* (2018) 8:16185. doi: 10.1038/s41598-018-33450-7
37. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol.* (1976) 4:97–110.
38. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. *J Biol Rhythms.* (2003) 18:80–90. doi: 10.1177/0748730402239679
39. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep.* (1991) 14:540–5. doi: 10.1093/sleep/14.6.540
40. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* (1989) 28:193–213. doi: 10.1016/0165-1781(89)90047-4
41. Rosenthal NE. *Winter Blues: Seasonal Affective Disorder: What It Is and How to Overcome It.* New York, NY:Guildford Press (1993).
42. Beck AT, Steer RA, Ball R, Ranieri W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess.* (1996) 67:588–97. doi: 10.1207/s15327752jpa6703_13
43. Münch M, Kourti P, Brouzas D, Kawasaki A. Variation in the pupil light reflex between winter and summer seasons. *Acta Ophthalmol.* (2016) 94:e244–6. doi: 10.1111/aos.12966
44. Bromundt V, Wirz-Justice A, Kyburz S, Opwis K, Dammann G, Cajochen C. Circadian sleep-wake cyles, well-being, and light therapy in borderline personality disorder. *J Pers Disord.* (2012) 27:680–96. doi: 10.1521/pedi_2012_26_057
45. Van Someren EJ, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. *Chronobiol Int.* (1999) 16:505–18. doi: 10.3109/07420529908998724
46. Eklund NH, Boyce PR. The development of a reliable, valid and simple office lighting survey. *J Illum Eng Soc.* (1996) 25:25–40. doi: 10.1080/00994480.1996.10748145
47. Münch M, Ladaique M, Roemer S, Hashemi K, Kawasaki A. Melanopsin-mediated acute light responses measured in winter and in summer: seasonal variations in adults with and without cataracts. *Front Neurol.* (2017) 8:464. doi: 10.3389/fneur.2017.00464
48. Della Monica C, Johnsen S, Atzori G, Groeger JA, Dijk DJ. Rapid eye movement sleep, sleep continuity and slow wave sleep as predictors of cognition, mood, and subjective sleep quality in healthy men and women, aged 20-84 years. *Front Psychiatry.* (2018) 9:255. doi: 10.3389/fpsyt.2018.00255
49. Keklund G, Akerstedt T. Objective components of individual differences in subjective sleep quality. *J Sleep Res.* (1997) 6:217–20. doi: 10.1111/j.1365-2869.1997.00217.x
50. Benedetti F, Dallaspezia S, Fulgosi MC, Barbini B, Colombo C, Smeraldi E. Phase advance is an actimetric correlate of antidepressant response to sleep deprivation and light therapy in bipolar depression. *Chronobiol Int.* (2007) 24:921–37. doi: 10.1080/07420520701649455
51. Herbst K, Sander B, Lund-Andersen H, Broendsted AE, Kessel L, Hansen MS, et al. Intrinsically photosensitive retinal ganglion cell function in relation to age: a pupillometric study in humans with special reference to the age-related optic properties of the lens. *BMC ophthalmol.* (2012) 12:4. doi: 10.1186/1471-2415-12-4
52. ICNIRP guidelines on limits of exposure to incoherent visible and infrared radiation. *Health Phys.* (2013) 105:74–96. doi: 10.1097/HP.0b013e318289a611
53. IEC 62471:2006/CIE S 009:2002. Photobiological Safety of Lamps and Lamp Systems. *International Commission on Illumination (CIE)* Division 6, Vienna (2006).
54. CIE Position Statement on the Blue Light Hazard. International Commission on Illumination (CIE) Central Bureau. Vienna (2019).

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

REVIEW
published: 27 January 2021
doi: 10.3389/fneur.2021.630553

Human-Centric Lighting: Foundational Considerations and a Five-Step Design Process

Kevin W. Houser[1,2]* *and Tony Esposito*[3]

[1] School of Civil and Construction Engineering, Oregon State University, Corvallis, OR, United States, [2] Advanced Lighting Team, Pacific Northwest National Laboratory, Portland, OR, United States, [3] Lighting Research Solutions LLC, Cambridge, MA, United States

At its best, human-centric lighting considers the visual and non-visual effects of light in support of positive human outcomes. At its worst, it is a marketing phrase used to healthwash lighting products or lighting design solutions. There is no doubt that environmental lighting contributes to human health, but how might one practice human-centric lighting given both the credible potential and the implausible hype? Marketing literature is filled with promises. Technical lighting societies have summarized the science but have not yet offered design guidance. Meanwhile, designers are in the middle, attempting to distinguish credible knowledge from that which is dubious to make design decisions that affect people directly. This article is intended to: (1) empower the reader with fundamental understandings of ways in which light affects health; (2) provide a process for human-centric lighting design that can dovetail with the decision-making process that is already a part of a designer's workflow.

Keywords: human-centric lighting, lighting quality, non-visual action of light, circadian light effects, alertness

OPEN ACCESS

Edited by:
Manuel Spitschan,
University of Oxford, United Kingdom

Reviewed by:
Oliver Stefani,
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Katharina Wulff,
Umeå University, Sweden
Ruth Kelly Waskett,
Hoare Lea, United Kingdom

***Correspondence:**
Kevin W. Houser
kevin.houser@oregonstate.edu

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *17 November 2020*
Accepted: *06 January 2021*
Published: *27 January 2021*

Citation:
Houser KW and Esposito T (2021) Human-Centric Lighting: Foundational Considerations and a Five-Step Design Process. Front. Neurol. 12:630553. doi: 10.3389/fneur.2021.630553

INTRODUCTION

Human-centric lighting is an idiom intended to describe lighting solutions that considers the traditional elements of lighting quality that are rooted in human vision while simultaneously incorporating new insights about the non-visual effects of light. Recently, Houser et al. (1) outlined the rise of human-centric lighting and its current status in lighting. That manuscript describes a range of visual and non-visual responses and the eye-brain pathways that drive them, outlines the agreed upon science that can inform the practice of human-centric lighting, and offers general guidance for the practice of human-centric lighting. That work asserts that human-centric lighting, also called integrative lighting (2, 3), is not a product feature, and that lighting products that claim to improve sleep or performance should be met with skepticism.

Instead, human-centric lighting begins with effective prioritization of design goals and is an outcome of good decision making at every step in the lighting design process. Human-centric lighting begins with project conceptualization, and continues through prioritization of design goals, architectural design (including daylighting design), lighting equipment specification, commissioning, and operation of the lighting systems. Successful implementation requires buy-in from all stakeholders involved in building design, construction, and operation, including occupants.

The stakeholders with the most at stake are occupants, since it is their health, well-being, and cognitive performance that we are concerned about. Yet, designers cannot influence light exposure

when occupants are elsewhere, such as at home, in transit, or in other buildings. Individual outcomes will vary based on individual lifestyles and habits. As will be expanded upon later, the best that a designer can do is to provide the proper light at the proper time for the projects that they design.

Since many American adults spend about 90% of their time in buildings (4, 5), we believe that human-centric lighting should almost always be a part of a designer's scope and a building owner's desires. For the many people that spend their time indoors, days are dimmer and nights are brighter than would be experienced in nature (6–8). Thus, while natural light from the sky and sun is ideal for many health outcomes associated with light, electric lighting has a critical role in supporting human health in the modern world.

Implementing a successful human-centric lighting solution is complex. The goal of this work, therefore, is to outline the early stages of the design process for projects where human-centric lighting is deemed important. We offer a five-step process that will help organize information gathering and decision-making. Though no standard for biologically effective lighting has gone through the full consensus process required by ANSI, ISO, or IEC, we consider compliance with WELL v2 (9) and UL Design Guideline 24480 (10) as they may be appropriate for some projects. In support of informed decision making, we summarize relevant responses of people to light and lighting. Our review is intended to not overwhelm the reader with every study that links light with human physiology. Instead, we offer enough background to describe the unequivocal physiological responses to light, including why such responses may not always affect real-world outcomes. The content covered in this manuscript is complemented by reviews by others (1, 11–16).

FOUNDATIONAL CONSIDERATIONS

The goal of this section is to succinctly introduce the principles of human-centric lighting in support of conversational understanding of the most relevant considerations for lighting design.

The Stimulus Response Relationship Between Light and Human Outcomes

Figure 1 is a schematic of the stimulus response relationship between light and human outcomes. The top four categories are lighting variables that can be manipulated in the design of a light stimulus, whether in lighting experiments or in the design of the built environment. These categories are temporal pattern, light level, light spectrum, and spatial pattern.

These four aspects of the light stimulus can be manipulated to influence two categories of human responses to light, visual and non-visual, as illustrated in the middle of **Figure 1**. A *visual response* is an eye-brain response that enables sight, contributing to visual performance, the visual experience including emotional responses, and visual comfort (or discomfort, as with glare). Non-visual responses might also be called non-image forming (NIF) effects of light, biological responses, or physiological responses. CIE adopted the phrase ipRGC-influenced light responses (18) and IES uses the phrase visual, circadian, neuroendocrine, and neurobehavioral responses (19). *Circadian responses* are internal biological processes that occur on a roughly 24-h period, such as the sleep-wake cycle. *Neuroendocrine responses* refer to how the brain regulates hormones, such as expression of melatonin. *Neurobehavioral responses* refer to the relationship between the action of the nervous system and human behavior. The IES terminology is adopted in the bottom of **Figure 1** because this collection of responses encompasses all the ways that light may affect people through the eyes. **Table 1** provides examples of physiological and psychophysical responses that are important to human health, well-being, cognition, and performance, and which may be influenced by light and lighting over different time periods.

The four categories of lighting variables identified in the top row of **Figure 1** have unequal influences on non-visual responses to light, and thus contribute unequally to the biological potency of a light stimulus. *Biological potency* refers to the strength of influence of a light stimulus on a human biological response.

The temporal pattern is most important to non-visual responses because the brain tells time by observing nature's daily pattern of light and dark (20). The same light stimulus may be beneficial at one time of day but detrimental at another. For day-active people—that is, people who are active during the day, relatively less active in the evening, and sleep at night—bright light in the morning and during the day will support health, whereas bright light in the evening may delay the onset of sleep and be detrimental to health.

At any given time, the biological potency of a light stimulus can be altered first by adjusting light level and second by adjusting light spectrum. Brighter light with proportionally more short-wavelength radiation, coinciding with the melanopsin action spectrum, is more biologically potent than dimmer light with proportionally less short-wavelength radiation that coincides with the melanopsin action spectrum. At constant illuminance, broadband sources with a higher CCT are often more biologically potent than those with a lower CCT, though this depends upon how biological potency is quantified. CCT, however, is a one-dimensional reduction of a light source SPD that cannot reliably predict biological potency, a limitation that is especially apparent for color-mixed LEDs (21, 22) or any other SPD with pronounced peaks and valleys.

With respect to the spatial pattern of light, light exposure on the lower retina more effectively suppresses melatonin than light on the upper retina (23), and light exposure on the nasal side of the retina is more biologically potent than light exposure on the temporal side of the retina (24, 25). In building interiors, these effects are likely dwarfed by gaze direction. Looking toward a bright window will produce a more biologically potent light stimulus than looking toward a dimly lit interior wall. Lighting criteria for vision are at task locations and are often oriented horizontally (26), whereas criteria for circadian lighting design are at the plane of the occupant's eyes and are oriented vertically (9, 10, 27). There are opportunities for novel lighting solutions

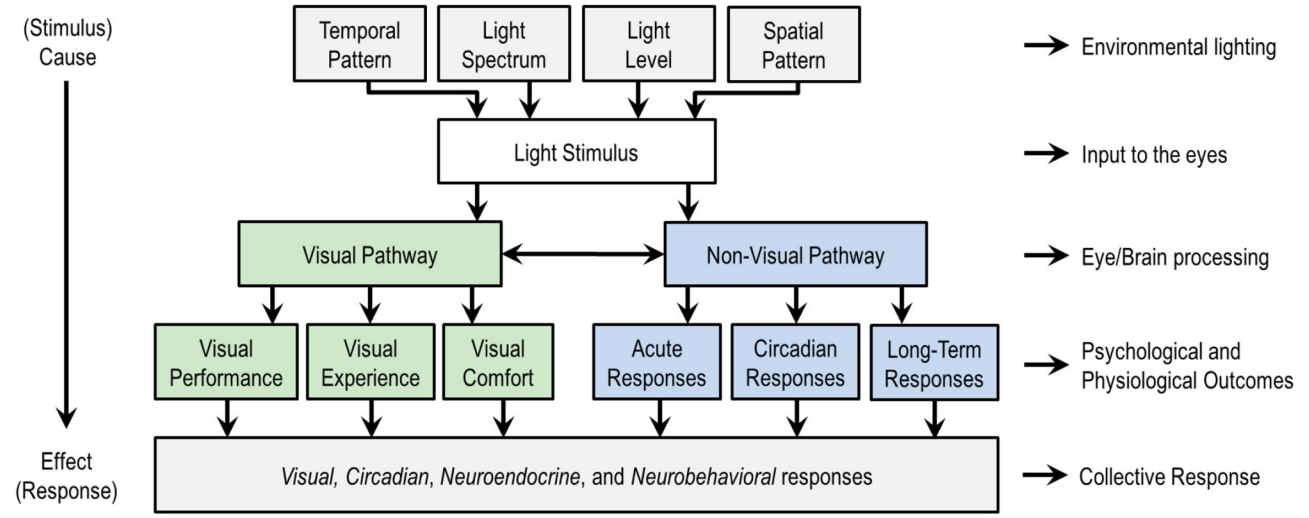

FIGURE 1 | An overview of the stimulus (top) response (bottom) relationship between light and human responses, with a schematic subdivision of the visual and non-visual responses. At the top level, the temporal pattern relates the timing and duration of exposure to a light stimulus, spatial pattern refers to the spatial distribution of light in the three-dimensional light field, light spectrum refers to the spectral power distribution (SPD) that governs color qualities, and light level refers to the quantity of light in radiometric or photometric units. These four factors contribute to the biological potency of the light stimulus. Designers often vary factors together, though any of the factors can be disengaged from any other. Researchers usually vary just a small number of factors, sometimes only one, to isolate cause and effect. Non-lighting factors not shown such as age and chronotype moderate the effects of light on people and are important in practice. This figure only considers the effects of light through the eyes; the effects of optical radiation on or through the skin are not considered here. Figure inspired by de Kort and Veitch (17).

TABLE 1 | Examples of psychophysical and physiological responses that are influenced by light and lighting.

Time Course	Psychophysical	Physiological
Immediate (seconds or minutes)	• Brightness perception • Visual amenity • Visual discomfort • Attention response	• Pupil size • Acute melatonin suppression • Luminance adaptation • Short-term chromatic adaptation
Delayed (hours, days, or weeks)	• Mood • Cognition • Motivation	• Circadian phase shift • Sleep quality • Long-term chromatic adaptation
Long-Term (months or years)	• Productivity • Depression	• Stress • Poor health • Seasonal affective disorder • Depression

*Psychophysical responses are largely though not exclusively driven by the visual pathway and physiological responses are largely though not exclusively driven by the non-visual pathway (see also **Figure 1**). Every item in this table is subject to influence by non-lighting factors. For example, even an immediate effect like pupil size may be affected by signals from other senses. The longer the delay between the stimulus and the response, the more opportunity there is for other factors to influence the response.*

that balance the need to deliver light at the plane of an observer's eyes without causing visual discomfort.

Quantifying the Biological Potency of Light for Human-Centric Lighting Design

Visual and non-visual responses are driven by photoreceptors that reside in the retina and send signals to the brain. Visual responses in humans are largely driven by the rods and cones. Non-visual responses are largely driven by intrinsically photosensitive retinal ganglion cells (ipRGCs) (28, 29). The ipRGCs combine their own intrinsic response to light with extrinsic inputs from the rods and cones (30). Though the understanding of the balance between extrinsic and intrinsic signaling in the various ipRGC subtypes (31) is incomplete, it is known that all photoreceptors contribute to both visual and non-visual responses.

There are two prevalent methods for quantifying light as a non-visual stimulus: (1) based on the spectral response of the photopigments in the rods, cones, and ipRGCs (18, 32), (2) based on nocturnal suppression of the hormone melatonin (33–36).

Equivalent Melanopic Lux (EML) introduced by Lucas et al. (32) is computed as the product of Photopic Illuminance, E, and

the Melanopic Ratio, R, where:

$$\text{EML} = (\text{E})(\text{R}) \quad (1)$$

EML is expressed in units of melanopic lux (m-lux), which is not recognized by the International System of Units (SI). R is computed as the ratio of a light source's melanopsin-activating radiation to its photopic-activating radiation, multiplied by 1.218, which guarantees that R = 1.0 for the Equal Energy illuminant. R is unitless and ranges from ∼0.45 to 1.70. On average, light sources with a higher CCT also have a higher value for R.

CIE has proscribed the use of EML and has proposed an SI compliant quantity as its replacement (18). Melanopic Equivalent Daylight Illuminance, melanopic EDI, $E_{\text{v,mel}}^{\text{D65}}$, or "mel-EDI" for short, is the illuminance of standard daylight (D65), at a point, that provides equal melanopic irradiance as the test source. For example, a mel-EDI of 100 lx means that the light source under evaluation produces the same amount of melanopsin-activating radiation as 100 lx of daylight at 6,500 K. It is computed as the product of photopic illuminance, E_{v}, and the melanopic Daylight Efficacy Ratio, melanopic DER, $\gamma_{\text{v,mel}}^{\text{D65}}$, or "mel-DER" for short, where:

$$E_{\text{v,mel}}^{\text{D65}} = (E_{\text{v}})\left(\gamma_{\text{mel,v}}^{\text{D65}}\right) \quad (2)$$

$E_{\text{v,mel}}^{\text{D65}}$ (mel-EDI) is expressed in units of lux, which is an SI-compliant unit. $\gamma_{\text{v,mel}}^{\text{D65}}$ (mel-DER) is computed as the ratio of a test source's melanopic efficacy of luminous radiation to the melanopic efficacy of luminous radiation of D65. Melanopic DER is unitless and ranges from ∼0.40 to 1.60. EML and mel-EDI are related by a scalar multiplier, where

$$\text{EML} \approx (\text{mel-EDI})(1.103) \quad (3)$$

A limitation of the Lucas et al. and CIE methods is that they are based solely on photopigment signals, yet it is unknown how photopigment signals are combined by photoreceptors and processed by the brain. Understanding of how EML and mel-EDI relate to non-visual outcomes in real-world settings is incomplete. The CIE method is the only consensus standard for characterizing the instantaneous biological potency of a light stimulus, but it comes with the caveat that quantities derived using the CIE system may not necessarily represent how light influences non-visual responses in real-world settings.

Circadian Stimulus (CS) is a non-linear model of human nocturnal melatonin suppression that is based on the quantity and spectrum of light and assumes 1-h of exposure time (33–36). CS models a hypothesized relationship between the retina and pineal gland as operating on a spectral opponency between the "blue" and "yellow" channels, from which Circadian Light, CL_{a}, values are determined. CS is calculated by fitting CL_{a} values to a four-parameter logistic function. CS is expressed as a decimal percentage of melatonin suppression. CS ranges from 0.00 (0%) to 0.70 (70%). Though CS is a measure of melatonin suppression, it is intended to be relevant for the regulation of circadian rhythms. A limitation of CS is that melatonin suppression and circadian phase shift are not proxies for each other (37), and CS does not necessarily represent how light influences other non-visual responses.

There are publicly available calculators for computing the above quantities. For a Microsoft Excel calculator to compute EML see IWBI (38). For a Microsoft Excel calculator to compute mel-EDI see CIE (39). CS can be computed using an online calculator (40) or Microsoft Excel (41). Computation of EML, mel-EDI and CS require two inputs, a light source's spectral power distribution (SPD) and photopic illuminance at the plane of the eye. In principal, these quantities can be measured on site—though the complexity and cost of doing so in a non-cursory way must be acknowledged. It should also be understood that instantaneous measurements may not be representative of mean light exposure, which is almost always transitory and varies with things such as view direction, instantaneous daylight exposure, and lamp aging factors such as lumen depreciation and spectral changes with time. There are recommendations for measurement and reporting of light exposure in experiments (42, 43), with some recommendations being transferable to field settings. Spectral lighting software is available to investigate some of these measures via simulation (44, 45).

Responses to Light That Matter

Humans have a wide range of visual and non-visual responses to light. Human outcomes most commonly relevant in applied lighting are visual performance, visual experience, visual comfort, circadian phase-shifting, and alertness. This prioritization is similar to the response headings in **Figure 1**, but not identical since here we are endeavoring to be more explicit. For example, while alertness, melatonin suppression, and pupil size can all be acute responses, we believe that alertness is more tangible in daily life.

Visual performance refers to the ability of the eye/brain system to gather and process visual information to perform a task. *Visual experience* refers to the perceptual response to illuminated environments, including evoked perceptions and emotions such as feelings of relaxation, tension, spaciousness, closure, and the like. *Visual comfort* is a reference to the perceptual response to the qualities and quantities of light in an environment, and whether they result in comfortable or uncomfortable seeing. Since visual discomfort is usually simpler to define than visual comfort, a visually comfortable environment is often defined as one that does not create visual discomfort. *Circadian phase-shifting* refers to the ability of light to advance or delay the circadian clock, and so sleep timing. *Alertness* refers to light's potential to moderate a person's state of sensory awareness and active attention. These five categories are not exhaustive and may not receive equal prioritization in a design solution. For example, the primary goal in retail lighting may be to encourage sales (46) and in open surgery visual performance will dominate the design criteria (47).

Visual performance, visual experience, and visual comfort have undergone decades of research and are the basis for lighting industry standards, recommended practices, and design guidelines. Of the various non-visual responses to light, alertness (48, 49), melatonin suppression (50, 51), and circadian phase shifting (51, 52) have been most extensively studied, mostly

in laboratory settings. Pupillary response (53, 54), heart rate (55, 56), mood state (57, 58), and body temperature (55, 56) have also been studied, as has student performance in school settings (59–62), workspaces (63–66), and senior living centers (67). In this section we summarize considerations related to circadian entrainment, alertness, and performance of students and office workers since these considerations are especially relevant to how lighting design influences human health.

Circadian Entrainment

Circadian rhythms are biological rhythms with predictable changes in magnitude that repeat on a period of about 24 h. Examples include core body temperature, alertness, and the concentration of melatonin. *Circadian entrainment* is a stable relationship between a biological rhythm and an external environmental cue. A *circadian phase shift* is a change in the timing of circadian rhythms, where a *phase-advance* means that bedtime and wake-up time will move earlier in the day, and a *phase-delay* means that bedtime and wake-up time will move later in the day. The light/ dark cycle is the most important exogenous cue for entraining circadian rhythms. Reduced contrast between day and night can weaken circadian entrainment (6).

Acute suppression of the hormone melatonin is much easier to measure than changes in circadian rhythms and has been treated as a proxy measure in some of the studies endeavoring to study the effects of light on circadian health (33, 34, 36, 68, 69). Unfortunately, acute melatonin suppression by light may not be a suitable proxy for other physiological responses, such as circadian phase shifting and alertness (37, 70). The human circadian system adapts to prior light exposure (71), light exposure earlier in the day affects the biological potency of light later in the day (72–75), extended periods under dim light may negatively impact subsequent sleep (76), and there is considerable interindividual variability in the response to evening light (77). Collectively, these findings suggest nuance in the human circadian response to light, raising questions about the veracity of numerical design targets for circadian lighting design. For example, the CS targets in UL 24480 (10) are based on research that characterizes acute melatonin suppression after a 1-h exposure to light, yet compliance with the UL standard requires a minimum of a 2-h exposure of CS $>$ 0.30 between 7 a.m. and 4 p.m., and is intended to support a broad range of health outcomes for all day-shift workers.

Daylight naturally provides bright days and dark nights, creating both the cycle and light/dark ratio that is essential for circadian entrainment (78, 79). Daylight offers other psychological and physiological benefits (80–82) and, whenever possible, should play a key role in human-centric lighting design (83).

Alertness

The potential to use light to enhance alertness and improve cognitive performance is of interest to educational institutions and knowledge-work environments. Organizations that operate 24/7 may also aspire to reduce errors and increase safety by using light to enhance nighttime alertness of employees.

The alerting response has largely been characterized in laboratory settings during nighttime. The acute alerting properties of light have been compared to that observed after caffeine consumption (84). In laboratory settings, light has reduced attentional lapses, decreased subjective sleepiness, improved alertness, and enhanced performance on some cognitive tests (11, 15, 85–87). The alerting effects of light may be stronger at night than during the day (87). During daytime hours, improvements in alertness may be minimal for well-rested people (88). Many studies of alertness and cognition have shown mixed results where some measures have improved but others have not (15, 89–92).

Alertness is commonly characterized using an objective assessment of sustained attention (93) or a subjective alertness measure (94). An advantage of these standardized tests is that they are sensitive to sleep deprivation and have a large base of prior literature for comparison. More complex tasks, however, may be resistant to the short-term alerting effects of light. The generalizability of simplistic assessments of attention to real-world outcomes is questionable.

In their review, Souman et al. (15) concluded that increasing the intensity of white light sometimes increases subjective ratings of alertness, that the effect of CCT on subjective alertness is unclear, and that no studies show a systematic relationship between alertness and wavelength. The meta-analysis by Brown (95) suggests a sigmoidal relationship between subjective sleepiness and mel-EDI (18). Others have shown that light without short-wavelength content can maintain subjective alertness without suppressing melatonin (96, 97), and that long-wavelength (red) light can elicit an alerting response during daytime hours without acute melatonin suppression (98). Few studies have tested the potential alerting benefits of architectural lighting relative to operational outcomes in real-world settings. Despite the consensus that light influences alertness, especially in controlled settings, and general guidance in the peer-reviewed literature (99), no consensus body has offered lighting criteria that implies a direct stimulus response relationship between light and alertness.

Student and Office Worker Performance

There is some evidence that lighting influences student performance. Early evidence demonstrated a positive connection between the presence of daylight in classrooms and student performance (100). More recently, classroom lighting with relatively more short-wavelength radiation was shown to improve cognitive processing speed in high school students (59), improve concentration in elementary school children (60), and improve oral reading fluency performance in third-grade students (61). Lighting that varies in color temperature and illuminance was shown to increase attention and reading speed of elementary and high school students (62). Collectively, these studies suggest that architectural lighting, including daylight, has the potential to positively impact student learning.

There is also some evidence that lighting influences knowledge worker performance. Lighting with a very high CCT of 17,000 K was employed within a shift-working call center

(63) and in an office setting (64). Mills et al. found mean improvements on fatigue, alertness, daytime sleepiness, and work performance. Viola et al. found mean improvements on alertness, mood, daytime sleepiness, evening fatigue, and work performance. Office workers in windowless environments self-reported poorer well-being and sleep quality in comparison to workers with access to windows (65). View very likely plays a role, but the reported positive effects of daylight and view have not yet been disentangled (101). Figueiro et al. (66) linked daytime light exposure to the sleep quality and mood of office workers. Collectively, these studies suggest that architectural lighting, including daylight (and view), has the potential to influence office worker well-being and performance.

External Validity

External validity is the extent to which results can be applied to other people or contexts that differ from the specific circumstances of the experiment (102). Outside of laboratory settings, responses to light do not occur in isolation. The same light stimulus may simultaneously influence none or many biological responses. A biological response may or may not translate to a change in performance or overall health. Any of the outcomes mentioned above can be influenced by factors other than light, such as age, climate, diet, disease, exercise, genetics, medications, mental health, pregnancy, sleep habits, stress, and travel. Responses evoked by light may also be evoked by other sensory inputs, such as an attention response evoked by auditory (103) or olfactory (104) stimuli. While light is indeed potent, it is important to consider the effects of light in a broader context that includes other sensory and non-sensory inputs.

Of the field studies available, many have used participants with limited or less-common exposure to light, such as elders with limited mobility (67, 105–107), night shift workers (108–111), fatigued cancer survivors (112), hospitalized patients (113), and infants (114). In most of these studies, the benefit of lighting was modest or the outcome was mixed.

The least studied group are healthy adults that work during the day, sleep at night, and who have exposure to daylight. For this group, if electric light exposure is compliant with guidelines for vison (26, 115), it is unclear the degree to which changing the lighting will affect non-visual outcomes. While laboratory studies have demonstrated the capacity and potential of light to influence non-visual outcomes, more field studies are needed to understand the veracity of the effects in real-world settings.

A FIVE-STEP DESIGN PROCESS FOR HUMAN-CENTRIC LIGHTING

Though there is still much to learn, enough is known today to at least offer a framework for addressing visual and non-visual outcomes through lighting design decisions. The five-step process outlined below augments the already well-established lighting design processes (116) and can be used to integrate human-centric lighting design concepts into design practice. **Table 2** provides an overview of the process with examples for select application types.

Step 1: Characterize the Lighting Application

The first step is to establish the application's primary tasks and activities, when they occur, and the desired outcomes or operational goals. For example, healthcare environments are typified by the intent to prevent, cure, or treat illness. Operational goals will likely include improving patient health and well-being, which often requires visual evaluation, diagnostic testing, and interpersonal communication. Architectural design and human-centric lighting strategies should support these activities.

A thorough understanding of desired outcomes will guide prioritization of design criteria and facilitate rational design decisions when all outcomes cannot be simultaneously achieved. For example, in a healthcare environment, temporary visual discomfort of a patient may be acceptable if it increases the speed and efficacy with which a caregiver can diagnose and administer treatment (e.g., over-bed patient exam lights tends to be very bright when viewed from the perspective of a patient, but are necessary for the caregiver). In an office environment, such visual discomfort would likely affect productivity and would therefore be unacceptable. There is no one-size-fits all approach.

Step 2: Determine the Likely Sleep-Wake Cycle(s) of Occupants

Determine if the application includes day-active people, night-active people, or both. Occupants that are day-active/night-inactive have wake-sleep cycles that are largely synchronous with the day-night cycle. Their lighting needs may conflict with occupants that are night-active/day-inactive and who have wake-sleep cycles that are largely asynchronous with the day-night cycle. Day-active people benefit from light with high biological potency during the morning and daytime, low biological potency in the evening, and as little biological potency as possible at night. Night-active people need nighttime illumination to adequately and safely perform personal or professional tasks while minimizing the potential negative human outcomes associated with an asynchronous sleep-wake cycle and nighttime light exposure.

For example, education settings are likely to have predominately day-active occupants, 24/7 industrial facilities may have day-active or night-active people sequentially throughout the day as the work shift changes from day-shift, to second-shift, to night-shift, and healthcare facilities may include both day-active and night-active occupants simultaneously. Applications with both day-active and night-active people—either sequentially or simultaneously—may require specialized design solutions that include advanced lighting controls (e.g., controlling zones, scenes, intensity, spectrum) and/or architectural interventions (e.g., barriers to block obtrusive light, temporary or permanent partitions to create zonal workspaces) that rethink traditional architectural and spatial relationships.

TABLE 2 | A sample of representative application-specific examples.

Application	Characteristics	Step 1	Step 2	Step 3	Step 4
		Operational goals	**Likely occupant sleep-wake cycle**	**Occupant sleep needs**	**Human-centric lighting principles[a,b]**
Military and Maritime	• Demanding environment with low tolerance for errors	• Safety • Achieve mission objectives • Maximize energy efficiency • Maximum system reliability	• Applications are likely to have both day-active and night-active people, or both simultaneously	• Application is likely to have a mix of sleeping and active occupants	A, B, C, D, E, F
Healthcare	• Environments intended to prevent, cure, or treat illness	• Safety • Save lives • Improve patients' quality of life • Minimize suffering	• Patients: likely to be day-active, but may be night-night active as well • Care providers: doctors and nurses are likely to be both day-active and night-active depending on the shift and the type of healthcare environment	• Application is likely to have a mix of sleeping and active occupants • Sleeping occupants consists of patients using inpatient services	A, B, C, D, E, F
Hotel	• Strong need for aesthetic considerations and brand-conscious design	• Create mood and atmosphere consistent with brand identity • Accommodate guest sleeping and waking needs	• Guests: Quite variable, with many suffering from jet lag • Employees: 24/7 operation requires some day and some night workers	• Guest have a variety of sleep needs due to circadian phase shifts from different time zones	B, D, E
Education	• Environments dedicated principally to teaching and learning	• Learning	• Mostly day-active people, though likely working/studying into evening hours	• Application is unlikely to have sleeping occupants	A, B, C, D, E
Industrial and Commercial	• Productivity is important • May be non-specific productivity, such as increasing attentiveness of office workers • May be task-specific productivity, such as minimizing assembly line errors	• Safety • Productivity	• Day-active • Some applications, such as 24/7 industrial facilities, may include night-active workers	• Application is unlikely to have sleeping occupants	A, B, C, D, E, F

There could be many sub-categories within each row that are not shown.
[a] *All applicable codes and standards must also be addressed, including those related to safety and energy.*
[b] *Refer to* ***Table 3*** *for published WELL v2 and UL guidelines.*

Key for human-centric lighting principles

A *Comply with recommended practice for light level and quality, as from CIBSE (115) or IES (26).*
B *Address lighting quality (e.g., low glare, no flicker, good color rendition).*
C *Maximize daylight exposure/outside view while controlling for possible glare from the sun and sky.*
D *Consider psychological reinforcement (e.g., positive distraction in healthcare, color tuning in classrooms, aesthetics in hotels).*
E *Evaluate/consider WELL and/or UL guidelines for day-active people where applicable.*
F *Provide light to promote visual performance for nighttime activities where applicable.*

It is especially challenging to address the needs of workers with rotating shifts, such as nurses that cycle between dayshifts and nightshifts. During periods of rotation, the circadian pacemaker is playing perpetual catch-up to the changing timing of the light exposure rhythms. The optimal lighting solution for an occupant with a rotating shift may differ from the optimal lighting solution for an occupant with a permanent shift, even when both people occupy the same environment at the same time and are performing comparable work tasks.

Step 3: Determine the Sleep Needs of Occupants

Determine if the application includes sleeping occupants. If yes, determine if the sleeping occupants will be day-active, night-active, or both. Darkness promotes sleep and spaces where occupants will sleep should be as dark as possible while providing enough light for safe navigation (27). For day-active people sleep occurs primarily in the evening and throughout the night—this may require window treatments to block intrusive light. For night

active people, sleep occurs primarily during daytime hours and window treatments or eye coverings are almost certainly necessary.

For example, healthcare environments are likely to have a combination of occupant needs: day-active (or night-active) patients seeking treatment during the day (outpatient); day-active (or night-active) patients seeking treatment over several days (inpatient), with both active and sleep requirements; and the healthcare workers that provide care, and who may be day-active or night-active, depending on the shift. These groups have separate and distinct needs that change throughout the day and the interplay between conflicting needs by different occupants demands careful examination, including prioritization and a considered understanding of tradeoffs. See Zee and Goldstein (117) for guidance about using light and sleep hygiene practices to improve outcomes for people with non-traditional work schedules.

Step 4: Review Published Human-Centric Lighting Guidance

Human-centric lighting design can be informed by industry guidelines that endeavor to bridge the gap between scientific understanding and guidance for application. Organizations like the Society for Light and Lighting and the Illuminating Engineering Society (IES) provide standards that focus of visual outcomes (26, 115). The WELL Building Standard (WELL) (9) and Underwriters Laboratory (UL) (10) each recommend quantitative design targets for circadian lighting design. The WELL standard has changed threshold values over time; to our knowledge, the rationale for the threshold design targets and changes to the thresholds has never been disclosed. The IES maintains that, to ensure transparency and involvement of relevant constituencies, any recommended practice related to light and health should be a consensus document developed through an accredited ANSI process (118), which was not done with either the WELL or UL standards. DIN SPEC 67600 also provides design guidelines for biologically effective illumination (119). DIN SPEC 67600 was not developed through the full ISO consensus standards process, which is why it bears the "SPEC" modifier in the title (120).

The WELL criteria are based primarily on EML (32), while also allowing compliance with mel-EDI (18) or CS (36). UL's criteria are primarily expressed using CS while also allowing compliance using EML or photopic illuminance. Both WELL and UL have exposure times and durations associated with their recommendations. The UL standard is limited to promoting circadian entrainment for day-active and night-inactive people in commercial, educational, and industrial settings, while encouraging consideration of other legitimate design goals such as glare and color quality. The WELL standard is also intended to promote circadian entrainment, while also including explicit pass/fail criteria related to illuminance, glare, visual comfort, access to daylight, views, color quality, flicker, and personal control. Both systems endeavor to promote a comprehensive approach to human-centric lighting. The criteria related to circadian entrainment for the UL and WELL systems are provided in **Table 3**. The criteria are based on the temporal pattern of light exposure (time of day and duration of exposure), light level, spectrum, and the location where the light is delivered. While **Table 3** is current as of the date of this article, it is prudent to check for updates since recommendations may change.

Brown et al. (27) provide recent and noteworthy light exposure recommendations for healthy adults with regular daytime schedule. They recommend mel-EDI $\geq$ 250 lx throughout the day, mel-EDI $\leq$ 10 lx in the 3 h before bedtime, and sleep environments as dark as possible (mel-EDI $\leq$ 1 lx). All measurements are at the plane of an observer's eye, simplified as a vertical plane at $\approx$ 1.2 m height. Insofar as possible, these recommendations should be applied daily at the same time of day. These recommendations are not intended to supersede existing guidelines related to visual function and safety; rather, they are additional criteria to be considered by lighting specifiers. The daytime criteria of Brown et al. (27) are higher in quantity and longer in duration than both the WELL and UL criteria.

WELL v2 (9), UL 24480 (10), and Brown et al. (27) provide pass/fail criteria that facilitate ease-of-use in practice but may promote a false sense of precision. Considerations of precision and accuracy are relevant (121) since they can complicate the debate about how to establish metrics and thresholds that serve both vision and autonomic body functions that are influenced by light. It is already appreciated that under many circumstances great precision is not needed when designing lighting systems. The IES Lighting Handbook (26) suggest that, at time of occupancy, illuminance measurements within 30% of the target are sufficiently accurate for most applications. Similar considerations are likely relevant for non-visual effects, since physiological responses are modulated by large relative changes more so than by small fractional changes. The key considerations in Well v2 (9), UL 24480 (10), and Brown et al. (27) focus on defining the magnitude of photic stimulation at the plane of the eye, linked to time of day, for a given length of time. While the recommendations available today are not identical, they are comparable in manner and degree, which we view as progress toward consensus. The fuzziness that exists today is not a problem since estimates need not be overly precise or accurate to be useful in lighting practice. We believe WELL v2 (9), UL 24480 (10), and Brown et al. (27) all move lighting practice in a positive direction by encouraging consideration of non-visual responses to light and by providing quantitative target that can inform the development of design criteria.

Step 5: Put It All Together

Once the application characteristics and operational goals have been defined, the occupants' sleep-wake cycles established, occupant sleep requirements have been determined, and published guidance has been reviewed, design criteria and numerical design targets can be established. Recommendations from WELL, UL, CIBSE, and IES provide guidance, but it is ultimately the duty of the design team, in consultation with the building owner, to balance the relative importance of visual and non-visual needs. The WELL and UL circadian lighting guidelines are specifically for day-active

TABLE 3 | Guidelines published by the WELL Building Standard v2 and Underwriter's Laboratory 24480.

Standard	Temporal pattern		Lighting quantity (Note: these are a function of light level and spectrum)				Location
	Timing of exposure	Duration of exposure	Circadian stimulus (CS) (Percent)	Equivalent melanopic Lux (EML) (Melanopic Lux)	Melanopic equivalent daylight illuminance (Melanopic EDI) (Lux)	Photopic Illuminance (Lux)	
WELL v2.0 Requirements for 1 point	At least between the hours of 9 a.m. and 1 p.m. Light levels may be lowered after 8 p.m.	Minimum of 4 h.	≥0.30 (if electric light only)	≥150 (if electric light only) ≥120 from electric lighting (if certain daylighting criteria are met)	≥ 136 (if electric light only) ≥109 from electric lighting (if certain daylighting criteria are met)	N/A	Vertical plane at eye level
WELL v2.0 Requirements for 3 points	At least between the hours of 9 a.m. and 1 p.m. Light levels may be lowered after 8 p.m.	Minimum of 4 h.	N/A	≥240 (if electric light only) ≥180 from electric lighting (if certain daylighting criteria are met)	≥ 218 (if electric light only) ≥163 from electric lighting (if certain daylighting criteria are met)	N/A	Vertical plane at eye level
UL 24480	7 a.m.–4 p.m.	Minimum of 2 h, morning If not full period	≥0.30	Comply with WELL criteria shown above based on desire for 1 point or 3 points	N/A	≥500	Vertical plane at eye level
	5–7 p.m.	During full period	≤0.20		N/A	N/A	
	After 8 p.m.	During full period	≤0.10		N/A	N/A	

Note that where alternative compliance paths are offered, either CS, EML, EDI, or Photopic Illuminance can meet the criteria.

(night-inactive) people; stakeholders will need to use their best judgement when designing lighting systems for people who do not fit this profile or where multiple populations or people with different schedules occupy the same spaces.

Lighting design demands consideration of competing criteria. Prioritization may be needed to balance tradeoffs between visual and non-visual design goals. For example, bright light during the day is expected to better support non-visual outcomes for day-active people. But, if the light is so bright that it creates glare, then productivity may suffer. Higher light levels also require more energy, which may not be compatible with required energy codes (122). As another example, darkness is most desirable at nighttime for circadian health, but light may be needed to support safe navigation. Such difficult tradeoffs are best addressed by competent professionals and thoughtful clients that are willing to prioritize needs and define expected outcomes.

Design teams work on projects on a per-project basis, where a typical project may be a building or a subset of spaces within a building. Yet, people transition through many buildings and spaces through the course of a day, week, month, and year. Coworkers or classmates may have very different spectral diets (123), which will moderate the manner with which comparable workplace or school lighting affects individual outcomes. Individuals have different levels of control over their exposure to light. Some people may be able to take morning and lunchtime walks and limit screen use at night. Others may have limited mobility or inflexible work schedules. Even longitudinal position within a time zone affects cancer risk, likely due to varying degrees of circadian disruption (124). Though the design team cannot control these and other pertinent factors, designers have some control over the light stimulus received by occupants in the spaces they design. By providing light with appropriate qualities (e.g., intensity, spectrum, spatial pattern, controllability) at the right time of day, designers can support good outcomes even against this background of uncertainty.

CONCLUSIONS

For day-active people, visual and non-visual needs are generally synchronized. While light from the sun and sky naturally provides the cycle and light/dark ratio that supports circadian health, electric lighting has a critical role since most of us spend most of our time indoors (4, 5). Architecture, glazing, lighting equipment, and lighting control solutions can be used in combination to deliver biologically potent light during the day while minimizing light exposure as night, all while balancing traditional factors such as color quality, flicker, glare, psychological reinforcement, and visibility (26, 115). Perhaps the simplest guidance to support the health of day-active people is to provide light of high biologically potency during the day and low biological potency at night.

For night-active people, visual and non-visual needs are in conflict, requiring explicit prioritization. Many night-active people provide critical societal functions, such as night shift nurses and doctors, with visually demanding jobs that have a low tolerance for errors. For these people, factors like visibility and alertness may be prioritized over circadian entrainment, even while recognizing that circadian disruption from an asynchronous sleep-wake cycle is associated with long-term negative health outcomes (125). Lighting design solutions for night-active people demands complete consideration of tradeoffs.

Good outcomes are most likely when a knowledgeable team that includes designers, owners, and equipment manufacturers prioritize visual and non-visual design outcomes. Such teams are well-positioned to develop lighting solutions that deliver light of an appropriate amount and spectrum at the right time of day, for an appropriate length of time.

Because there is no one-size-fits-all solution, we suggested a framework to guide lighting design: characterize the lighting application, determine the likely sleep-wake cycle(s) of occupants, determine the sleep needs of the occupants, review published guidance to develop goals and design criteria that support visual and non-visual outcomes, then use this information to establish design criteria that will guide decisions in the latter stages of the design process. We hope that implementation of this process facilitates realization of what we all want—lighting solutions that support human outcomes.

AUTHOR CONTRIBUTIONS

KH and TE contributed to conceptualization, writing, reviewing, and editing of this manuscript, including figures and tables. KH managed project administration. All authors contributed to the article and approved the submitted version.

FUNDING

The authors received funding from Energy Focus, Inc. for the preparation of this manuscript. Energy Focus, Inc. had no role in the conceptualization, preparation, or approval of this manuscript.

REFERENCES

1. Houser KW, Boyce PR, Zeitzer J, Herf M. Human centric lighting: myth, magic, or metaphor? *Lighting Res Technol.* (2020) Online before print. doi: 10.1177/1477153520958448
2. CIE. *Commission Internationale de l'Eclairage. CIE Position Statement on Non-Visual Effects of Light: Recommending Proper Light and the Proper Time*. 2nd ed. Vienna: CIE (2019).
3. ISO/CIE. *International Standards Organization and Commission Internationale de l'Eclairage. Light and lighting—Integrative lighting—Non-visual effects*. Under development (2020). Report No. ISO/CIE TR 21783.
4. Beyer KMM, Szabo A, Hoormann Stolley M. Time spent outdoors, activity levels, and chronic disease among American adults. *J Behav Med*. (2018) 41:494–503. doi: 10.1007/s10865-018-9911-1
5. Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, et al. The national human activity pattern survey (NHAPS): a resource for assessing exposure to environmental pollutants. *J Expo Anal Environ Epidemiol.* (2001) 11:231–52. doi: 10.1038/sj.jea.7500165
6. Wright KP, McHill AW, Birks BR, Griffin BR, Rusterholz T, Chinoy ED. Entrainment of the human circadian clock to the natural light-dark cycle. *Curr Biol.* (2013) 23:1554–8. doi: 10.1016/j.cub.2013.06.039
7. Stothard ER, McHill AW, Depner CM, Birks BR, Moehlman TM, Ritchie HK, et al. Circadian entrainment to the natural light-dark cycle across seasons and the weekend. *Curr Biol.* (2017) 27:508–13. doi: 10.1016/j.cub.2016.12.041
8. Chen S, Wei M, Dai Q, Huang Y. Estimation of possible suppression of melatonin production caused by exterior lighting in commercial business districts in metropolises. *LEUKOS.* (2020) 16:137–44. doi: 10.1080/15502724.2018.1523013
9. International Well Building Institute. *WELL Building Standard v2, Q3 2020 Version. Section L03: Circadian Lighting Design.* (2020). Available online at: https://v2.wellcertified.com/v/en/light/feature/3 (accessed November 2, 2020).
10. Underwriters Laboratory. *Design Guidelines for Promoting Circadian Entrainment with Light for Day-Active People.* UL Design Guideline 24480, Edition 1. Northbrook, IL: UL (2019).
11. Cajochen C. Alerting effects of light. *Sleep Med Rev.* (2007) 11:453–64. doi: 10.1016/j.smrv.2007.07.009
12. Golombek D, Rosenstein RE. Physiology of circadian entrainment. *Physiol Rev.* (2010) 90:1063–102. doi: 10.1152/physrev.00009.2009
13. Khademagha P, Aries MBC, Rosemann ALP, van Loenen EJ. Implementing non-image-forming effects of light in the built environment: a review of what we need. *Build Environ.* (2016) 108:263–272. doi: 10.1016/j.buildenv.2016.08.035
14. Münch M, Brøndsted AE, Brown SE, Gjedde A, Kantermann T, Martiny K, et al. Chapter 3. The effect of light on humans. In: Sanders S, Oberst J, editors. *Changing Perspectives on Daylight: Science, Technology, and Culture*. Washington, DC: Science/AAAS. (2017). p. 16–23.
15. Souman JL, Tinga AM, te Pas SF, van Ee R, Vlaskamp BNS. Acute alerting effects of light: a systematic literature review. *Behav Brain Res.* (2018) 337:228–39. doi: 10.1016/j.bbr.2017.09.016
16. Vetter C, Pattison PM, Houser KW, Herf M, Phillips AJK, Wright KPM, et al. *A Review of Human Physiological Responses to Light: Implications for the Development of Integrative Lighting Solutions*. LEUKOS. doi: 10.1080/15502724.2021.1872383
17. de Kort YAW, Veitch JA. From blind spot into the spotlight: introduction to the special issue 'Light, lighting, and human behaviour'. *J Environ Psychol.* (2014) 39:1–4. doi: 10.1016/j.jenvp.2014.06.005
18. Commission Internationale de l'Eclairage. *CIE S 026/E:2018 CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light*. Vienna: CIE (2018).
19. Illuminating Engineering Society. TM-18-18. *Light and Human Health: An Overview of the Impact of Optical Radiation on Visual, Circadian, Neuroendocrine and Neurobehavioral Responses*. New York, NY: IES (2018).
20. Roenneberg T, Kumar CJ, Merrow M. The human circadian clock entrains to sun time. *Curr Biol.* (2006) 17:R44–5. doi: 10.1016/j.cub.2006.12.011
21. Houser KW. Editorial: human centric lighting and semantic drift. *LEUKOS.* (2018) 14:213–4. doi: 10.1080/15502724.2018.1501234
22. Souman JL, Borra T, de Goijer I, Schlangen LJM, Vlaskamp BNS, Lucassen MP. Spectral tuning of white light allows for strong reduction in melatonin suppression without changing illumination level or color temperature. *J Biol Rhythms.* (2018) 33:420–31. doi: 10.1177%2F0748730418784041

23. Glickman G, Hanifin JP, Rollag MD, Wang J, Cooper H, Brainard GC. Inferior retinal light exposure is more effective than superior retinal exposure in suppressing melatonin in humans. *J Biol Rhythms.* (2003) 18:71–9. doi: 10.1177/0748730402239678
24. Visser EK, Beersma DGM, Daan S. Melatonin suppression by light in humans is maximum when the nasal part of the retina is illuminated. *J Biol Rhythms.* (1999) 14:116–21. doi: 10.1177/074873099129000498
25. Ruger M, Gordijn MCM, Beersma DGM, de Vries B, Daan S. Nasal versus temporal illumination of the human retina: effects on core body temperature, melatonin, and circadian phase. *J Biol Rhythms.* (2005) 20:60–70. doi: 10.1177/0748730404270539
26. DiLaura DL, Houser KW, Mistrick RG, Steffy GR, editors. *The Lighting Handbook, Reference and Application*. 10th ed. New York, NY: Illuminating Engineering Society (2011).
27. Brown T, Brainard G, Cajochen C, Czeisler C, Hanifin J, Lockley S, et al. Recommendations for healthy daytime, evening, and night-time indoor light exposure. *Preprints.* (2020) doi: 10.20944/preprints202012.0037.v1
28. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* (2002) 295:1070–3. doi: 10.1126/science.1067262
29. Hattar S, Liao HW, Takao M, Derson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science.* (2002) 296:1065–70. doi: 10.1126/science.1069609
30. Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature.* (2005) 433:749–54. doi: 10.1038/nature03387
31. Mure LS, Vinberg F, Hanneken A, Panda S. Functional diversity of human intrinsically photosensitive retinal ganglion cells. *Science.* (2019) 366:1251–5. doi: 10.1126/science.aaz0898
32. Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, et al. Measuring and using light in the melanopsin age. *Trends Neurosci.* (2014) 37:1–9. doi: 10.1016/j.tins.2013.10.004
33. Rea MS, Figueiro MG, Bullough JD, Bierman A. A model of phototransduction by the human circadian system. *Brain Res Rev.* (2005) 50:213–28. doi: 10.1016/j.brainresrev.2005.07.002
34. Rea MS, Figueiro MG, Bierman A, Bullough JD. Circadian light. *J Circadian Rhythms.* (2010) 8:10. doi: 10.1186/1740-3391-8-2
35. Rea MS, Figueiro MG, Bierman A, Hamner R. Modelling the spectral sensitivity of the human circadian system. *Lighting Res Technol.* (2012) 44:386–96. doi: 10.1177/1477153511430474
36. Rea MS, Figueiro MG. Light as a circadian stimulus for architectural lighting. *Lighting Res Technol.* (2018) 50:497–510. doi: 10.1177/1477153516682368
37. Rahman SA, St Hilaire MA, Gronfier C, Chang AM, Santhi N, Czeisler CA, et al. Functional decoupling of melatonin suppression and circadian phase resetting in humans. *J Physiol.* (2018) 596:2147–57. doi: 10.1113/JP275501
38. International Well Building Institute. *Melanopic Ratio (Microsoft Excel).* (2020). Available online at: https://standard.wellcertified.com/sites/default/files/Melanopic Ratio.xlsx (accessed November 2, 2020).
39. Commission Internationale de l'Eclairage. *Alpha Opic Toolbox (Microsoft Excel).* (2020). Available online at: http://files.cie.co.at/CIE%20S%20026%20alpha-opic%20Toolbox.xlsx (accessed November 2, 2020)
40. Lighting Research Center. *CS Calculator (Web Based).* (2020). Available online at: https://www.lrc.rpi.edu/cscalculator/ (accessed November 2, 2020).
41. Lighting Research Center. *CS Calculator (Microsoft Excel).* (2020). Available online at: http://www.lrc.rpi.edu/resources/CSCalculator_2017_10_03_Mac.xlsm (accessed November 2, 2020)
42. Knoop M, Broszio K, Diakite A, Liedtke C, Niedling M, Rothert I, et al. Methods to describe and measure lighting conditions in experiments on non-image-forming aspects. *LEUKOS.* (2019) 15:163–79. doi: 10.1080/15502724.2018.1518716
43. Spitschan M, Stefani O, Blattner P, Gronfier C, Lockley SW, Lucas RJ. How to report light exposure in human chronobiology and sleep research experiments. *Clocks Sleep.* (2019) 1:280–9. doi: 10.3390/clockssleep1030024
44. University of Washington and ZGF, Architects LLP. *Lark Spectral Lighting Software.* (2020). Available online at: https://faculty.washington.edu/inanici/Lark/Lark_home_page.html (accessed November 16, 2020).
45. Solemma Inc. *Alfa Software*. (2020). Avaliable online at: https://www.solemma.com/Alfa.html (accessed November 16, 2020).
46. Cuttle C, Brandston H. Evaluation of retail lighting. *J Illum Eng Soc.* (1995) 24:33–49. doi: 10.1080/00994480.1995.10748117
47. Mundinger JJ, Houser KW. Adjustable correlated colour temperature for surgical lighting. *Lighting Res Technol.* (2017) 51:280–90. doi: 10.1177/1477153517742682
48. Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. *Behav Brain Res.* (2000) 115:75–83. doi: 10.1016/S0166-4328(00)00236-9
49. Lockley SW, Evans EE, Scheer FAJL, Brainard GC, Czeisler CA, Aeschbach D. Short-wavelength sensitivity for the direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans. *Sleep Physiol.* (2006) 29:161–8. doi: 10.1093/sleep/29.2.161
50. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. *Science.* (1980) 210:1267–9. doi: 10.1126/science.7434030
51. Zeitzer JM, Dijk DJ, Kronauer RE, Brown EN, Czeisler CA. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J Physiol.* (2000) 526:695–702. doi: 10.1111/j.1469-7793.2000.00695.x
52. Czeisler CA, Kronauer RE, Allan JS, Duffy JF, Jewett ME, Brown EN, et al. Bright light induction of strong (type 0) resetting of the human circadian pacemaker. *Science.* (1989) 244:1328–33. doi: 10.1126/science.2734611
53. Gooley JJ, Ho Mien I, St Hilaire MA, Yeo SC, Chua EC, van Reen E, et al. Melanopsin and rod-cone photoreceptors play different roles in mediating pupillary light responses during exposure to continuous light in humans. *J Neurosci.* (2012) 32:14242–53. doi: 10.1523/JNEUROSCI.1321-12.2012
54. Spitschan M, Jain S, Brainard DH, Aguirre GK. Opponent melanopsin and S-cone signals in the human pupillary light response. *PNAS.* (2014) 111:15568–72. doi: 10.1073/pnas.1400942111
55. Cajochen C, Munch M, Kobialka S, Krauchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. *J Clin Endocrinol Metab.* (2005) 90:1311–6. doi: 10.1210/jc.2004-0957
56. Prayag AS, Jost S, Avouac P, Dumortier D, Gronfier C. Dynamics of non-visual responses in humans: as fast as lightning? *Front Neurosci*. (2019) 13:16. doi: 10.3389/fnins.2019.00126
57. Golden RN, Gaynes BN, Ekstrom RD, Hamer RM, Jacobsen FM, Suppes T, et al. The efficacy of light therapy in the treatment of mood disorders: a review and meta-analysis of the evidence. *Am J Psychiatry.* (2005) 162:656–63. doi: 10.1176/appi.ajp.162.4.656
58. Bedrosian TA, Nelson RJ. Influence of the modern light environment on mood. *Mol Psychiatry.* (2013) 18:751–7. doi: 10.1038/mp.2013.70
59. Keis O, Helbig H, Streb J, Hille K. Influence of blue-enriched classroom lighting on students' cognitive performance. *Trends Neurosci Educ.* (2014) 3:86–92. doi: 10.1016/j.tine.2014.09.001
60. Sleegers PJC, Moolenaar NM, Galetzka M, Pruyn A, Sarroukh BE, van der Zande B. Lighting affects students' concentration positively: findings from three Dutch studies. *Lighting Res Technol.* (2013) 45:169–75. doi: 10.1177/1477153512446099
61. Mott MS, Robinson DH, Walden A, Burnette J, Rutherford AS. Illuminating the effects of dynamic lighting on student learning. *Sage Open.* (2012) 2:1–9. doi: 10.1177/2F2158244012445585
62. Barkmann C, Wessolowski N, Schulte-Markwort M. Applicability and efficacy of variable light in schools. *Physiol Behav.* (2012) 105:621–7. doi: 10.1016/j.physbeh.2011.09.020
63. Mills PR, Tomkins S, Schlangen LJM. The effect of high correlated colour temperature office lighting on employee wellbeing and work performance. *J Circadian Rhythms.* (2007) 5:9. doi: 10.1186/1740-3391-5-2
64. Viola AU, James LM, Schlangen LJM, Dijk DJ. Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. *Scand J Work Environ Health.* (2008) 34:297–306. doi: 10.5271/sjweh.1268

65. Boubekri M, Cheung IN, Reid KJ, Wang CH, Zee PC. Impact of windows and daylight exposure on overall health and sleep quality of office workers: a case-control pilot study. *J Clin Sleep Med.* (2014) 10:603–11. doi: 10.5664/jcsm.3780
66. Figueiro MG, Steverson B, Heerwagen J, Kampschroer K, Hunter CM, Gonzales K, et al. The impact of daytime light exposures on sleep and mood in office workers. *Sleep Health.* (2017) 3:204–15. doi: 10.1016/j.sleh.2017.03.005
67. Royer M, Ballentine NH, Eslinger PJ, Houser K, Mistrick R, Behr R, et al. Light therapy for seniors in long term care. *JAMDA.* (2012) 13:100–2. doi: 10.1016/j.jamda.2011.05.006
68. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci.* (2001) 21:6405–12. doi: 10.1523/JNEUROSCI.21-16-06405.2001
69. Najjar RP, Chiquet C, Teikari P, Cornut PL, Claustrat B, Denis P, et al. Aging of non-visual spectral sensitivity to light in humans: compensatory mechanisms? *PLoS ONE.* (2014) 9:e85837. doi: 10.1371/journal.pone.0085837
70. Zeitzer JM. When is a proxy not a proxy? The foibles of studying non-image forming light. *J Physiol.* (2018) 596:2029–30. doi: 10.1113/JP276076
71. Chang A-M, Scheer FAJL, Czeisler CA. The human circadian system adapts to prior photic history. *J Physiol.* (2011) 589:1095–102. doi: 10.1113/jphysiol.2010.201194
72. Hébert M, Martin SK, Lee C, Eastman CI. The effects of prior light history on the suppression of melatonin by light in humans. *J Pineal Res.* (2002) 33:198–203. doi: 10.1034/j.1600-079X.2002.01885.x
73. Zeitzer JM, Friedman L, Yesavage JA. Effectiveness of evening phototherapy for insomnia is reduced by bright daytime light exposure. *Sleep Med.* (2011) 12:805–7. doi: 10.1016/j.sleep.2011.02.005
74. Kozaki T, Kubokawa A, Taketomi R, Hatae K. Effects of day-time exposure to different light intensities on light-induced melatonin suppression at night. *J Physiol Anthropol.* (2015) 34:5. doi: 10.1186/s40101-015-0067-1
75. te Kulve M, Schlangen LJM, van Marken Lichtenbelt WD. Early evening light mitigates sleep compromising physiological and alerting responses to subsequent late evening light. *Sci Rep*. (2019) 9:16064. doi: 10.1038/s41598-019-52352-w
76. Cajochen C, Reichert C, Maire M, Schlangen LJM, Schmidt C, Viola AU, et al. Evidence that homeostatic sleep regulation depends on ambient lighting conditions during wakefulness. *Clocks Sleep.* (2019) 1:517–31. doi: 10.3390/clockssleep1040040
77. Phillips AJK, Vidafar P, Burns AC, McGlashan EM, Anderson C, Rajaratnam SMW, et al. High sensitivity and interindividual variability in the response of the human circadian system to evening light. *PNAS.* (2019) 116:12019–24. doi: 10.1073/pnas.1901824116
78. Roenneberg T, Foster RG. Twilight times: light and the circadian system. *Photochem Photobiol.* (1997) 66:549–61. doi: 10.1111/j.1751-1097.1997.tb03188.x
79. Roenneberg T, Daan S, Merrow M. The art of entrainment. *J Biol Rhythms.* (2003) 18:183–94. doi: 10.1177/0748730403018003001
80. Boyce PR, Hunter C, Howlett O. *The Benefits of Daylight Through Windows*. Troy, NY: Rensselaer Polytechnic Institute (2003).
81. Solt J, Aarts MPJ, Andersen M, Appelt S, Bodart M, Kaempf J, et al. Chapter 4. Daylight in the built environment. In: Sanders S, Oberst J, editors. *Changing Perspectives on Daylight: Science, Technology, and Culture*. Washington, DC: Science/AAAS. (2017). p. 24–32.
82. Knoop M, Stefani O, Bueno B, Matusiak B, Hobday R, Wirz-Justice A, et al. Daylight: what makes the difference? *Lighting Res Technol.* (2020) 52:423–42. doi: 10.1177/1477153519869758
83. Aarts MPJ, Brown SA, Bueno B, Gjedde A, Mersch D, Münch M, et al. Chapter 5. Reinventing daylight. In: Sanders S, Oberst J, editors. *Changing Perspectives on Daylight: Science, Technology, and Culture*. Washington, DC: Science/AAAS (2017). p. 33–7.
84. Wright KP, Badia P, Myers BL, Plenzler SC, Hakel M. Caffeine and light effects on nighttime melatonin and temperature levels in sleep-deprived humans. *Brain Res.* (1997) 747:78–84. doi: 10.1016/S0006-8993(96)01268-1
85. Badia P, Myers B, Boecker M, Culpepper J, Harsh JR. Bright light effects on body temperature, alertness, EEG and behavior. *Physiol Behav.* (1991) 50:583–8. doi: 10.1016/0031-9384(91)90549-4
86. Cajochen C, Frey S, Anders D, Spati J, Bues M, Pross A, et al. Evening exposure to a light-emitting diodes (led)-backlit computer screen affects circadian physiology and cognitive performance. *J Appl Physiol.* (2011) 110:1432–8. doi: 10.1152/japplphysiol.00165.2011
87. Rahman SA, Flynn-Evans EE, Aeschbach D, Brainard GC, Czeisler CA, Lockley SW. Diurnal spectral sensitivity of the acute alerting effects of light. *Sleep.* (2014) 37:271–81. doi: 10.5665/sleep.3396
88. Lok R, Woelders T, Gordijn MCM, Hut RA, Beersma DGM. White light during daytime does not improve alertness in well-rested individuals. *J Biol Rhythms.* (2018) 33:637–48. doi: 10.1177/0748730418796036
89. Rahman SA, St. Hilaire MA, Lockley SW. The effects of spectral tuning of evening ambient light on melatonin suppression, alertness and sleep. *Physiol Behav.* (2017) 177:221–9. doi: 10.1016/j.physbeh.2017.05.002
90. Segal AY, Sletten TL, Flynn-Evans EE, Lockley SW, Rajaratnam SMW. Daytime exposure to short- and medium-wavelength light did not improve alertness and neurobehavioral performance. *J Biol Rhythms.* (2016) 31:470–82. doi: 10.1177/0748730416659953
91. Sletten TL, Ftouni S, Nicholas CL, Magee M, Grunstein RR, Ferguson S, et al. Randomised controlled trial of the efficacy of a blue-enriched light intervention to improve alertness and performance in night shift workers. *Occup Environ Med*. (2017) 74:792–801. doi: 10.1136/oemed-2016-103818
92. Rodriguez-Morilla B, Madrid JA, Molina E, Perez-Navarro Correa A. Blue-enriched light enhances alertness but impairs accurate performance in evening chronotypes driving in the morning. *Front Psychol.* (2018) 9:688. doi: 10.3389/fpsyg.2018.00688
93. Dorrian J, Rogers NL, Dinges DF. Psychomotor vigilance performance: neurocognitive assay sensitive to sleep loss. In: Kushida CA, editor. *Sleep Deprivation: Clinical Issues Pharmacology, and Sleep Loss Effects*. Boca Raton, FL: CRC Press (2004). p. 67–98.
94. Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. *Int J Neurosci*. (1990) 52:29–37. doi: 10.3109/00207459008994241
95. Brown T. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res.* (2020) 69:e12655. doi: 10.1111/jpi.12655
96. Papamichael C, Skene DJ, Revell VL. Human nonvisual responses to simultaneous presentation of blue and red monochromatic light. *J Biol Rhythms.* (2012) 27:70–8. doi: 10.1177/0748730411431447
97. van de Werken M, Gimenez MC, de Vries B, Beersma DG, Gordijn MC. Short-wavelength attenuated polychromatic white light during work at night: limited melatonin suppression without substantial decline of alertness. *Chronobiol Int.* (2013) 30:843–54. doi: 10.3109/07420528.2013.773440
98. Sahin L, Figueiro MG. Alerting effects of short-wavelength (blue) and long-wavelength (red) lights in the afternoon. *Physiol Behav.* (2013) 116–117:1–7. doi: 10.1016/j.physbeh.2013.03.014
99. Figueiro MG, Nagare R, Price LLA. Non-visual effects of light: how to use light to promote circadian entrainment and elicit alertness. *Lighting Res Technol.* (2018) 50:38–62. doi: 10.1177/1477153517721598
100. Heschong L, Wright RL, Okura S. Daylighting impacts on human performance in school. *J Illum Eng Soc.* (2002) 31:101–14. doi: 10.1080/00994480.2002.10748396
101. Boubekri M, Lee J, MacNaughton P, Woo M, Schuyler L, Tinianov B, et al. The impact of optimized daylight and views on the sleep duration and cognitive performance of office workers. *Int J Environ Res Public Health.* (2020) 17:3219. doi: 10.3390/ijerph17093219
102. Veitch JA, Fotios SA, Houser KW. Judging the scientific quality of applied lighting research. *LEUKOS.* (2019) 15:97–114. doi: 10.1080/15502724.2018.1550365
103. Snyder JS, Gregg MK, Weintraub DM, Alain C. Attention, awareness, and the perception of auditory scenes. *Front Psychol.* (2012) 3:17. doi: 10.3389/fpsyg.2012.00015
104. Keller A. Attention and olfactory consciousness. *Front Psychol.* (2011) 2:13. doi: 10.3389/fpsyg.2011.00380
105. Figueiro MG, Plitnick BA, Lok A, Jones GE, Higgins P, Hornick TR, et al. Tailored lighting intervention improves measures of sleep, depression, and agitation in persons with Alzheimer's disease and related dementia

living in long-term care facilities. *Clin Interv Aging.* (2014) 9:1527–37. doi: 10.2147/CIA.S68557
106. Hopkins S, Morgan PL, Schlangen LJM, Williams P, Skene DJ, Middleton B. Blue-enriched lighting for older people living in care homes: effect on activity, actigraphic sleep, mood and alertness. *Curr Alzheimer Res.* (2017) 14:1053–62. doi: 10.2174/1567205014666170608091119
107. Giggins OM, Doyle J, Hogan K, George M. The impact of cycled lighting intervention on nursing home residents: a pilot study. *Gerontol Geriatr Med.* (2019) 5:1–6. doi: 10.1177/2333721419897453
108. Lunn RM, Blask DE, Coogan AN, Figueiro MG, Gorman MR, Hall JE, et al. Health consequences of electric lighting practices in the modern world: a report on the National Toxicology Program's workshop on shift work at night, artificial light at night, and circadian disruption. *Sci Total Environ.* (2017) 607–608:1073–84. doi: 10.1016/j.scitotenv.2017.07.056
109. Hunter CM, Figueiro MG. Measuring light and night and melatonin levels in shift workers: a review of the literature. *Biol Res Nurs.* (2017) 19:365–74. doi: 10.1177/1099800417714069
110. Motamedzadeh M, Golmohammadi R, Kazemi R, Heidarimoghadam R. The effect of blue-enriched white light on cognitive performances and sleepiness of night-shift workers: a field study. *Physiol Behav.* (2017) 177:208–14. doi: 10.1016/j.physbeh.2017.05.008
111. Griepentrog JE, Labiner HE, Gunn SR, Rosengart MR. Bright environmental light improves the sleepiness of nightshift ICU nurses. *Crit Care.* (2018) 22:1–9. doi: 10.1186/s13054-018-2233-4
112. Wu LM, Amidi A, Valdimarsodittir H, Ancoli-Israel S, Liu L, Winkey G, et al. The effect of systematic light exposure on sleep in a mixed group of fatigued cancer survivors. *J Clin Sleep Med.* (2018) 14:31–9. doi: 10.5664/jcsm.6874
113. Giménez MC, Geerdinck LM, Versteylen M, Leffers P, Meekes GJBM, Herremans H, et al. Patient room lighting influences on sleep, appraisal and mood in hospitalized people. *J Sleep Res.* (2017) 26:236–46. doi: 10.1111/jsr.12470
114. Tsai SY, Thomas KA, Lentz MJ, Barnard KE. Light is beneficial for infant circadian entrainment: an actigraphic study. *J Adv Nurs.* (2011) 68:1738–47. doi: 10.1111/j.1365-2648.2011.05857.x
115. Chartered Institution of Building Service Engineers. *SLL Lighting Handbook*. London: CIBSE (2018). p. 519.
116. Illuminating Engineering Society. *PS-12-19. IES Position on UL RP 24480 Regarding Light and Circadian Entrainment.* (2020). Available online at: https://www.ies.org/about-outreach/position-statements/ (accessed November 2, 2020).
117. Zee PC, Goldstein CA. Treatment of shift work disorder and jet lag. *Curr Treat Options Neurol.* (2010) 12:396–411. doi: 10.1007/s11940-010-0090-9
118. American National Standards Institute/Illuminating Engineering Society. *LP-7-20 Lighting Practice: The Lighting Design and Construction Process*. New York, NY: IES (2020). p. 46.
119. Deutsches Institut für Normung. *DIN SPEC 67600:2013-04 Biologically Effective Illumination – Design Guidelines*. Berlin: DIN (2013).
120. Deutsches Institut für Normung. *Standards Deliverables.* (2020). Available online at: https://www.din.de/en/about-standards/din-standards/deliverables (accessed December 21, 2020).
121. Houser KW. Editorial: estimates, precision, accuracy, and adaptation in applied lighting. *LEUKOS.* (2020) 17:1–2. doi: 10.1080/15502724.2020.1834283
122. Safranek S, Collier JM, Wilkerson A, Davis RG. Energy impact of human health and wellness lighting recommendations for office and classroom applications. *Energy Build.* (2020) 226:110365. doi: 10.1016/j.enbuild.2020.110365
123. Webler FS, Spitschan M, Foster RG, Andersen M, Peirson SN. What is the 'spectral diet' of humans? *Curr Opin Behav Sci.* (2019) 30:80–6. doi: 10.1016/j.cobeha.2019.06.006
124. Gu F, Xu S, Devesa SS, Zhang F, Kierman EB, Graubard BI, et al. Longitudinal position in a time zone and cancer risk in the United States. *Cancer Epidemiol Biomark Prev.* (2017) 26:1306–11. doi: 10.1158/1055-9965.EPI-16-1029
125. Stevens RG, Blask DE, Brainard GC, Hanses J, Lockley SW, Provencio I, et al. Meeting report: the role of environmental lighting and circadian disruption in cancer and other diseases. *Environ Health Perspect.* (2007) 115:1357–62. doi: 10.1289/ehp.10200

Conflict of Interest: KH is Editor in Chief of LEUKOS, the journal of the Illuminating Engineering Society, founder of Loucetios, LLC, and co-founder of Lyralux, Inc. TE is founder of Lighting Research Solutions, LLC.

frontiers in Neurology

REVIEW
published: 01 February 2021
doi: 10.3389/fneur.2021.625334

Naturalistic Intensities of Light at Night: A Review of the Potent Effects of Very Dim Light on Circadian Responses and Considerations for Translational Research

Thijs J. Walbeek[1,2†‡], *Elizabeth M. Harrison*[1†‡], *Michael R. Gorman*[1,3*†] and *Gena L. Glickman*[1,4†]

[1] Center for Circadian Biology, University of California, San Diego, La Jolla, CA, United States, [2] Oregon Institute of Occupational Health Sciences, Oregon Health and Science University, Portland, OR, United States, [3] Department of Psychology, University of California, San Diego, San Diego, CA, United States, [4] Departments of Psychiatry and Neuroscience, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

In this review, we discuss the remarkable potency and potential applications of a form of light that is often overlooked in a circadian context: naturalistic levels of dim light at night (nLAN), equivalent to intensities produced by the moon and stars. It is often assumed that such low levels of light do not produce circadian responses typically associated with brighter light levels. A solid understanding of the impacts of very low light levels is complicated further by the broad use of the somewhat ambiguous term "dim light," which has been used to describe light levels ranging seven orders of magnitude. Here, we lay out the argument that nLAN exerts potent circadian effects on numerous mammalian species, and that given conservation of anatomy and function, the efficacy of light in this range in humans warrants further investigation. We also provide recommendations for the field of chronobiological research, including minimum requirements for the measurement and reporting of light, standardization of terminology (specifically as it pertains to "dim" light), and ideas for reconsidering old data and designing new studies.

Keywords: entrainment, physiology, translation, parametric effects, circadian, dim light, nLAN

OPEN ACCESS

Edited by:
Manuel Spitschan,
University of Oxford, United Kingdom

Reviewed by:
Randy J. Nelson,
West Virginia University, United States
Stuart N. Peirson,
University of Oxford, United Kingdom
Travis Longcore,
UCLA Institute of the Environment and Sustainability, United States

***Correspondence:**
Michael R. Gorman
mgorman@ucsd.edu

†ORCID:
Thijs J. Walbeek
orcid.org/0000-0002-2907-2126
Elizabeth M. Harrison
orcid.org/0000-0002-0482-589X
Michael R. Gorman
orcid.org/0000-0001-6122-8437
Gena L. Glickman
orcid.org/0000-0001-7064-7238

‡*These authors have contributed equally to this work and share first authorship*

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *02 November 2020*
Accepted: *06 January 2021*
Published: *01 February 2021*

Citation:
Walbeek TJ, Harrison EM, Gorman MR and Glickman GL (2021) Naturalistic Intensities of Light at Night: A Review of the Potent Effects of Very Dim Light on Circadian Responses and Considerations for Translational Research. Front. Neurol. 12:625334. doi: 10.3389/fneur.2021.625334

INTRODUCTION

Light has a profound influence on the biological health of mammals, including humans. In addition to illuminating the environment for vision (i.e., image forming), light also regulates non-image forming processes, such as the circadian timing system, neuroendocrine fluctuations, and acute alerting effects, just to name a few (1, 2). All of the non-image forming effects of light studied to date appear to be most sensitive to short-wavelength light and at intensities greater than what is required for visual illumination (3). As current architectural lighting strategies have been developed around the human visual system, most do not fully support biological health and well-being. Yet, the timing of photic delivery is also of utmost importance: one major non-image forming function of light is to synchronize internal biological rhythms—orchestrated by a master pacemaker in the suprachiasmatic nucleus (SCN) in the hypothalamus—to environmental light-dark cycles.

This process is called entrainment. To provide a daily photic temporal cue in lieu of exposure to the solar light dark cycle, a biologically potent light stimulus should be used to signal daytime (4), whereas a relatively less potent stimulus (dimmer and depleted in shorter wavelengths) should be utilized during the evening and night. It has been proposed that the current suboptimal patterns of photic exposure, including insufficient light during the day and excessive light at night, are at least partly responsible for a variety of negative health and safety consequences (5, 6).

Interestingly, much of the seminal work characterizing non-image forming responses to light has taken place during the biological night, yet the focus of lighting countermeasures in humans has been on optimizing photic exposure during the day. By far, the majority of these studies assesses the responses elicited by exposure to a short duration pulse of light (typically <2 h) against a background of complete darkness or dim light. Using this approach, investigators have operationally defined the lower limits of light sensitivity with respect to these biological responses to acute light pulses. A growing body of literature, however, demonstrates that light far below these putative thresholds for circadian sensitivity nevertheless exerts potent biological effects in non-human mammals, particularly if the illumination is present over several hours (7, 8).

Compared to complete darkness (to the extent that this can be feasibly created in the laboratory), dim illumination in the range approximately of moonlight and starlight markedly alters the fundamental properties of circadian rhythms in model organisms. Specifically, the addition of a relatively small number of photons throughout the biological night enhances the flexibility of behavioral entrainment by traditionally bright lighting regimens. These rarely recognized, and highly potent actions of very low light doses have been particularly well-documented in nocturnal mammals. Given the broad conservation of circadian (neuro)biology (e.g., molecular feedback loops, brain structures) and function (e.g., phase-shifting, light sensitivity) across taxa and ecological niche (see below for more detail), it is critical to ask whether comparable effects could be elicited in humans. If so, dim light treatments would represent a novel and more efficient approach for correcting or preventing circadian disturbances, particularly in more extreme cases, such as is common with jet lag and shift work. This review first aims to clarify terminology on different definitions of "dim light" and summarize the effects of very dim light in model systems. We limit the scope of our review to circadian research in mammalian organisms because of the focus on potential translational value for human circadian studies. Next, we discuss why the biological potency of very low levels of dim light should be considered and examined in humans. Although some reports showing effects of lunar cycles on human sleep exist (9–12), there are few experiments examining the effects of comparable dim light levels on circadian endpoints in humans. Therefore, we identify a range of opportunities for future human work and include recommendations for the field going forward.

DIFFERENT LEVELS AND DEFINITIONS OF DIM LIGHT AND DARK

Light levels may be reported in photometric (e.g., lux), radiometric (e.g., $\mu W/cm^2$ or photons/cm^2/s) and the emergent melanopic EDI (also in lux). If sufficient information is provided, which must include the spectral power distribution (SPD), these measures can all be converted into each other (13, 14). Because of very different scales, however, comparing across metrics may not always be intuitive, especially with polychromatic light. Throughout this review, we prioritize presenting measures as reported in the original work, but have converted to melanopic lux when possible and when useful for comparison.

Across the circadian literature, studies have used dim light for different purposes and defined a wide range of light levels as "dim" (**Figure 1**). These studies can generally be divided into three categories. The first group aims to eliminate any form of temporal information by exposing subjects to equal amounts of light throughout the night and day. It is worth noting that despite constant levels of environmental light, orientation of the head as well as opening and closing of the eyes inevitably leads to varied photic exposure patterns. In humans, dim constant light is more commonly used to study intrinsic clock properties that are not directly driven (or masked) by light (20).

The second body of work aims to mimic urban levels of artificial light at night to study its effects on sleep or health (21, 22). These studies usually focus on the ramifications of our modern lifestyle and the ubiquity of artificial light on natural processes, such as exposure to electronic devices, indoor LED lighting, or light derived from artificial light sources outside, such as street lights. Thus, such studies typically use 5–30 lux during the "night," as opposed to near darkness. These protocols lead to all kinds of health decrements, including loss of sleep and metabolic syndrome in both rodents [reviewed in (23)] and humans [reviewed in (24)].

The last group of studies uses much lower levels of light (commonly 0.01–0.1 lux) that are more consistent with natural light at night (nLAN) cast by the moon or stars (9–12, 25–38). In contrast to the relatively brighter light used in constant light or studies of urban nighttime light, nLAN seemingly does not induce circadian health disruption, though data are limited. It does, however, have marked effects on circadian organization of behavior, despite being reported as below threshold for phase shifting or melatonin suppression. These changes in circadian behavior, which generally indicate a higher degree of circadian flexibility, have so far been primarily studied in rodents, but yield strong translational potential in aiding the adjustment of the human circadian clock to either rapid travel across multiple time zones or irregular schedules such as in shiftwork.

To illustrate the wide range of light levels referred to as "dim light" by circadian researchers, we performed a literature review and gathered light parameters summarized in **Figure 1** (see legend for search methodology). When reviewing these articles, we noticed that, often, investigators reported extensive detail about the bright light stimuli employed in their studies but did not quantify or qualify the "dim light." Specifically, 39/71

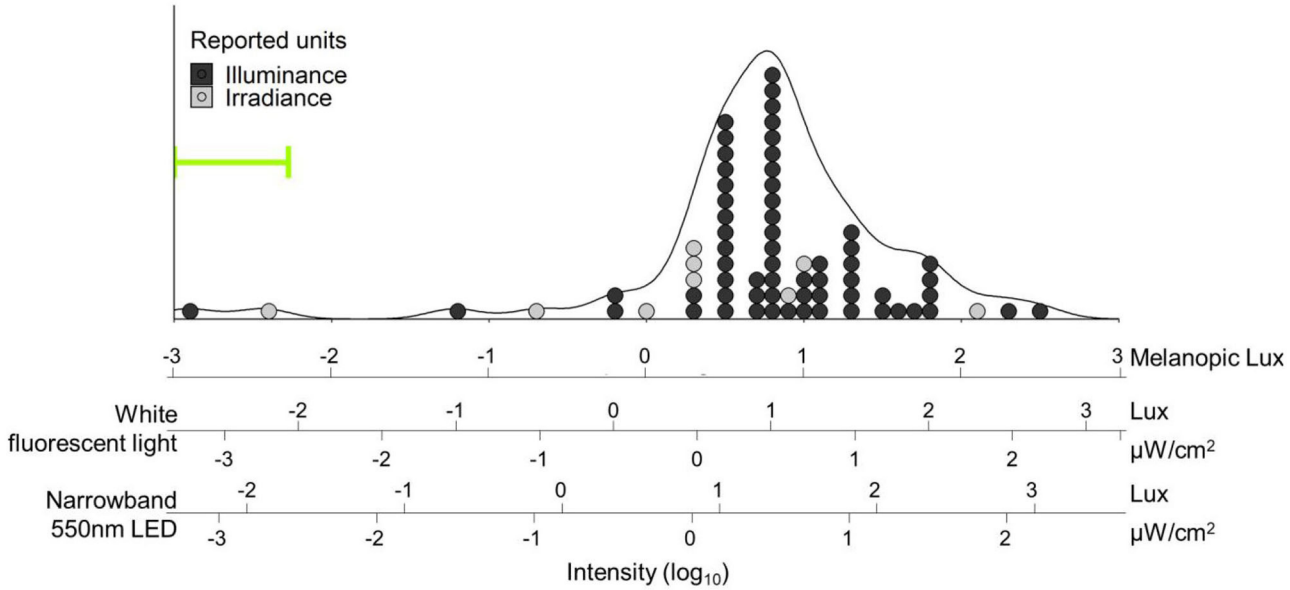

FIGURE 1 | Distribution of light intensities referred to as "dim light" in human circadian research papers. Light intensities were converted into melanopic lux [using the Lucas toolbox 2014 (13)] and plotted on log units. Additional scales with white fluorescent light and narrowband green (550 ± 23 nm) light are provided and aligned for equivalent biological potency [e.g., "10 lux" (1.5 μW/cm2) narrowband green LED light has the same biological potency as 4.7 lux while fluorescent light: 2.92 melanopic lux]. Fifty-five percent of the papers (39/71) did not report any spectral or color information about their light, so SPD for fluorescent white light was assumed for conversion. Overall density is represented by the black line. Individual papers are plotted as dots, and color coded for the reported unit type; 62/71 (87%) reported light intensities in lux alone. Both mode (tallest stack) and median intensities reported as "dim light" in our search were 10 lux fluorescent white light (6.2 melanopic lux) – bright enough for phase shifting (15), entrainment (16), or melatonin suppression (17) in humans. The three papers below 0.1 melanopic lux all reported the use of red dim light; all other papers used either white light or did not report color information. Range of intensities used in Gorman lab rodent studies is indicated by the green bracket, and extends to lower values outside the bounds of the figure (see **Table 1**). Search strategy: A pubmed search was performed on October 23, 2020 with search terms: "human circadian dim light." Results were filtered for "2,000-present" and "Journal Article," which resulted in a total of 509 articles, and sorted for "Best Match." The first 100 results were downloaded and screened for light intensity information. In total, 29 papers were excluded because they were either review papers ($n = 11$), used non-human subjects ($n = 13$), or reported neither intensity nor source ($n = 5$). From each paper the brightest light referred to as "dim light" was recorded. If the paper reported a range (e.g., 1–5 lux, or <5 lux), the highest value within the range was used. If multiple units were reported (e.g., Lux and μW/cm^2) irradiance was used over illuminance.

(55%) only reported "dim light" levels in lux (only relevant for the image-forming system), without any information on spectral quality or source; a further 5/76 (6%) were excluded from our analyses because they did not even report intensity.

Because absolute darkness in the laboratory often cannot be feasibly achieved or measured with any confidence, it is difficult to define precisely where the "threshold" is between (very) dim light and darkness. Walls, doors and ceilings are rarely completely light-tight, and equipment in or near animal housing may also generate light. The QA-4 modules in the VitalView data collection system commonly used for circadian monitoring, for example, have red indicator lights that fluctuate when an activity signal is received and can produce feedback (39). Moreover, investigators may intentionally put in place constant, low levels of illumination for practical reasons, such as animal husbandry (6), sometimes without reporting. Careful exclusion or attenuation of all such light sources can, of course, diminish light exposure.

It is our impression that many circadian researchers share the assumption that (very) dim light is biologically equivalent to actual darkness. Such beliefs might be founded on carefully executed experiments demonstrating minimal light levels that are required to induce phase shifts or melatonin suppression (40–42). As we will lay out in this review, however, there is a multitude of evidence, within the circadian literature, as well as from other fields, that light well below 1 lux can have dramatic effects on circadian oscillators or their outputs, challenging these common assumptions. By summarizing these (perhaps unintentionally) overlooked effects of very dim light levels within circadian research, we hope to provide a framework to aid researchers in designing and reporting on their experimental lighting conditions.

HISTORICAL VIEW OF nLAN STUDIES

Laboratory studies cannot escape trade-offs between simplicity and experimental control on one hand and real-word validity on the other. In the domain of circadian entrainment, this trade-off has been operationally resolved in favor of exposure to an alternation between just two photic conditions, each of unvarying spectrum, intensity and geometry, to represent "day" and "night." Twilight transitions, lunar cycles, and variability due to cloud cover are just a few examples that reflect naturally-occurring changes in intensity and spectrum that are rarely simulated, although each of these photic dimensions may be highly

relevant to circadian timing systems of particular organisms, and it could be adaptive for such organisms to be sensitive to them (43). The work in the 1960's of zoologist JL Kavanau (44–46) on rest/activity cycles of mammals provided myriad demonstrations of the behavioral importance of additional domains of photic stimuli. For example, running speed, perhaps a reflection of motivation, was sensitive to light intensity during twilight; rodents of various genera operantly manipulated light intensity in relation to their endogenous rest/activity cycles; and animals expressed reliable (but non-homogenous) orientation preferences toward light sources, such as always running toward a dim night-time light source, conceptualized by the investigator as a simulated moon. In short, there emerged a rich ecology of environmental lighting that did not, for whatever historical reason, strongly imprint on the emergent field of chronobiology, though the zoological tradition remains important (30, 37, 47).

SERENDIPITOUS DISCOVERY OF nLAN EFFECTS IN AN EXPERIMENTAL SETTING

It came as a great surprise when we accidentally "rediscovered" the fundamental importance of dim illumination on circadian entrainment, when we thought we knew it to be of little consequence. In preparing for a time series collection of Syrian hamster (*Mesocricetus auratus*) brains for a study of SCN rhythmicity in a paradigm termed "bifurcation," we discovered notable and nearly categorical entrainment differences between the first and second cohort of animals. We had thought that each set of 20 hamsters was exposed to identical conditions, which included a very dim green light that was continuously illuminated—day and night—to aid in working with animals at night. These lights (see **Table 1** for intensities) had been incorporated after thorough (but perhaps not thorough enough) consultation of the literature suggested their inefficacy (e.g., thresholds for melatonin suppression and phase shifting) and some further experimental validations that they did not induce classic circadian responses. Confronted by the statistically improbable result that chance alone caused animals 1–20 to respond one way and animals 21–40 another, we looked for other explanations, only to discover that the electrical power for dim nocturnal illumination had been disrupted in half of the animals. Although we approached this explanation with skepticism, a *de novo*-test confirmed a potent entrainment role of this very dim light source (48). Since that time, we have come to appreciate that the effects of nLAN are more generalizable than we initially imagined.

Demonstrations of a potent circadian role for nLAN both preceded and ran parallel to our work [reviewed in (30, 37, 47)]. Working with bats, for example, Erkert demonstrated systematic variation in circadian entrainment parameters with variations in light intensity in the range of starlight (32–35, 49). Readers interested in the ecological breadth of the biological effects of nighttime light could probably not do better than to consult a comprehensive resource, such as that from Rich and Longcore (47), or this recent review (50). But in the circadian domain specifically, our lab has accrued a critical mass of studies contrasting fundamental properties of circadian rhythms in animals under dimly illuminated vs. conditions without detectable light. We present a synthesis here that is not easily derived from reading the publications as they individually appeared.

TABLE 1 | Range of reported Gorman lab nLAN values.

560 + 23 nm LED	Lowest reported value (18)	Highest reported intensity (19)
Illuminance (Lux)[a,b]	0.004	0.03
Irradiance ($\mu W/cm^2$)	0.00062	0.00390
Photon Flux (Photons/cm^2/s)	1.75×10^9	1.09×10^{10}
S Cone (α opic lux)[a,b]	0.0000001	0.000014
M Cone (α opic lux)[a,b]	0.00367	0.02370
L Cone (α opic lux)[a,b]	0.00352	0.02150
Rod (α opic lux)[a]	0.00193	0.01440
Melanopsin (α opic lux)[a]	0.000614	0.0053

[a] *Values are calculated based on reported irradiance using the Lucas toolbox 2014 (13).*
[b] *Values based on human cone sensitivity.*

Range of Parameters Used in our Lab Over the Years

Over the years, we have used a variety of nLAN sources. Our initial studies assessed the efficacy of a single green narrowband LED (560 nm, full width at half maximum of 23 nm) mounted on the wall of a light-tight enclosure containing a single opaque plastic cage that provided some diffusion. Other studies employed panelescent LED nightlights ∼1 m from free-standing caging racks, with animals housed in clear cages. Most of our studies have used a single green LED mounted in a standard position ∼10–30 cm from the orthogonally oriented running wheel in transparent cages (**Table 1**).

Unlike highly controlled human studies that can employ Ganzfeld domes, pharmacological pupil dilation etc., the animal housing environment allows ample opportunity for variation in effective light exposure. Although in various enclosures we have taken pains to minimize variability in light exposure via diffusers, positioning of cages, etc., conventionally, we have reported light intensity at the brightest location in the cage. But as intensity falls off by the inverse square law, it can vary considerably within the cage. Moreover, animals oriented directly toward the light [as in Kavanau's rodents (44)] might, depending on the experimental setup, be sampling several orders of magnitude more light than animals with their gaze diverted from the point source. Conversely, effective illumination of the retina could be attenuated with pupillary constriction.

In short, it is not yet possible to define with any precision the range of parameters of nLAN that produce a substantial effect on rodent rest/activity cycles, but the sources and configurations used do not appear to be near the limits of an operating range. Effects appear to be robust with respect to some variation in caging configuration and light orientation, although systematic assessments of these factors have not been completed. Finally, the narrowband green light is likely to activate multiple photoreceptor mechanisms. In limited studies, white light proved

also to have efficacy, so we have little reason to believe that narrowband stimulation is a requirement, although this remains to be thoroughly assessed.

Despite a variety of enclosures as well as light sources and intensities (see **Table 1**) used throughout two decades of experiments to investigate the effect of simulated natural night light levels, the nLAN conditions were always deliberately contrasted with darkness, created by careful exclusion or attenuation of all light sources. As mentioned above, typical laboratory conditions do not achieve complete darkness. In our lab, we made every effort to exclude light to approximate complete darkness. Even after covering all equipment LEDs with black electrical tape and blocking all visible light coming into the room through doors or ventilation, however, our dark conditions may not be absolute. Nevertheless, they were always below the limits of detection (using an IL1700 radiometer; International Light, Inc., Newburyport, MA).

HOW DOES nLAN AFFECT CIRCADIAN RHYTHMS IN MODEL SYSTEMS?

Historically, the actions of light on circadian systems have been commonly categorized as either parametric or non-parametric. A non-parametric effect is exemplified by an acute resetting of circadian phase induced by a brief light pulse, and an absence of any sustained changes in any other parameters of the underlying clock oscillation. Such effects are the basis for much entrainment theory, particularly as it applies to nocturnal animals that are exposed to short intervals of bright light at dawn and dusk. Parametric actions, in contrast, are typified by ongoing modulations of clock function, such as a change in the free-running period, often caused by chronic light exposure. In reality, this conceptual distinction can be problematic, because parametric and non-parametric effects cannot always be completely distinguished. A brief light pulse, for example, can both acutely reset circadian phase and alter free-running period (51). Further, shifts in the light:dark cycle induce rapid shifts in the phase of activity but the SCN oscillatory network may be perturbed for several cycles before returning to steady state (52, 53).

Circadian rhythms in behavior are described using several common metrics, including period and amplitude. Even though different circadian assays are often used to measure one or more of these metrics, basic circadian theory suggests that these parameters are interrelated. Here we describe the effects of nLAN on some of the most common circadian measures. Throughout this review, we will use "nLAN" to refer to light levels below one lux, whether it is used to illuminate strictly during the night, as a constant condition, or as a brief pulse, and regardless of the source. For an overview of definitions of the various parameters, see these reviews: (54–56).

Effects of nLAN on Common Rhythm Parameters

Period

Period is the time it takes for the circadian oscillator to complete one cycle. The period of the internal circadian pacemaker can only be measured in conditions absent external time cues (zeitgebers) such as light or temperature cycles. Across multiple species, nLAN changes the free-running period (8, 25, 57, 58). For example, Syrian hamsters exposed to continuous complete darkness have a free running period (FRP) of just under 24 h. In contrast, in constant dim illumination (<0.01 lux, nLAN) these same animals have FRPs of ∼0.3 h longer (8). These findings are consistent with one of "Aschoff's rules" (more accurately characterized as generalizations), which notes the tendency of FRPs to increase with light intensity in nocturnal rodents (59, 60).

Waveform

Each full cycle in behavioral rhythms can be divided into biological day and night, which can be assessed by monitoring the relative lengths of an organism's active (alpha, α) and rest (rho, ρ) phases. In nocturnal rodents, alpha and rho correspond to (biological) night and (biological) day, respectively, and vice versa in diurnal humans. Although less commonly assayed and reported than is free-running period, waveform (relative length of alpha and rho) is a fundamental property of a circadian rhythm and is encoded in the SCN (56, 61–65). Aschoff observed that alpha in nocturnal rodents tends to decrease with increasing light intensity. In stark contradiction of this rule, the active phase of Syrian hamsters increases by 3 h in nLAN vs. darkness (8). Effects of nLAN on alpha are discernible even under entrained conditions with varying photoperiods. In most nocturnal species examined, exposure to long winter nights – or short days – eventually yields a waveform with an elongated biological night as assessed by lengthened activity duration, melatonin secretion or SCN pattern of electrical firing and gene expression (61, 66). Upon transfer of male Siberian (*Phodopus sungorus*) and Syrian hamsters from summer to winter photoperiods, nLAN accelerates short-photoperiod entrainment as well as its downstream sequelae (e.g., photoperiodic regulation of reproduction) (8, 67–70).

Amplitude

Whereas the free-running rhythm of a measured rhythm unambiguously reflects the period of an underlying pacemaker, the same is not necessarily true for rhythm amplitude. The amplitude of a rhythm describes the magnitude of the change in an output signal (for example body temperature, wheel-running or SCN electrical firing) throughout one cycle. Mathematically, pacemaker amplitude has been conceptualized as related to its phase perturbability by external zeitgebers, with small amplitude pacemakers having larger amplitude phase shifting responses (71, 72). Paradoxically, short photoperiods increase the amplitude of phase shifting by bright light pulses in rodents (68, 69), suggesting a smaller pacemaker amplitude while simultaneously increasing the daily variation in SCN electrical firing (73, 74). With respect to nLAN, there is sporadic evidence of increased wheel-running amplitude in Syrian hamsters (48). In subsequent studies using slightly different running wheels, the amount of wheel-running was indistinguishable in dark vs. nLAN conditions (8). To our knowledge, wheel-running amplitude has never been observed to decrease with nLAN. Importantly from the perspective that wheel-running intensity may have feedback effects on circadian pacemakers (75), the matched activity levels

in dark vs. dim nLAN excludes this feedback as the basis for observed differences in circadian period and waveform among male Syrian hamsters (8). In other contexts, nLAN-effects can be documented in the complete absence of running wheels (70).

Together, characterization of basic circadian parameters clearly demonstrates that feasibly attainable levels of darkness (e.g., $<10^{-6}$ lux) and nLAN are not biologically equivalent despite the common belief that such "dim" intensities fall below the threshold for markedly altering circadian function.

nLAN as a Zeitgeber

Dim Light PRC

Brief exposure to light (i.e., light pulses) can induce phase shifts in free running organisms. Depending on the timing of these light pulses, the phase shifts can be either advancing (shifting earlier), delaying (shifting later), or of minimal magnitude. The relationship between timing of the stimulus and the resulting phase shifts is described in a phase-response curve (PRC). While the amplitude of the light PRC can vary depending on the intensity, duration, and spectral properties of light, the shape is very consistent across species (76). Typically, light induces advances at the end of the (biological) night, delays at the beginning of the biological night, and minimal phase shifts during the middle of the biological day, in both diurnal and nocturnal animals. Against a dark background, 2-h pulses of nLAN in male Syrian hamsters yielded a statistically significant PRC with an amplitude of 45 min that was generally similar in shape to that from a 15-min bright light PRC (8).

Entrainment

The ability of nLAN light pulses to induce phase shifts suggests that such illumination might be a strong enough zeitgeber to entrain mammals. The Gorman lab at UC San Diego, however, has never directly tested entrainment in a light-dark cycle alternating nLAN and complete darkness (dim-dark). Efforts by others, on the other hand, did demonstrate that mice (C57Bl/6) exposed to 12 h dim−12 h dark light cycles were able to entrain with lights as dim as 0.0005 lux, depending on the wavelength (77). Similarly, Molossid bats (*Molossus molossus*) were able to entrain to 0.1 lux, with half of the animals entraining to light as dim as 0.00001 lux (33). Lastly, daily 2 h pulses of 0.07 lux of "deep red" light was enough to reliably entrain female albino rats (78). In sum, even relatively short exposure to nLAN levels of light can be a zeitgeber in at least four different species.

Acute Effects of nLAN

In addition to affecting the underlying circadian pacemaker as described above, low levels of dim light can affect the output behaviors directly.

Masking + Melatonin Suppression

Masking refers to the phenomena where a stimulus directly suppresses (negative masking) or enhances (positive masking) a certain behavior below or above levels expected based on circadian phase. These changes are direct effects on clock outputs and might not necessarily change the underlying circadian pacemaker. In rodents, a common example of negative masking is the suppression of activity levels by light during the biological night. Positive masking is often seen following a cage change during the day, where activity levels are temporarily higher than typical for the time of day because of novelty-induced activity that does not reflect the underlying circadian biology (79). Common examples of masking in humans are activity-induced changes in core body temperature, light-induced increased alertness, or suppression of plasma melatonin levels by light. Even if masked responses do not necessarily originate from or affect the underlying circadian pacemaker (although in the example of light-suppressed activity in rodents or melatonin in humans, it might do both), masking is important to every circadian researcher as it might affect inferences made about the underlying pacemaker if not appropriately controlled (80, 81). Furthermore, masked behavioral or physiological signals may feed back to the core clock and alter it in subsequent cycles.

In rodents, thresholds are different for positive (increased activity by darkness) and negative (decreased activity by light) masking, and depend on wavelength (79, 82–86). In mice, the lowest intensity at which positive and negative masking was observed with 518 nm light was 10^9 and 10^{12} photon flux, respectively (77). In Siberian hamsters, nLAN was sufficient to induce strong positive and negative masking, depending on the timing and duration, although effects cannot be completely separated from entrainment effects (87).

Melatonin suppression, often considered a form of negative masking, is the most commonly measured effect of light in human research (88) and relies on the SCN (66). The threshold intensity to acutely suppress pineal melatonin in Syrian hamsters was determined to be between 0.019 and 0.186 $\mu W/cm^2$, or 0.11 and 1.08 lux (89), similar to a lower threshold for 300 s 503 nm light of 3.5×10^{10} photons/cm^2/s (41). In an entrainment study in Syrian hamsters, 8 h of nLAN (0.1 lux) significantly suppressed melatonin expression compared to darkness (8). Because 2 or 5 h of nLAN was not sufficient to suppress melatonin in the same animals, the effects seen with 8 h of exposure might result from differences in entrainment itself, rather than acute suppression (masking). Similarly, in young Sprague-Dawley rats maintained in light-dark cycles with various amounts of dim light at night, 0.08 $\mu W/cm^2$ (0.2 lux) light suppressed plasma melatonin, while 0.06 $\mu W/cm^2$ did not (90), and in female nude rats, 0.08 $\mu W/cm^2$ (0.2 lux) of light at night was sufficient to attenuate nightly melatonin levels (91). Again, entrainment effects cannot be separated from acute masking. Although the relative contribution of acute and chronic exposure to (or parametric vs. non-parametric effects of) nLAN might not be definitive, it should be apparent that nLAN affects both levels of behavior and melatonin in a variety of rodent species.

Pupil Responses

Because many circadian responses are slow, difficult, invasive, and/or expensive to measure, pupillometry is commonly used as a proxy for circadian response. Light-induced pupil constriction relies on the same non-image forming visual system (e.g., photoreceptors and synaptic connections) as the circadian clock, and can therefore be a useful substitute psychophysical measure (92). Just like most measures, pupillary light responses are graded

responses that depend on intensity and wavelength. In mice, reliable pupil constrictions were observed at $10^{10.4}$, $10^{11.4}$, and $10^{12.4}$ photon flux for 470, 517, and 626 nm light, respectively (77). These thresholds are roughly comparable to levels reported by others (93–95), corroborating the overall conclusion that nLAN elicits biologically relevant responses (77, 96).

nLAN as a Potentiator for Other Stimuli

The research summarized above demonstrates that very low levels of light influence both internal circadian rhythms as well as the behavioral and physiological outputs we measure to infer internal circadian conditions. Furthermore, nLAN can interact with, or potentiate, other light and non-visual stimuli. In this section, the research on indirect effects of nLAN is summarized.

Phase Angle of Entrainment

A phase angle is the relative timing between two phase markers. For the phase angle of entrainment in nocturnal animals, the timing of onset of activity is often expressed relative to lights-off transition. Non-parametric entrainment predicts that oscillators with different free-running rhythms entrain to the same zeitgeber signal with different phase angles of entrainment (97). Following the lengthening of FRP with dim light, this would predict a relatively later activity onset in a light-dim compared to a light-dark cycle. On the contrary, activity onset was observed to occur 0.8 h earlier in Siberian hamsters exposed to nLAN compared to dark controls (87).

Resetting

The phase response curve of a 15-min bright light (100 lux) pulse in male Syrian hamsters free-running in low levels of dim light does not statistically differ in shape or amplitude from the same PRC obtained in constant darkness (8). Notwithstanding this similar bright-light PRC, nLAN does facilitate re-entrainment following a phase shift (simulated jet-lag experiment) (87, 98). For example, in both Syrian and Siberian hamsters, nLAN significantly accelerated re-entrainment recovery following a 4- or 8-h phase shift compared to completely dark nights, and was observed for advances as well as delays and among animals of different ages. In the most robust case, animals exposed to dim light re-aligned activity up to 68% faster (98).

Resetting to Non-photic Cues

While light is the most potent and dominant zeitgeber for mammalian circadian rhythms, non-photic cues can also phase shift and/or entrain rodent behavior. For example, introducing a wheel-naive animal to a running wheel triggers activity. The phase shift that can be induced by this novel wheel running is amplified by nLAN (99). Similarly, a cage change can induce activity levels atypical for the circadian phase which produces a significant PRC. Amplitude of the PRC of cage-changing, however, was not significantly altered by nLAN (8).

Extreme Entrainment: T-Cycles and Bifurcation

Non-parametric entrainment theory explains entrainment by positing daily discrete phase corrections of the same magnitude as the difference between the internal and external rhythms. Real-life entrainment, however, is undoubtedly more complex than just a daily non-parametric phase resetting (100, 101). As mentioned above, the shape of the bright light PRC was not affected by an nLAN background, but re-entrainment to simulated jetlag is reliably accelerated. This observation indicates that nLAN influences re-etrainment by bright light regimes in a manner that goes beyond simple non-parametric resetting.

A near-defining feature of circadian rhythms is their narrow range of entrainment. In most mammals, entrainment to light-dark cycles much more than 2 h longer or shorter than 24 h would be considered exceptional. This range, however, can be very markedly expanded by incorporation of nLAN and/or twilight transitions. Once again, Kavanau raised the possibility of such an effect, but provided only sketchy data in support of the claim (46). More definitively, in a study of twilight transition effects in Syrian hamsters, Boulus et al. (102) demonstrated a markedly increased range of entrainment to both short and long (non-24 h) cycles (T-cycles), compared to typical instantaneous transitions between day and night conditions; however, besides ramping up or down the intensity gradually, the experimental manipulations also included a tonic exposure to nLAN throughout most of the scotophase. Without explicitly engaging the issue of this nighttime illumination, the authors attributed the enhanced range of entrainment to the twilight transitions. Having demonstrated tremendous efficacy of nLAN, we assessed whether its presence, without twilight transitions, was sufficient to extend the upper range of entrainment (we did not conduct a parallel assessment of short T-cycles). Indeed, the upper range of entrainment was significantly extended with nLAN, and some animals met entrainment criteria at cycles as long as 30 h (103). Using dim light at night, various rodent species have demonstrated reliable entrainment to cycles as extreme as 6 h on either side of 24 h (19, 103–105). This type of circadian flexibility is unprecedented in genetically intact mammals with dark nights.

As referenced above, nLAN facilitates a bifurcated pattern of entrainment in mice and two species of hamsters exposed to particular 24 h LDLD cycles, which of course is not a zeitgeber condition experienced in nature. For example, mice (C57Bl/6j) exposed to LDLD 7:5:7:5 with dark nights will generally entrain with a unimodal pattern of wheel running and treat the alternate scotophases as biological day (remain mainly inactive). In the presence of nLAN, however, most mice reorganize their behavior and divide activity between the two scotophases nearly symmetrically. Although some T24 LDLD conditions could be equivalently described as a T12 LD cycle, non-T12 LDLD conditions such as LDLD9:5:5:5 etc. also induce and maintain bifurcation. Systematic investigations of bifurcated entrainment clearly establish that it reflects temporally reorganized 24 h underlying oscillations rather than a 12 h clock (48, 106–110).

Both of these consequences of nLAN – enhanced T-cycle entrainment and behavioral rhythm bifurcation – cannot be explained by simple masking (19, 104, 106, 110–119). By the same token, neither entrainment pattern is readily understood by non-parametric entrainment theory that accurately models entrainment to more traditional experimental regimens. For example, the extended upper range of entrainment in nLAN is not explicable in terms of a proportionately increased delay

portion of the light pulse PRC, nor a sufficiently large lengthening of free-running period (beyond the 0.3 h effect described above). Phase angles in dim-facilitated entrainment in non-24 h lighting conditions do not consistently follow the patterns predicted by relative length of the LD-cycle and the FRP, suggesting more complex entrainment mechanisms must play a role (19, 104, 112, 120). In addition, the acceleration of re-entrainment following simulated jetlag, which occurs for phase advances and delays of various sizes, cannot be explained by changes in free-running period without concomitant changes in the PRC amplitude.

The mechanisms by which nLAN affects circadian behavior remain to be much more deeply investigated, but nLAN is known to acutely affect electrical activity in the SCN. Specifically, electrophysiological recordings in awake Wistar rats show acute responses in SCN cellular activity to light levels as low as 0.15 lux (lowest intensity reported) (121). Additionally, some nLAN effects are mediated by the intergeniculate leaflet (122), a thalamic area with neuropeptide Y projections to the SCN and which has been implicated in integration of non-visual feedback to the SCN (75). Despite the lack of a clear mechanistic understanding, a recurring theme, beyond the scope of this review, is that nLAN may alter coupling interactions among multiple oscillators comprising the circadian pacemaker (67, 123, 124), and this change in coupling results in a more flexibly entrained oscillator to a host of conditions. Coupling remains a poorly understood dimension of circadian organization, but it is central to the functioning of a complex pacemaker. It is worth mentioning that the behavioral effects of nLAN appear often to be categorical (e.g., bifurcated or not) rather than modest extensions of entrainment parameters based on bright light entrainment theory. Thus, nLAN seems to be doing something fundamentally different than tweaking known dose-response relationships between light and phase-shifting. Collectively, these experiments demonstrate that in the right conditions, the circadian system can be much more flexible than traditional circadian theory predicts. Such flexibility is unprecedented in intact animals without genetic or pharmacological intervention or with completely dark nights.

COMPARING RODENTS TO HUMANS

To properly assess how relevant the summarized research on nLAN in model organisms is for human circadian rhythms, in this section we compare the pertinent neurobiology across species. First, the mammalian circadian system is very well-conserved (76, 125), including fundamental properties of the primary oscillators. While the main body of mechanistic basic research involved nocturnal rodents (mice, rats, hamsters), circadian rhythms have been well-described in diurnal rodent species, including grass rats, degus and squirrels as well as larger mammals, including sheep and several species of non-human primates [e.g., baboon (126)]. In humans, the possibilities for mechanistic, anatomical, molecular, or neuronal studies are limited, but available data from post-mortem studies, for example, [e.g., (127–129)], confirm homology with other species. Circadian rhythms in all these species rely on a similar transcription-translation feedback loop and a functionally equivalent retinohypothalamic tract, and are orchestrated by an anatomically similar SCN with comparable cell subtypes (127, 130). Furthermore, human circadian rhythms in behavior and physiology match those of other mammalian species rather closely. Functionally, rhythms in the SCN and melatonin levels (as well as suppression and resetting by light) are similarly phased in nocturnal and diurnal species, including humans, (i.e., they are tied to environmental day/night rather than the rest/activity cycle) (131–133). Because of the similarities in circadian clock functions across species (76, 134, 135), the presumption should be that the human biological clock might, as in rodents, exhibit un(der)studied responses to low levels of light that are generally accepted to be ineffective. We will discuss the field's use of dim and dark as a control in more depth below.

Biological potency of light does not solely depend on the molecular and neurological foundations in the SCN, but also on the retina. Most mammals have three classes of photoreceptors (136). Cones are the photoreceptors used for color vision under photopic conditions (brighter light) and are the most variable between species. While primates, including humans, have three types of cones (S, M, and L, for short, medium and long wavelengths, respectively) (137), mice only have two types, lacking a long-wavelength cone found in primates and rendering mice incapable of distinguishing between green and red light (138). Albeit with less sensitivity, mice can still respond to long-wavelength light and are not blind to "red light" [reviewed in (96)]. In primates, 99% of cones are in the fovea, providing us with high-acuity vision in a small part of our visual field (139). Mice also lack a fovea, making the entire retina much more similar to the peripheral retina in primates. The majority of photoreceptors, in both primates and rodents, are the much more sensitive rods, which are primarily at play under low light conditions (140, 141). Because a primate eye is bigger than a mouse eye, the total number of photoreceptors is larger in primates. Both cones and rods have been demonstrated to contribute to circadian responses to light (142–144).

Furthermore, the mammalian retina contains a third type of photoreceptor: intrinsically photoreceptive retinal ganglion cells, or ipRGCs (143, 145–148). The much more recently-discovered ipRGCs are present in both humans and rodents, are highly conserved across species (149, 150) and are primarily responsible for the majority of non-image forming photic responses (85). In addition to their intrinsic photoreception through melanopsin, ipRGCs also receive input from other photoreceptor types (151). Combined intrinsic and extrinsic input makes ipRGCs sensitive, albeit with an increased latency, to nLAN lower than 10^7 photons/cm^2/s, close to absolute detection limits in human vision (146, 152). This suggests that nLAN effects rely at least in part on rod-mediated photoreception, which may be routed through ipRGCs. The similarities in these two types of photoreceptors between mammalian species [review: (153, 154)] corroborate the idea that humans could be sensitive to such light levels as well.

Even if retinal circuitry for dim light reception were comparable between rodents and primates, one might question whether the inverted rest/activity cycles would render dim light at night irrelevant to diurnal animals who are sleeping with closed eyelids through much of the night. First, there is no data in rodents suggesting dim light needs to be delivered uninterrupted throughout the night. In fact, outside, under natural conditions, rodents might spend parts of their night underground, only experiencing ecological light at night during a limited window (30, 155, 156). Second, light has been delivered in sleeping humans (157–159), demonstrating that closed eyes do not fully block light or its effects on circadian biology. For example, although the purpose of the experiment was to study traditional bright light responses, brief pulses of light flashed through closed eyes while sleeping induced significant phase shifts in melatonin rhythms (159). Total transmission through adult eyelids is estimated at 0.3–3%, with more transmission for longer wavelengths (160, 161). Even if this means intensities for sleeping humans may need to be adjusted to correct for closed eyes, given the range of light levels described in nLAN studies (0.01–0.1 lux), that would still include very dim light (<3 lux), significantly below intensities of most night lights and electronic devices as well as most "dim" light used in human studies (**Figure 1**).

Together, comparisons of circadian neurobiology and ocular neuroanatomy provide ample evidence to believe the effects of nLAN in rodents could be relevant to humans, and that they would not be impractical or unfeasible as targets of circadian manipulation. While closed eyelids are a valid factor to consider when optimizing delivery of light, we believe the cross-species similarities are strong enough to warrant careful study of the effects of nLAN in humans.

DIM LIGHT STUDIES IN HUMANS

Most physiological effects of light, albeit not all (e.g., alpha, as discussed above), in both humans and model systems, demonstrate a characteristic intensity-dependent response, with increasing photic intensities yielding relatively greater responses. Examining the magnitude of these responses across a range of light intensities allows for the construction of a fluence response curve, with a reportable goodness of fit of the data to a sigmoid function, which provides various parameters of interest. Threshold sensitivity represents the lowest intensity of light required to elicit a detectable physiological response, and saturation occurs at the intensity at which additional light cannot increase the response further. The photic dose eliciting a half-maximum response (ED_{50}) is also a useful point of comparison, as it occurs along the steep portion of the curve, comfortably within the range of responsiveness. Consequently, when relatively lower light levels are capable of eliciting an equivalent (ED_{50}) response, greater photic sensitivity can be inferred.

In comparing human fluence-response curves for phase shifting and melatonin suppression to those in some of the most commonly employed model systems [but see (162) for greater taxonomic consideration], there appear to be species differences in photic sensitivity, as indicated by significantly lower values of ED_{50} in hamsters 0.04–1.64 melanopic lux (41, 68, 163) vs. humans ∼4–60 melanopic lux (40, 42, 164–168) (see **Table 2**). When human studies did not optimize other elements known to exert an influence, such as spectrum and pupil dilation, relatively greater intensities of light (60–75 melanopic lux) were required to achieve a comparable half-saturation response, and this is true across different physiological effects of light (2, 40). Yet, the light levels required to elicit a response in those studies are also more likely to map on to real world applications, where white polychromatic light exposures to eyes with freely responding pupils are the norm. Most animal work with complete fluence-response curves has not optimized spectrum or pupil dilation and thus, the ∼2 orders of magnitude difference in ED_{50}s between species is likely to represent an underestimation. Nonetheless, the 560 nm narrowband nLAN stimulus typically used to illuminate the scotophase in Gorman lab experiments is orders of magnitude lower than the calculated ED_{50} (or even minimum threshold) for melatonin suppression in the same species. By the same logic, even after considering species differences in ED_{50}, humans might be sensitive to light well below 1 lux. Therefore, the presented effects of nLAN underscore the idea that light in the tail of the fluence response curves could still be potent enough to elicit circadian responses, but might be overlooked by focusing on effects at ED_{50}.

Most commonly, dim light in the nLAN (or even brighter) range of intensities appears in human studies only as a control condition and/or in contrast to a "bright light" condition [e.g., (15, 169)—see **Figure 1**]. But the assumption that there is no effect of nLAN on circadian physiology should be recognized as such—an assumption. First, without a no-light control group, we cannot say so definitively. Second, given the fact that ipRGCs also contribute to brightness discrimination (149), it is quite conceivable that a circadian response to a light stimulus might be affected by the contrast (fold increase) with the background, or light history, rather than absolute intensity. For example, switching between a background of 0.1 and 1 lux could conceivably be as important as the difference between a 100 and 1,000 lux stimulus. Lastly, there is emerging evidence that the precise mechanisms and pathways differ across various circadian responses (69, 77, 170–174). This also applies to the shape of the fluence response curve—for example, while effects of light may be largely linear for phase-shifting or melatonin suppression, the effects of nLAN, which appear to be largely parametric, are likely non-linear. The field of circadian research is littered with designs based on these assumptions, whether implicit or explicit.

In classic studies of the physiological effects of light in humans, participants are often maintained under dim light conditions or darkness prior to administration of a light pulse in order to control for the potential effects of photic history. Though the intensity and duration of that period of adaptation varies across studies, research that has systematically examined the effects of photic history in humans has shown robust effects. Most of these studies have altered daytime photic exposure and then, examined subsequent response to a test pulse during the biological night. For example, Chang et al. (169, 175) had

TABLE 2 | ED_{50} for melatonin suppression and phase shifting in humans and hamsters.

	Wavelength (nm)	FWHM[a] (nm)	Photon flux (Photons/cm^2/sec)	Photopic lux	Melanopic lux
Melatonin suppression					
Syrian hamster[b] (41)	503	20	$10^{10.11}$	0.01	0.04
Human[c] (42)	555	10–14	$10^{12.94}$	21.14	4.39
Phase shifting					
Syrian hamster[b] (41)	503	20	$10^{11.49}$	0.33	0.93
Human[c] (42)	555	10–14	$10^{13.07}$	28.57	5.85

[a] *Full width at half maximum.*
[b] *Study employed a 5 min light pulse.*
[c] *Study employed a 6.5 h light pulse.*

participants spend 3 days in the laboratory under either dim light (1 lux) or relatively brighter light (90 lux) during waking hours, while scotopic levels were kept very dim (<0.1 lux) across both conditions. When participants were subsequently exposed to a 6.5 h light pulse (90 lux) ∼1 h prior to habitual bedtime, a 40 min greater circadian phase delay and increased alertness occurred with pre-exposure to daytime dim vs. brighter light. Earlier studies similarly had participants exposed to more or less daytime light in the week preceding a bright light test pulse during the biological night, and there was significantly increased melatonin suppression and phase shifting after the dimmer daytime condition (176–178). In contrast, within a single, much shorter time frame (2 h), early in the scotophase and during the biological night, dim light served to attenuate the melatonin suppression response to a subsequent 90 min test pulse as compared to a completely dark adaptation period (179). Together, these findings are consistent with the recommendation of a high day-night contrast, in terms of biological potency, in order to clearly signal time of day to the circadian timing system and maximize physiological responses to light [e.g., (14, 180)]; however, they leave open the question as to whether complete darkness or nLAN is an optimal scotophase condition.

Two unique examples of studies that included human exposure to nLAN include work from Wright et al. (181, 182), in which participants were exposed to only natural sources of light (including moon and starlight) while tent camping. In the first paper, circadian outputs for this week were compared, within participants, to a more typical week that included work, school, self-selected sleep schedules, and time spent in built environments with electrical lighting. The smaller phase angle of entrainment to the solar day during the camping period of only natural light exposure was primarily attributed to less electrical light at night and more light (from the sun) during the day, yet nLAN cannot be discounted as a potential contributing factor. Indeed, in the second study, nocturnal light while camping is characterized in more detail, and they report sensor-derived night light levels in the 500–600 nm range between 0.1 and 1 lux (in addition to intermittent light from a campfire) (182).

To our knowledge, there has never been a direct examination of the effects of very dim vs. complete darkness throughout the scotophase in human studies of the physiological effects of light. Considering the varied background conditions in the seminal human work on the topic, it may be possible to retrospectively compare a variety of "dark" scotophase conditions across experiments in order to glean whether or not there are potential effects of nLAN in humans that are similar to what has been established in model systems.

SUMMARY AND RECOMMENDATIONS TO THE FIELD

While it remains to be rigorously examined, there is good reason to suspect that the potent effects of very dim light (akin to moon and starlight intensities) summarized here have translational potential for human circadian research, including the fact that the tremendous overlap in circadian physiology and responses between species make it likely that such effects are possible in humans. However, even if these effects do not directly translate (i.e., if it is established later that these lower levels of light do not markedly affect the human circadian system in these ways, or indeed, in any discernible way), they nevertheless provide a window into the potential for a latent plasticity that may exist in humans and may be inducible via other mechanisms, which have yet to be determined (119). Keeping both of these notions in mind, but focusing on the former, the following section includes some recommendations for future work for circadian researchers focused on mammalian systems.

Measurement and Reporting of Light

This review focuses on photic intensity; however, light can vary along several other important dimensions, including spectral properties, duration, and directionality. All of these parameters have been shown to affect biological potency. Recent progress has been made, via an internationally balloted consensus-based process, in developing a standardized method of measuring and reporting light for non-image forming physiological effects of light [CIE S 026:2018 (14)]. Essentially, the intensity for each of the five α -opic photoreceptors (S-cone-opic, M-cone-opic, L-cone-opic, Rhodopic; Melanopic) is determined, allowing for the assessment of the relative contribution to a given response; this is conceptually similar to and builds upon the introduction of melanopic lux (13). Under typical lighting conditions, melanopic contribution is the dominant photoreceptor mediating photic input for the physiological effects of light in humans, such as melatonin suppression and phase shifting (183–185). Thus, when quantifying photic

stimuli for these physiological effects of light, stimuli should be characterized in terms of melanopic Equivalent Daylight Illuminance (EDI), which is also reported in units of lux (14). While we support these new metrics for quantification of light for non-visual responses, converting previously reported photopic lux values into precise melanopic lux/EDI is often not feasible due to insufficient information about the spectral quality of the light stimulus. The Lucas and CIE toolboxes (13, 14), however, support such conversions by providing standardized or estimated SPD for several light sources. (**Figure 1**; see below for recommendations for reporting light in research papers). Furthermore, the primary purpose of this review is to emphasize the potency of nLAN, which has largely been assumed to be subthreshold for affecting the mammalian circadian system, regardless of spectrum. The research summarized above should make it abundantly clear that light levels multiple orders of magnitude below 1 lux are sufficient to alter circadian rhythms both directly and indirectly.

In all work going forward, researchers should take pains to ensure that lighting conditions are reproducible and convertible. This requires detailed reporting of the set up, the distance and gaze of the organism, placement of sensors, the measurement instruments used (including their ranges of accuracy, the exact sensor-heads used, the ability to set a 0-reference point, etc.), and any other factor that could influence the effect of light (e.g., directionality, dilated pupils, etc.), see (186). Many labs, however, are likely ill-equipped to accurately measure light levels in the nLAN range. At a minimum, researchers should state that light levels were below levels of detection by their equipment, state what those levels are, and report details on any effort taken to reduce light levels, such as removing or blocking light sources. The availability of open access and supplemental options mean a much more detailed account of lighting set ups need not be subject to space or word limit concerns. At a minimum, the specifications of the light source need to be described. For example, light sources that can be described as "white light" or "white fluorescent" can span at least an order of magnitude in their biological potency (183, 187). Further, as discussed above, light levels should be reported in units that can be later converted (e.g., spectral power distributions of all light sources) if reporting metrics change, as is likely as more is learned (14). For example, the bulk of the nLAN work described here was conducted before the emergence of melanopic lux (13, 14). However, because the spectral composition and irradiance are reported in our work, we can now calculate the melanopic lux, or any other metric that emerges from the field subsequently (see **Table 1** and **Figure 1**).

Standardization of Terminology

Standardization of language both within the circadian and lighting communities surrounding light levels, especially lower levels, is crucial, both in our science communications as well as public health outreach and messaging. In the future, it might be useful to develop a set of agreed upon terms to further narrow the range of intensities currently termed as "dim light." For example, "dim" has been used variously to describe values between 0.0001 and 500 lux (see **Figure 1**), which spans many orders of magnitude. In this paper, we have used the term "nLAN" throughout to describe intensities of light no brighter than naturally occurring nighttime light levels.

As a field, our current recommendation that "nights should be completely dark," may warrant further consideration. Importantly, solar night is not naturally completely dark, and while the total absence of light is perhaps a more simple aim than prescribing a sweet-spot range of dim photic intensities that do more good than harm, absolutely no light at night may not always be feasible, optimal, or desirable. Furthermore, the lighting industry may play an important role in helping to optimize not just our days but our nights as well, with the development of novel technologies that capitalize on these more nuanced understandings.

Reconsidering Old Data and Designing New Studies

Existing data sets and conclusions drawn from them may be reconsidered in light of the evidence presented here. It may be possible to conduct a systematic review and/or meta-analysis of data from near-scotopic conditions, either within or across species.

Additionally, while painstaking, new studies of the biological effects of light in humans need to include sufficient data points to measure responses at different intensities, including no detectable light, and time courses.

Recent discoveries show that not all physiological effects of light rely on identical mechanisms [e.g., alertness vs. melatonin suppression (41, 42, 69, 77, 85, 95, 170–174)]. Thus, ED_{50}s of one circadian response should not be assumed to generalize to a different response. The same caution applies to sensitivity values reflected in the tails of fluence response curves, where estimation is less reliable. To better understand these response differences, further studies aiming to better understand relative photoreceptor contribution and to identify practically important dose response parameters should ideally include measurements across multiple responses (e.g., pupillary constriction, entrainment) in the same subject under the same experimental conditions. Where collection of multiple endpoints might not be feasible, the use of standardized, and replicable, methodologies becomes even more critical to enable between study comparisons.

Finally, should investigators need dim illumination for practical purposes, given the relative biological potency of short wavelengths, including at relatively "dim" levels (**Figure 1**), we recommend the use of the dimmest light, most-depleted of short wavelengths, possible. For example, 10 lux with a 650 nm LED, more than is needed for comfortable vision, is only 0.01 melanopic lux, as opposed to 10 lux fluorescent light (∼3,000 K), which is 6.2 melanopic lux (**Figure 1**). While the former light source is undoubtedly less potent, researchers should nevertheless bear in mind that, in at least one case, "deep red" light levels as low as 0.07 lux were sufficient to elicit behavioral entrainment in albino rats (78). Furthermore, this same lighting stimulus was described by the authors as

insufficient to aid researchers with animal handling. Thus, as with many experimental design considerations, there are trade-offs between rigor and practicality; however, there may be no such thing as "dim enough" when trying to minimize effects of background light – any amount of light may potentially affect the circadian system. Therefore, investigators should both consider and report light levels in every condition, including those that may have otherwise been simply described as "dim" or "dark."

AUTHOR CONTRIBUTIONS

GG, TW, EH, and MG: conceptualization. TW, EH, and GG: analysis. TW, EH, GG, and MG: writing. GG: funding acquisition. All authors contributed to the article and approved the submitted version.

FUNDING

Conversions of light levels and associated analyses were supported by the Department of Energy #DE-EE0008206 (GG). Analyses and writing associated with background, review and translational concepts, as well as open access publication fees, were supported by the Office of Naval Research #N6290919MP00030 (GG) and by NIH/NINDS #R01NS102962 (TW).

ACKNOWLEDGMENTS

The authors would like to acknowledge Sara Bessman and Michael Herf for their continued support with photic conversions to alpha-opic illuminance values, particularly for the much lower intensities that required modification to Toolbox settings.

REFERENCES

1. Duffy JF, Czeisler CA. Effect of light on human circadian physiology. *Sleep Med Clin*. (2009) 4:165–77. doi: 10.1016/j.jsmc.2009.01.004
2. Cajochen C. Alerting effects of light. *Sleep Med Rev*. (2007) 11:453–64. doi: 10.1016/j.smrv.2007.07.009
3. Brainard GC, Sliney D, Hanifin JP, Glickman G, Byrne B, Greeson JM, et al. Sensitivity of the human circadian system to short-wavelength (420-nm) light. *J Biol Rhythms*. (2008) 23:379–86. doi: 10.1177/0748730408323089
4. Münch M, Wirz-Justice A, Brown SA, Kantermann T, Martiny K, Stefani O, et al. The role of daylight for humans: gaps in current knowledge. *Clocks Sleep*. (2020) 2:61–85. doi: 10.3390/clockssleep2010008
5. Plano SA, Casiraghi LP, García Moro P, Paladino N, Golombek DA, Chiesa JJ. Circadian and metabolic effects of light: implications in weight homeostasis and health. *Front Neurol*. (2017) 8:558. doi: 10.3389/fneur.2017.00558
6. González MMC. Dim light at night and constant darkness: two frequently used lighting conditions that jeopardize the health and well-being of laboratory rodents. *Front Neurol*. (2018) 9:609. doi: 10.3389/fneur.2018.00609
7. Evans JA, Elliott JA, Gorman MR. Individual differences in circadian waveform of Siberian hamsters under multiple lighting conditions. *J Biol Rhythms*. (2012) 27:410–9. doi: 10.1177/0748730412455915
8. Evans JA, Elliott JA, Gorman MR. Circadian effects of light no brighter than moonlight. *J Biol Rhythms*. (2007) 22:356–67. doi: 10.1177/0748730407301988
9. Cajochen C, Altanay-Ekici S, Münch M, Frey S, Knoblauch V, Wirz-Justice A. Evidence that the lunar cycle influences human sleep. *Curr Biol*. (2013) 23:1485–8. doi: 10.1016/j.cub.2013.06.029
10. Della Monica C, Atzori G, Dijk D-J. Effects of lunar phase on sleep in men and women in Surrey. *J Sleep Res*. (2015) 24:687–94. doi: 10.1111/jsr.12312
11. Casiraghi L, Spiousas I, Dunster G, McGlothlen K, Fernández-Duque E, Valeggia C, et al. Moonstruck sleep: synchronization of human sleep with the moon cycle under natural conditions. *bioRxiv*. (2020) doi: 10.1101/2020.06.01.128728
12. de la Iglesia HO, Fernández-Duque E, Golombek DA, Lanza N, Duffy JF, Czeisler CA, et al. Access to electric light is associated with shorter sleep duration in a traditionally hunter-gatherer community. *J Biol Rhythms*. (2015) 30:342–50. doi: 10.1177/0748730415590702
13. Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, et al. Measuring and using light in the melanopsin age. *Trends Neurosci*. (2014) 37:1–9. doi: 10.1016/j.tins.2013.10.004
14. *CIE S 026:2018: Cie System For Metrology Of Optical Radiation For Iprgc-influenced Responses To Light*. Vienna (2018). Available online at: http://www.cie.co.at/publications/cie-system-metrology-optical-radiation-iprgc-influenced-responses-light-0
15. St Hilaire MA, Gooley JJ, Khalsa SBS, Kronauer RE, Czeisler CA, Lockley SW. Human phase response curve to a 1 h pulse of bright white light. *J Physiol*. (2012) 590:3035–45. doi: 10.1113/jphysiol.2012.227892
16. Wright KP, Hughes RJ, Kronauer RE, Dijk DJ, Czeisler CA. Intrinsic near-24-h pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. *Proc Natl Acad Sci USA*. (2001) 98:14027–32. doi: 10.1073/pnas.201530198
17. Phillips AJK, Vidafar P, Burns AC, McGlashan EM, Anderson C, Rajaratnam SMW, et al. High sensitivity and interindividual variability in the response of the human circadian system to evening light. *Proc Natl Acad Sci*. (2019) 116:201901824. doi: 10.1073/pnas.1901824116
18. Evans JA, Elliott JA, Gorman MR. Dynamic interactions between coupled oscillators within the hamster circadian pacemaker. *Behav Neurosci*. (2010) 124:87–96. doi: 10.1037/a0018088
19. Walbeek TJ, Gorman MR. Simple lighting manipulations facilitate behavioral entrainment of mice to 18-h days. *J Biol Rhythms*. (2017) 32:309–22. doi: 10.1177/0748730417718347
20. Duffy JF, Dijk D-J. Getting through to circadian oscillators: why use constant routines? *J Biol Rhythms*. (2002) 17:4–13. doi: 10.1177/074873002129002294
21. Bedrosian TA, Fonken LK, Walton JC, Nelson RJ. Chronic exposure to dim light at night suppresses immune responses in Siberian hamsters. *Biol Lett*. (2011) 7:468–71. doi: 10.1098/rsbl.2010.1108
22. Stenvers DJ, van Dorp R, Foppen E, Mendoza J, Opperhuizen A-L, Fliers E, et al. Dim light at night disturbs the daily sleep-wake cycle in the rat. *Sci Rep*. (2016) 6:35662. doi: 10.1038/srep35662
23. Opperhuizen A-L, van Kerkhof LWM, Proper KI, Rodenburg W, Kalsbeek A. Rodent models to study the metabolic effects of shiftwork in humans. *Front Pharmacol*. (2015) 6:50. doi: 10.3389/fphar.2015.00050
24. Smolensky MH, Sackett-Lundeen LL, Portaluppi F. Nocturnal light pollution and underexposure to daytime sunlight: complementary mechanisms of circadian disruption and related diseases. *Chronobiol Int*. (2015) 32:1029–48. doi: 10.3109/07420528.2015.1072002
25. Aschoff J. Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. *Z Tierpsychol*. (1979) 49:225–49. doi: 10.1111/j.1439-0310.1979.tb00290.x
26. Scherbarth F, Steinlechner S. The annual activity pattern of djungarian hamsters (*Phodopus sungorus*) is affected by wheel-running activity. *Chronobiol Int*. (2008) 25:905–22. doi: 10.1080/07420520802544514
27. Wolfe JL, Tan Summerlin C. The influence of lunar light on nocturnal activity of the old-field mouse. *Anim Behav*. (1989) 37:410–4. doi: 10.1016/0003-3472(89)90088-2
28. Daly M, Behrends PR, Wilson MI, Jacobs LF. Behavioural modulation of predation risk: moonlight avoidance and crepuscular compensation in a

nocturnal desert rodent, *Dipodomys merriami*. *Anim Behav*. (1992) 44:1–9. doi: 10.1016/S0003-3472(05)80748-1
29. Fernández-Duque E, de la Iglesia H, Erkert HG. Moonstruck primates: owl monkeys (Aotus) need moonlight for nocturnal activity in their natural environment. *PLoS ONE*. (2010) 5:e12572. doi: 10.1371/journal.pone.0012572
30. Smarr BL, Schwartz MD, Wotus C, de la Iglesia HO. Re-examining "Temporal Niche." *Integr Comp Biol*. (2013) 53:165–74. doi: 10.1093/icb/ict055
31. Hoffmann J, Palme R, Eccard JA. Long-term dim light during nighttime changes activity patterns and space use in experimental small mammal populations. *Environ Pollut*. (2018) 238:844–51. doi: 10.1016/j.envpol.2018.03.107
32. Erkert HG. Der Einfluß des Mondlichtes auf die Aktivitätsperiodik nachtaktiver Säugetiere. *Oecologia*. (1974) 14:269–87. doi: 10.1007/BF01039797
33. Erkert HG. Extremely low threshold for photic entrainment of circadian activity rhythms in molossid bats (*Molossus molossus*; Chiroptera—Molossidae). *Mamm Biol*. (2004) 69:361–74. doi: 10.1078/1616-5047-00158
34. Erkert HG, Bay FA, Kracht S. Zeitgeber induced modulation of activity patterns in nocturnal mammals (Chiroptera). *Experientia*. (1976) 32:560–2. doi: 10.1007/BF01990160
35. Erkert HG, Gröber J. Direct modulation of activity and body temperature of owl monkeys (*Aotus lemurinus griseimembra*) by low light intensities. *Folia Primatol*. (1986) 47:171–88. doi: 10.1159/000156276
36. Lin MC, Kripke DF, Perry BL, Berga SL. Night light alters menstrual cycles. *Psychiatry Res*. (1990) 33:135–8. doi: 10.1016/0165-1781(90)90067-F
37. Kronfeld-Schor N, Dominoni D, de la Iglesia H, Levy O, Herzog ED, Dayan T, et al. Chronobiology by moonlight. *Proc R Soc B Biol Sci*. (2013) 280:20123088. doi: 10.1098/rspb.2012.3088
38. Kyba CCM, Mohar A, Posch T. How bright is moonlight? *Astron Geophys*. (2017) 58:1.31–2. doi: 10.1093/astrogeo/atx025
39. Hofstetter JR, Hofstetter AR, Hughes AM, Mayeda AR. Intermittent long-wavelength red light increases the period of daily locomotor activity in mice. *J Circadian Rhythms*. (2005) 3:8. doi: 10.1186/1740-3391-3-8
40. Zeitzer JM, Dijk DJ, Kronauer RE, Brown EN, Czeisler CA. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J Physiol*. (2000) 526:695–702. doi: 10.1111/j.1469-7793.2000.00695.x
41. Nelson DE, Takahashi JS. Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. *Brain Res*. (1991) 554:272–7. doi: 10.1016/0006-8993(91)90200-F
42. Gooley JJ, Rajaratnam SMW, Brainard GC, Kronauer RE, Czeisler CA, Lockley SW. Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Sci Transl Med*. (2010) 2:31ra33. doi: 10.1126/scitranslmed.3000741
43. Walmsley L, Hanna L, Mouland J, Martial F, West A, Smedley AR, et al. Colour as a signal for entraining the mammalian circadian clock. *PLoS Biol*. (2015) 13:e1002127. doi: 10.1371/journal.pbio.1002127
44. Kavanau JL. Activity and orientational responses of white-footed mice to light. *Nature*. (1968) 218:245–52. doi: 10.1038/218245a0
45. Kavanau JL. Behavior of captive white-footed mice. *Science*. (1967) 155:1623–39. doi: 10.1126/science.155.3770.1623
46. Kavanau JL. Activity patterns on regimes employing artificial twilight transitions. *Experientia*. (1962) 18:382–4. doi: 10.1007/BF02172265
47. Rich C, Longcore T. *Ecological Consequences of Artificial Night Lighting*. Washington, DC: Island Press (2006).
48. Gorman MR, Elliott JA, Evans JA. Plasticity of hamster circadian entrainment patterns depends on light intensity. *Chronobiol Int*. (2003) 20:233–48. doi: 10.1081/CBI-120018576
49. Erkert HG, Kracht S. Evidence for ecological adaptation of circadian systems. *Oecologia*. (1978) 32:71–8. doi: 10.1007/BF00344690
50. Sanders D, Frago E, Kehoe R, Patterson C, Gaston KJ. A meta-analysis of biological impacts of artificial light at night. *Nat Ecol Evol*. (2020) 5:74–81. doi: 10.1038/s41559-020-01322-x
51. Comas M, Beersma DGM, Spoelstra K, Daan S. Phase and period responses of the circadian system of mice (*Mus musculus*) to light stimuli of different duration. *J Biol Rhythms*. (2006) 21:362–72. doi: 10.1177/0748730406292446
52. Nicholls SK, Casiraghi LP, Wang W, Weber ET, Harrington ME. Evidence for internal desynchrony caused by circadian clock resetting. *Yale J Biol Med*. (2019) 92:259–70.
53. Reddy AB, Field MD, Maywood ES, Hastings MH. Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. *J Neurosci*. (2002) 22:7326–30. doi: 10.1523/JNEUROSCI.22-17-07326.2002
54. Kao K, Loi J, Sanderson H, Kim J, Lee J, Nudell V, et al. *An Introduction to Chronobiology*. The BioClock Studio (2016). Available online at: https://ccb.ucsd.edu/the-bioclock-studio/education-resources/basics/index.html (accessed November 2, 2020).
55. Wirz-Justice A. How to measure circadian rhythms in humans. *Medicographia*. (2007) 29:84–90. doi: 10.1111/j.1479-8425.2006.00195.x
56. Gorman MR, Harrison EM, Evans JA. Circadian waveform and its significance for clock organization and plasticity. In: Kumar V, editor. *Biological Timekeeping: Clocks, Rhythms and Behaviour*. New Delhi: Springer India (2017). p. 59–79. doi: 10.1007/978-81-322-3688-7_4
57. Butler MP, Karatsoreos IN, LeSauter J, Silver R. Dose-dependent effects of androgens on the circadian timing system and its response to light. *Endocrinology*. (2012) 153:2344–52. doi: 10.1210/en.2011-1842
58. Boulos Z. Wavelength dependence of light-induced phase shifts and period changes in hamsters. *Physiol Behav*. (1995) 57:1025–33. doi: 10.1016/0031-9384(95)00015-B
59. Aschoff J. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb Symp Quant Biol*. (1960) 25:11–28. doi: 10.1101/SQB.1960.025.01.004
60. Pittendrigh CS. Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb Symp Quant Biol*. (1960) 25:159–84. doi: 10.1101/SQB.1960.025.01.015
61. Coomans CP, Ramkisoensing A, Meijer JH. The suprachiasmatic nuclei as a seasonal clock. *Front Neuroendocrinol*. (2015) 37:29–42. doi: 10.1016/j.yfrne.2014.11.002
62. Sumova A, Travnickova Z, Peters R, Schwartz WJ, Illnerova H. The rat suprachiasmatic nucleus is a clock for all seasons. *Proc Natl Acad Sci*. (1995) 92:7754–8. doi: 10.1073/pnas.92.17.7754
63. Schwartz WJ, de la Iglesia HO, Zlomanczuk P, Illnerová H. Encoding le quattro stagioni within the mammalian brain: photoperiodic orchestration through the suprachiasmatic nucleus. *J Biol Rhythms*. (2001) 16:302–11. doi: 10.1177/074873001129002024
64. Wehr TA, Moul DE, Barbato G, Giesen HA, Seidel JA, Barker C, et al. Conservation of photoperiod-responsive mechanisms in humans. *Am J Physiol Integr Comp Physiol*. (1993) 265:R846–57. doi: 10.1152/ajpregu.1993.265.4.R846
65. Illnerová H, Hoffmann K, Vanecek J. Adjustment of pineal melatonin and N-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster *Phodopus sungorus*. *Neuroendocrinology*. (1984) 38:226–31. doi: 10.1159/000123895
66. Arendt J. Managing jet lag: some of the problems and possible new solutions. *Sleep Med Rev*. (2009) 13:249–56. doi: 10.1016/j.smrv.2008.07.011
67. Evans JA, Elliott JA, Gorman MR. Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. *Am J Physiol Regul Integr Comp Physiol*. (2004) 286:R539–46. doi: 10.1152/ajpregu.00456.2003
68. Glickman GL, Webb IC, Elliott JA, Baltazar RM, Reale ME, Lehman MN, et al. Photic sensitivity for circadian response to light varies with photoperiod. *J Biol Rhythms*. (2012) 27:308–18. doi: 10.1177/0748730412450826
69. Glickman GL, Harrison EM, Elliott JA, Gorman MR. Increased photic sensitivity for phase resetting but not melatonin suppression in Siberian hamsters under short photoperiods. *Horm Behav*. (2014) 65:301–7. doi: 10.1016/j.yhbeh.2014.01.002
70. Gorman MR, Elliott JA. Dim nocturnal illumination alters coupling of circadian pacemakers in Siberian hamsters, *Phodopus sungorus*. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. (2004) 190:631–9. doi: 10.1007/s00359-004-0522-7
71. Abraham U, Granada AE, Westermark PO, Heine M, Kramer A, Herzel H. Coupling governs entrainment range of circadian clocks. *Mol Syst Biol*. (2010) 6:438. doi: 10.1038/msb.2010.92

72. Schmal C, Myung J, Herzel H, Bordyugov G. A theoretical study on seasonality. *Front Neurol*. (2015) 6:94. doi: 10.3389/fneur.2015.00159
73. vanderLeest HT, Rohling JHT, Michel S, Meijer JH. Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLoS ONE*. (2009) 4:e4976. doi: 10.1371/journal.pone.0004976
74. VanderLeest HT, Houben T, Michel S, Deboer T, Albus H, Vansteensel MJ, et al. Seasonal encoding by the circadian pacemaker of the SCN. *Curr Biol*. (2007) 17:468–73. doi: 10.1016/j.cub.2007.01.048
75. Janik D, Mikkelsen JD, Mrosovsky N. Cellular colocalization of Fos and neuropeptide Y in the intergeniculate leaflet after nonphotic phase-shifting events. *Brain Res*. (1995) 698:137–45. doi: 10.1016/0006-8993(95)00878-T
76. Kronauer RE, Hilaire MA, Rahman SA, Czeisler CA, Klerman EB. An exploration of the temporal dynamics of circadian resetting responses to short- and long-duration light exposures: cross-species consistencies and differences. *J Biol Rhythms*. (2019) 34:497–514. doi: 10.1177/0748730419862702
77. Butler MP, Silver R. Divergent photic thresholds in the non-image-forming visual system: entrainment, masking and pupillary light reflex. *Proc Biol Sci*. (2011) 278:745–50. doi: 10.1098/rspb.2010.1509
78. McCormack CE, Sontag CR. Entrainment by red light of running activity and ovulation rhythms of rats. *Am J Physiol*. (1980) 239:R450–3. doi: 10.1152/ajpregu.1980.239.5.R450
79. Mrosovsky N. In praise of masking: behavioural responses of retinally degenerate mice to dim light. *Chronobiol Int*. (1994) 11:343–8. doi: 10.3109/07420529409057251
80. Marques MD, Waterhouse JM. Masking and the evolution of circadian rhythmicity. *Chronobiol Int*. (1994) 11:146–55. doi: 10.3109/07420529409057234
81. Waterhouse J, Minors D, Akerstedt T, Hume K, Kerkhof G. Circadian rhythm adjustment: difficulties in assessment caused by masking. *Pathol Biol (Paris)*. (1996) 44:205–7.
82. Edelstein K, Mrosovsky N. Behavioral responses to light in mice with dorsal lateral geniculate lesions. *Brain Res*. (2001) 918:107–12. doi: 10.1016/S0006-8993(01)02966-3
83. Redlin U, Mrosovsky N. Masking of locomotor activity in hamsters. *J Comp Physiol A*. (1999) 184:429–37. doi: 10.1007/s003590050342
84. Redlin U, Vrang N, Mrosovsky N. Enhanced masking response to light in hamsters with IGL lesions. *J Comp Physiol A*. (1999) 184:449–56. doi: 10.1007/s003590050344
85. Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, et al. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*. (2003) 424:76–81. doi: 10.1038/nature01761
86. Thompson S, Foster RG, Stone EM, Sheffield VC, Mrosovsky N. Classical and melanopsin photoreception in irradiance detection: negative masking of locomotor activity by light. *Eur J Neurosci*. (2008) 27:1973–9. doi: 10.1111/j.1460-9568.2008.06168.x
87. Frank DW, Evans JA, Gorman MR. Time-dependent effects of dim light at night on re-entrainment and masking of hamster activity rhythms. *J Biol Rhythms*. (2010) 25:103–12. doi: 10.1177/0748730409360890
88. Tähkämö L, Partonen T, Pesonen A-K. Systematic review of light exposure impact on human circadian rhythm. *Chronobiol Int*. (2019) 36:151–70. doi: 10.1080/07420528.2018.1527773
89. Brainard GC, Richardson BA, Petterborg LJ, Reiter RJ. The effect of different light intensities on pineal melatonin content. *Brain Res*. (1982) 233:75–81. doi: 10.1016/0006-8993(82)90931-3
90. Dauchy RT, Dauchy EM, Tirrell RP, Hill CR, Davidson LK, Greene MW, et al. Dark-phase light contamination disrupts circadian rhythms in plasma measures of endocrine physiology and metabolism in rats. *Comp Med*. (2010) 60:348–56.
91. Blask DE, Dauchy RT, Dauchy EM, Mao L, Hill SM, Greene MW, et al. Light exposure at night disrupts host/cancer circadian regulatory dynamics: impact on the warburg effect, lipid signaling and tumor growth prevention. *PLoS ONE*. (2014) 9:e102776. doi: 10.1371/journal.pone.0102776
92. Münch M, Léon L, Collomb S, Kawasaki A. Comparison of acute non-visual bright light responses in patients with optic nerve disease, glaucoma and healthy controls. *Sci Rep*. (2015) 5:15185. doi: 10.1038/srep15185
93. Jain V, Srivastava I, Palchaudhuri S, Goel M, Sinha-Mahapatra SK, Dhingra NK. Classical photoreceptors are primarily responsible for the pupillary light reflex in mouse. *PLoS ONE*. (2016) 11:e0157226. doi: 10.1371/journal.pone.0157226
94. Keenan WT, Rupp AC, Ross RA, Somasundaram P, Hiriyanna S, Wu Z, et al. A visual circuit uses complementary mechanisms to support transient and sustained pupil constriction. *Elife*. (2016) 5:15392. doi: 10.7554/eLife.15392
95. Lucas RJ, Douglas RH, Foster RG. Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat Neurosci*. (2001) 4:621–6. doi: 10.1038/88443
96. Peirson SN, Brown LA, Pothecary CA, Benson LA, Fisk AS. Light and the laboratory mouse. *J Neurosci Methods*. (2018) 300:26–36. doi: 10.1016/j.jneumeth.2017.04.007
97. Granada AE, Bordyugov G, Kramer A, Herzel H. Human chronotypes from a theoretical perspective. *PLoS ONE*. (2013) 8:e59464. doi: 10.1371/journal.pone.0059464
98. Evans JA, Elliott JA, Gorman MR. Dim nighttime illumination accelerates adjustment to timezone travel in an animal model. *Curr Biol*. (2009) 19:R156–7. doi: 10.1016/j.cub.2009.01.023
99. Evans JA, Elliott JA, Gorman MR. Circadian entrainment and phase resetting differ markedly under dimly illuminated versus completely dark nights. *Behav Brain Res*. (2005) 162:116–26. doi: 10.1016/j.bbr.2005.03.014
100. Pohl H. Characteristics and variability in entrainment of circadian rhythms to light in diurnal rodents. In: Aschoff J, Daan S, Groos GA, editors. *Vertebrate Circadian Systems. Proceedings in Life Sciences*. Berlin; Heidelberg: Springer (1982). doi: 10.1007/978-3-642-68651-1_36
101. Pittendrigh CS, Daan S. A functional analysis of circadian pacemakers in nocturnal rodents—IV. Entrainment: pacemaker as clock. *J Comp Physiol A*. (1976) 106:291–331. doi: 10.1007/BF01417859
102. Boulos Z, Macchi MM, Terman M. Twilights widen the range of photic entrainment in hamsters. *J Biol Rhythms*. (2002) 17:353–63. doi: 10.1177/074873002129002654
103. Gorman MR, Kendall M, Elliott JA. Scotopic illumination enhances entrainment of circadian rhythms to lengthening light:dark cycles. *J Biol Rhythms*. (2005) 20:38–48. doi: 10.1177/0748730404271573
104. Harrison EM, Walbeek TJ, Sun J, Johnson J, Poonawala Q, Gorman MR. Extraordinary behavioral entrainment following circadian rhythm bifurcation in mice. *Sci Rep*. (2016) 6:38479. doi: 10.1038/srep38479
105. Chiesa JJ, Anglès-Pujolràs M, Díez-Noguera A, Cambras T. Activity rhythm of golden hamster (*Mesocricetus auratus*) can be entrained to a 19-h light-dark cycle. *Am J Physiol Regul Integr Comp Physiol*. (2005) 289:R998–1005. doi: 10.1152/ajpregu.00139.2005
106. Gorman MR, Elliott JA. Entrainment of 2 subjective nights by daily light:dark:light:dark cycles in 3 rodent species. *J Biol Rhythms*. (2003) 18:502–12. doi: 10.1177/0748730403260219
107. Gorman MR, Steele NA. Phase angle difference alters coupling relations of functionally distinct circadian oscillators revealed by rhythm splitting. *J Biol Rhythms*. (2006) 21:195–205. doi: 10.1177/0748730406287665
108. Gorman MR, Evans JA, Elliott JA. Potent circadian effects of dim illumination at night in hamsters. *Chronobiol Int*. (2006) 23:245–50. doi: 10.1080/07420520500521905
109. Walbeek TJ, Joye DAM, Mishra I, Gorman MR. Physiological, behavioral and environmental factors influence bifurcated circadian entrainment in mice. *Physiol Behav*. (2019) 210:112625. doi: 10.1016/j.physbeh.2019.112625
110. Raiewski EE, Elliott JA, Evans JA, Glickman GL, Gorman MR. Twice daily melatonin peaks in Siberian but not Syrian hamsters under 24 h light:dark:light:dark cycles. *Chronobiol Int*. (2012) 29:1206–15. doi: 10.3109/07420528.2012.719965
111. Sun J, Joye DAM, Farkas AH, Gorman MR. Photoperiodic requirements for induction and maintenance of rhythm bifurcation and extraordinary entrainment in male mice. *Clocks Sleep*. (2019) 1:290–305. doi: 10.3390/clockssleep1030025

112. Walbeek TJ, Harrison EM, Soler RR, Gorman MR. Enhanced circadian entrainment in mice and its utility under human shiftwork schedules. *Clocks Sleep*. (2019) 1:394–413. doi: 10.3390/clockssleep1030032
113. Rosenthal SL, Vakili MM, Evans JA, Elliott JA, Gorman MR. Influence of photoperiod and running wheel access on the entrainment of split circadian rhythms in hamsters. *BMC Neurosci*. (2005) 6:41. doi: 10.1186/1471-2202-6-41
114. Harrison EM, Gorman MR. Rapid adjustment of circadian clocks to simulated travel to time zones across the globe. *J Biol Rhythms*. (2015) 30:557–62. doi: 10.1177/0748730415598875
115. Noguchi T, Harrison EM, Sun J, May D, Ng A, Welsh DK, et al. Circadian rhythm bifurcation induces flexible phase resetting by reducing circadian amplitude. *Eur J Neurosci*. (2018) 51:2329–42. doi: 10.1111/ejn.14086
116. Gorman MR, Yellon SM, Lee TM. Temporal reorganization of the suprachiasmatic nuclei in hamsters with split circadian rhythms. *J Biol Rhythms*. (2001) 16:552–63. doi: 10.1177/0748730011290 02240
117. Yan L, Silver R, Gorman MR. Reorganization of suprachiasmatic nucleus networks under 24-h LDLD conditions. *J Biol Rhythms*. (2010) 25:19–27. doi: 10.1177/0748730409352054
118. Watanabe T, Naito E, Nakao N, Tei H, Yoshimura T, Ebihara S. Bimodal clock gene expression in mouse suprachiasmatic nucleus and peripheral tissues under a 7-hour light and 5-hour dark schedule. *J Biol Rhythms*. (2007) 22:58–68. doi: 10.1177/0748730406295435
119. Harrison EM, Gorman MR. Changing the waveform of circadian rhythms: considerations for shift-work. *Front Neurol*. (2012) 3:72. doi: 10.3389/fneur.2012.00072
120. Gorman MR, Elliott JA. Exceptional entrainment of circadian activity rhythms with manipulations of rhythm waveform in male syrian hamsters. *Yale J Biol Med*. (2019) 92:187–99.
121. Meijer JH, Watanabe K, Schaap J, Albus H, Détári L. Light responsiveness of the suprachiasmatic nucleus: long-term multiunit and single-unit recordings in freely moving rats. *J Neurosci*. (1998) 18:9078–87. doi: 10.1523/JNEUROSCI.18-21-09078.1998
122. Evans JA, Carter SN, Freeman D a, Gorman MR. Dim nighttime illumination alters photoperiodic responses of hamsters through the intergeniculate leaflet and other photic pathways. *Neuroscience*. (2012) 202:300–8. doi: 10.1016/j.neuroscience.2011.11.037
123. Evans JA, Gorman MR. In synch but not in step: circadian clock circuits regulating plasticity in daily rhythms. *Neuroscience*. (2016) 320:259–80. doi: 10.1016/j.neuroscience.2016.01.072
124. Chiesa JJ, Anglès-Pujolràs M, Díez-Noguera A, Cambras T. History-dependent changes in entrainment of the activity rhythm in the Syrian hamster (*Mesocricetus auratus*). *J Biol Rhythms*. (2006) 21:45–57. doi: 10.1177/0748730405283654
125. Foster RG, Hughes S, Peirson SN. Circadian photoentrainment in mice and humans. *Biology (Basel)*. (2020) 9:180. doi: 10.3390/biology9070180
126. Mure LS, Le HD, Benegiamo G, Chang MW, Rios L, Jillani N, et al. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. *Science*. (2018) 359:eaao0318. doi: 10.1126/science.aao0318
127. Sadun AA, Schaechter JD, Smith LEH. A retinohypothalamic pathway in man: light mediation of circadian rhythms. *Brain Res*. (1984) 302:371–7. doi: 10.1016/0006-8993(84)90252-X
128. McGlashan EM, Poudel GR, Vidafar P, Drummond SPA, Cain SW. Imaging individual differences in the response of the human suprachiasmatic area to light. *Front Neurol*. (2018) 9:1022. doi: 10.3389/fneur.2018. 01022
129. Li JZ, Bunney BG, Meng F, Hagenauer MH, Walsh DM, Vawter MP, et al. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proc Natl Acad Sci*. (2013) 110:9950–5. doi: 10.1073/pnas.13058 14110
130. Hofman MA, Zhou J-N, Swaab DF. No evidence for a diurnal vasoactive intestinal polypeptide (VIP) rhythm in the human suprachiasmatic nucleus. *Brain Res*. (1996) 722:78–82. doi: 10.1016/0006-8993(96)00180-1
131. Yan L, Smale L, Nunez AA. Circadian and photic modulation of daily rhythms in diurnal mammals. *Eur J Neurosci*. (2020) 51:551–66. doi: 10.1111/ejn.14172
132. Duffy JF, Wright KP. Entrainment of the human circadian system by light. *J Biol Rhythms*. (2005) 20:326–38. doi: 10.1177/0748730405277983
133. Smale L, Lee T, Nunez AA. Mammalian diurnality: some facts and gaps. *J Biol Rhythms*. (2003) 18:356–66. doi: 10.1177/0748730403256651
134. Panda S, Hogenesch JB, Kay SA. Circadian rhythms from flies to human. *Nature*. (2002) 417:329–35. doi: 10.1038/417329a
135. Vansteensel MJ, Michel S, Meijer JH. Organization of cell and tissue circadian pacemakers: a comparison among species. *Brain Res Rev*. (2008) 58:18–47. doi: 10.1016/j.brainresrev.2007.10.009
136. Peichl L. Diversity of mammalian photoreceptor properties: adaptations to habitat and lifestyle? *Anat Rec Part A Discov Mol Cell Evol Biol*. (2005) 287A:1001–12. doi: 10.1002/ar.a.20262
137. Roorda A, Williams DR. The arrangement of the three cone classes in the living human eye. *Nature*. (1999) 397:520–2. doi: 10.1038/17383
138. Calderone JB, Jacobs GH. Regional variations in the relative sensitivity to UV light in the mouse retina. *Vis Neurosci*. (1995) 12:463–8. doi: 10.1017/S0952523800008361
139. Perry VH, Cowey A. The ganglion cell and cone distributions in the monkey's retina: implications for central magnification factors. *Vision Res*. (1985) 25:1795–810. doi: 10.1016/0042-6989(85)90004-5
140. Jeon C-J, Strettoi E, Masland RH. The major cell populations of the mouse retina. *J Neurosci*. (1998) 18:8936–46. doi: 10.1523/JNEUROSCI.18-21-08936.1998
141. Zele AJ, Cao D. Vision under mesopic and scotopic illumination. *Front Psychol*. (2015) 5:1594. doi: 10.3389/fpsyg.2014.01594
142. Altimus CM, Guler AD, Villa KL, McNeill DS, LeGates TA, Hattar S, et al. Rods-cones and melanopsin detect light and dark to modulate sleep independent of image formation. *Proc Natl Acad Sci*. (2008) 105:19998–20003. doi: 10.1073/pnas.0808312105
143. Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, et al. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science*. (2002) 298:2213–6. doi: 10.1126/science. 1076848
144. Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, et al. Role of melanopsin in circadian responses to light. *Science*. (2002) 298:2211–3. doi: 10.1126/science.1076701
145. Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. *J Neurosci*. (2000) 20:600–5. doi: 10.1523/JNEUROSCI.20-02-00600.2000
146. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. (2002) 295:1070–3. doi: 10.1126/science.1067262
147. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. (2002) 295:1065–70. doi: 10.1126/science.1069609
148. Graham DM, Wong KY. *Melanopsin-expressing, Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCs)*. Salt Lake City, UT: The Organization of the Retina and Visual System (1995).
149. Brown TM, Tsujimura S, Allen AE, Wynne J, Bedford R, Vickery G, et al. Melanopsin-based brightness discrimination in mice and humans. *Curr Biol*. (2012) 22:1134–41. doi: 10.1016/j.cub.2012.04.039
150. Mure LS, Vinberg F, Hanneken A, Panda S. Functional diversity of human intrinsically photosensitive retinal ganglion cells. *Science*. (2019) 366:1251–5. doi: 10.1126/science.aaz0898
151. Wong KY, Dunn FA, Graham DM, Berson DM. Synaptic influences on rat ganglion-cell photoreceptors. *J Physiol*. (2007) 582:279–96. doi: 10.1113/jphysiol.2007.133751
152. Dacey DM, Liao H-W, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. (2005) 433:749–54. doi: 10.1038/nature03387
153. Huberman AD, Niell CM. What can mice tell us about how vision works? *Trends Neurosci*. (2011) 34:464–73. doi: 10.1016/j.tins.2011.07.002
154. Kolb H. *Facts and Figures Concerning the Human Retina*. Salt Lake City, UT: The Organization of the Retina and Visual System (1995).
155. Hut RA, van Oort BEH, Daan S. Natural entrainment without dawn and dusk: the case of the european ground squirrel (*Spermophilus citellus*). *J Biol Rhythms*. (1999) 14:290–9. doi: 10.1177/074873099129000704

156. DeCoursey PJ. Light-sampling behavior in photoentrainment of a rodent circadian rhythm. *J Comp Physiol A*. (1986) 159:161–9. doi: 10.1007/BF00612299
157. Harrison EM, Gorman MR, Mednick SC. The effect of narrowband 500 nm light on daytime sleep in humans. *Physiol Behav*. (2011) 103:197–202. doi: 10.1016/j.physbeh.2011.01.020
158. Cole RJ, Smith JS, Alcalá YC, Elliott JA, Kripke DF. Bright-light mask treatment of delayed sleep phase syndrome. *J Biol Rhythms*. (2002) 17:89–101. doi: 10.1177/074873002129002366
159. Zeitzer JM, Fisicaro RA, Ruby NF, Heller HC. Millisecond flashes of light phase delay the human circadian clock during sleep. *J Biol Rhythms*. (2014) 29:370–6. doi: 10.1177/0748730414546532
160. Robinson J, Bayliss SC, Fielder AR. Transmission of light across the adult and neonatal eyelid *in vivo*. *Vision Res*. (1991) 31:1837–40. doi: 10.1016/0042-6989(91)90031-Y
161. Ando K, Kripke DF. Light attenuation by the human eyelid. *Biol Psychiatry*. (1996) 39:22–5. doi: 10.1016/0006-3223(95)00109-3
162. Grubisic M, Haim A, Bhusal P, Dominoni DM, Gabriel KMA, Jechow A, et al. Light pollution, circadian photoreception, and melatonin in vertebrates. *Sustainability*. (2019) 11:6400. doi: 10.3390/su11226400
163. Brainard GC, Richardson BA, King TS, Matthews SA, Reiter RJ. The suppression of pineal melatonin content and N-acetyltransferase activity by different light irradiances in the Syrian hamster: a dose-response relationship. *Endocrinology*. (1983) 113:293–6. doi: 10.1210/endo-113-1-293
164. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci*. (2001) 21:6405–12. doi: 10.1523/JNEUROSCI.21-16-06405.2001
165. Brainard GC, Hanifin JP, Warfield B, Stone MK, James ME, Ayers M, et al. Short-wavelength enrichment of polychromatic light enhances human melatonin suppression potency. *J Pineal Res*. (2015) 58:352–61. doi: 10.1111/jpi.12221
166. Cajochen C, Zeitzer JM, Czeisler CA, Dijk D-J. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. *Behav Brain Res*. (2000) 115:75–83. doi: 10.1016/S0166-4328(00)00236-9
167. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J Physiol*. (2001) 535:261–7. doi: 10.1111/j.1469-7793.2001.t01-1-00261.x
168. West KE, Jablonski MR, Warfield B, Cecil KS, James M, Ayers MA, et al. Blue light from light-emitting diodes elicits a dose-dependent suppression of melatonin in humans. *J Appl Physiol*. (2011) 110:619–26. doi: 10.1152/japplphysiol.01413.2009
169. Chang A-M, Scheer FAJL, Czeisler CA. The human circadian system adapts to prior photic history. *J Physiol*. (2011) 589:1095–102. doi: 10.1113/jphysiol.2010.201194
170. Hut RA, Oklejewicz M, Rieux C, Cooper HM. Photic sensitivity ranges of hamster pupillary and circadian phase responses do not overlap. *J Biol Rhythms*. (2008) 23:37–48. doi: 10.1177/0748730407311851
171. Zeitzer JM. When is a proxy not a proxy? The foibles of studying non-image forming light. *J Physiol*. (2018) 596:2029–30. doi: 10.1113/JP276076
172. Najjar RP, Zeitzer JM. Temporal integration of light flashes by the human circadian system. *J Clin Invest*. (2016) 126:938–47. doi: 10.1172/JCI82306
173. Hanifin JP, Lockley SW, Cecil K, West K, Jablonski M, Warfield B, et al. Randomized trial of polychromatic blue-enriched light for circadian phase shifting, melatonin suppression, and alerting responses. *Physiol Behav*. (2019) 198:57–66. doi: 10.1016/j.physbeh.2018.10.004
174. Rahman SA, Hilaire MA, Lockley SW. The effects of spectral tuning of evening ambient light on melatonin suppression, alertness and sleep. *Physiol Behav*. (2017) 177:221–9. doi: 10.1016/j.physbeh.2017.05.002
175. Chang A-M, Scheer FAJL, Czeisler CA, Aeschbach D. Direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans depend on prior light history. *Sleep*. (2013) 36:1239–46. doi: 10.5665/sleep.2894
176. Hébert M, Martin SK, Lee C, Eastman CI. The effects of prior light history on the suppression of melatonin by light in humans. *J Pineal Res*. (2002) 33:198–203. doi: 10.1034/j.1600-079X.2002.01885.x
177. Smith KA, Schoen MW, Czeisler CA. Adaptation of human pineal melatonin suppression by recent photic history. *J Clin Endocrinol Metab*. (2004) 8:3610–4. doi: 10.1210/jc.2003-032100
178. Zeitzer JM, Friedman L, Yesavage JA. Effectiveness of evening phototherapy for insomnia is reduced by bright daytime light exposure. *Sleep Med*. (2011) 12:805–7. doi: 10.1016/j.sleep.2011.02.005
179. Jasser SA, Hanifin JP, Rollag MD, Brainard GC. Dim light adaptation attenuates acute melatonin suppression in humans. *J Biol Rhythms*. (2006) 21:394–404. doi: 10.1177/0748730406292391
180. Vetter C, Phillips AJK, Silva A, Lockley SW, Glickman G. *Light Me up? Why, When, and How Much Light We Need*. *J Biol Rhythms*. (2019) 34:573–5. doi: 10.1177/0748730419892111
181. Wright KP, McHill AW, Birks BR, Griffin BR, Rusterholz T, Chinoy ED. Entrainment of the human circadian clock to the natural light-dark cycle. *Curr Biol*. (2013) 23:1554–8. doi: 10.1016/j.cub.2013.06.039
182. Stothard ER, McHill AW, Depner CM, Birks BR, Moehlman TM, Ritchie HK, et al. Circadian entrainment to the natural light-dark cycle across seasons and the weekend. *Curr Biol*. (2017) 27:508–13. doi: 10.1016/j.cub.2016.12.041
183. Prayag AS, Najjar RP, Gronfier C. Melatonin suppression is exquisitely sensitive to light and primarily driven by melanopsin in humans. *J Pineal Res*. (2019) 66:e12562. doi: 10.1111/jpi.12562
184. Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res*. (2020) 69:12655. doi: 10.1111/jpi.12655
185. Spitschan M. Melanopsin contributions to non-visual and visual function. *Curr Opin Behav Sci*. (2019) 30:67–72. doi: 10.1016/j.cobeha.2019.06.004
186. Spitschan M, Stefani O, Blattner P, Gronfier C, Lockley S, Lucas R. How to report light exposure in human chronobiology and sleep research experiments. *Clocks Sleep*. (2019) 1:280–9. doi: 10.3390/clockssleep1030024
187. *Fluxometer*. Available online at: https://fluxometer.com/rainbow/

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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: 05 February 2021
doi: 10.3389/fneur.2021.625431

Daily Morning Blue Light Therapy for Post-mTBI Sleep Disruption: Effects on Brain Structure and Function

Adam C. Raikes[1], Natalie S. Dailey[2], Brittany Forbeck[2], Anna Alkozei[2] and William D. S. Killgore[2]*

[1] Center for Innovation in Brain Science, University of Arizona, Tucson, AZ, United States, [2] Social, Cognitive, and Affective Neuroscience Lab, University of Arizona, Tucson, AZ, United States

Background: Mild traumatic brain injuries (mTBIs) are associated with novel or worsened sleep disruption. Several studies indicate that daily morning blue light therapy (BLT) is effective for reducing post-mTBI daytime sleepiness and fatigue. Studies demonstrating changes in brain structure and function following BLT are limited. The present study's purpose is to identify the effect of daily morning BLT on brain structure and functional connectivity and the association between these changes and self-reported change in post-mTBI daytime sleepiness.

Methods: A total of 62 individuals recovering from a mTBI were recruited from two US cities to participate in a double-blind placebo-controlled trial. Eligible individuals were randomly assigned to undergo 6 weeks of 30 min daily morning blue or placebo amber light therapy (ALT). Prior to and following treatment all individuals completed a comprehensive battery that included the Epworth Sleepiness Scale as a measure of self-reported daytime sleepiness. All individuals underwent a multimodal neuroimaging battery that included anatomical and resting-state functional magnetic resonance imaging. Atlas-based regional change in gray matter volume (GMV) and region-to-region functional connectivity from baseline to post-treatment were the primary endpoints for this study.

Results: After adjusting for pre-treatment GMV, individuals receiving BLT had greater GMV than those receiving amber light in 15 regions of interest, including the right thalamus and bilateral prefrontal and orbitofrontal cortices. Improved daytime sleepiness was associated with greater GMV in 74 ROIs, covering many of the same general regions. Likewise, BLT was associated with increased functional connectivity between the thalamus and both prefrontal and orbitofrontal cortices. Improved daytime sleepiness was associated with increased functional connectivity between attention and cognitive control networks as well as decreased connectivity between visual, motor, and attention networks (all FDR corrected $p < 0.05$).

Conclusions: Following daily morning BLT, moderate to large increases in both gray matter volume and functional connectivity were observed in areas and networks previously associated with both sleep regulation and daytime cognitive function, alertness, and attention. Additionally, these findings were associated with improvements

OPEN ACCESS

Edited by:
Shadab Rahman, Harvard Medical School, United States

Reviewed by:
Elise Maree McGlashan, Monash University, Australia
Marco Zucconi, San Raffaele Hospital (IRCCS), Italy

****Correspondence:***
Adam C. Raikes
raikes.research@gmail.com

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *03 November 2020*
Accepted: *15 January 2021*
Published: *05 February 2021*

Citation:
Raikes AC, Dailey NS, Forbeck B, Alkozei A and Killgore WDS (2021) Daily Morning Blue Light Therapy for Post-mTBI Sleep Disruption: Effects on Brain Structure and Function. Front. Neurol. 12:625431. doi: 10.3389/fneur.2021.625431

in self-reported daytime sleepiness. Further work is needed to identify the personal characteristics that may selectively identify individuals recovering from a mTBI for whom BLT may be optimally beneficial.

Keywords: concussion, phototherapy, daytime sleepiness, fatigue, gray matter volume, functional connectivity

INTRODUCTION

Mild traumatic brain injury (mTBI), or concussion, occurs when an individual experiences a blow to the head or other mechanical force that results in altered cognition and/or brief loss of consciousness. The short- and long-term effects of mTBIs are of significant concern across the spectrum of sports, military, and public health. In light of the conservatively estimated 3 million mTBIs reported to emergency rooms each year (1, 2) those sustained by athletes (3) and military Service members (4) that are cared for by embedded medical teams, the need for effective treatments is great. While considerable work is being done to identify biomarkers of injury (5–7) and improve clinical diagnoses (8–10), there is comparatively little in the way of efficacious treatments (11–14).

Among the myriad of neurobiological consequences faced by individuals who have sustained a mild traumatic brain injury, sleep disruption is among the most common and the most persistent (15–19). Up to 90% of individuals who sustain a mTBI report some form of sleep disruption—including insomnia, frequent wakefulness after sleep onset, and a general sense of poor sleep quality (19–24)—or associated complaints of increased daytime fatigue or sleepiness that interferes with one or more activities of daily living (25–28). These sleep-related complaints often go untreated and may persist for months to years post-injury.

Post-mTBI sleep disruption is also associated with slowed recovery (29–32), exacerbated symptom presentation (29, 31), and degraded overall functioning (cognitive, motor, emotional) (33–37). Notably, a reciprocal cycle appears to exist where daytime fatigue and sleepiness predispose individuals to future mTBIs, while mTBIs predispose individuals to future daytime fatigue and sleepiness (27). Consequently, identifying and developing effective treatments to deal with sleep-related disruptions is necessary to support high-quality academic, job, and sport performance. While there is evidence that pharmacologic intervention may be beneficial for some individuals following a mTBI (38–40), there is some suggestion that these may not be the best first course of treatment (41) and it is likely that non-pharmacologically driven treatments offer the added benefit of lower risk for substance dependence.

Among available non-pharmacologic treatments for sleep-related disruptions, morning blue light therapy (BLT) has been shown to be effective in reducing daytime fatigue in individuals recovering from a mTBI (42–44). Additionally, this work suggests that these effects on daytime fatigue may be associated with shifts in circadian phase in some patients (43). While these outward effects on daytime functioning are encouraging, the associated effects of blue light therapy on the brain in those recovering from a mTBI has not been extensively examined.

Work from our group has focused on using multimodal neuroimaging to examine both the acute effects of blue light on cognitive function and alertness, as well as the effects of treatment in those recovering from a mTBI. Our work suggests that – in comparison to amber light exposure—a single, short duration (30 min) blue light exposure modulates anterior cingulate activation in anticipation tasks (45), dorso- and ventrolateral prefrontal cortex activation in working memory tasks (46), and may improve neural efficiency during interference tasks (47) while being associated with improved task performance on anticipation, working memory, and verbal memory tasks (45, 46, 48).

Recently, we completed two separate randomized clinical trials using morning blue light therapy (BLT) as a treatment for mTBI-related sleep disruption vs. an amber light control condition. Across both trials, BLT was associated with reduced self-reported daytime sleepiness at the end of six of weeks of treatment (43, 44); a circadian phase advance and increased midday sleep onset latency (43); and improved actigraphically-measured nighttime sleep quality (44). Neuroimaging evidence from the first trial indicated that BLT was associated with increases in bilateral thalamic volume, particularly in the pulvinar region (43); improved functional and structural connectivity between the thalamus and areas of the parietal cortex (43); and altered white matter diffusion characteristics in white matter tracts passing the through the thalamus, corpus callosum, and left anterior corona radiata (43, 49). These neuroimaging findings suggest that altered brain structure and function in regions and circuits subserving alertness, attention, and cognitive control may be facilitated by improved nighttime sleep and reduced daytime sleepiness.

What remains unknown are the broader effects of BLT on brain structure and function following a mTBI. The effects on thalamic volume and connectivity described above were observed in a small sample (total $n = 31$) using targeted follow-up analyses (e.g., thalamic volume changes were observed using voxel-based morphometry and subsequently used as a seed region for examining function and structural connectivity changes) (43). The purpose of the present study is to expand these neuroimaging findings by (A) combining the samples from both trials and (B) applying an atlas-based approach to both gray matter volumetry and functional connectivity to explore the effects of BLT, as opposed to amber light therapy (ALT), in individuals recovering from a mTBI. We hypothesized that, consistent with prior findings, BLT would be associated with increased volume in and

connectivity between cortical and subcortical regions involved in task-related attention and cognitive control.

MATERIALS AND METHODS

Participants

Sixty-two individuals were recruited across two conceptually linked studies on the effects of BLT on sleep-related outcomes following mTBI. These studies took place in Boston, MA (study 1; $n = 31$) and Tucson, AZ (study 2; $n = 31$). Data on these two samples have been reported previously for individual samples (43, 44, 49), but the findings reported here are novel and have not been previously presented. Inclusion and exclusion criteria were the same across both studies and have been described elsewhere (43, 44, 50). Briefly, all individuals sustained a mTBI within 18 months of enrollment according to the mTBI definition consistent with the Veteran's Administration/Department of Defense guidelines (51). Individuals provided documentation of their head injury either from a medical provider, qualified third-party witness, or first responder. Qualified witness reports—including those from a coach, allied health professional, or emergency personnel—were accepted, as many mTBIs are not evaluated in a physician's office or emergency department. mTBI reports were further corroborated by the participants via the Ohio State University Traumatic Brain Injury ID self-report form (52, 53). Individuals were excluded on the basis of pre-existing medical or neuropsychiatric disorders, a history of a moderate to severe TBI, alcohol or illicit substance abuse, or contra-indications for neuroimaging. None of the participants were undergoing treatment or taking medications for sleep disorders. Complete exclusionary criteria have been previously described (43, 44). Prior to study initiation, all procedures were reviewed and approved by the Institutional Review Boards for Partner's Healthcare (study 1), the University of Arizona (study 2), and the Human Research Protections Office of the U.S. Army (both studies). All participants were fully informed of all study procedures and provided written informed consent.

Study Procedures

Participants completed three in-lab visits separated by 1- and 6-weeks, respectively. Individuals were first screened to confirm meeting eligibility criteria and given a wrist-worn accelerometer used to quantify 24-h sleep patterns for the duration of the study [Philips Respironics Actiwatch Spectrum; data previously reported and not included here (43, 44, 50)]. Participants returned to the lab 1 week later and completed a comprehensive neuropsychological and self-report assessment battery, as well as a multimodal imaging protocol (described below). Following these procedures, individuals were randomized to either the BLT ($\lambda \sim 469$ nm; total illuminance 214 lux; total irradiance: 248 $\mu W/cm^2$ at 50 cm; Philips goLITE BLUE, Philips Electronics, Stamford Connecticut) or ALT ($\lambda \sim 578$ nm; total illuminance:188 lux; total irradiance: 35 $\mu W/cm^2$ at 50 cm; Philips Electronics custom light box) treatment groups. Participants were provided a corresponding light box to be used at home, based on treatment group. Treatment consisted of 6 weeks of at-home daily light box use (30 min per day within 2 h of waking). The light device was placed at arm's length at a slight angle from the participant's direction of gaze, but facing the participant enough to bathe the face and eyes with the light. Participants returned to the lab after 6 weeks of light therapy and completed identical testing and neuroimaging procedures.

Image Acquisition

The multimodal neuroimaging protocol included the collection of structural, functional, and diffusion weighted imaging. Neuroimaging was conducted pre- and post-treatment, resulting in two sets of images for each participant. The results presented here include only the structural and functional imaging findings. With a few minor exceptions, imaging protocols were nearly identical between the two studies.

Study 1: Boston, MA

All imaging data were collected on a 3.0T Siemens Tim Trio (Erlanger, Germany) magnetic resonance imaging (MRI) scanner located at McLean Hospital, Belmont, MA. Imaging included a T1-weighted 3D magnetization-prepared rapid acquisition gradient echo sequence (MPRAGE; TE: 2.3 ms; TR: 2.1 s; flip angle: 12°; acquisition matrix: 256 × 256; slice thickness: 1 mm, voxel size: 1 mm^3) and a resting-state functional MRI sequence (rsFMRI; TE: 30 ms; TR: 2 s; flip angle: 90°; acquisition matrix: 64 × 64; slice thickness: 3.5 mm; voxel size: 3.5 mm^3). For distortion correction, a gradient echo field map sequence was also collected (TE: 4.92/7.38 ms; TR: 625 ms; flip angle: 90°; acquisition matrix: 64 × 64; slice thickness: 3.5 mm; voxel size: 3.5 mm^3) resulting in two magnitude images and a phase difference map. The set of baseline scans were collected during the in-lab visit, prior to light therapy. The set of post-treatment scans were collected 6 weeks later during the final in-lab visit, following the completion of either BLT or ALT.

Study 2: Tucson, AZ

Imaging data were collected on a 3.0 Siemens Skyra (Erlanger, Germany) MRI scanner located at the University of Arizona. Imaging included a T1-weighted MPRAGE sequence (TE: 2.3 ms; TR: 2.1 s; flip angle: 12°; acquisition matrix: 256 × 256; slice thickness: 1 mm, voxel size: 1 mm) and a rsFMRI sequence (TE: 25 ms; TR: 2 s; flip angle: 90°; acquisition matrix: 84 × 84; voxel size: 2 mm^3). For distortion correction, a gradient echo field map sequence was also collected (TE: 4.92/7.38 ms; TR: 625 ms; flip angle: 90°; acquisition matrix: 64 × 64; slice thickness: 3.5 mm; voxel size: 3.5 mm^3) resulting in two magnitude images and a phase difference map. Baseline scans were collected prior to light therapy and post-treatment scans were collected 6 weeks later, following the completion of at-home light therapy.

Imaging Processing

Imaging from both studies underwent the same pre- and post-processing protocol. Prior to pre-processing all data were converted from DICOM to NIFTI format using HeuDiConv (v. 0.6.0) into a Brain Imaging Dataset (BIDS) compliant format. Image quality was initially assessed using MRIQC (v 0.15.1). All participants in the present analyses had complete structural imaging datasets (one usable T1-weighted image).

Structural Image Processing

All T1-weighted images were processed using the Computation Anatomy Toolbox 12 (CAT12 r1450; http://www.neuro.uni-jena.de/cat/) implemented through Statistical Parametric Mapping 12 (SPM12 r7219; https://www.fil.ion.ucl.ac.uk/spm/) using MATLAB R2016A (The MathWorks Inc, Natick, MA). Prior to processing, all images were realigned to the anterior-posterior commissure axis using Convert3D (http://www.itksnap.org/pmwiki/pmwiki.php?n=Convert3D.Documentation). These realigned data were subsequently segmented using CAT12's longitudinal pipeline, which included denoising, skull stripping, three tissue type segmentation, and normalizing to Montreal Neurological Institute (MNI) space with an output resolution of 1 mm^3. Total intracranial volume (ICV) was additionally computed using CAT12. Following normalization, gray matter volume estimates were extracted for each of 400 cortical (54), 36 subcortical (55), and 37 cerebellar (56) regions of interest (ROIs) using a concatenation of three well-validated atlases that has been previously used for similar purposes (57). Each of the ROIs was assigned to one of nine networks based on the Yeo 17-network parcellation [using overarching network names when multiple sub-networks exist; e.g., the default mode A, B, and C networks were labels as DMN; (58)] for the cortical ROIs, one subcortical network, and a cerebellar network.

rsFMRI Image Pre-processing

Results included in this manuscript come from preprocessing performed using fMRIPrep 20.1.3 [RRID:SCR_016216, (59, 60)], which is based on Nipype 1.5.1 [RRID:SCR_002502, (61, 62)]. The descriptions of the pre-processing steps in fMRIPrep are provided by the creators of the software under a CC0 license and reproduced here without changes (aside from formatting for references).

Anatomical data preprocessing

A total of 2 T1-weighted (T1w) images were found within the input BIDS dataset. All of them were corrected for intensity non-uniformity (INU) with *N4BiasFieldCorrection* (63), distributed with ANTs 2.2.0 [RRID:SCR_004757, (64)]. The T1w-reference was then skull-stripped with a Nipype implementation of the *antsBrainExtraction.sh* workflow (from ANTs), using OASIS30ANTs as the target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using *fast* [FSL 5.0.9, RRID:SCR_002823, (65)]. A T1w-reference map was computed after registration of 3 T1w images (after INU-correction) using *mri_robust_template* [FreeSurfer 6.0.1, (66)]. Brain surfaces were reconstructed using *recon-all* [FreeSurfer 6.0.1, RRID:SCR_001847, (67)], and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of Mindboggle (RRID:SCR_002438) (68). Volume-based spatial normalization to two standard spaces (MNI152NLin2009cAsym, MNI152NLin6Asym) was performed through non-linear registration with *antsRegistration* (ANTs 2.2.0), using brain-extracted versions of both T1w reference and the T1w template. The following templates were selected for spatial normalization: *ICBM 152 Non-linear Asymmetrical template version 2009c* [RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym, (69)], *FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model* [RRID:SCR_002823; TemplateFlow ID: MNI152NLin6Asym, (70)].

Functional data processing

For each of the two BOLD rsFMRI runs found per subject (across all sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using *mcflirt* [FSL 5.0.9, (71)]. BOLD runs were slice-time corrected using *3dTshift* from AFNI 20160207 [RRID:SCR_005927, (72)]. A B0-non-uniformity map (or fieldmap) was estimated based on a phase-difference map calculated with a dual-echo GRE (gradient-recall echo) sequence, processed with a custom workflow of SDCFlows inspired by the *epidewarp.fsl* script and further improvements in HCP Pipelines (73). The fieldmap was then co-registered to the target EPI (echo-planar imaging) reference run and converted to a displacements field map (amenable to registration tools such as ANTs) with FSL's *fugue* and other SDCflows tools. Based on the estimated susceptibility distortion, a corrected EPI (echo-planar imaging) reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using *bbregister* (FreeSurfer) which implements boundary-based registration (74). Co-registration was configured with six degrees of freedom. The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These resampled BOLD time-series will be referred to as preprocessed BOLD in original space, or just preprocessed BOLD.

The BOLD time-series were resampled into several standard spaces, correspondingly generating the following spatially-normalized, preprocessed BOLD runs: MNI152NLin2009cAsym, MNI152NLin6Asym. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power [absolute sum of relative motions, (75)], and Jenkinson [relative root mean square displacement between affines, (71)]. FD and DVARS are calculated for each functional run, both using their implementations in Nipype [following the definitions by Power et al. (75)]. The three global signals are extracted within the CSF, the WM, and the whole-brain masks.

Additionally, a set of physiological regressors were extracted to allow for component-based noise correction [CompCor, (76)]. Principal components are estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine

filter with 128 s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 5% variable voxels within a mask covering the subcortical regions. This subcortical mask is obtained by heavily eroding the brain mask, which ensures it does not include cortical GM regions. For aCompCor, components are calculated within the intersection of the aforementioned mask and the union of CSF and WM masks calculated in T1w space, after their projection to the native space of each functional run (using the inverse BOLD-to-T1w transformation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration.

The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (77). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardized DVARS were annotated as motion outliers. All resamplings can be performed with a single interpolation step by composing all the pertinent transformations (i.e., head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using *antsApplyTransforms* (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (78). Non-gridded (surface) resamplings were performed using *mri_vol2surf* (FreeSurfer).

Many internal operations of fMRIPrep use Nilearn 0.6.2 [RRID:SCR_001362, (79)], mostly within the functional processing workflow. For more details of the pipeline, see the section corresponding to workflows in fMRIPrep's documentation.

Functional Connectivity

Post-processing and functional connectivity estimation from the preprocessed BOLD time series (in MNI152NLin2009cAsym space) was accomplished for each subject and session using the eXtensible Connectivity Pipeline (XCP Engine, v 1.2.3; https://github.com/PennBBL/xcpEngine). Confound regressors were collected from fMRIPrep and included the mean global, cerebrospinal fluid, and white matter signals, framewise motion (x-, y-, and z- axis translation and rotation) as well as the derivatives and quadratic expansions of these terms [36 parameter confound regressors, (80)]. Processing steps included demeaning, detrending, and temporal filtering (0.01–0.08 Hz Butterworth filter) of the time series and regressors, as well as despiking of the BOLD time-series using *3dDespike* from AFNI [RRID:SCR_005927, (72)] followed by regression using the 36 parameters. The residual BOLD time-series following regression was the averaged in each of the 473 ROIs used in estimating the gray matter volume. However, due to varying levels of field of view coverage for the cerebellum during the resting state imaging, the 37 cerebellar ROIs were ultimately excluded. Functional connectivity was then computed as the Pearson *r* correlation between each ROI, yielding a 436 × 436 functional connectivity matrix per subject per session (400 cortical ROIs, 36 subcortical ROIs). These connectivity matrices were subsequently Fisher *r-to-z* transformed to improve the normality of the distribution of the correlation coefficients. Additional quality metrics provided by XCP Engine included mean root mean square (RMS) motion estimates and node coverage.

Data Harmonization

A necessary consideration in the analyses of these data is the collection of imaging on different systems in different locations over the course of several years. To ensure that the present findings were not confounded by site effects, the GMV and functional connectivity data were individually harmonized using *neuroCombat* [https://github.com/Jfortin1/ComBatHarmonization, (81)] implemented in R [v. 3.6.1, (82)]. *neuroCombat* uses an Empirical Bayes approach to minimize site-level effects in neuroimaging data while preserving biological effects. This approach has successfully been used to harmonize structural, functional, and diffusion-weighted data (81, 83, 84). To ensure that effects of interest and potentially meaningful regressors were preserved, we included age, sex, number of previous mTBIs, days post-injury, and group (BLT, ALT) in the modeling. For the functional connectivity data, we additionally included mean root mean square motion from XCP Engine as a potential covariate.

Statistical Analyses

All post-processing statistical analyses were conducted in R and Python (v. 3.7.6). All between-groups analyses were ultimately conducted using DABEST [v 0.3.0, (85)] in Python. DABEST computes effect sizes for both between-group and within-group comparisons as well as bootstrapped, bias-corrected, accelerated (BCa) 95% confidence intervals (using 20,000 bootstrap resamples) around these effect sizes. Additionally, DABEST provides traditional hypothesis testing using Welch's *t*, Student's *t*, and Mann-Whitney *U* test *p*-values as well as a permutation-based *p*-value (5,000 permutations).

Treatment-Related Effects on GMV

We fit an initial regression model to both baseline and post-treatment harmonized ROI GMV estimates separately to remove the effects of total intracranial volume, age, and sex and extracted the residuals to estimate covariate-adjusted ROI volumes. We then regressed baseline covariate-adjusted values against post-treatment covariate-adjusted values. The residuals from this model were then passed to DABEST to compare BLT to ALT. Results are reported as Hedges' *g* effect sizes. In reporting these findings, we employed a hierarchical approach to controlling family-wise error. First, we included only ROIs whose 95% confidence intervals did not include 0. Second, we applied false discovery rate (FDR) correction to the permutation *p*-values from DABEST and thresholded the findings at FDR corrected $p < 0.05$. Treatment-related effects on functional connectivity.

Univariate correlations were fit for each edge at baseline in the harmonized dataset in the adjusted baseline models to additionally assess age, sex, total number of prior mTBIs, days post-injury, and relative RMS motion as potential covariates. Approximately 6% of the edges were associated with either age, total number of prior mTBIs, or days post-injury and none of these correlations survived multiple comparisons correction and so no further covariate adjustment was considered. Post-treatment functional connectivity data were regressed on the baseline data to remove baseline effects. Residuals from these baseline-adjusted models were passed to DABEST to compare BLT to ALT on an edge-by-edge basis. Similar to the GMV models we report Hedges *g* effect sizes for each edge thresholded by (1) 95% confidence intervals not including 0 and (2) FDR corrected permutation $p < 0.05$. Results were plotted using in Python using the *plot_connectome* function from Nilearn [v 0.6.2; RRID:SCR_001362; (79)].

Exploratory Analyses

In order to further explore the neuroimaging findings in relationship to previously identified behavior outcomes, we conducted several exploratory analyses. These analyses included correlations between baseline and baseline-adjusted daytime sleepiness (ESS scores; the primary behavioral outcome previously reported) and both GMV and functional connectivity. For baseline-adjusted ESS scores, these were computed as the residual ESS scores after regressing post-treatment values on baseline values. Consistent with our prior findings, these baseline-adjusted values were computed without adjusting for additional covariates (e.g., sex, number of mTBIs, days post-injury) as none improved the models in stepwise model selection. These correlations were fit in Python using the *bootstrap-stat* (https://github.com/rwilson4/bootstrap-stat) package to compute 95% BCa confidence intervals. Findings were initially thresholded to include only correlations with 95% BCa confidence intervals not including 0. Results were visualized using BrainNetViewer [v. 1.7; RRID:SCR_009446; (86)] for GMV correlations and *plot_connectome* for functional connectivity.

These exploratory analyses were executed to provide comprehensive estimates of effects for planning future similar studies.

RESULTS

A total of 62 individuals (25 males) completed all study procedures across both cohorts. In general, participants were young adults (mean age: 24.7 ± 7.8 years) who were in the chronic phase of mTBI recovery (mean weeks from index injury: 36.4 ± 20.9). Across the combined sample, ESS scores approached, on average, the threshold for excessive daytime sleepiness (mean ESS: 9.2 ± 3.4; threshold for EDS = 10) (87). We previously reported baseline balance between blue and amber groups in each of the cohorts separately (43, 44).

Treatment Effects on GMV

After controlling for total intracranial volume, age, sex, and baseline GMV, a total of 17 ROIs survived multiple comparisons correction (all FDR corrected $p < 0.05$) and demonstrated moderate to large differences, including greater post-treatment GMV after BLT ($n = 15$) and ALT ($n = 2$; **Figure 1**, **Table 1**). Compared to ALT, greater GMV following BLT was observed primarily in the left hemisphere in regions associated with the attention, default mode, and limbic networks. Consistent with prior findings in the Boston subset of this sample, greater GMV was also observed in the right caudal temporal and right occipital thalamic ROIs. Greater GMV following ALT compared to BLT was observed in two areas of the cerebellum.

Treatment Effects on Functional Connectivity

After controlling for baseline functional connectivity, a total of 3,276 edges exhibited differences in connectivity (all FDR corrected $p < 0.05$) between the BLT and ALT groups. Greater connectivity following BLT was observed in $n = 2{,}028$ edges while lower connectivity was observed in $n = 1{,}248$. Based on the network labeling for the associated ROIs, these differences included a widespread network for connections between the default mode, somatomotor, cognitive control, subcortical gray matter, as well as dorsal and ventral attention networks.

The largest differences (FDR corrected $p < 0.01$; **Figure 2**) were observed in a total of 16 edges linking these same networks. These edges specifically include connections with the right thalamus, prefrontal and orbitofrontal cortices, and numerous somatomotor regions.

Exploratory Analyses

Relationship Between ESS and GMV

At baseline, ESS scores were inversely correlated with GMV (i.e., greater ESS, lower GMV) in 20 cortical ROIs and positively associated with GMV 5 ROIs, including four subcortical ROIs. After adjusting for baseline GMV and ESS, decreased ESS scores at post-treatment were correlated with increased GMV in 15.6% of the 473 ROIs ($n = 74$; **Figure 3**) including the right caudal temporal thalamus. These ROIs were associated with the bilateral attention, cognitive control, visual, and default mode networks.

Relationship Between ESS and Functional Connectivity

Prior to treatment, ESS scores were associated with functional connectivity across a total of 5,656 edges. Those with the strongest correlations ($r > |0.40|$, $n = 144$ edges) included positive correlations (greater ESS and greater connectivity; $n = 72$ edges) within the DMN as well as between the DMN, salience and cognitive control networks ($n = 27/72$ edges) and negative correlations (greater ESS, lower connectivity; $n = 72$ edges) primarily between the DMN and subcortical gray matter as well as between the temporoparietal network and the somatomotor and ventral attention networks ($n = 27/72$ edges across these connections).

A total 5,039 edges exhibited a change in connectivity that was associated with a change in daytime sleepiness, including 3,072 edges with increased connectivity and 1,967 with decreased connectivity. Examining those edges with the strongest correlations ($r > |0.40|$, $n = 121$ edges; **Figure 4**) revealed

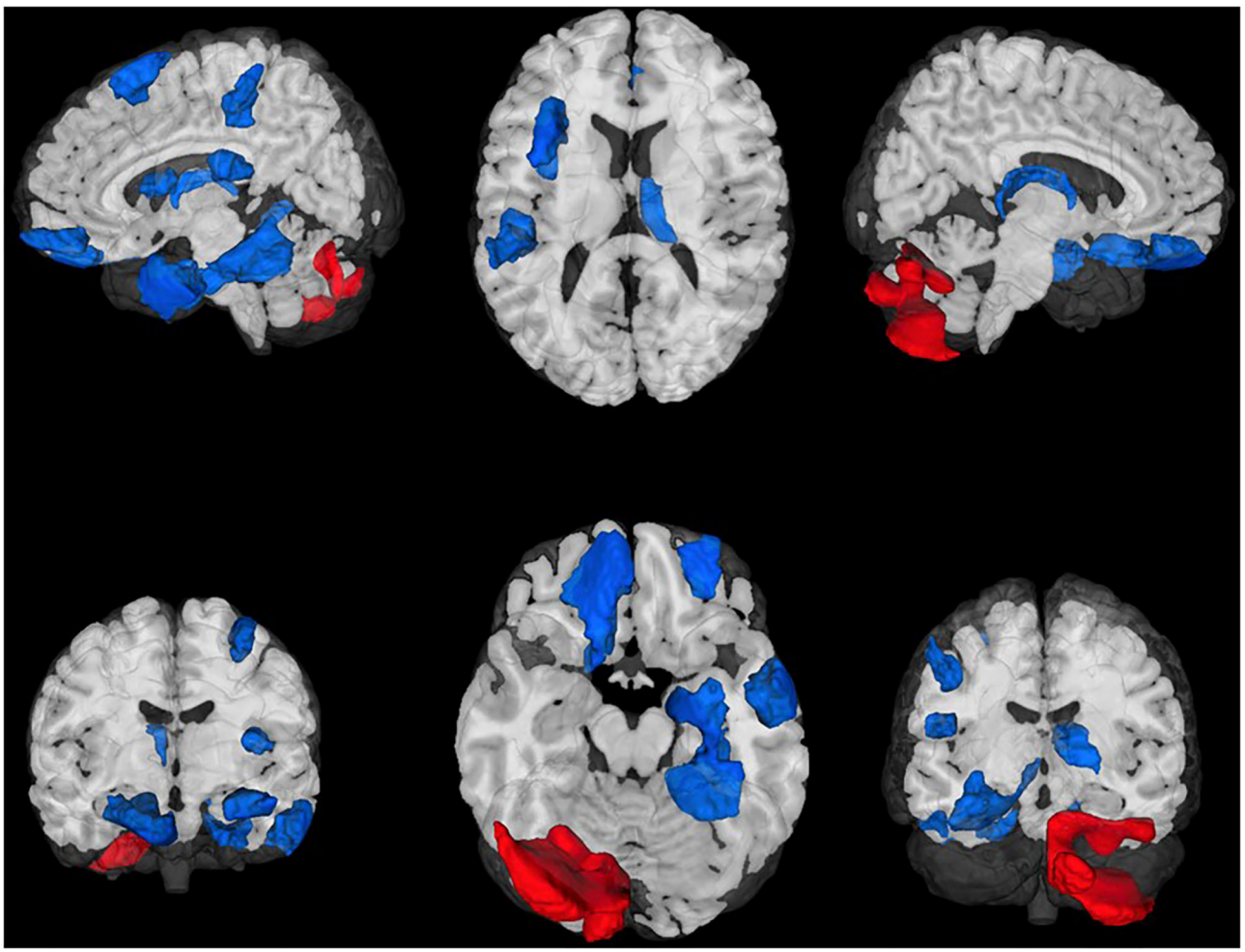

FIGURE 1 | Regions of interest (ROIs) exhibiting moderate-to-large baseline adjusted differences in gray matter volume (GMV). Greater GMV was observed following blue light treatment (ROIs in blue) in 15 cortical and subcortical ROIs. Greater GMV was observed following amber light (red ROIs) in two cerebellar regions. All ROIs were false discovery rate corrected $p < 0.05$. Top Left: Lateral left view, Top Middle: Superior view; Top Right, Lateral right view. Bottom Left, anterior view; Bottom Middle: Inferior view; Bottom Right: Posterior view.

increased connectivity primarily associated with decreased ESS scores (negative correlation) in edges connecting the dorsal attention, somatomotor, and visual networks as well as the default mode, and subcortical networks ($n = 48$ edges total across these network connections; 82 total edges with negative correlations; **Figure 4** bottom). Decreased connectivity was associated with decreased ESS scores in 39 edges (**Figure 4** top).

DISCUSSION

The primary purpose of this study was to extend our prior findings on the effects of 6 weeks of 30 min daily morning BLT on brain structure and function in individuals recovering from a mTBI. Consistent with our hypotheses and prior findings, we observed increased gray matter volume and modulated functional connectivity in and between areas of the brain associated with attention, cognitive control, salience, and visual processing among those who received BLT. Increased GMV after adjusting for baseline GMV was also observed in the right thalamus, consistent with the earlier voxel-based analysis of a subset of these data (43). These changes in both GMV and functional connectivity were additionally correlated with improvement in subjective daytime sleepiness.

In particular, individuals who reported improved daytime sleepiness exhibited increased GMV in the thalamus as well as orbitofrontal and prefrontal cortices and the precuneus, among other regions. Furthermore, increased functional connectivity associated with decreases in daytime sleepiness were observed in ROI-to-ROI connections linking these regions to other areas in the attention, default mode, visual, and somatomotor networks. This is consistent with other findings suggesting that daytime sleepiness is associated with reduced thalamocortical connectivity (88). These data provide evidence that, particularly for individuals experiencing daytime sleepiness following a

mTBI, blue light therapy may confer beneficial effects, in part due to changes to both structure and function in brain networks associated with alertness and attention as well as linkage between these and the default mode network.

Blue Light Effects on GMV

The present findings extend our previous work on blue light therapy and GMV in several ways. Specifically, we provide further evidence that reduced thalamic volume is associated with daytime sleepiness following mTBI and that this volume increases following blue light therapy (as opposed to similar exposure to amber light). We additionally identified several other regions that were not previously observed in our voxel-wise analyses that may respond positively (i.e., increased GMV) to blue light therapy, including the orbitofrontal and prefrontal cortices. For both mTBI recovery and daytime sleepiness, these regions have important implications.

TABLE 1 | Regions of interest demonstrating baseline-adjusted differences in gray matter volume.

Atlas	Region	Effect size	Interpretation
Buckner cerebellar atlas	Cerebellum #22	0.563	Amber > Blue
	Cerebellum #34	0.554	Amber > Blue
Brainnetome atlas	Right occipital thalamus	−0.552	Blue > Amber
	Right caudal temporal thalamus	−0.557	Blue > Amber
Schaefer 400	LH_ContB_PFCd_1	−0.538	Blue > Amber
	LH_DefaultB_Temp_2	−0.615	Blue > Amber
	LH_DefaultC_PHC_2	−0.572	Blue > Amber
	LH_DorsAttnA_TempOcc_2	−0.788	Blue > Amber
	LH_DorsAttnB_PostC_4	−0.514	Blue > Amber
	LH_Limbic_TempPole_3	−0.522	Blue > Amber
	LH_SalVentAttnA_Ins_4	−0.574	Blue > Amber
	LH_SalVentAttnB_OFC_1	−0.575	Blue > Amber
	LH_SomMotB_Aud_10	−0.554	Blue > Amber
	RH_Limbic_OFC_1	−0.644	Blue > Amber
	RH_Limbic_OFC_3	−0.541	Blue > Amber
	RH_Limbic_OFC_4	−0.552	Blue > Amber
	RH_SalVentAttnA_ParMed_6	−0.485	Blue > Amber

Regions of interest defined in the Buckner cerebellar atlas (56), Brainnetome atlas (55), and Schaefer 400 ROI 17-Network parcellation (54). Effect sizes as Hedges g.

First, decreased thalamic volume has been observed following mTBIs, specifically in individuals with post-mTBI fatigue (89), while increased thalamic volume is positively associated with more rapid recovery from post-mTBI cognitive impairment (90). Second, both decreased thalamic volume and decreased volume in orbitofrontal and prefrontal regions has been observed in individuals with chronic insomnia, sleep loss, daytime fatigue, and daytime sleepiness (91–93). Relatedly, previous studies show that sleep loss is associated with decreases in glucose metabolism in pre-/orbitofrontal regions (94) as well as changes in the upregulation of A1 adenosine receptors which may underpin homeostatic sleep regulation (95). Given the metabolic crisis that accompanies an mTBI (96), it is possible that reduced GMV in these regions may be associated with specifically compromised glucose metabolism and sleep regulation, while increases in volume may either facilitate or be facilitated by improved sleep. Collectively, findings from those studies, as well as our present findings of decreased volume and increased ESS scores at baseline, suggest that post-mTBI sleep disruption and thalamic, orbitofrontal, and prefrontal volume may be closely linked. However, prospective studies are lacking to identify the directionality of this relationship as well as the effect of mTBI on factors associated with sleep regulation (e.g., glucose metabolism) in these areas.

Blue Light Effects on Functional Connectivity

The present work also extends prior findings on the effects of BLT and functional connectivity. Acute exposure to blue light (even as short as 1 min) modulates task-dependent fMRI activation in regions critical to cognitive control, attention, and memory including the anterior cingulate (45) and dorso- and ventrolateral prefrontal cortex (46, 97, 98) as well as the thalamus and anterior insula (97). These effects have also been observed in completely blind individuals with brief (<1 min) exposures to blue light, indicating that these effects are not dependent on visual perception of blue light (99). Task-dependent modulation is also evident for acute blue light exposure during emotion

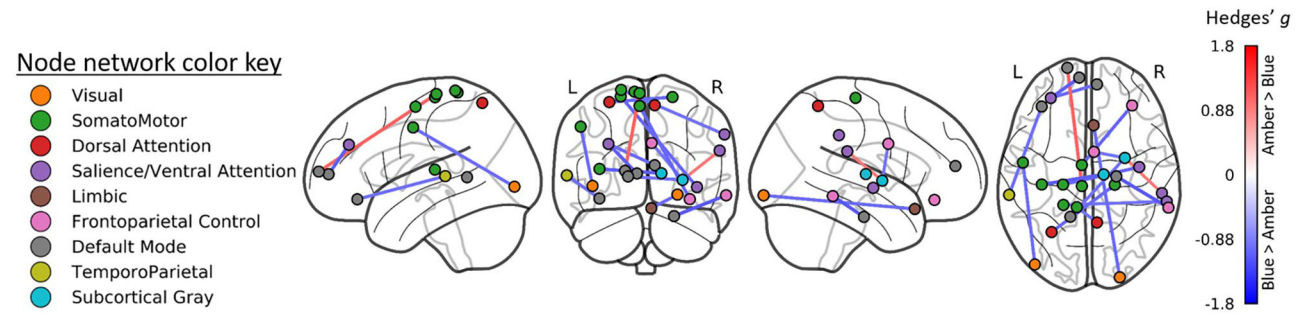

FIGURE 2 | Region-to-region functional connectivity differences following treatment. Blue light treatment was associated with greater functional connectivity in 14 ROI-to-ROI connections linking the visual, default mode, somatomotor, and dorsal attention networks (all FDR $p < 0.01$).

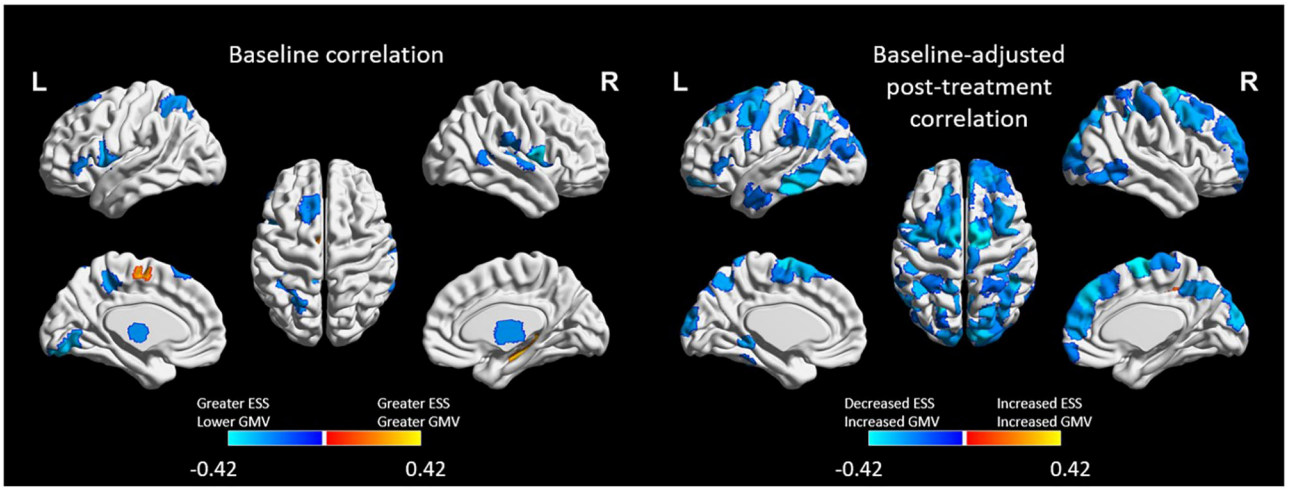

FIGURE 3 | Regions of interest (ROIs) exhibiting moderate-to-large correlations ($|r| > 0.4$) between Epworth Sleepiness Scale scores and gray matter volume (GMV) at baseline (left) and at post-treatment (right). Treatment-related decreases in daytime sleepiness were associated with moderate-to-large increases in GMV in 74 ROIs. All ROIs were FDR corrected at $p < 0.05$.

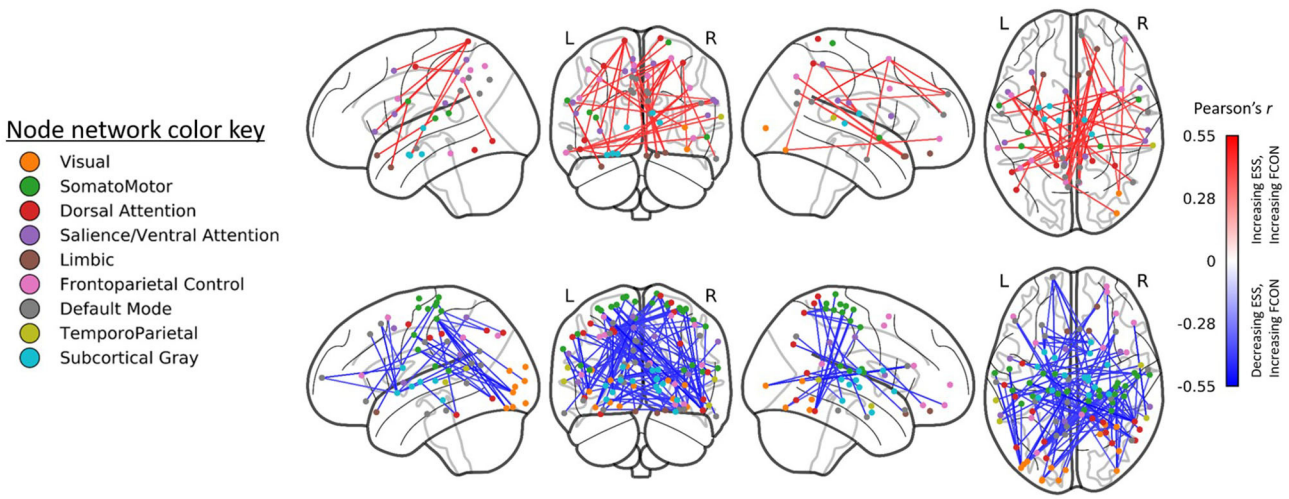

FIGURE 4 | Edges (region-to-region connections) exhibiting moderate-to-large correlations ($|r| > 0.4$) between Epworth Sleepiness Scale scores and gray matter volume (GMV) at post-treatment (right). Treatment-related decreases in daytime sleepiness were associated with moderate-to-large increases in functional connectivity in 82 edges and with decreases in functional connectivity in 39 edges. All edgewise correlations were FDR corrected at $p < 0.05$.

regulation tasks (100). Additionally, and highly relevant to the present work, these acute, task-dependent modulations may be strongly influenced by both circadian phase and sleep pressure (97). Collectively, these prior findings indicate that acute blue light exposure enhances functional activation during cognitive tasks and repeated or prolonged exposure may confer lasting effects. Future work should specifically examine the similarities and differences between acute and repeated blue light exposure on both resting-state and task-dependent activation.

Further, we previously reported increased functional connectivity specifically between the left thalamus and both frontal and parietal cortical areas that was associated with decreased daytime sleepiness following treatment in this population (43). The present findings demonstrate wider influences of blue light therapy on increased coherence between visual, attention, and subcortical gray matter networks, including bilateral thalamocortical connectivity. We further demonstrate greater dissociation between internally and externally oriented networks (i.e., the dorsal vs. ventral attention networks; default mode vs. frontoparietal control networks). Critically, these effects of BLT were associated with observed decreases in daytime sleepiness.

These findings are broadly in line with prior neuroimaging findings linking mTBI and sleep disrupted states. Decreased

and disrupted thalamocortical connectivity—including dorsal attention and frontoparietal control networks—has previously been reported in individuals recovering from a mTBI who report fatigue (25, 101, 102). Additional work indicates that poor quality sleep and fatigue is associated with decreased functional connectivity within the default mode network and increased limbic network functional connectivity (including thalamocortical connectivity) in pediatric mTBI (103). Those studies also indicate that connectivity in the limbic and default mode networks normalizes in parallel with the recovery of cognitive function and sleep.

Sleep Treatments Facilitate mTBI Recovery

The present findings agree both with our prior analyses and more broadly with a recent randomized controlled trial investigating exogenous melatonin supplementation for adolescents recovering from a mTBI (38, 43). Findings from that study on melatonin also demonstrated increases in GMV following treatment that was associated with increased sleep quality (specifically, decreased wake after sleep onset as measured with actigraphy), particularly in the posterior cingulate cortex. Changes in WASO were also associated with increased functional connectivity between the default mode network and attention, visual, and somatosensory networks as well as an overall increase in whole brain functional connectivity (38).

While the exact mechanisms by which either blue light therapy or melatonin would alter brain structure and function remain to be fully elucidated, we posit that this is due primarily to effects on sleep, rather than directly the result of treatment. Recent work suggests that sleep is a critical component of synaptic plasticity (104, 105), and sleep is critical for the formation of oligodendrocyte precursor cells, which form the basis of the myelin sheath (106), which is often damaged in mTBI. Both blue light therapy (as a daytime melatonin suppressor) and melatonin supplementation (as a nighttime sleep-promotor) exert potent effects on circadian rhythm and sleep regulation (107–111). Therefore, it is likely that treatment-mediated changes in circadian rhythm and sleep enable plasticity and modulation of resting-state connectivity in the recovery from a mTBI, which may in turn facilitate clinical recovery, in keeping with the observed relationship between clinical outcomes and functional connectivity normalization. Future work is necessary to more conclusively determine the mechanisms by which these effects are observed.

Limitations

There are several limitations that should be noted in interpreting the present findings. First, we had limited capability to fully ensure treatment compliance and dose. Treatment was completed by participants in their home and required them to position the light box within their peripheral vision each day for 6 weeks. However, across both studies self-reported compliance was high (>80% of treatment sessions completed) and the individuals with the lowest compliance were those who had missing or late recording of treatments. These lapses in self-reporting do not necessarily mean that treatment was not completed, nor does high reporting compliance necessarily indicate strict adherence or truthful reporting. The data here were analyzed with an intention-to-treat design, so that all cases with available data were included, regardless of treatment compliance. Future work in this area should endeavor to develop methods for quantitatively and objectively determining light box treatment compliance.

Second, we enrolled participants ranging from 5 to 80 weeks post-injury. Spontaneous recovery from a mTBI has been noted at least up to 12 weeks post-injury (112) and so some changes here may be attributable to post-injury timing. However, the relationship between weeks post-injury and ESS scores was neither strong nor statistically significant and so the relative impact of treatment timing may be small. Third, no clinical reads were performed and so the presence of unidentified brain pathology (e.g., vascular lesions) may have affected the present findings. However, our sample is comprised of young adults all with diagnosed injury no more severe than mild, so we consider the likelihood of this potential confound to be small.

Fourth, this was not a prospective study and we did not include a non-injured control group as a baseline or post-treatment reference. Therefore, we are unable to determine whether BLT brings individuals recovering from a mTBI closer to a pre-injury or uninjured state from a neuroimaging perspective. However, the purpose the trial was neither to establish the effects of mTBIs on brain structure or function (a baseline comparison to self or an uninjured control group) nor to demonstrate that blue light therapy normalizes to a pre-injury or non-injured state. Rather, the overall purpose of the trial was to demonstrate that, in those individuals self-reporting adverse sleep-related outcomes following injury, blue light therapy is a viable treatment alternative for reducing daytime sleepiness and improving sleep (quantity and quality) as we have previously reported (43, 44), with the present findings as secondary outcomes. Future prospective studies should examine the capacity of BLT to minimize both the neurophysiological effects and long-term sleep disruption stemming from mTBIs relative to pre-injury or uninjured states.

CONCLUSION

For individuals reporting daytime sleepiness following a mild traumatic brain injury, BLT is a non-invasive treatment option that may help to facilitate neural plasticity and hasten recovery. Here, we demonstrated moderate to large increases in both gray matter volume and functional connectivity following blue light therapy in areas previously associated with both sleep regulation and daytime cognitive function, alertness, and attention. Further work is needed to more completely elucidate the exact mechanisms of these changes as well as precision medicine factors that may selectively identify individuals in whom the greatest benefits may be seen. These data add to a growing body of research suggesting that morning BLT may facilitate structural and functional brain recovery among some individuals who have sustained a mTBI.

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 Partners Healthcare, The University of Arizona, and The U.S. Army Human Protections Office. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AR conducted the analyses and drafted the initial manuscript. ND revised the manuscript. BF and AA assisted with data collection, study implementation, and manuscript preparation. WDSK designed the study, analyzed the data, and assisted with manuscript preparation and revision. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by multiple grants to WK from the US Army Medical Research and Development Command (USAMRDC) including W81XWH-11-1-0056 and W81XWH-14-1-0571. The content, opinions, interpretations, conclusions, and recommendations are solely the responsibility of the authors and do not necessarily represent the views of Partners Healthcare, the University of Arizona College of Medicine, the Department of Defense, or the US Army Medical Research and Development Command.

REFERENCES

1. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil*. (2006) 21:375–8. doi: 10.1097/00001199-200609000-00001
2. Faul M, Xu L, Wald MM, Coronado VG. *Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002-2006*. Atlanta (GA): Centers for Disease Control and Prevention, National Center for Injury Prevention and Control (2010). Available online at: https://www.cdc.gov/traumaticbraininjury/tbi_ed.html (accessed April 25, 2015). doi: 10.15620/cdc.5571
3. Kerr Z, Roos K, Dompier T, Dalton S, Djoko A, Broglio S, et al. Estimating concussion incidence in college sports: rates, risks, average per team and proportion of teams with concussions. In: *Brain Injury*. Philadelphia, PA: Taylor & Francis Inc. (2016), p. 504.
4. Defense and Veterans Brain Injury Center. *DoD Worldwide TBI Numbers 2000-2018 Q1*. (2018). Available online at: https://health.mil/Reference-Center/Publications/2020/09/30/2000-2019-DoD-Worldwide-Numbers-for-TBI (accessed August 27, 2019).
5. Pattinson CL, Meier TB, Guedes VA, Lai C, Devoto C, Haight T, et al. Plasma biomarker concentrations associated with return to sport following sport-related concussion in collegiate athletes—A Concussion Assessment, Research, and Education (CARE) Consortium Study. *JAMA Netw Open*. (2020) 3:e2013191. doi: 10.1001/jamanetworkopen.2020.13191
6. Master CL, Podolak OE, Ciuffreda KJ, Metzger KB, Joshi NR, McDonald CC, et al. Utility of pupillary light reflex metrics as a physiologic biomarker for adolescent sport-related concussion. *JAMA Ophthalmol*. (2020) 138:1135–41. doi: 10.1001/jamaophthalmol.2020.3466
7. Meyer J, Bartolomei C, Sauer A, Sajatovic M, Bailey CM. The relationship between fluid biomarkers and clinical outcomes in sports-related concussions: a systematic review. *Brain Inj*. (2020) 34:1435–45. doi: 10.1080/02699052.2020.1802780
8. Collins MW, Kontos AP, Okonkwo DO, Almquist J, Bailes J, Barisa M, et al. Statements of agreement from the Targeted Evaluation and Active Management (TEAM) approaches to treating concussion meeting held in Pittsburgh, October 15–16, 2015. *Neurosurgery*. (2016) 79:912–29. doi: 10.1227/NEU.0000000000001447
9. Kontos AP, Sufrinko A, Sandel N, Emami K, Collins MW. Sport-related concussion clinical profiles: clinical characteristics, targeted treatments, and preliminary evidence. *Curr Sports Med Rep*. (2019) 18:82–92. doi: 10.1249/JSR.0000000000000573
10. Garcia G-GP, Lavieri MS, Jiang R, McAllister TW, McCrea MA, Broglio SP. A data-driven approach to unlikely, possible, probable, and definite acute concussion assessment. *J Neurotrauma*. (2018) 36:1571–83. doi: 10.1089/neu.2018.6098
11. Kontos AP, Collins MW, Holland CL, Reeves VL, Edelman K, Benso S, et al. Preliminary evidence for improvement in symptoms, cognitive, vestibular, and oculomotor outcomes following targeted intervention with chronic mTBI patients. *Mil Med*. (2018) 183:333–8. doi: 10.1093/milmed/usx172
12. Dobney DM, Grilli L, Kocilowicz H, Beaulieu C, Straub M, Friedman D, et al. Evaluation of an active rehabilitation program for concussion management in children and adolescents. *Brain Inj*. (2017) 31:1753–9. doi: 10.1080/02699052.2017.1346294
13. Dobney DM, Miller MB, Tufts E. Non-pharmacological rehabilitation interventions for concussion in children: a scoping review. *Disabil Rehabil*. (2019) 41:727–39. doi: 10.1080/09638288.2017.1400595
14. Leddy JJ, Haider MN, Ellis MJ, Mannix R, Darling SR, Freitas MS, et al. Early subthreshold aerobic exercise for sport-related concussion: a randomized clinical trial. *JAMA Pediatr*. (2019) 173:319–25. doi: 10.1001/jamapediatrics.2018.4397
15. Mantua J, Grillakis A, Mahfouz SH, Taylor MR, Brager AJ, Yarnell AM, et al. A systematic review and meta-analysis of sleep architecture and chronic traumatic brain injury. *Sleep Med Rev*. (2018) 41:61–77. doi: 10.1016/j.smrv.2018.01.004
16. Theadom A, Cropley M, Parmar P, Barker-Collo S, Starkey N, Jones K, et al. Sleep difficulties one year following mild traumatic brain injury in a population-based study. *Sleep Med*. (2015) 16:926–32. doi: 10.1016/j.sleep.2015.04.013
17. Farrell-Carnahan L, Franke L, Graham C, McNamee S. Subjective sleep disturbance in veterans receiving care in the veterans affairs polytrauma system following blast-related mild traumatic brain injury. *Mil Med*. (2013) 178:951–6. doi: 10.7205/MILMED-D-13-00037
18. Farrell-Carnahan L, Barnett S, Lamberty G, Hammond FM, Kretzmer TS, Franke LM, et al. Insomnia symptoms and behavioural health symptoms in veterans 1 year after traumatic brain injury. *Brain Inj*. (2015) 29:1400–8. doi: 10.3109/02699052.2015.1063161
19. Wiseman-Hakes C, Gosselin N, Sharma B, Langer L, Gagnon I. A longitudinal investigation of sleep and daytime wakefulness in children and youth with concussion. *ASN Neuro*. (2019) 11:1759091418822405. doi: 10.1177/1759091418822405
20. Brooks BL, Sayers P, Virani S, Rajaram A, Tomfohr L. Insomnia in adolescents with slow recovery from concussion. *J Neurotrauma*. (2019) 36:2391–9. doi: 10.1089/neu.2018.6257
21. Ouellet M-C, Beaulieu-Bonneau S, Morin CM. Insomnia in patients with traumatic brain injury: frequency, characteristics, and risk factors. *J Head Trauma Rehabil*. (2006) 21:199–212. doi: 10.1097/00001199-200605000-00001

22. Williams BR, Lazic SE, Ogilvie RD. Polysomnographic and quantitative EEG analysis of subjects with long-term insomnia complaints associated with mild traumatic brain injury. *Clin Neurophysiol*. (2008) 119:429–38. doi: 10.1016/j.clinph.2007.11.003
23. Raikes AC, Schaefer SY. Sleep quantity and quality during acute concussion: a pilot study. *Sleep*. (2016) 39:2141–7. doi: 10.5665/sleep.6314
24. Hoffman NL, O'Connor PJ, Schmidt MD, Lynall RC, Schmidt JD. Differences in sleep between concussed and nonconcussed college students: a matched case–control study. *Sleep*. (2018) 42:1–11. doi: 10.1093/sleep/zsy222
25. Nordin LE, Möller MC, Julin P, Bartfai A, Hashim F, Li T-Q. Post mTBI fatigue is associated with abnormal brain functional connectivity. *Sci Rep*. (2016) 6:21183. doi: 10.1038/srep21183
26. Combs PR, Ford CB, Campbell KR, Carneiro KA, Mihalik JP. Influence of self-reported fatigue and sex on baseline concussion assessment scores. *Orthop J Sports Med*. (2019) 7:2325967118817515. doi: 10.1177/2325967118817515
27. Raikes AC, Athey A, Alfonso-Miller P, Killgore WDS, Grandner MA. Insomnia and daytime sleepiness: risk factors for sports-related concussion. *Sleep Med*. (2019) 58:66–74. doi: 10.1016/j.sleep.2019.03.008
28. Sullivan KA, Blaine H, Kaye S-A, Theadom A, Haden C, Smith SS. A systematic review of psychological interventions for sleep and fatigue after mild traumatic brain injury. *J Neurotrauma*. (2017) 35:195–209. doi: 10.1089/neu.2016.4958
29. Ludwig R, D'Silva L, Vaduvathiriyan P, Rippee MA, Siengsukon C. Sleep disturbances in the acute stage of concussion are associated with poorer long-term recovery: a systematic review. *PM&R*. (2020) 12:500–11. doi: 10.1002/pmrj.12309
30. Chung JS, Zynda AJ, Didehbani N, Hicks C, Hynan LS, Miller SM, et al. Association between sleep quality and recovery following sport-related concussion in pediatrics. *J Child Neurol*. (2019) 34:639–45. doi: 10.1177/0883073819849741
31. Howell DR, Oldham JR, Brilliant AN, Meehan WP. Trouble falling asleep after concussion is associated with higher symptom burden among children and adolescents. *J Child Neurol*. (2019) 34: 256–61. doi: 10.1177/0883073818824000
32. Oyegbile TO, Dougherty A, Tanveer S, Zecavati N, Delasobera BE. High sleep disturbance and longer concussion duration in repeat concussions. *Behav Sleep Med*. (2020) 18:241–8. doi: 10.1080/15402002.2019.1578223
33. Bloomfield ILM, Espie CA, Evans JJ. Do sleep difficulties exacerbate deficits in sustained attention following traumatic brain injury? *J Int Neuropsychol Soc*. (2010) 16:17–25. doi: 10.1017/S1355617709990798
34. Mantua J, Mahan KM, Henry OS, Spencer RMC. Altered sleep composition after traumatic brain injury does not affect declarative sleep-dependent memory consolidation. *Front Hum Neurosci*. (2015) 9:328. doi: 10.3389/fnhum.2015.00328
35. Mantua J, Henry OS, Garskovas NF, Spencer RMC. Mild traumatic brain injury chronically impairs sleep- and wake-dependent emotional processing. *Sleep*. (2017) 40:zsx062. doi: 10.1093/sleep/zsx062
36. Kostyun RO, Milewski MD, Hafeez I. Sleep disturbance and neurocognitive function during the recovery from a sport-related concussion in adolescents. *Am J Sports Med*. (2015) 43:633–40. doi: 10.1177/0363546514560727
37. Sufrinko A, Pearce K, Elbin RJ, Covassin T, Johnson E, Collins M, et al. The effect of preinjury sleep difficulties on neurocognitive impairment and symptoms after sport-related concussion. *Am J Sports Med*. (2015) 43:830–8. doi: 10.1177/0363546514566193
38. Iyer KK, Zalesky A, Cocchi L, Barlow KM. Neural correlates of sleep recovery following melatonin treatment for pediatric concussion: a randomized controlled trial. *J Neurotrauma*. (2020) 37:2647–55. doi: 10.1089/neu.2020.7200
39. Jha A, Weintraub A, Allshouse A, Morey C, Cusick C, Kittelson J, et al. A randomized trial of modafinil for the treatment of fatigue and excessive daytime sleepiness in individuals with chronic traumatic brain injury. *J Head Trauma Rehabil*. (2008) 23:52. doi: 10.1097/01.HTR.0000308721.77911.ea
40. Lequerica A, Jasey N, Portelli Tremont JN, Chiaravalloti ND. Pilot study on the effect of ramelteon on sleep disturbance after traumatic brain injury: preliminary evidence from a clinical trial. *Arch Phys Med Rehabil*. (2015) 96:1802–9. doi: 10.1016/j.apmr.2015.05.011
41. Killgore WDS. Lightening the mood: evidence for blue light exposure in the treatment of post-concussion depression. *Expert Rev Neurother*. (2020) 20:1081–3. doi: 10.1080/14737175.2020.1814147
42. Sinclair KL, Ponsford JL, Rajaratnam SM. Actigraphic assessment of sleep disturbances following traumatic brain injury. *Behav Sleep Med*. (2014) 12:13–27. doi: 10.1080/15402002.2012.726203
43. Killgore WDS, Vanuk JR, Shane BR, Weber M, Bajaj S. A randomized, double-blind, placebo-controlled trial of blue wavelength light exposure on sleep and recovery of brain structure, function, and cognition following mild traumatic brain injury. *Neurobiol Dis*. (2020) 134:104679. doi: 10.1016/j.nbd.2019.104679
44. Raikes AC, Dailey NS, Shane BR, Forbeck B, Alkozei A, Killgore WDS. Daily morning blue light therapy improves daytime sleepiness, sleep quality, and quality of life following a mild traumatic brain injury. *J Head Trauma Rehabil*. (2020) 35:E405–21. doi: 10.1097/HTR.0000000000000579
45. Alkozei A, Smith R, Killgore WDS. Exposure to blue wavelength light modulates anterior cingulate cortex activation in response to 'uncertain' versus 'certain' anticipation of positive stimuli. *Neurosci Lett*. (2016) 616:5–10. doi: 10.1016/j.neulet.2016.01.034
46. Alkozei A, Smith R, Pisner DA, Vanuk JR, Berryhill SM, Fridman A, et al. Exposure to blue light increases subsequent functional activation of the prefrontal cortex during performance of a working memory task. *Sleep*. (2016) 39:1671–80. doi: 10.5665/sleep.6090
47. Killgore WDS, Dailey NS, Raikes AC, Vanuk JR, Taylor E, Alkozei A. Blue light exposure enhances neural efficiency of the task positive network during a cognitive interference task. *Neurosci Lett*. (2020) 735:135242. doi: 10.1016/j.neulet.2020.135242
48. Alkozei A, Smith R, Dailey NS, Bajaj S, Killgore WDS. Acute exposure to blue wavelength light during memory consolidation improves verbal memory performance. *PLoS ONE*. (2017) 12:e0184884. doi: 10.1371/journal.pone.0184884
49. Bajaj S, Vanuk JR, Smith R, Dailey NS, Killgore WDS. Blue-light therapy following mild traumatic brain injury: effects on white matter water diffusion in the brain. *Front Neurol*. (2017) 8:616. doi: 10.3389/fneur.2017.00616
50. Raikes AC, Satterfield BC, Killgore WDS. Evidence of actigraphic and subjective sleep disruption following mild traumatic brain injury. *Sleep Med*. (2019) 54:62–9. doi: 10.1016/j.sleep.2018.09.018
51. Management of Concussion/mTBI Working Group. V*A/DoD Clinical Practice Guideline for Management of Concussion-Mild Traumatic Brain Injury*. (2016). Available online at: https://www.healthquality.va.gov/guidelines/Rehab/mtbi/mTBICPGFullCPG50821816.pdf (accessed July 27, 2018).
52. Bogner J, Corrigan JD. Reliability and predictive validity of the Ohio State University TBI identification method with prisoners. *J Head Trauma Rehabil*. (2009) 24:279. doi: 10.1097/HTR.0b013e3181a66356
53. Corrigan JD, Bogner J. Initial reliability and validity of the Ohio State University TBI identification method. *J Head Trauma Rehabil*. (2007) 22:318. doi: 10.1097/01.HTR.0000300227.67748.77
54. Schaefer A, Kong R, Gordon EM, Laumann TO, Zuo X-N, Holmes AJ, et al. Local-global parcellation of the human cerebral cortex from intrinsic functional connectivity MRI. *Cereb Cortex*. (2018) 28:3095–114. doi: 10.1093/cercor/bhx179
55. Fan L, Li H, Zhuo J, Zhang Y, Wang J, Chen L, et al. The human brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb Cortex*. (2016) 26:3508–26. doi: 10.1093/cercor/bhw157
56. Buckner RL, Krienen FM, Castellanos A, Diaz JC, Yeo BTT. The organization of the human cerebellum estimated by intrinsic functional connectivity. *J Neurophysiol*. (2011) 106:2322–45. doi: 10.1152/jn.00339.2011
57. Mohajer B, Abbasi N, Mohammadi E, Khazaie H, Osorio RS, Rosenzweig I, et al. Gray matter volume and estimated brain age gap are not linked with sleep-disordered breathing. *Hum Brain Mapp*. (2020) 41:3034–44. doi: 10.1002/hbm.24995
58. Yeo BTT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol*. (2011) 106:1125–65. doi: 10.1152/jn.00338.2011

59. Esteban O, Markiewicz CJ, Blair RW, Moodie CA, Isik AI, Erramuzpe A, et al. fMRIPrep: a robust preprocessing pipeline for functional MRI. *Nat Methods*. (2019) 16:111. doi: 10.1038/s41592-018-0235-4
60. Esteban O, Blair R, Markiewicz CJ, Berleant SL, Moodie C, Ma F, et al. *fMRIPrep: A Robust Preprocessing Pipeline for Functional MRI*. Zenodo (2019). doi: 10.1101/306951
61. Gorgolewski K, Burns CD, Madison C, Clark D, Halchenko YO, Waskom ML, et al. Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python. *Front Neuroinform*. (2011) 5:13. doi: 10.3389/fninf.2011.00013
62. Esteban O, Markiewicz CJ, Burns C, Goncalves M, Jarecka D, Ziegler E, et al. *nipy/nipype: 1.5.1*. Zenodo (2020).
63. Tustison NJ, Avants BB, Cook PA, Zheng Y, Egan A, Yushkevich PA, et al. N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging*. (2010) 29:1310–20. doi: 10.1109/TMI.2010.2046908
64. Avants BB, Epstein CL, Grossman M, Gee JC. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Anal*. (2008) 12:26–41. doi: 10.1016/j.media.2007.06.004
65. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging*. (2001) 20:45–57. doi: 10.1109/42.906424
66. Reuter M, Rosas HD, Fischl B. Highly accurate inverse consistent registration: a robust approach. *NeuroImage*. (2010) 53:1181–96. doi: 10.1016/j.neuroimage.2010.07.020
67. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis: I. Segmentation and surface reconstruction. *NeuroImage*. (1999) 9:179–94. doi: 10.1006/nimg.1998.0395
68. Klein A, Ghosh SS, Bao FS, Giard J, Häme Y, Stavsky E, et al. Mindboggling morphometry of human brains. *PLoS Comput Biol*. (2017) 13:e1005350. doi: 10.1371/journal.pcbi.1005350
69. Fonov V, Evans A, McKinstry R, Almli C, Collins D. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *NeuroImage*. (2009) 47:S102. doi: 10.1016/S1053-8119(09)70884-5
70. Evans AC, Janke AL, Collins DL, Baillet S. Brain templates and atlases. *NeuroImage*. (2012) 62:911–22. doi: 10.1016/j.neuroimage.2012.01.024
71. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*. (2002) 17:825–41. doi: 10.1006/nimg.2002.1132
72. Cox RW, Hyde JS. Software tools for analysis and visualization of fMRI data. *NMR Biomed*. (1997) 10:171–8
73. Glasser MF, Sotiropoulos SN, Wilson JA, Coalson TS, Fischl B, Andersson JL, et al. The minimal preprocessing pipelines for the Human Connectome Project. *NeuroImage*. (2013) 80:105–24. doi: 10.1016/j.neuroimage.2013.04.127
74. Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. *NeuroImage*. (2009) 48:63–72. doi: 10.1016/j.neuroimage.2009.06.060
75. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. *NeuroImage*. (2014) 84:320–41. doi: 10.1016/j.neuroimage.2013.08.048
76. Behzadi Y, Restom K, Liau J, Liu TT. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *NeuroImage*. (2007) 37:90–101. doi: 10.1016/j.neuroimage.2007.04.042
77. Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughead J, Calkins ME, et al. An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. *NeuroImage*. (2013) 64:240–56. doi: 10.1016/j.neuroimage.2012.08.052
78. Lanczos C. Evaluation of noisy data. *J Soc Ind Appl Math Ser B Numerical Anal*. (1964) 1:76–85. doi: 10.1137/0701007
79. Abraham A, Pedregosa F, Eickenberg M, Gervais P, Mueller A, Kossaifi J, et al. Machine learning for neuroimaging with scikit-learn. *Front Neuroinform*. (2014) 8:14. doi: 10.3389/fninf.2014.00014
80. Ciric R, Wolf DH, Power JD, Roalf DR, Baum GL, Ruparel K, et al. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. *NeuroImage*. (2017) 154:174–87. doi: 10.1016/j.neuroimage.2017.03.020
81. Fortin J-P, Cullen N, Sheline YI, Taylor WD, Aselcioglu I, Cook PA, et al. Harmonization of cortical thickness measurements across scanners and sites. *NeuroImage*. (2018) 167:104–20. doi: 10.1016/j.neuroimage.2017.11.024
82. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing (2018). Available online at: https://www.R-project.org/ (accessed November 30, 2020).
83. Fortin J-P, Parker D, Tunç B, Watanabe T, Elliott MA, Ruparel K, et al. Harmonization of multi-site diffusion tensor imaging data. *NeuroImage*. (2017) 161:149–70. doi: 10.1016/j.neuroimage.2017.08.047
84. Yu M, Linn KA, Cook PA, Phillips ML, McInnis M, Fava M, et al. Statistical harmonization corrects site effects in functional connectivity measurements from multi-site fMRI data. *Hum Brain Mapp*. (2018) 39:4213–27. doi: 10.1002/hbm.24241
85. Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. Moving beyond P values: data analysis with estimation graphics. *Nat Methods*. (2019) 16:565–6. doi: 10.1101/377978
86. Xia M, Wang J, He Y. BrainNet viewer: a network visualization tool for human brain connectomics. *PLoS ONE*. (2013) 8:e68910. doi: 10.1371/journal.pone.0068910
87. Johns M, Hocking B. Daytime sleepiness and sleep habits of Australian workers. *Sleep*. (1997) 20:844–7. doi: 10.1093/sleep/20.10.844
88. Killgore WD, Vanuk JR, Knight SA, Markowski SM, Pisner D, Shane B, et al. Daytime sleepiness is associated with altered resting thalamocortical connectivity. *Neuroreport*. (2015) 26:779–84. doi: 10.1097/WNR.0000000000000418
89. Clark AL, Sorg SF, Holiday K, Bigler ED, Bangen KJ, Evangelista ND, et al. Fatigue is associated with global and regional thalamic morphometry in veterans with a history of mild traumatic brain injury. *J Head Trauma Rehabil*. (2018) 33:382–92. doi: 10.1097/HTR.0000000000000377
90. Munivenkatappa A, Devi BI, Shukla DP, Rajeswaran J. Role of the thalamus in natural recovery of cognitive impairment in patients with mild traumatic brain injury. *Brain Inj*. (2016) 30:388–92. doi: 10.3109/02699052.2015.1089599
91. Altena E, Vrenken H, Werf YDVD, Heuvel OA van den, Someren EJWV. Reduced orbitofrontal and parietal gray matter in chronic insomnia: a voxel-based morphometric study. *Biol Psychiatry*. (2010) 67:182–5. doi: 10.1016/j.biopsych.2009.08.003
92. Stoffers D, Moens S, Benjamins J, van Tol M-J, Penninx BWHJ, Veltman DJ, et al. Orbitofrontal gray matter relates to early morning awakening: a neural correlate of insomnia complaints? *Front Neurol*. (2012) 3:105. doi: 10.3389/fneur.2012.00105
93. Dai X-J, Jiang J, Zhang Z, Nie X, Liu B-X, Pei L, et al. Plasticity and susceptibility of brain morphometry alterations to insufficient sleep. *Front Psychiatry*. (2018) 9:266. doi: 10.3389/fpsyt.2018.00266
94. Thomas M, Sing H, Belenky G, Holcomb H, Mayberg H, Dannals R, et al. Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J Sleep Res*. (2000) 9:335–52. doi: 10.1046/j.1365-2869.2000.00225.x
95. Elmenhorst D, Meyer PT, Winz OH, Matusch A, Ermert J, Coenen HH, et al. Sleep deprivation increases A1 adenosine receptor binding in the human brain: a positron emission tomography study. *J Neurosci*. (2007) 27:2410–5. doi: 10.1523/JNEUROSCI.5066-06.2007
96. Giza CC, Hovda DA. The new neurometabolic cascade of concussion. *Neurosurgery*. (2014) 75:S24–33. doi: 10.1227/NEU.0000000000000505
97. Vandewalle G, Archer SN, Wuillaume C, Balteau E, Degueldre C, Luxen A, et al. Effects of light on cognitive brain responses depend on circadian phase and sleep homeostasis. *J Biol Rhythms*. (2011) 26:249–59. doi: 10.1177/0748730411401736
98. Vandewalle G, Gais S, Schabus M, Balteau E, Carrier J, Darsaud A, et al. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure. *Cereb Cortex*. (2007) 17:2788–95. doi: 10.1093/cercor/bhm007
99. Vandewalle G, Collignon O, Hull JT, Daneault V, Albouy G, Lepore F, et al. Blue light stimulates cognitive brain activity in visually blind individuals. *J Cogn Neurosci*. (2013) 25:2072–85. doi: 10.1162/jocn_a_00450

100. Vandewalle G, Schwartz S, Grandjean D, Wuillaume C, Balteau E, Degueldre C, et al. Spectral quality of light modulates emotional brain responses in humans. *PNAS*. (2010) 107:19549–54. doi: 10.1073/pnas.1010180107
101. Tang L, Ge Y, Sodickson DK, Miles L, Zhou Y, Reaume J, et al. Thalamic resting-state functional networks: disruption in patients with mild traumatic brain injury. *Radiology*. (2011) 260:831–40. doi: 10.1148/radiol.11110014
102. Banks SD, Coronado RA, Clemons LR, Abraham CM, Pruthi S, Conrad BN, et al. Thalamic functional connectivity in mild traumatic brain injury: longitudinal associations with patient-reported outcomes and neuropsychological tests. *Arch Phys Med Rehabil*. (2016) 97:1254–61. doi: 10.1016/j.apmr.2016.03.013
103. Iyer KK, Barlow KM, Brooks B, Ofoghi Z, Zalesky A, Cocchi L. Relating brain connectivity with persistent symptoms in pediatric concussion. *Ann Clin Transl Neurol*. (2019) 6:954–61. doi: 10.1002/acn3.764
104. Puentes-Mestril C, Aton SJ. Linking network activity to synaptic plasticity during sleep: hypotheses and recent data. *Front Neural Circuits*. (2017) 11:61. doi: 10.3389/fncir.2017.00061
105. Cirelli C, Tononi G. Linking the need to sleep with synaptic function. *Science*. (2019) 366:189–90. doi: 10.1126/science.aay5304
106. Bellesi M, Pfister-Genskow M, Maret S, Keles S, Tononi G, Cirelli C. Effects of Sleep and wake on oligodendrocytes and their precursors. *J Neurosci*. (2013) 33:14288–300. doi: 10.1523/JNEUROSCI.5102-12.2013
107. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. (2002) 295:1070–3. doi: 10.1126/science.1067262
108. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau K-W, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. (2006) 497:326–49. doi: 10.1002/cne.20970
109. Dewan K, Benloucif S, Reid K, Wolfe LF, Zee PC. Light-induced changes of the circadian clock of humans: increasing duration is more effective than increasing light intensity. *Sleep*. (2011) 34:593–9. doi: 10.1093/sleep/34.5.593
110. Cajochen C, Kräuchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol*. (2003) 15:432–7. doi: 10.1046/j.1365-2826.2003.00989.x
111. Williams WP (Trey), McLin DE, Dressman MA, Neubauer DN. comparative review of approved melatonin agonists for the treatment of circadian rhythm sleep-wake disorders. *Pharmacotherapy*. (2016) 36:1028–41. doi: 10.1002/phar.1822
112. Williams RM, Puetz TW, Giza CC, Broglio SP. Concussion recovery time among high school and collegiate athletes: a systematic review and meta-analysis. *Sports Med*. (2015) 45:893–903. doi: 10.1007/s40279-015-0325-8

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

HYPOTHESIS AND THEORY
published: 11 February 2021
doi: 10.3389/fneur.2021.627550

Circadian Responses to Light-Flash Exposure: Conceptualization and New Data Guiding Future Directions

Kwoon Y. Wong[1,2*] *and Fabian-Xosé Fernandez*[3,4*]

[1] Department of Molecular, Cellular, & Developmental Biology, University of Michigan, Ann Arbor, MI, United States, [2] Department of Ophthalmology & Visual Sciences, University of Michigan, Ann Arbor, MI, United States, [3] Department of Psychology, BIO5 Research Institute, University of Arizona, Tucson, AZ, United States, [4] Department of Neurology, McKnight Brain Research Institute, University of Arizona, Tucson, AZ, United States

A growing number of studies document circadian phase-shifting after exposure to millisecond light flashes. When strung together by intervening periods of darkness, these stimuli evoke pacemaker responses rivaling or outmatching those created by steady luminance, suggesting that the circadian system's relationship to light can be contextualized outside the principle of simple dose-dependence. In the current review, we present a brief chronology of this work. We then develop a conceptual model around it that attempts to relate the circadian effects of flashes to a natural integrative process the pacemaker uses to intermittently sample the photic information available at dawn and dusk. Presumably, these snapshots are employed as building blocks in the construction of a coherent representation of twilight the pacemaker consults to orient the next day's physiology (in that way, flash-resetting of pacemaker rhythms might be less an example of a circadian visual illusion and more an example of the kinds of gestalt inferences that the image-forming system routinely makes when identifying objects within the visual field; i.e., closure). We conclude our review with a discussion on the role of cones in the pacemaker's twilight predictions, providing new electrophysiological data suggesting that classical photoreceptors—but not melanopsin—are necessary for millisecond, intermediate-intensity flash responses in ipRGCs (intrinsically photosensitive retinal ganglion cells). Future investigations are necessary to confirm this "Cone Sentinel Model" of circadian flash-integration and twilight-prediction, and to further define the contribution of cones vs. rods in transducing pacemaker flash signals.

Keywords: light, circadian, rhythms, photostimulation, flash, retina, photoreceptors, ipRGC

OPEN ACCESS

Edited by:
Victoria Susan Pelak, University of Colorado, United States

Reviewed by:
Chiara La Morgia, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Institute of Neurological Sciences of Bologna (ISNB), Italy
Christophe P. Ribelayga, University of Texas Health Science Center at Houston, United States
Kenkichi Baba, Morehouse School of Medicine, United States

****Correspondence:***
Kwoon Y. Wong
kwoon@umich.edu
Fabian-Xosé Fernandez
FabianF@email.arizona.edu

Specialty section:
This article was submitted to Neuro-Ophthalmology, a section of the journal Frontiers in Neurology

Received: *09 November 2020*
Accepted: *21 January 2021*
Published: *11 February 2021*

Citation:
Wong KY and Fernandez F-X (2021) Circadian Responses to Light-Flash Exposure: Conceptualization and New Data Guiding Future Directions. Front. Neurol. 12:627550. doi: 10.3389/fneur.2021.627550

INTRODUCTION

The retina integrates light signals detected across a tripartite network of photoreceptors to convey time-of-day information related to the Earth's rotation and solar cycle directly to the brain's circadian pacemaker, the suprachiasmatic nucleus (SCN) (1–5). Grounded within the crossroads of this light-detection system is a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) containing the vitamin A-based photopigment, melanopsin (6–14). ipRGCs are recurrently configured in the eye. By virtue of melanopsin expression, these cells are themselves photo-excitable and can operate as independent relays to the SCN, but—nevertheless—also receive synaptic connections from rods and cones (15–24), and participate in a centrifugal feedback

pathway involving several types of retinal amacrine interneurons (25, 26). As an emergent unit, the whole of the retinal circuitry that comprises the (non-image forming) input to the SCN is extremely flexible in its reading of ambient light across various intensities, spectra, and patterns of contrast. While it is appreciated that all photoreceptor classes make contributions to irradiance detection and are activated across an overlapping range of wavelengths (27–31), there is particular specialization with regards to each's role in signaling contact with steady (i.e., non-flickering) vs. intermittent light.

For example, ipRGCs provide a sustained signal to the SCN throughout the duration of exposure to a discrete light stimulus lasting up to several hours (32–38); cones, on the other hand, amplify signaling only at the beginning of the exposure owing to their transient response within the first <1 s of light-onset (39–44). This functional dissociation is evident in electrophysiological retinal recordings of ipRGCs (8, 17, 19), single-unit recordings of SCN neurons (33, 41), as well as behavioral comparisons between rodents with selective loss of cones vs. wildtype animals. In the latter case, cone-deficient mice exhibit full-magnitude phase shifts to 15-min but not 1-min light administration (480 nm) (40). Conversely, cone-activating light fails to phase-shift the rodent pacemaker as a *continuous* 15-min stimulus but does so when presented *intermittently* along 15 separated 1-min steps over an hour (42). These aggregated data suggest a wider truth about the circadian pacemaker's timekeeping estimates. They are based on two superimposed changes in incident light: (1) the slow intensity variation of sunlight that marks the day's movement through the morning and afternoon and that which separates the day from the night (∼10–12 h; weighted toward melanopsin function); and (2) the higher-frequency changes in irradiance and spectrum that punctuate twilight interludes at dawn/dusk (∼30–60 min; weighted toward cone function).

The pacemaker's phase responses to the same light stimulus (e.g., a 15-min pulse) change systematically across the subjective night along a sigmoidal-like wave (45). In the vast majority of species that have been surveyed, light administration in the first half of the night will produce phase delays in behavioral-physiological rhythms commensurate with the difference in timing between the photic stimulation and the timing of dusk in the solar cycle or lights-out within an indoor light-dark schedule [e.g., in humans, lab rodents and *Drosophila*, introduction of the light stimulus 2 h after subjective sunset will delay rhythms by up to 2 h; (46–48)]. The reciprocal is observed in the second half of the night, where light administration will advance rhythms in proportion to how much earlier the light was seen with respect to expected sunrise [e.g., stimulation 2 h before sunrise or lights-on will fast-forward the onset of diurnal physiology and behavior by up to 2 h; (46–48)]. When describing the circadian pacemaker's *phase response curve* (PRC) to light, many commentators note the technical shape of the PRC in passing or the relationship it might bear to a biological phenomenon of interest. Few point out the bigger picture: the circadian PRC to light is arguably the most demonstrable example of the brain's prediction coding.

Predictive processing is a mature field of inquiry in psychology and cognitive neuroscience (49–52), where diverse methodologies have established the brain as a prospection device that interprets sensory information with the express purpose of generating expectations—and thereby obtaining a level of preparedness—for the immediately relevant future (49–61). Early studies of prediction coding or "sensory anticipation" were primarily motivated by experiments that attempted to resolve fundamental questions about how the visual field manages to remain stable with the constant image-displacement introduced by physical activity, head and eye movements, and blinking (62, 63). At about the same time as these models of primary vision were conceived, species-generalizable PRCs-to-light had been developed across several experimental organisms occupying different ecological and temporal niches within the biosphere (45, 64, 65). Ironically, study of the non-image forming visual system had produced a wealth of empirical data (not to mention the resounding image of the PRC itself) attesting to the brain's prediction-making capabilities and its raison d'être in reducing the ongoing discrepancies occurring between expectation and actual experience. Yet, it was in the field of perceptual vision research that inference, prediction, and information-seeking became topics of intense scrutiny and now look to embody cutting-edge algorithms for machine vision and artificial intelligence [e.g., (66)].

Organisms were pressured to evolve a circadian timekeeping system that could make predictions about the environment because, ultimately, an inability to do so meant life or death vis-à-vis finding food, staying temperature-regulated, and avoiding predators. While direct responses to light independent of such a timekeeping mechanism (e.g., masking) would effectively restrict animals to a nocturnal or diurnal niche (67), they would not be sufficient for preparing and optimizing vast, interconnected areas of organismal physiology for times-of-day when—for example—food might be most readily available and digested or sleep might be most biologically restorative. Regarding entrainment, we have lost sight of these stakes and the inferences that came along with them—namely, that photodetection mechanisms in the service of the circadian pacemaker are likely to be highly flexible in the light information they use to localize sunset or sunrise. Evolutionary pressure not only coaxed the advent of an entrainable clock but also created a race to the bottom for sunlight detection. Organisms who won-out were able to use the least amount of light information in the service of entrainment and could interpret that information accurately whether it resulted from consistent or erratic contact with sunlight. Successful entrainment did not require a prolonged "sitting" audience with midday or twilight and, for some animals, could be achieved (well-enough) within their natural habitats by a few minutes' exposure once or twice a day (68, 69).

DYNAMIC LIGHT AND THE CIRCADIAN PACEMAKER

Research has established the lower floors of circadian photoentrainment in laboratory models such as *Drosophila* and mice (70–72). Data suggest that most animals can synchronize and maintain a stable phase relationship to a 12-h light-dark schedule with an irradiance of <1 nW/cm^2 or with

skeleton photoperiods consisting of ∼11-h intervals of darkness bookended by a pair of 30-min light pulses simulating dusk and dawn (70–75). Despite the appreciation that entrainment requires little in the way of photic energy, there is still a lingering assumption among chronobiologists that light-induced phase shifting demands a relatively large energy investment to trigger complete resetting of endogenous rhythmicity. This perspective is best couched by the reciprocity hypothesis, which asserts that the size of any phase-shift is directly proportional to the time-integrated illuminance the pacemaker registers from a light signal (76, 77). However, extant data suggest that light's association with circadian timekeeping is more complicated.

Emission technology in the 20th and early 21st centuries rarely offered control systems equipped to deliver light in a rapid, intermittent, and multidimensional fashion, where all physical exposure variables could be manipulated at once in quick successive steps. Within the technology milieu, however, were movie/photography studio devices that could produce microsecond and millisecond xenon flashes at fixed frequencies. These devices are still used today in order to illuminate and visualize color in dark scenes (e.g., Metz Mecablitz and DynaLite units). Over the past 60 years, several investigators have also used them to examine the phase-shifting effects of flashes in organisms as diverse as *Drosophila*, hamsters, rats, mice, and humans (78–86). The findings collated from these studies have established that the metazoan circadian pacemaker responds to intermittent millisecond flashes with phase shifts comparable to those that would have been generated with continuous, uninterrupted light administration, provided that the stimuli are delivered at regular intervals every few seconds or each minute. What's more, each circadian hour of the subjective night is equally amenable to flash stimulation; xenon-flash PRCs have been compiled for the eclosion rhythm of *Drosophila pseudoobscura* (79) and the flight activity of the Schneider's roundleaf bat (*Hipposideros speoris*) (80, 81), and this patterned stimulation has proven effective in both the delay and advance zones of C57BL/6 mice (82), the most common mouse strain bred in biomedical science.

Employing electrophysiology amplifiers and LED Ganzfeld lamps, researchers have summarized a few other observations germane to the pacemaker's reaction to (sub)millisecond light. First, the energy-efficiency with which flashes phase-shift the clock are maximized by shortening exposure, with optimization accruing all the way down to at least 10 μs (87–89). Ergo, it is likely that the circadian system responds to instantaneous light contact, habituates immediately thereafter, and then cycles through a rapid re-sensitization process. Second, flashes that reset the clock do so with a combinatorial logic that integrates the responses of these flashes with shorter and longer episodes of light (87). This means that flashes do not require delivery in some invariant or artificial sequence (e.g., with a fixed pulse duration, metered along a specific frequency) to impact the circadian system's timekeeping (90, 91). Third, the action spectra for flash resetting of circadian rhythms follows the action spectra that's been documented for visible light. Analogous to broad-spectrum xenon flashes, narrowband blue and green LED flashes can operate as stand-ins for continuous blue/green light exposure (92). Finally, the lower energy bounds for photic induction of circadian resetting reside within the micro-to-nanojoule range (92).

These observations lend support to a model where the pacemaker creates wholistic representations of twilight—thereby predicting the timing of the next day's dawn and dusk—by intermittently sampling bits of photic information that strike the retina as an organism navigates its environment. Presumably, sampling is done in rapid succession by capturing snapshots of incident light, integrating these snapshots across seconds/minutes, and favoring this integration process for the parts of the day when the sun's movement in the sky will invoke the greatest rates of change in ambient illumination intensity and spectral composition (i.e., the 30–60 min of twilight perceived when the sun is ascending or descending the horizon). Under this scenario, the pacemaker's intermittent reading frame is (1) optimized with photic information in its most dynamic state, and (2) withstands stochastic changes in light quantity and quality transiently introduced by clouds, wind and atmospheric turbidity (e.g., light scattering from wind-borne particles, haze), and by the behavior of the organism itself as it moves back-and-forth underground or underneath a discontinuous awning of trees and green vegetation (93).

It is worth noting that this model of circadian photoreception recapitulates an important gestalt principle of the image-forming visual system referred to as *closure*, which describes the brain's ability to perceive objects as a whole in their completeness even when the objects appear in the visual field lacking one or more constituent parts (94). The image-forming brain is not a stickler for the discrepancies that arise in detecting and identifying figures when they are obstructed, appear at an alternative angle, or when constituent parts may be physically absent. It compensates for the lack of information, interpolates what is missing, makes (mostly) correct deductions about the object or person in front of it, and actively "re-creates" the image of it. Compression algorithms are applied as soon as light contacts the retina, continue their processing as the signal traverses the thalamus and visual cortex, and culminate as the signal breaches the visual streams (95–97). Gestalt principles of visual perception detail how the image-forming system creates structure—and structure within space—by default. Analogous "gestalt" principles might be valuable toward explaining how the pacemaker creates automatic representations of time using twilight as a palette and compression algorithms requiring operation only within the circumscribed circuitry binding the retina and SCN.

FLASHES, CONES, AND CIRCADIAN PREDICTIONS ABOUT TWILIGHT

Throughout millennia, the pacemaker has been conditioned to track light transitions enveloping sunrise and sunset. The aggregated literature on flash-induction of circadian resetting suggests that this timekeeping mechanism occurs

intermittently, using but a quantum of contact with light, to set in motion an integrative process that results in the synthetic construction of a ∼60-min episode of twilight from just milliseconds of photic information; integration might occur throughout all stages of signal transduction across the retina and retinohypothalamic tract (RHT), SCN, and SCN outputs. With an engram of this temporarily stored in the SCN circuitry, the representation is then consulted to orient the synchronization of the SCN's output signal to the rest of the brain and periphery, thus phase-locking the next day's physiology and behavior. The literature that has burgeoned from Van den Pol and Heller's original observations (82)—that the circadian pacemaker orients to sequenced intermittent light exposures and not just individual flashes of oversaturating light (78, 80)—was facilitated by co-opting studio equipment but has since been enabled by the advent of semiconductor LEDs. These luminaires provide spectral/temporal photoemission control at the microsecond-level and offer the prospect of engineering dynamic patterns of flash exposure that quickly (and repeatedly) transition from one set of physical-exposure variables to another (98, 99). Despite this technology breakthrough, which is now ushering a fundamental shift in lighting practices around the world, many investigators still regard flash induction of circadian resetting as a lab curiosity—an example of a "circadian visual illusion" worth noting but not necessarily formalizing within studies of circadian photoreception (100). Perhaps what is needed is a better mechanistic characterization of the phenomenon and identification of the relative importance of each photoreceptor class to these types of physiological responses.

Prior to any experiment, one might hypothesize that classic photoreceptor cells would be important conduits for transducing flashes that will feed the pacemaker's twilight predictions. Both rods and cones contribute to RHT responses at light onset and provide short-latency inputs to ipRGCs. If the relative contribution of rods vs. cones were weighed, however, more data than not would suggest that cones are the more relevant photoreceptor class for flash conveyance. Cones with overlapping ranges of wavelength sensitivity: (1) Are the chief contributors to retinal responses driven by short-duration light, generating transient signals that account for most of the RHT activity evoked by a series of brief light pulses (8, 17–19, 33, 40–43); (2) Are disproportionately responsible for the upstream ability of the SCN to track one or more sudden fluctuations in light intensity and spectral contrast (e.g., akin to the salient changes in ambient illumination that characterize dawn and dusk) (33, 42, 44); (3) Differ from melanopsin or rods in that cones cannot drive sustained RHT activity during continuous, uninterrupted light exposure; accordingly, they are neither necessary nor sufficient for photoentrainment of behavioral activity rhythms to recurring solar or electric light-dark cycles (where light is presented in a relatively unwavering fashion for 10–12 straight hours) (27, 29, 33–37, 42); and (4) Are better than melanopsin or rods at adjusting their sensitivity to background illuminance (101–103), thus enabling them to operate as twilight detectors irrespective of how bright or long the photoperiod feeding into dusk. In short, cones are not "circadian-alignment" tools à la melanopsin or rods, which signal the enduring presence of light with fidelity thereby marking the day from the night and providing an estimation of daylength. Rather, they are critical sentinels for detecting the flickering kinds of light that signal the initiation of a sunset or sunrise has migrated to a time later or earlier than anticipated. In this role, they might or might not work together with rods, which already have established roles supporting photoentrainment and quantitative assessments of irradiance alongside melanopsin (10, 11, 27, 29, 42, 104).

Retinally degenerate and knockout mice offer powerful platforms for gauging the relative contribution of each photoreceptor class to a clock light-response of interest. As a proof-of-concept test of our suggestion that classic photoreceptors mediate the circadian effects of millisecond light flashes, one of the authors (KYW) recorded *ex vivo* ipRGC responses from $Opn4^{Cre/Cre}$ melanopsin-knockout (105) vs. $Pde6b^{rd1/rd1}$ rod/cone-degenerate mice. The stimuli were 150-s trains of 2-ms flashes (full-field; 470 nm, 14.0 log photons cm^{-2} s^{-1} or roughly 300 lux) with various interstimulus intervals (ISI, 2.5–10 s; **Figure 1**). These experiments were motivated by previous observations suggesting that: (1) Melanopsin responds better to 20 1-s flashes with certain ISIs than to a continuous 20-s light step, indicating temporal summation (106); and (2) Flashes 50-ms in duration could, occasionally, evoke a melanopsin response in ipRGCs (34), indicating that melanopsin might respond to short as well as prolonged illumination. Given these properties, and the lack of any preexisting data on temporal summation of shorter flashes (i.e., <1 s) by melanopsin, it was not immediately obvious that there would be a clear dichotomy between an ipRGC's outer retinal photoreceptor-driven and melanopsin-mediated responses to millisecond flash stimulation. Such a dichotomy did emerge, however. Upon flash exposure, wildtype mouse ipRGCs with fully-intact rod/cone input showed increases in ipRGC spiking that scaled inversely with ISI (**Figure 1B**, left panel). Melanopsin-deficient ipRGCs ($Opn4^{Cre/Cre}$) exhibited similar ISI-dependent patterns of flash response, but ipRGCs in retinas largely devoid of rods and cones ($Pde6b^{rd1/rd1}$) mounted virtually no response (**Figure 1B**, middle and right panels, respectively). Subsequent head-to-head analysis of wildtype and $Opn4^{Cre/Cre}$ ipRGC light-evoked spiking indicated that the decay in the spike rate over the stimulation window was not statistically different for any of the ISI conditions, suggesting that melanopsin did not contribute to any temporal summation of the 2-ms flashes (**Figure 1C**).

Like all data collected from retinal-degenerate mice, these results need to be interpreted with caution. These models do not allow us to visualize what would occur within an intact system where all photoreceptor classes influence the activity of one another, nor control for the possibility of compensatory reorganization of the circuit loops in which these photoreceptors operate [e.g., (107)]. There is also the caveat that melanopsin might have responded to flashes had they been delivered with higher-intensity stimulation protocols [moderate regimens were tested here, instead, because they are more physiologically relevant for nocturnal rodents and better conform to the flash intensities that have been studied in humans; (91)]. All that

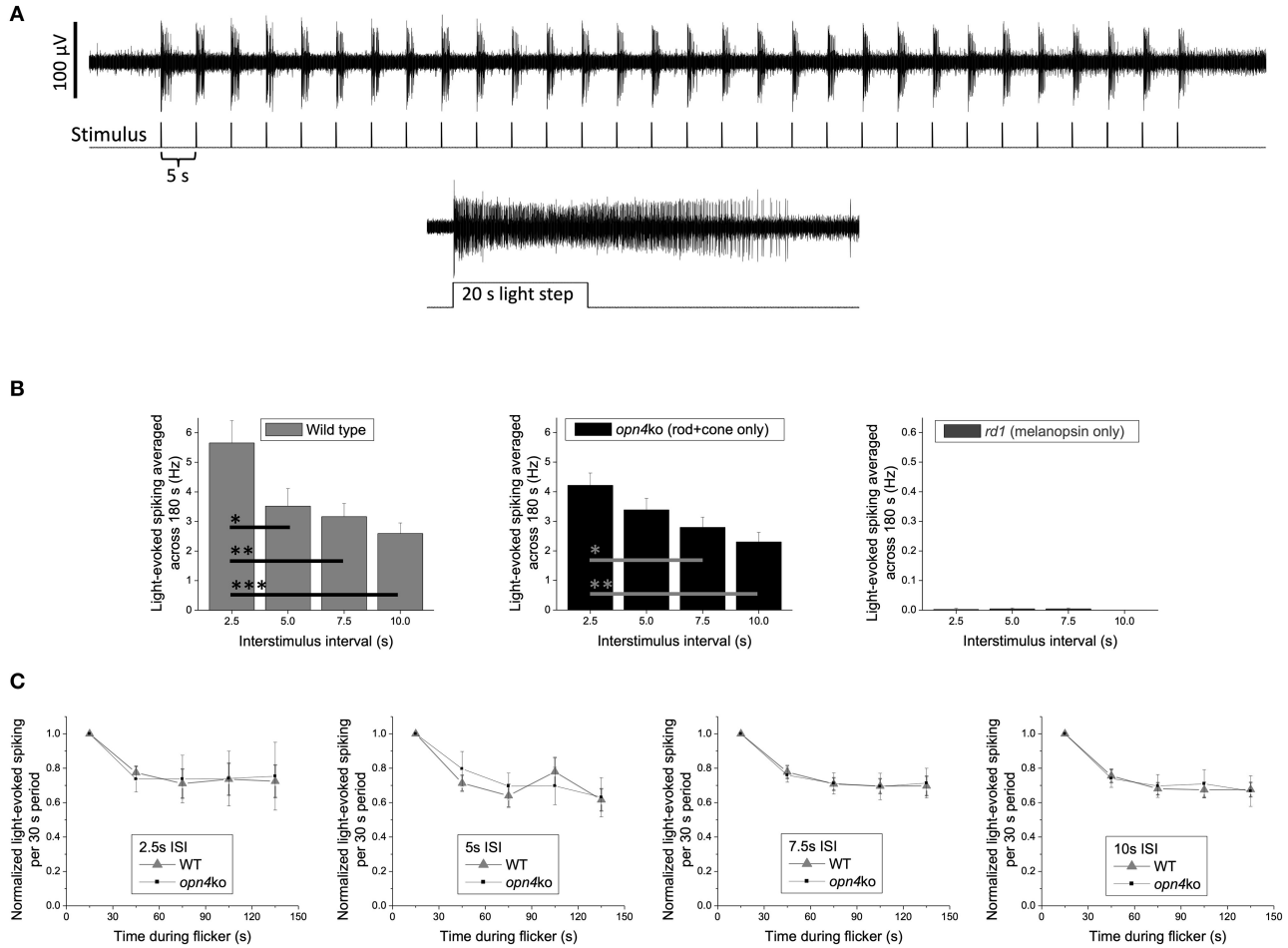

FIGURE 1 | Spiking responses of mouse ipRGCs to a 150 s train of 2 ms flashes (2.5–10 s intervals) are mediated almost exclusively by rod/cone input. **(A)** Example response of a wild-type ipRGC to 30 flashes with a 5 s interstimulus interval (upper recording). This cell was identified as an ipRGC by its sustained response to a subsequent prolonged light step (lower recording), which evokes transient responses in all non-intrinsically photosensitive RGCs [Wong 2012; (35)]. Spike recording methods were identical to those described in the "Multielectrode-array recording" section in Wong 2012 except that all stimuli, including the 20 s light step, were 14.0 log photons cm^{-2} s^{-1} (~300 lux) full-field light produced by an LED with peak emission at 470 nm. **(B)** The light-induced elevation in spike rate averaged across the flicker plus 30 s of post-flicker darkness (to include responses outlasting the flicker) for C57BL/6 wild-type mice ($n = 26$ ipRGCs; left plot), *Opn4*$^{Cre/Cre}$ melanopsin-knockout mice originally created by Ecker et al. (105) ($n = 24$ ipRGCs; middle plot), and *Pde6b*$^{rd1/rd1}$ rod/cone-degenerate mice ($n = 43$ ipRGCs; right plot); note the expanded y-axis in the right plot. All mice in each group were about 7 months old and included both sexes. Though flashes delivered with a 2.5 s interstimulus interval appear to cause a greater spike rate increase in wild-type vs. melanopsin-knockout mice, this difference is not statistically significant ($p = 0.156$). The wild-type vs. melanopsin-knockout differences for the other three intervals are also statistically insignificant (p-values between 0.689 and 0.818), suggesting that melanopsin does not enhance the flicker responses. Asterisks represent p-values calculated using one-way ANOVA with *post-hoc* Tukey test: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **(C)** For all four interstimulus intervals, the gradual decay in light-evoked spike rate during the 150 s flicker is comparable between wild-type and melanopsin-knockout mice, suggesting that melanopsin does not contribute to any temporal summation of successive flash responses. Error bars are S.E.M.

said, the clarity of the data and the conclusion they offer remain striking: rods and/or cones enable ipRGC detection and integration of light flashes independent of melanopsin, whose contribution—if any—is predicated on initial processing by the outer retinal photoreceptors. The results are consistent with the suppositions made in the current review and with the idea that cones (with or without the input of rods) will prove to be key regulators of flash-induced resetting of pacemaker rhythms. Future experiments using mice with intact retinal circuitry (e.g., *Opn1mw*^{R}) or with selective loss in rod vs. cone photosensitivity [e.g., *Gnat1*$^{-/-}$ and *Gnat2*^{cpfl3} mice (108–110)] will be necessary to isolate the relative contributions of cones to this phenomenon and to determine whether the retina processes staccatos of narrowband light with any circadian phase-dependence. Using principles of silent substitution, flash stimuli differing in the amount of cone-and-rod excitation might also be probed for their circadian effects in retinally-intact humans compared to humans with congenital achromatopsia (i.e., people without a functional cone system; prevalence one in 30,000–50,000) (111).

CONCLUSION

The way we illuminate our world is changing, moving away from unidimensional forms of illumination provided by gas-discharge fluorescent lamps to highly customizable solid-state lighting with semiconductor LEDs. Data suggest that this watershed is material to understanding the circadian pacemaker's phase-responses to electric light exposure, which are likely rooted within a natural process where the pacemaker "flash-samples" the photic information available at twilight to create predictions about the timing of the next day's dusk and dawn. While the mechanisms subserving flash photoreception and the SCN's twilight predictions require further study, it is becoming clear that classical photoreceptors, including cones, operate as sensors in this process. The "Cone Sentinel Model" we articulate here [and hinted at by Zeitzer; (91)] raises many considerations for how different combinations of narrowband LED stimulation can be strung together in phototherapy protocols to improve mental and physical health (112). The gestalt inferences made in this model will be challenging to demonstrate experimentally. However, prudent first steps might include the design of studies examining the differential phase-shifting effects of flashes patterned after twilight progressions—vs. more randomly generated sequences—in parallel to those examining the neurophysiological responses arising from flash regimens highly-optimized (or ill-suited) for driving cone input to ipRGCs.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/supplementary material, further inquiries can be directed to Kwoon Y. Wong, kwoon@umich.edu.

ETHICS STATEMENT

The animal study was reviewed and approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC).

AUTHOR CONTRIBUTIONS

F-XF conceptualized the Cone Sentinel model, which was further refined by KYW. KYW developed and carried out the retinal electrophysiology experiments. All authors contributed to writing the manuscript.

FUNDING

F-XF was supported by Project No. 1360 from the Velux Stiftung (Switzerland). KYW acknowledges support from a Brain Research Foundation Fay/Frank Seed Grant and an Alliance for Vision Research grant.

REFERENCES

1. Lucas RJ, Lall GS, Allen AE, Brown TM. How rod, cone, and melanopsin photoreceptors come together to enlighten the mammalian circadian clock. *Prog Brain Res*. (2012) 199:1–18. doi: 10.1016/B978-0-444-59427-3.00001-0
2. Freedman MS, Lucas RJ, Soni B, von Schantz M, Muñoz M, David-Gray Z, et al. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science*. (1999) 284:502–4. doi: 10.1126/science.284.5413.502
3. Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, et al. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*. (2003) 424:76–81. doi: 10.1038/nature01761
4. Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature*. (2008) 453:102–5. doi: 10.1038/nature06829
5. Güler AD, Altimus CM, Ecker JL, Hattar S. Multiple photoreceptors contribute to nonimage-forming visual functions predominantly through melanopsin-containing retinal ganglion cells. *Cold Spring Harb Symp Quant Biol*. (2007) 72:509–15. doi: 10.1101/sqb.2007.72.074
6. Fu Y, Zhong H, Wang MH, Luo DG, Liao HW, Maeda H, et al. Intrinsically photosensitive retinal ganglion cells detect light with a vitamin A-based photopigment, melanopsin. *Proc Natl Acad Sci USA*. (2005) 102:10339–44. doi: 10.1073/pnas.0501866102
7. Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: an opsin in melanophores, brain, and eye. *Proc Natl Acad Sci USA*. (1998) 95:340–5. doi: 10.1073/pnas.95.1.340
8. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. (2002) 295:1070–3. doi: 10.1126/science.1067262
9. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat Neurosci*. (2001) 4:1165. doi: 10.1038/nn768
10. Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, et al. Role of melanopsin in circadian responses to light. *Science*. (2002) 298:2211–3. doi: 10.1126/science.1076701
11. Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, et al. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science*. (2002) 298:2213–6. doi: 10.1126/science.1076848
12. Sollars PJ, Smeraski CA, Kaufman JD, Ogilvie MD, Provencio I, Pickard GE. Melanopsin and non-melanopsin expressing retinal ganglion cells innervate the hypothalamic suprachiasmatic nucleus. *Vis Neurosci*. (2003) 20:601–10. doi: 10.1017/S0952523803206027
13. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. (2006) 497:326–49. doi: 10.1002/cne.20970
14. Hannibal J, Fahrenkrug J. Target areas innervated by PACAP-immunoreactive retinal ganglion cells. *Cell Tissue Res*. (2004) 316:99–113. doi: 10.1007/s00441-004-0858-x
15. Zhao X, Stafford BK, Godin AL, King WM, Wong KY. Photoresponse diversity among the five types of intrinsically photosensitive retinal ganglion cells. *J Physiol*. (2014) 592:1619–36. doi: 10.1113/jphysiol.2013.262782
16. Ekesten B, Gouras P. Cone and rod inputs to murine retinal ganglion cells: evidence of cone opsin specific channels. *Vis Neurosci*. (2005) 22:893–903. doi: 10.1017/S0952523805226172
17. Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. (2005) 433:749–54. doi: 10.1038/nature03387
18. Wong KY, Dunn FA, Graham DM, Berson DM. Synaptic influences on rat ganglion-cell photoreceptors. *J Physiol*. (2007) 582:279–96. doi: 10.1113/jphysiol.2007.133751
19. Schmidt TM, Taniguchi K, Kofuji P. Intrinsic and extrinsic light responses in melanopsin-expressing ganglion cells during mouse development. *J Neurophysiol*. (2008) 100:371–84. doi: 10.1152/jn.00062.2008

20. Dumitrescu ON, Pucci FG, Wong KY, Berson DM. Ectopic retinal ON bipolar cell synapses in the OFF inner plexiform layer: contacts with dopaminergic amacrine cells and melanopsin ganglion cells. *J Comp Neurol*. (2009) 517:226–44. doi: 10.1002/cne.22158
21. Hoshi H, Liu WL, Massey SC, Mills SL. ON inputs to the OFF layer: bipolar cells that break the stratification rules of the retina. *J Neurosci*. (2009) 29:8875–83. doi: 10.1523/JNEUROSCI.0912-09.2009
22. Schmidt TM, Kofuji P. Differential cone pathway influence on intrinsically photosensitive retinal ganglion cell subtypes. *J Neurosci*. (2010) 30:16262–71. doi: 10.1523/JNEUROSCI.3656-10.2010
23. Weng S, Estevez ME, Berson DM. Mouse ganglion-cell photoreceptors are driven by the most sensitive rod pathway and by both types of cones. *PLoS ONE*. (2013) 8:e66480. doi: 10.1371/journal.pone.0066480
24. Belenky MA, Smeraski CA, Provencio I, Sollars PJ, Pickard GE. Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *J Comp Neurol*. (2003) 460:380–93. doi: 10.1002/cne.10652
25. Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG. Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proc Natl Acad Sci USA*. (2008) 105:14181–6. doi: 10.1073/pnas.0803893105
26. Reifler AN, Chervenak AP, Dolikian ME, Benenati BA, Li BY, Wachter RD, et al. All spiking, sustained ON displaced amacrine cells receive gap-junction input from melanopsin ganglion cells. *Curr Biol*. (2015) 25:2763–73. doi: 10.1016/j.cub.2015.09.018
27. Altimus CM, Güler AD, Alam NM, Arman AC, Prusky GT, Sampath AP, et al. Rod photoreceptors drive circadian photoentrainment across a wide range of light intensities. *Nat Neurosci*. (2010) 13:1107–12. doi: 10.1038/nn.2617
28. van Oosterhout F, Fisher SP, van Diepen HC, Watson TS, Houben T, VanderLeest HT, et al. Ultraviolet light provides a major input to non-image-forming light detection in mice. *Curr Biol*. (2012) 22:1397–402. doi: 10.1016/j.cub.2012.05.032
29. van Diepen HC, Ramkisoensing A, Peirson SN, Foster RG, Meijer JH. Irradiance encoding in the suprachiasmatic nuclei by rod and cone photoreceptors. *FASEB J*. (2013) 27:4204–12. doi: 10.1096/fj.13-233098
30. Walmsley L, Hanna L, Mouland J, Martial F, West A, Smedley AR, et al. Colour as a signal for entraining the mammalian circadian clock. *PLoS Biol*. (2015) 13:e1002127. doi: 10.1371/journal.pbio.1002127
31. Mouland JW, Martial F, Watson A, Lucas RJ, Brown TM. Cones support alignment to an inconsistent world by suppressing mouse circadian responses to the blue colors associated with twilight. *Curr Biol*. (2019) 29:4260–7. doi: 10.1016/j.cub.2019.10.028
32. Hannibal J, Vrang N, Card JP, Fahrenkrug J. Light-dependent induction of cFos during subjective day and night in PACAP-containing ganglion cells of the retinohypothalamic tract. *J Biol Rhythms*. (2001) 16:457–70. doi: 10.1177/074873001129002132
33. Mure LS, Rieux C, Hattar S, Cooper HM. Melanopsin-dependent nonvisual responses: evidence for photopigment bistability in vivo. *J Biol Rhythms*. (2007) 22:411–24. doi: 10.1177/0748730407306043
34. Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, et al. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature*. (2009) 457:281–7. doi: 10.1038/nature07682
35. Wong KY. A retinal ganglion cell that can signal irradiance continuously for 10 hours. *J Neurosci*. (2012) 32:11478–85. doi: 10.1523/JNEUROSCI.1423-12.2012
36. Emanuel AJ, Do MT. Melanopsin tristability for sustained and broadband phototransduction. *Neuron*. (2015) 85:1043–55. doi: 10.1016/j.neuron.2015.02.011
37. Zhao X, Pack W, Khan NW, Wong KY. Prolonged inner retinal photoreception depends on the visual retinoid cycle. *J Neurosci*. (2016) 36:4209–17. doi: 10.1523/JNEUROSCI.2629-14.2016
38. Harrison KR, Reifler AN, Chervenak AP, Wong KY. Prolonged melanopsin-based photoresponses depend in part on RPE65 and cellular retinaldehyde-binding protein (CRALBP). *Curr Eye Res*. (2020) 7:1–9. doi: 10.1080/02713683.2020.1815793
39. Aggelopoulos NC, Meissl H. Responses of neurones of the rat suprachiasmatic nucleus to retinal illumination under photopic and scotopic conditions. *J Physiol*. (2000) 523:211–22. doi: 10.1111/j.1469-7793.2000.t01-1-00211.x
40. Dkhissi-Benyahya O, Gronfier C, De Vanssay W, Flamant F, Cooper HM. Modeling the role of mid-wavelength cones in circadian responses to light. *Neuron*. (2007) 53:677–87. doi: 10.1016/j.neuron.2007.02.005
41. Drouyer E, Rieux C, Hut RA, Cooper HM. Responses of suprachiasmatic nucleus neurons to light and dark adaptation: relative contributions of melanopsin and rod-cone inputs. *J Neurosci*. (2007) 27:9623–31. doi: 10.1523/JNEUROSCI.1391-07.2007
42. Lall GS, Revell VL, Momiji H, Al Enezi J, Altimus CM, Güler AD, et al. Distinct contributions of rod, cone, and melanopsin photoreceptors to encoding irradiance. *Neuron*. (2010) 66:417–28. doi: 10.1016/j.neuron.2010.04.037
43. Dollet A, Albrecht U, Cooper HM, Dkhissi-Benyahya O. Cones are required for normal temporal responses to light of phase shifts and clock gene expression. *Chronobiol Int*. (2010) 27:768–81. doi: 10.3109/07420521003695704
44. Brown TM, Wynne J, Piggins HD, Lucas RJ. Multiple hypothalamic cell populations encoding distinct visual information. *J Physiol*. (2011) 589:1173–94. doi: 10.1113/jphysiol.2010.199877
45. Johnson CH. Forty years of PRCs–what have we learned? *Chronobiol Int*. (1999) 16:711–43. doi: 10.3109/07420529909016940
46. Hall JC, Rosbash M. Genes and biological rhythms. *Trends Genet*. (1987) 3:185–91. doi: 10.1016/0168-9525(87)90231-9
47. Schwartz WJ, Zimmerman P. Circadian timekeeping in BALB/c and C57BL/6 inbred mouse strains. *J Neurosci*. (1990) 10:3685–94. doi: 10.1523/JNEUROSCI.10-11-03685.1990
48. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. A phase response curve to single bright light pulses in human subjects. *J Physiol*. (2003) 549:945–52. doi: 10.1113/jphysiol.2003.040477
49. Clark A. Whatever next? Predictive brains, situated agents, and the future of cognitive science. *Behav Brain Sci*. (2013) 36:181–204. doi: 10.1017/S0140525X12000477
50. Friston K. A theory of cortical responses. *Philos Trans R Soc Lond B Biol Sci*. (2005) 360:815–36. doi: 10.1098/rstb.2005.1622
51. Schacter DL, Addis DR, Buckner RL. Episodic simulation of future events: concepts, data, and applications. *Ann N Y Acad Sci*. (2008) 1124:39–60. doi: 10.1196/annals.1440.001
52. Molinari M, Masciullo M. The implementation of predictions during sequencing. *Front Cell Neurosci*. (2019) 13:439. doi: 10.3389/fncel.2019.00439
53. Alink A, Schwiedrzik CM, Kohler A, Singer W, Muckli L. Stimulus predictability reduces responses in primary visual cortex. *J Neurosci*. (2010) 30:2960–6. doi: 10.1523/JNEUROSCI.3730-10.2010
54. Vetter P, Edwards G, Muckli L. Transfer of predictive signals across saccades. *Front Psychol*. (2012) 3:176. doi: 10.3389/fpsyg.2012.00176
55. Sigman M, Cecchi GA, Gilbert CD, Magnasco MO. On a common circle: natural scenes and Gestalt rules. *Proc Natl Acad Sci USA*. (2001) 98:1935–40. doi: 10.1073/pnas.98.4.1935
56. Elder JH, Goldberg RM. Ecological statistics of Gestalt laws for the perceptual organization of contours. *J Vis*. (2002) 2:324–53. doi: 10.1167/2.4.5
57. Coggan DD, Giannakopoulou A, Ali S, Goz B, Watson DM, Hartley T, et al. A data-driven approach to stimulus selection reveals an image-based representation of objects in high-level visual areas. *Hum Brain Mapp*. (2019) 40:4716–31. doi: 10.1002/hbm.24732
58. Criado-Boado F, Alonso-Pablos D, Blanco MJ, Porto Y, Rodríguez-Paz A, Cabrejas E, et al. Coevolution of visual behaviour, the material world and social complexity, depicted by the eye-tracking of archaeological objects in humans. *Sci Rep*. (2019) 9:3985. doi: 10.1038/s41598-019-39661-w
59. Iacaruso MF, Gasler IT, Hofer SB. Synaptic organization of visual space in primary visual cortex. *Nature*. (2017) 547:449–52. doi: 10.1038/nature23019
60. Desolneux A, Moisan L, Morel JM. Computational gestalts and perception thresholds. *J Physiol Paris*. (2003) 97:311–24. doi: 10.1016/j.jphysparis.2003.09.006
61. Kourtzi Z, Welchman AE. Adaptive shape coding for perceptual decisions in the human brain. *J Vis*. (2015) 15:2. doi: 10.1167/15.7.2
62. Merriam EP, Colby CL. Active vision in parietal and extrastriate cortex. *Neuroscientist*. (2005) 11:484–93. doi: 10.1177/1073858405276871
63. Bubic A, von Cramon DY, Schubotz RI. Prediction, cognition and the brain. *Front Hum Neurosci*. (2010) 4:25. doi: 10.3389/fnhum.2010.00025

64. Hastings JW, Sweeney BM. A persistent diurnal rhythm of luminescence in Gonyaulax polyedra. *Biol Bull*. (1958) 115:440–58. doi: 10.2307/1539108
65. De Coursey PJ. Daily light sensitivity rhythm in a rodent. *Science*. (1960) 131:33–5. doi: 10.1126/science.131.3392.33
66. Kim B, Reif E, Wattenberg M, Bengio S, Mozer MC. Neural networks trained on natural scenes exhibit gestalt closure. *arXiv:[Preprint]* arXiv:1903.01069v4.
67. Redlin U, Mrosovsky N. Masking by light in hamsters with SCN lesions. *J Comp Physiol A*. (1999) 184:439–48. doi: 10.1007/s003590050343
68. Hut RA, van Oort BE, Daan S. Natural entrainment without dawn and dusk: the case of the European ground squirrel (Spermophilus citellus). *J Biol Rhythms*. (1999) 14:290–9. doi: 10.1177/074873099129000704
69. Flôres DE, Jannetti MG, Valentinuzzi VS, Oda GA. Entrainment of circadian rhythms to irregular light/dark cycles: a subterranean perspective. *Sci Rep*. (2016) 6:34264. doi: 10.1038/srep34264
70. Ebihara S, Tsuji K. Entrainment of the circadian activity rhythm to the light cycle: effective light intensity for a Zeitgeber in the retinal degenerate C3H mouse and the normal C57BL mouse. *Physiol Behav*. (1980) 24:523–7. doi: 10.1016/0031-9384(80)90246-2
71. Hirsh J, Riemensperger T, Coulom H, Iché M, Coupar J, Birman S. Roles of dopamine in circadian rhythmicity and extreme light sensitivity of circadian entrainment. *Curr Biol*. (2010) 20:209–14. doi: 10.1016/j.cub.2009.11.037
72. Butler MP, Silver R. Divergent photic thresholds in the non-image-forming visual system: entrainment, masking and pupillary light reflex. *Proc Biol Sci*. (2011) 278:745–50. doi: 10.1098/rspb.2010.1509
73. Pittendrigh CS, Daan S. A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock. *J Comp Physiol*. (1976) 106:291–331. doi: 10.1007/BF01417859
74. Stephan FK. Circadian rhythms in the rat: constant darkness, entrainment to T cycles and to skeleton photoperiods. *Physiol Behav*. (1983) 30:451–62. doi: 10.1016/0031-9384(83)90152-X
75. Olde Engberink AHO, Huisman J, Michel S, Meijer JH. Brief light exposure at dawn and dusk can encode day-length in the neuronal network of the mammalian circadian pacemaker. *FASEB J*. (2020) 34:13685–95. doi: 10.1096/fj.202001133RR
76. Takahashi JS, DeCoursey PJ, Bauman L, Menaker M. Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature*. (1984) 308:186–8. doi: 10.1038/308186a0
77. Nelson DE, Takahashi JS. Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (Mesocricetus auratus). *J Physiol*. (1991) 439:115–45. doi: 10.1113/jphysiol.1991.sp018660
78. Bruce VG, Weight F, Pittendrigh CS. Resetting the sporulation rhythm in Pilobolus with short light flashes of high intensity. *Science*. (1960) 131:728–30. doi: 10.1126/science.131.3402.728
79. Pittendrigh CS. Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb Symp Quant Biol*. (1960) 25:159–84. doi: 10.1101/SQB.1960.025.01.015
80. Joshi D, Chandrashekaran MK. Bright light flashes of 0.5 milliseconds reset the circadian clock of a microchiropteran bat. *J Exp Zool*. (1984) 230:325–8. doi: 10.1002/jez.1402300219
81. Joshi D, Chandrashekaran MK. Light flashes of different durations (0.063-3.33 msec) phase shift the circadian flight activity of a bat. *J Exp Zool*. (1985) 233:187–92. doi: 10.1002/jez.1402330204
82. Van Den Pol AN, Cao V, Heller HC. Circadian system of mice integrates brief light stimuli. *Am J Physiol*. (1998) 275:R654–7. doi: 10.1152/ajpregu.1998.275.2.R654
83. Vidal L, Morin LP. Absence of normal photic integration in the circadian visual system: response to millisecond light flashes. *J Neurosci*. (2007) 27:3375–82. doi: 10.1523/JNEUROSCI.5496-06.2007
84. Zeitzer JM, Ruby NF, Fisicaro RA, Heller HC. Response of the human circadian system to millisecond flashes of light. *PLoS ONE*. (2011) 6:e22078. doi: 10.1371/journal.pone.0022078
85. Zeitzer JM, Fisicaro RA, Ruby NF, Heller HC. Millisecond flashes of light phase delay the human circadian clock during sleep. *J Biol Rhythms*. (2014) 29:370–6. doi: 10.1177/0748730414546532
86. Kaladchibachi S, Negelspach DC, Fernandez F. Circadian phase-shifting by light: beyond photons. *Neurobiol Sleep Circadian Rhythms*. (2018) 5:8–14. doi: 10.1016/j.nbscr.2018.03.003
87. Arvanitogiannis A, Amir S. Resetting the rat circadian clock by ultra-short light flashes. *Neurosci Lett*. (1999) 261:159–62. doi: 10.1016/S0304-3940(99)00021-X
88. Kaladchibachi S, Negelspach DC, Zeitzer JM, Fernandez F. Optimization of circadian responses with shorter and shorter millisecond flashes. *Biol Lett*. (2019) 15:20190371. doi: 10.1098/rsbl.2019.0371
89. Joyce DS, Spitschan M, Zeitzer JM. Integration of brief light flashes varying in intensity and duration by the human circadian system. *BioRxiv [Preprint]*. doi: 10.1101/759134
90. Vartanian GV, Zhao X, Wong KY. Using flickering light to enhance nonimage-forming visual stimulation in humans. *Invest Ophthalmol Vis Sci*. (2015) 56:4680–8. doi: 10.1167/iovs.15-16468
91. Najjar RP, Zeitzer JM. Temporal integration of light flashes by the human circadian system. *J Clin Invest*. (2016) 126:938–47. doi: 10.1172/JCI82306
92. Negelspach DC, Kaladchibachi S, Fernandez F. The circadian activity rhythm is reset by nanowatt pulses of ultraviolet light. *Proc Biol Sci*. (2018) 285:20181288. doi: 10.1098/rspb.2018.1288
93. Woelders T, Wams EJ, Gordijn MCM, Beersma DGM, Hut RA. Integration of color and intensity increases time signal stability for the human circadian system when sunlight is obscured by clouds. *Sci Rep*. (2018) 8:15214. doi: 10.1038/s41598-018-33606-5
94. Wagemans J, Elder JH, Kubovy M, Palmer SE, Peterson MA, Singh M, et al. A century of Gestalt psychology in visual perception: I. Perceptual grouping and figure-ground organization. *Psychol Bull*. (2012) 138:1172–217. doi: 10.1037/a0029333
95. Kveraga K, Ghuman AS, Bar M. Top-down predictions in the cognitive brain. *Brain Cogn*. (2007) 65:145–68. doi: 10.1016/j.bandc.2007.06.007
96. Gilbert CD, Sigman M. Brain states: top-down influences in sensory processing. *Neuron*. (2007) 54:677–96. doi: 10.1016/j.neuron.2007.05.019
97. Harrison LM, Stephan KE, Rees G, Friston KJ. Extra-classical receptive field effects measured in striate cortex with fMRI. *Neuroimage*. (2007) 34:1199–208. doi: 10.1016/j.neuroimage.2006.10.017
98. Katona TM, Pattison PM, Paolini S. Status of solid state lighting product development and future trends for general illumination. *Annu Rev Chem Biomol Eng*. (2016) 7:263–81. doi: 10.1146/annurev-chembioeng-080615-034625
99. Pattison PM, Tsao JY, Brainard GC, Bugbee B. LEDs for photons, physiology and food. *Nature*. (2018) 563:493–500. doi: 10.1038/s41586-018-0706-x
100. Dobb R, Martial F, Elijah D, Storchi R, Brown TM, Lucas RJ. The impact of temporal modulations in irradiance under light adapted conditions on the mouse suprachiasmatic nuclei (SCN). *Sci Rep*. (2017) 7:10582. doi: 10.1038/s41598-017-11184-2
101. Normann RA, Perlman I. The effects of background illumination on the photoresponses of red and green cones. *J Physiol*. (1979) 286:491–507. doi: 10.1113/jphysiol.1979.sp012633
102. Burkhardt DA. Light adaptation and photopigment bleaching in cone photoreceptors in situ in the retina of the turtle. *J Neurosci*. (1994) 14:1091–105. doi: 10.1523/JNEUROSCI.14-03-01091.1994
103. Nathan J, Reh R, Ankoudinova I, Ankoudinova G, Chang B, Heckenlively J, et al. Scotopic and photopic visual thresholds and spatial and temporal discrimination evaluated by behavior of mice in a water maze. *Photochem Photobiol*. (2006) 82:1489–94. doi: 10.1111/j.1751-1097.2006.tb09804.x
104. Demontis GC, Bisti S, Cervetto L. Light sensitivity, adaptation and saturation in mammalian rods. *Prog Brain Res*. (1993) 95:15–24. doi: 10.1016/S0079-6123(08)60353-2
105. Ecker JL, Dumitrescu ON, Wong KY, Alam NM, Chen SK, LeGates T, et al. Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. *Neuron*. (2010) 67:49–60. doi: 10.1016/j.neuron.2010.05.023
106. Walch OJ, Zhang LS, Reifler AN, Dolikian ME, Forger DB, Wong KY. Characterizing and modeling the intrinsic light response of rat ganglion-cell photoreceptors. *J Neurophysiol*. (2015) 114:2955–66. doi: 10.1152/jn.00544.2015
107. Lupi D, Cooper HM, Froehlich A, Standford L, McCall MA, Foster RG. Transgenic ablation of rod photoreceptors alters the circadian phenotype of mice. *Neuroscience*. (1999) 89:363–74. doi: 10.1016/S0306-4522(98)00353-4

108. Calvert PD, Krasnoperova NV, Lyubarsky AL, Isayama T, Nicoló M, Kosaras B, et al. Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha-subunit. *Proc Natl Acad Sci USA*. (2000) 97:13913–8. doi: 10.1073/pnas.250478897
109. Chang B, Dacey MS, Hawes NL, Hitchcock PF, Milam AH, Atmaca-Sonmez P, et al. Cone photoreceptor function loss-3, a novel mouse model of achromatopsia due to a mutation in Gnat2. *Invest Ophthalmol Vis Sci*. (2006) 47:5017–21. doi: 10.1167/iovs.05-1468
110. Schroeder MM, Harrison KR, Jaeckel ER, Berger HN, Zhao X, Flannery MP, et al. The roles of rods, cones, and melanopsin in photoresponses of M4 intrinsically photosensitive retinal ganglion cells (ipRGCs) and optokinetic visual behavior. *Front Cell Neurosci*. (2018) 12:203. doi: 10.3389/fncel.2018.00203
111. Genead MA, Fishman GA, Rha J, Dubis AM, Bonci DM, Dubra A, et al. Photoreceptor structure and function in patients with congenital achromatopsia. *Invest Ophthalmol Vis Sci*. (2011) 52:7298–308. doi: 10.1167/iovs.11-7762
112. Kaladchibachi S, Fernandez F. Precision light for the treatment of psychiatric disorders. *Neural Plast*. (2018) 2018:5868570. doi: 10.1155/2018/5868570

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: 22 February 2021
doi: 10.3389/fneur.2021.624217

Daytime Exposure to Short Wavelength-Enriched Light Improves Cognitive Performance in Sleep-Restricted College-Aged Adults

Leilah K. Grant[1,2], *Brianne A. Kent*[1,2], *Matthew D. Mayer*[1], *Robert Stickgold*[3,4], *Steven W. Lockley*[1,2] *and Shadab A. Rahman*[1,2*]

[1] Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, MA, United States, [2] Division of Sleep Medicine, Harvard Medical School, Boston, MA, United States, [3] Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA, United States, [4] Department of Psychiatry, Harvard Medical School, Boston, MA, United States

OPEN ACCESS

Edited by:
Maurice M. Ohayon,
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Viktor Stolc,
National Aeronautics and Space Administration (NASA), United States

***Correspondence:**
Shadab A. Rahman
sarahman@rics.bwh.harvard.edu

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: 02 November 2020
Accepted: 29 January 2021
Published: 22 February 2021

Citation:
Grant LK, Kent BA, Mayer MD, Stickgold R, Lockley SW and Rahman SA (2021) Daytime Exposure to Short Wavelength-Enriched Light Improves Cognitive Performance in Sleep-Restricted College-Aged Adults. Front. Neurol. 12:624217. doi: 10.3389/fneur.2021.624217

We tested the effect of daytime indoor light exposure with varying melanopic strength on cognitive performance in college-aged students who maintained an enforced nightly sleep opportunity of 7 h (i.e., nightly sleep duration no longer than 7 h) for 1 week immediately preceding the day of light exposure. Participants ($n = 39$; mean age ± SD = 24.5 ± 3.2 years; 21 F) were randomized to an 8 h daytime exposure to one of four white light conditions of equal photopic illuminance (~50 lux at eye level in the vertical plane) but different melanopic illuminance [24–45 melanopic-EDI lux (melEDI)] generated by varying correlated color temperatures [3000K (low-melEDI) or 5000K (high-melEDI)] and spectra [conventional or daylight-like]. Accuracy on a 2-min addition task was 5% better in the daylight-like high-melEDI condition (highest melEDI) compared to the conventional low-melEDI condition (lowest melEDI; $p < 0.01$). Performance speed on the motor sequence learning task was 3.2 times faster ($p < 0.05$) during the daylight-like high-melEDI condition compared to the conventional low-melEDI. Subjective sleepiness was 1.5 times lower in the conventional high-melEDI condition compared to the conventional low-melEDI condition, but levels were similar between conventional low- and daylight-like high-melEDI conditions. These results demonstrate that exposure to high-melanopic (short wavelength-enriched) white light improves processing speed, working memory, and procedural learning on a motor sequence task in modestly sleep restricted young adults, and have important implications for optimizing lighting conditions in schools, colleges, and other built environments.

Keywords: light, melanopsin, cognition, learning, melanopic light

INTRODUCTION

The physiological (non-visual) effects of light in humans range from changes in gene expression (1) to overt behavior (2–4). One of the characteristic non-visual responses to light is the stimulation of alertness and cognitive performance. These responses are mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs), primarily through stimulation of the photopigment melanopsin

that is most sensitive to higher intensity ∼480-nm light (5). Therefore, high intensity and short wavelength (blue)-enriched light with greater melanopic content is typically more effective in inducing physiologic responses relative to dimmer blue-depleted light with lower melanopic content (6–8).

The spectral sensitivity of non-visual responses to light, including alertness and cognitive performance, has predominantly been examined during evening and nighttime exposures (3, 4, 9–13). Relatively few studies have examined the effects of short-wavelength light on daytime alertness and performance. While comparison of monochromatic or narrow-bandwidth sources of different wavelengths have shown that short-wavelength light preferentially improves daytime alertness and performance (14–16), studies examining the effects of white light with different correlated color temperatures (CCT) have shown mixed results (17–19). As these differences may be a result of methodological inconsistencies, particularly with regard to differences in the duration of exposure (1–16 h) and photopic illuminance, more studies are needed to determine whether blue-enriched white light during the day has a beneficial effect on alertness and cognition.

While there is evidence for the benefits of blue-enriched light on alertness and cognitive performance, there is considerably less understanding of the effect of light spectra on learning and memory. A small number of studies suggest that declarative memory (9, 20), and procedural learning (21) are better under blue-enriched light in the evening, and one study has shown improved verbal memory recall under blue-enriched light during the day (20).

In the current study we aimed to examine the effects of an 8-h daytime light exposure (LE) to one of four polychromatic light emitting diode (LED) light sources with the same photopic (visual) illuminance but different spectral compositions, and therefore different melanopic content estimated by melanopic Equivalent Daylight Illuminance (melEDI) on cognition. It was hypothesized that learning and memory, sleepiness and alertness, and vigilance and concentration would improve with exposure to light with higher melEDI. Additionally, given recent evidence suggesting that daytime alertness is higher with exposure to light with daylight-like spectra compared to light with conventional LED spectra (17), we also compared the effects of conventional and daylight-like spectra within the high- and low-melEDI conditions.

MATERIALS AND METHODS

Participants

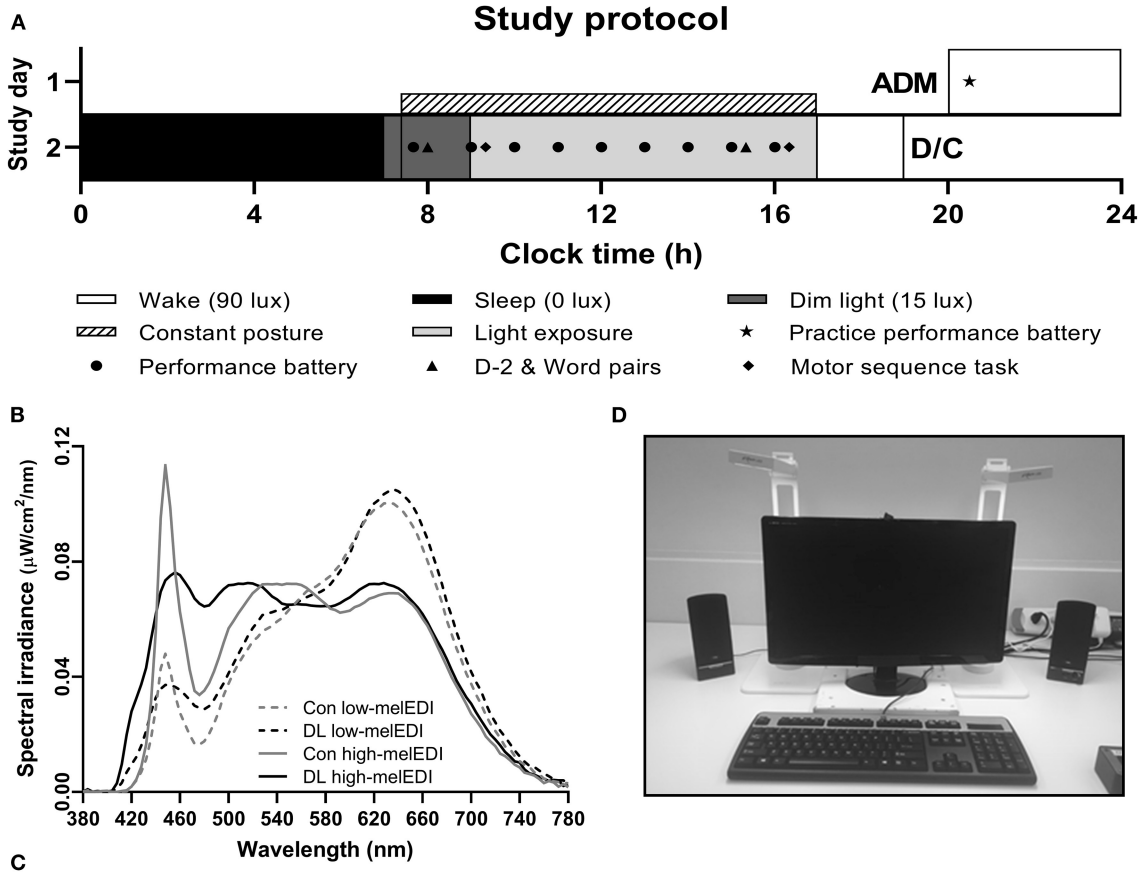
Thirty-nine healthy college-aged (18–30 years) participants [21 females; mean age (±SD): 24.5 ± 3.2 years)] were studied in the Intensive Physiological Monitoring (IPM) Unit in the Center for Clinical Investigation (CCI) at Brigham and Women's Hospital. The study was approved by the Partners Human Research Committee (IRB# 2019-P-000900), and participants provided written informed consent prior to study. All participants reported being free from medical and psychological conditions and had a negative Ishihara Color Blindness Test. Participants were either currently enrolled in college or had a college degree. For at least 1 week prior to entering the IPM Unit, participants maintained a consistent sleep/wake schedule that limited time in bed to 7 h (e.g., 23:00–06:00). Participants selected for themselves the 7-h interval for time in bed at the start of the study based on their own preference and schedule, but the same 7-h time in bed was then maintained every night for 7 consecutive nights leading up to the in-lab study. Adherence to the sleep/wake schedule was confirmed with (1) calls to a time- and date-stamped voicemail at bedtime and wake time, and (2) wrist actigraphy (Actiwatch, MiniMitter Company, Inc., Sunriver, OR, USA). The 7-h time in bed was selected based on the average sleep duration of college students being less than 7 h (22, 23). Participants were asked to refrain from use of any prescription or nonprescription medications, supplements, recreational drugs, caffeine, alcohol, or nicotine. Compliance was verified by urine toxicology upon entry to the IPM Unit. At the time of study, approximately half (11/21) of the women were using hormonal contraception (oral birth control $n = 4$; intrauterine device $n = 5$; Nexplanon implant $n = 2$). Of the naturally cycling women not using contraception ($n = 10$), six were in the follicular phase of their menstrual cycle, and they were approximately evenly distributed between the LE conditions.

Study Protocol

Participants were studied using a 2-day laboratory protocol (**Figure 1A**) in an environment free of time cues (no access to windows, clocks, watches, live TV, radio, internet, telephones, and newspapers and continually supervised by staff trained not to reveal information about the time of day). Participants were admitted to the Unit ∼4 h prior to bedtime and were oriented to their suite following examination by the clinical staff. A 7-h sleep opportunity (time in bed) was scheduled according to the centered average of sleep reported daily for 7 days immediately prior to admission. Upon waking, participants began a constant posture 25 min after wake until the end of the light exposure. Two hours after wake, participants began their experimental LE, which continued for 8 h followed by discharge from the Unit.

Light Exposure Conditions

On Day 1 (admission), maximum ambient light during scheduled wake was 48 $\mu W/cm^2$ (∼150 lux) when measured in the horizontal plane at a height of 187 cm and 23 $\mu W/cm^2$ or (∼89 lux) when measured in the vertical plane at a height of 137 cm. During the sleep episode, ambient lighting was switched off (0 lux). Following sleep, maximum ambient light was decreased to 0.05 $\mu W/cm^2$ (∼15 lux) in the horizontal plane at a height of 187 cm and 4.8 $\mu W/cm^2$ (∼3 lux) when measured in the vertical plane at a height of 137 cm, and maintained at that level until the beginning of the experimental light exposure (**Supplemental Table 1**). Ambient room lighting was generated using ceiling-mounted 4100K fluorescent lamps (F96T12/41U/HO/EW, 95W; F32T8/ADV841/A, 32W; F25T8/TL841, 25W; Philips Lighting, The Netherlands) with digital ballasts (Hi-Lume 1% and Eco-10 ballasts, Lutron Electronics Co., Inc., Coopersburg, PA) transmitted through a UV-stable filter (Lexan 9030 with prismatic lens, GE Plastics,



C

Lighting condition	Retinal photopigment weighted illuminances (α-opic EDI lux)					DER	CCT (K)
	S Cone	Melanopsin	Rod	M Cone	L Cone	Melanopsin	
Con low-melEDI	16.79	24.02	28.41	40.58	50.55	0.48	2984
DL low-melEDI	19.57	27.17	30.97	41.47	50.60	0.54	3127
Con high-melEDI	35.06	38.21	40.92	46.65	49.59	0.76	4864
DL high-melEDI	40.75	45.36	46.16	48.08	50.23	0.91	5251

FIGURE 1 | Study protocol and light source spectral characteristics and experimental configuration. The study protocol **(A)** consisted of one night in the laboratory where participants were admitted in the evening and maintained their pre-admit sleep-wake cycle including a 7-h sleep opportunity. On Day 2, participants underwent an 8-h light exposure where they were exposed to ∼50 lux of experimental light with differing spectra **(B)** 3000K or 5000K of standard (conventional) or full-spectrum (daylight-like) LED light. During the light exposure, two lamps were configured on either side of the testing station monitor at which participants maintained a constant posture **(C)**. α-opic EDI and DER values for each light source **(D)** were derived from the CIE S 026:2018 Toolbox V1.049. ADM, admission; CCT, correlated color temperature; Con, Conventional; D/C, discharge; DER, daylight (D65) efficacy ratio; DL, Daylight-like; EDI, equivalent daylight (D65) illuminance.

Pittsfield, MA). Routine illuminance and irradiance measures were conducted using an IL1400 radiometer/powermeter with an SEL-033/Y/W or SEL-033/F/W detector, respectively (International Light, Inc., Newburyport, MA).

Participants were randomized to one of four LE conditions with equal photopic illuminance (∼50 lux in the vertical plane at the level of the eye, and ∼150 lux in the horizontal plane at the level of the desk) but different melanopic illuminance (25–45 melEDI) generated using LED luminaires with either 5000K (high-melEDI) or 3000K (low-melEDI) CCT, and then further differentiated based on having a conventional or daylight-like spectra (**Figures 1B,C**). Luminaires were provided by Seoul Semiconductor Co., Ltd. (Ansan-si, Gyeonggi-do, Korea). During the 8-h LE, participants maintained a constant posture while seated at a testing station (**Figure 1D**), which maintained exposure of ∼50 lux in the vertical plane at the level of the eye, and ∼150 lux in the horizontal plane at level of the desk (**Table 1**). All light sources besides the experimental LED lamps remained turned off throughout the 8-h LE. The spectral profiles, CIE α-opic equivalent daylight (D65) illuminance (EDI) and melanopic daylight (D65) efficacy ratio (DER) (24) for each experimental light sources are shown in **Figures 1B,C**, and for the ambient lighting in **Supplemental Table 1**. Spectral measurements were conducted using a PR-650 SpectraScan Colorimeter with CR-650 cosine receptor (Photo Research Inc., Chatsworth, CA, USA).

TABLE 1 | Participant demographic and photopic illuminance and irradiance measures for each condition*.

Light condition	Age, years	Sex female (*n*, %)	Bedtime, hh:mm	Time in bed, hh:mm	Vertical plane		Horizontal plane	
					Photopic lux	Irradiance, μW/cm²	Photopic lux	Irradiance, μW/cm²
Conventional low-melEDI	24.20 (3.35)	5 (55.56)	23:25 (0:44)	07:00 (0:01)	50.13 (0.86)	15.11 (1.54)	150.97 (3.51)	47.81 (3.02)
Daylight-like low-melEDI	24.44 (3.94)	5 (55.56)	23:23 (1:13)	07:04 (0:04)	50.09 (0.71)	17.35 (4.36)	150.03 (2.05)	51.23 (3.09)
Conventional high-melEDI	25.30 (3.47)	5 (50)	23:50 (1:07)	07:07 (0:11)	50.35 (2.11)	18.06 (2.12)	151.19 (4.27)	49.92 (2.24)
Daylight-like high-melEDI	23.56 (1.8)	5 (55.56)	23:58 (0:52)	07:03 (0:02)	50.27 (0.74)	19.03 (2.15)	147.40 (5.55)	52.55 (2.48)

**Age, bedtime, time in bed, photopic lux, and irradiance are reported as mean ± SD. Sex is reported as number and percent of female participants. Light measurements in the vertical and horizontal planes were taken at the level of the eye and desk, respectively. Time-in-bed was derived from call-ins at bed and wake times.*

Sleepiness, Wellbeing, Performance, and Learning Assessments

The timing of assessments throughout the light exposure are shown in **Figure 1A**. The Performance Battery, which included the Psychomotor Vigilance Task [PVT (25)], Addition Task (26), Karolinska Sleepiness Scale [KSS (27)], and Visual Analog Scales [VAS, (26)], was administered once during dim light and then hourly throughout the light exposure. The battery assessed sustained attention (PVT), working memory and processing speed (Addition Task), subjective sleepiness (KSS), and alertness, health and wellbeing (VAS). A brief practice session to familiarize participants with the battery was administered at admit (**Figure 1A**). Approximately 1 h before lights on, participants completed the d-2 (28) and Word Pairs (29) tasks, which assessed concentration and declarative memory, respectively. These tasks were then repeated 7 h later, 6 h into the light exposure (**Figure 1A**). Approximately 30 min after lights on, participants completed the Motor Sequence Task [MST, (30)] to assess procedural learning and the Headache and Eye Strain Scale (31). These assessments were repeated 7 h later, 30 min before the end of the LE (**Figure 1A**). Detailed descriptions of the assessments are provided in the **Supplemental Materials and Methods**.

Data Analysis

Data from one female and one male participant (3906V, 3907V; neither reported in **Table 1**) were excluded from all analyses due to technical failure during the LE. For tests administered hourly during the LE, the median of each outcome measure was calculated across the LE for each individual. For the d-2 test, only the second session, which was performed during the LE, was included in the analysis comparing the different LE conditions. Data from the d-2 task was excluded for one participant (3923V) in the daylight-like low-melEDI condition as they did not adhere to testing instructions. MST task performance was analyzed across the LE such that the average of each trial across both sessions was used in the analysis. Errors on the MST task were square-root transformed ($\sqrt{x} + \sqrt{x+1}$) prior to analysis. Headache and Eye Strain Scale responses were dichotomized as None/Mild symptoms (scores of 0 and 1), and Moderate/Severe Symptoms (scores of 3 and 4).

The Shapiro–Wilk test was used to assess normal distribution of the data within each LE condition. Data points that were located more than ±1.5 times the interquartile range were considered outliers and removed from analyses (32). No more than one participant was removed from any LE condition (see **Table 2**). The effect of LE condition on each outcome variable was assessed by one-way ANOVA or the Kruskal–Wallis test, as appropriate. If a main effect was detected, *post-hoc* tests were performed to compare between the (1) conventional low-melEDI to daylight-like low-melEDI, conventional high-melEDI and daylight-like high-melEDI; and (2) conventional low- and high-melEDI to daylight-like low- and high-melEDI, respectively. Holm-Sidak and Dunn corrections for multiple comparisons were used for the ANOVA and Kruskal–Wallis tests, respectively. Dichotomized data from the Headache and Eye Strain Scale were analyzed using Fisher's Exact test for Session 1 (start of LE) and Session 2 (end of LE). All statistical analyses were conducted in GraphPad Prism (Version 8.4.0 for Windows, GraphPad Software, San Diego CA, USA).

RESULTS

There were no significant differences in age, bedtime, or pre-admission time-in-bed between the light condition groups ($p > 0.05$ for all; **Table 1**). Group mean (±SEM) and statistical test results for objective and subjective measures collected during the LE are presented in **Table 2**. There were no differences between light conditions in baseline performance for those tests and subjective ratings assessed under dim-light conditions prior to the LE, including the PVT, Addition Task, and d-2, and subjective sleepiness, alertness and general health and wellbeing ($p > 0.05$ for all; **Supplemental Table 2**).

Working Memory, Sustained Attention, and Concentration

The percentage correct responses on the Addition Task was significantly different between LE conditions (Kruskal–Wallis; $H = 13.36$, $p < 0.01$), such that participants exposed to

TABLE 2 | Mean ± SEM and ANOVA results for each test outcome*.

Test outcome	Conventional low-melEDI		Daylight-like low-melEDI		Conventional high-melEDI		Daylight-like high-melEDI		*F* (*p*-value)
	M (SEM)	*N*	*M* (SEM)	*N*	*M* (SEM)	*N*	*M* (SEM)	*N*	
OBJECTIVE MEASURES									
PVT reaction time (ms)	270.70 (6.54)	9	256.40 (8.69)	9	264.00 (12.23)	10	266.10 (10.28)	8	0.37 (0.78)
PVT attentional failures	1.33 (0.29)	9	1.11 (0.41)	9	2.25 (0.78)	10	0.81 (0.31)	8	2.84† (0.42)
Additions % correct	93.98 (1.02)	9	94.53 (1.09)	9	96.57 (1.11)	10	98.89 (0.42)	8	13.36† (0.004)
Additions # attempted	25.00 (2.26)	8	30.06 (3.74)	9	23.95 (2.34)	10	24.00 (2.77)	9	1.06 (0.38)
d-2 CP	255.9 (11.66)	9	262.0 (13.13)	8	264.6 (8.51)	10	254.9 (11.54)	9	0.19 (0.91)
d-2 % errors	1.49 (0.26)	9	1.81 (0.42)	8	1.95 (0.34)	10	2.24 (0.50)	9	0.65 (0.59)
MST % change speed	22.32 (9.6)	8	55.08 (14.03)	9	44.83 (7.32)	10	71.16 (9.59)	9	3.68 (0.02)
MST % change errors	54.70 (14.21)	9	3.09 (16.69)	9	15.45 (17.96)	10	−8.33 (14.0)	9	7.20† (0.06)
Word pairs % recall	94.42 (2.57)	9	95.12 (1.60)	9	92.00 (2.25)	10	93.17 (1.70)	9	0.84† (0.84)
SUBJECTIVE MEASURES									
KSS	5.00 (0.53)	9	3.72 (0.37)	9	3.35 (0.21)	10	5.22 (0.60)	9	11.30† (0.01)
Sleepy—Alert	60.37 (5.08)	9	76.04 (4.98)	9	71.16 (5.33)	10	63.84 (6.97)	9	4.60† (0.20)
Calm—Stressed	25.48 (4.02)	9	12.89 (3.91)	9	13.50 (3.96)	9	12.84 (3.32)	9	2.66 (0.07)
Sad—Happy	74.43 (5.30)	9	73.93 (5.33)	9	83.63 (4.95)	10	79.96 (3.86)	9	0.92 (0.44)
Healthy—Sick	16.49 (2.48)	9	7.78 (2.50)	9	6.11 (1.42)	9	23.32 (8.05)	9	3.25 (0.03)
Energetic—Exhausted	49.59 (4.52)	9	34.32 (6.22)	9	32.04 (5.14)	10	42.15 (8.02)	9	1.74 (0.18)
Exhausted—Sharp	56.12 (6.52)	9	67.52 (7.05)	9	66.37 (6.38)	10	67.37 (7.65)	9	0.62 (0.60)
Tired—Fresh	54.25 (5.81)	9	71.42 (7.17)	9	66.80 (5.51)	10	62.32 (6.10)	9	1.39 (0.26)
Motivated—Unmotivated	31.64 (2.01)	9	26.74 (6.46)	9	29.57 (5.76)	10	16.97 (4.89)	9	1.50 (0.23)

**M, mean; N, the number of participants in each group that were included in the analysis following removal of outliers; CP, concentration performance; † denotes Kruskal–Wallis statistic where data were not normally distributed.*

the daylight-like high-melEDI light performed better than participants exposed to the conventional low-melEDI light ($z = 3.3$, $p < 0.01$). Although there appeared to be a monotonic improvement in the percentage of correct responses with increasing melanopic illuminance, we did not detect a statistically significant difference between the intermediate melEDI conditions (daylight-like low-melEDI and conventional high-melEDI) and the conventional low-melEDI condition (**Figure 2B**). There were no significant differences between LE conditions in reaction time and attentional failures on the PVT, the number of attempted responses on the Addition task, or accuracy and percentage of errors on the d-2 task (**Table 2**).

Procedural Learning and Declarative Memory

Improvement in performance speed across trials (trials 10–12 relative to trial 1) on the MST task was significantly different between the groups (ANOVA; $F = 3.68$, $p < 0.05$; **Table 2**, **Figure 2C**). *Post-hoc* analyses showed that improvement in performance speed across trials was significantly greater in the daylight-like high-melEDI condition compared to the

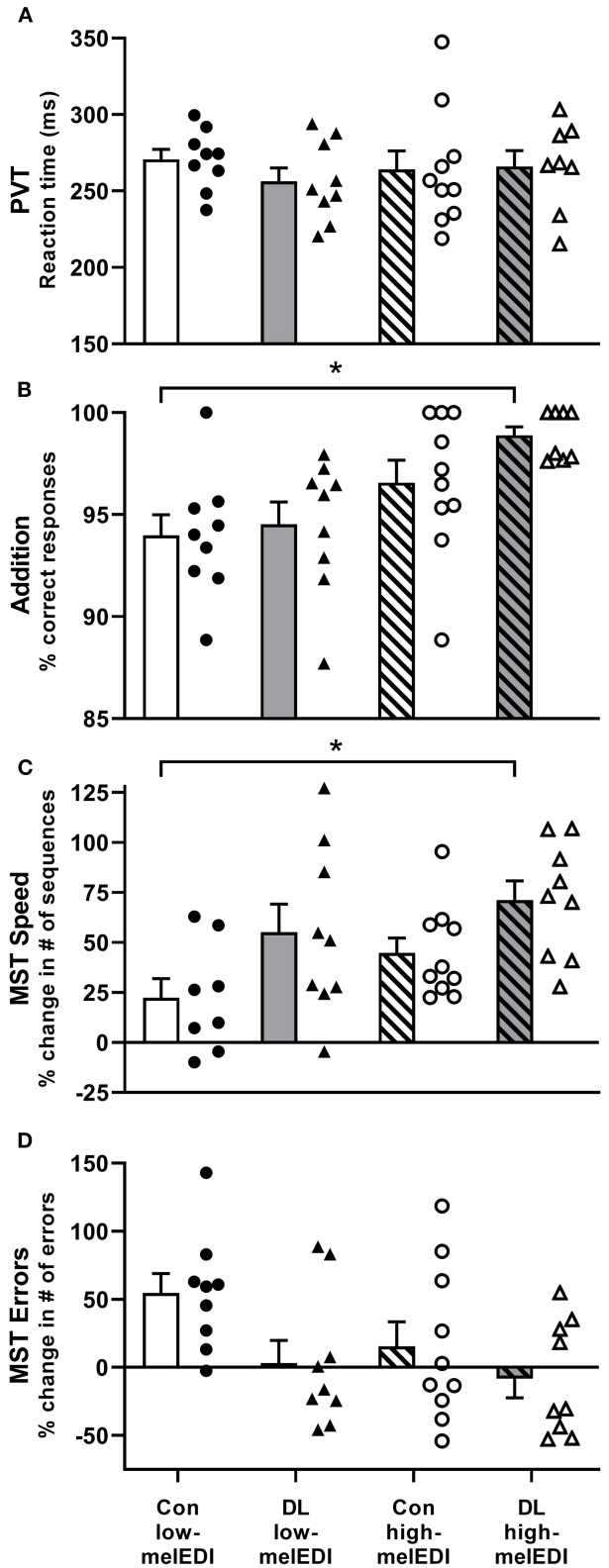


FIGURE 2 | Performance on the PVT, addition task, and motor sequence task for each light exposure condition. Mean ± SEM of PVT reaction time

(Continued)

FIGURE 2 | (A), addition task percent correct **(B)**, Motor Sequence Task percent change in the number of correct sequences **(C)**, and Motor Sequence Task percent change in the number of errors **(D)** for each light exposure condition. Individual participant data are shown for conventional low-melEDI (•), daylight-like low-melEDI (▲), conventional high-melEDI (○), and daylight-like high-melEDI (Δ) light conditions. PVT, psychomotor vigilance task; MST, Motor Sequence Task; Con, Conventional; DL, Daylight-like. *denotes a significant difference between light conditions.

conventional low-melEDI condition ($t = 3.24$, $p < 0.05$), but not for the intermediate conditions. Accuracy on the MST task increased with increasing melEDI exposure although this difference only approached statistical significance ($p = 0.06$, **Figure 2D**). There was no significant effect of light condition on percent recall on the word pairs task (**Table 2**).

Subjective Sleepiness, Health, and Wellbeing

There was a significant effect of light condition on KSS scores (Kruskal–Wallis; $H = 11.3$, $p < 0.05$; **Table 2**; **Figure 3A**). Participants in the conventional high-melEDI condition had lower KSS scores, indicating lower subjective sleepiness, compared to participants in the conventional low-melEDI condition ($z = 2.56$, $p < 0.05$). Conversely, participants in the daylight-like high-melEDI condition reported significantly greater subjective sleepiness than participants in the conventional high-melEDI condition ($z = 2.75$, $p = 0.02$). Additional *post-hoc* contrasts were not statistically significant (**Figure 3A**). In contrast to KSS ratings of sleepiness, the VAS for "sleepy-alert" was not different between conditions (**Figure 3B**).

There was a significant effect of LE condition on VAS ratings for "healthy-sick" (ANOVA; $F = 3.25$, $p < 0.05$). Participants in the daylight-like high-melEDI condition reported feeling significantly more sick than participants in the conventional low-melEDI condition ($t = 2.74$, $p < 0.05$); however, one participant in the daylight-like high-melEDI group, while not a statistical outlier, rated themselves consistently as more sick compared to other participants, and was clinically documented as displaying "Common Cold" symptoms during the LE, which were absent at admission to the laboratory. Additional sensitivity analysis with removal of this participant from the "healthy-sick" scale data showed that the *post-hoc* comparison was no longer significant ($p = 0.1$). VAS ratings on the "calm-stressed" scale trended toward being lower in all LE conditions compared to the conventional low-melEDI condition (**Figure 3C**) although this difference did not reach statistical significance ($p = 0.07$). There was no statistical difference between the LE conditions on any other VAS scales.

Headache and Eye Strain

There was no difference between any of the lighting conditions in irritability, headache, eye strain, eye discomfort, eye fatigue, or blurred vision assessed during the light exposure ($p > 0.3$ for all; **Supplemental Table 3**).

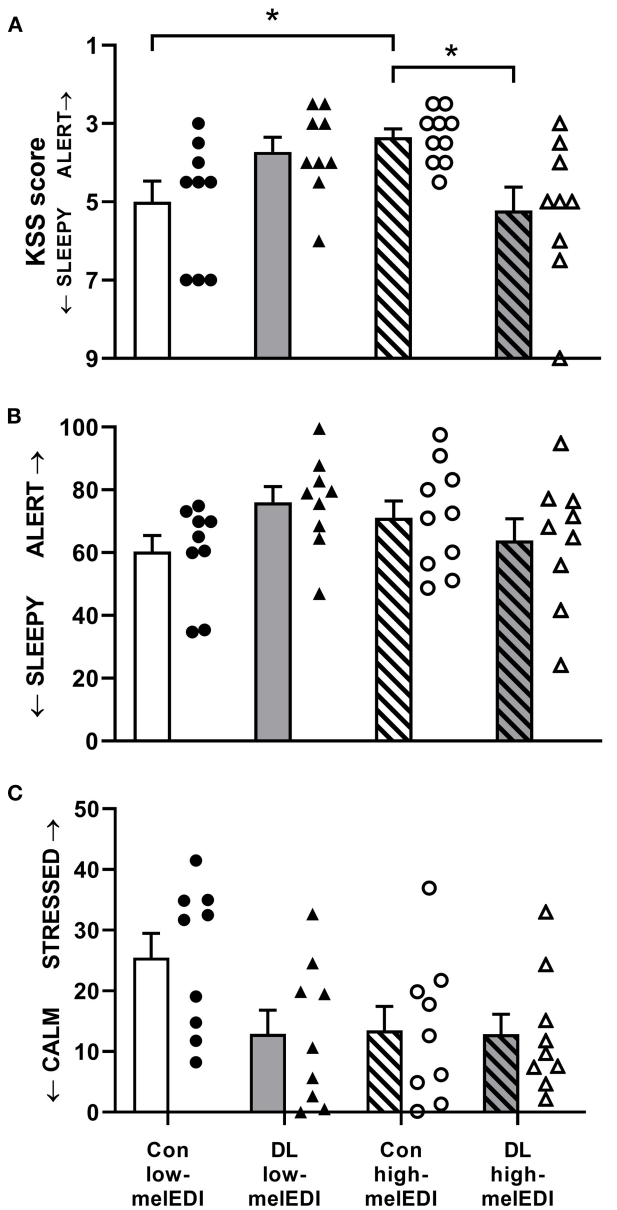


FIGURE 3 | Subjective sleepiness, alertness, and stress for each light exposure condition. Mean ± SEM of KSS sleepiness scores **(A)**, VAS alertness ratings **(B)**, and VAS stress ratings **(C)**. Individual participant data are shown for conventional low-melEDI (•), daylight-like high-melEDI (▲), conventional high-melEDI (○), and daylight-like melEDI (△) light conditions. KSS, Karolinska sleepiness scale; Con, Conventional; DL, Daylight-like. *denotes a significant difference between light conditions.

DISCUSSION

Our results show that compared to being exposed to lower melEDI light, exposure to short-wavelength enriched higher melEDI light during the daytime is associated with significantly less sleepiness, better working memory, processing speed, and procedural learning in moderately sleep-restricted college-aged adults. We did not, however, find a statistically significant difference between lighting conditions in vigilant attention, concentration, or declarative memory. These results provide preliminary evidence supporting the incorporation of short-wavelength (blue) enriched, higher melEDI lighting in the built environment to facilitate learning and task performance in young adults following modest sleep restriction.

To our knowledge, this is the first study in moderately sleep-restricted young healthy adults to find a robust improvement in procedural learning on a motor task, both in performance and accuracy, induced by higher melEDI light exposure during the day. Consistent with our findings, high melEDI light exposure (6500K) during the evening improved procedural learning in older adults (mean age > 60 years) who had UV-blocking (0% light transmission between 300 and 360 nm) intraocular lens (IOL) replacement compared to older adults with blue-blocking IOLs (0% of light transmission between 300 and 400 nm and ∼50% transmission between 410 and 480 nm) (21). Together, these results demonstrate that short-wavelength light exposure facilitates procedural learning, suggesting that the melanopic system is mediating the direct effects of light on learning, as has been shown previously for other cognitive domains [e.g., vigilance (3, 5, 14)].

Working memory and cognitive processing speed, as assessed by the Addition Task (26), was also better under higher melEDI light, exhibiting a clear linear dose-response relationship with melanopic illuminance. These results are consistent with previous studies showing improved working memory during exposure to short-wavelength enriched light (9, 33–35). Studies in similar age groups, but with shorter duration (≤30 min) and monochromatic exposures have also shown that short-wavelength light exposure activates the brain regions associated with working memory (35), including the prefrontal cortex [PFC (33)] whose activation is positively correlated with processing speed and accuracy on a working memory task. Our results extend these findings to show observed improvement in working memory and cognitive processing speed with short-wavelength light exposure in individuals following moderate sleep restriction, and under naturalistic long-duration exposures during the day.

Importantly, our results show that not all cognitive domains are responsive to short-wavelength enriched light to the same extent. In the current study, improvements were not observed in tests of vigilance and reaction time in these modestly sleep-restricted participants. While these findings are in contrast to previous reports of positive effects of short wavelength-enriched light exposure on sustained attention (3, 4, 9–13, 36), not all studies have shown positive effects, especially during daytime exposures (17, 37–40). The inconsistent findings may be due to differences in exposure characteristics including exposure duration and timing, and differences in spectra and intensity between experimental groups. Moreover, other factors such as prior sleep deficiency (40), light history (36), and pupillary constriction due to differences in light spectra and subsequently differences in retinal exposure (8) may have contributed to the differences in performance observed in our study compared to prior studies, especially given that the effects of light exposure on performance during the day are smaller compared to exposure

at night (14). Despite these differences, our results are internally consistent in that the two associated cognitive domains, namely sustained attention and concentration (PVT and d-2 tasks, respectively) were not different between lighting conditions. Importantly, while short wavelength-enriched light exposure did not improve all cognitive domains that were assessed, there was no evidence that light with lower melanopic illuminance was better. Future studies with higher statistical power are necessary to better understand the underlying relative photoreceptor contributions affecting different cognitive domains.

Interestingly, the subjective ratings of sleepiness in response to light were not consistent between different scales. Although we found that generally, higher melanopic illuminance was associated with less subjective sleepiness assessed by the KSS, which is consistent with some previous studies (2–4, 15, 34, 36), we did not see an effect of lighting condition on the self-rated sleepy-alert VAS. This may suggest that the different tests have differential sensitivity for detecting the effects of light on self-reported outcomes and may help to understand inconsistencies in the effect of light on subjective sleepiness and alertness between studies (8). Moreover, the inconsistency between subjective ratings of sleepiness and alertness and objective performance is in agreement with prior reports showing that subjectively reported sleepiness is often an unreliable indicator of objectively assessed neurobehavioral performance (40, 41). Surprisingly, subjective KSS sleepiness ratings did not increase with lower melEDI but was in fact highest under the highest melEDI exposure. This unexpected finding suggests that overall spectral composition of the light, besides only melanopic illuminance, may be influencing some neurobehavioral responses.

Our current study has several limitations. Given that the differences in spectra and melanopic illuminance between the light conditions may not have been large enough to differentiate their effects in some performance domains (e.g., sustained attention) and we only examined the effects of a single intensity, further studies are required to better test a broader range of melanopic illuminances and spectra. Similarly, future work is needed to better evaluate the time-course of light effects on performance. For example, declarative memory was assessed only once in the current study, several hours into the LE, although previous studies have shown a positive effect of light when declarative memory was assessed much sooner after light onset (9, 20). Furthermore, administering the word pairs task for the first time after lights on would have allowed us to assess the effects of light not only on recall but also on learning. Finally, future work is needed to examine the chronic (multiple days) vs. acute (single day) effects of light on performance, including the effects of light on sleep and the impact that this has on subsequent performance, for example sleep dependent learning and memory (29, 30).

The acute alerting response to light may be an effective non-invasive intervention for preventing the neurobehavioral performance impairment associated with inadequate sleep. Sleep restriction negatively influences many aspects of cognitive performance and mood (41, 42), even when only restricted to 7 h of sleep per night (43), as in the current study where participants could only sleep at most for 7 h given that their time in bed was fixed at 7 h. Furthermore, restricted and irregular sleep also impairs performance to a greater extent than stable sleep loss (40) and has also been shown to affect GPA in college students (23, 44), a population with a high prevalence of insufficient sleep. For example, in a study of college students ($n = 1{,}125$), more than 60% were categorized as poor-quality sleepers and a quarter reported getting <6.5 h of sleep per night (22). A sleep duration <7 h in college-aged adults is likely insufficient as when young adults are given an extended sleep opportunity (16 h per night for 9 nights) their total sleep duration has been shown to approach an asymptote of 8.7 h per night (45). Based on the findings of the current study, the acute alerting effects of light may be a useful countermeasure for performance impairment associated with sleep deficiency in this population; however, additional studies are required to evaluate the impact of sleep regularity and varying extents of sleep restriction (e.g., 5 vs. 7 h per night) on the acute alerting effects of light exposure. Blue-enriched light has been tested as an alertness countermeasure in school and college students and showed improvements in processing speed, concentration, and reading speed [e.g., (28, 46, 47)]. Similar benefits of short-wavelength enriched light exposure on performance and alertness have also been observed in office settings (31, 48). These studies, coupled with our results showing that short wavelength enriched long-duration light exposure during the daytime improves working memory/processing speed, procedural learning and subjective sleepiness, support the incorporation of short wavelength enriched white light in indoor environments to enhance learning and cognitive performance.

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 Partners Human Research Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SL and SR contributed to the initial concept of the study. LG, RS, SL, and SR contributed to the design of the study. LG, BK, MM, and SR contributed to, or oversaw, participant recruitment, and data collection. LG, RS, and SR contributed to the analysis of the data. All authors contributed to the interpretation of the data and drafting of the manuscript. All authors have contributed to and approved this manuscript.

FUNDING

This work was supported by an investigator-initiated grant from Seoul Semiconductor Co., Ltd. The lamps used in the study were provided by Seoul Semiconductor Co., Ltd. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication. The project described was supported by Clinical Translational Science Award 1UL1TR002541-01 to Harvard University and Brigham and Women's Hospital from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

ACKNOWLEDGMENTS

We thank the technical, dietary and laboratory staff, nurses and physicians, and the study participants at the Center for Clinical Investigation and Division of Sleep and Circadian Disorders, Brigham and Women's Hospital. We also thank Mr. Craig Poskanzer for providing macros for the learning and memory task analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.624217/full#supplementary-material

REFERENCES

1. Cajochen C, Jud C, Münch M, Kobialka S, Wirz-Justice A, Albrecht U. Evening exposure to blue light stimulates the expression of the clock gene PER2 in humans. *Eur J Neurosci*. (2006) 23:1082–6. doi: 10.1111/j.1460-9568.2006.04613.x
2. Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. *J Clin Endocrinol Metab*. (2005) 90:1311–6. doi: 10.1210/jc.2004-0957
3. Chellappa SL, Steiner R, Blattner P, Oelhafen P, Götz T, Cajochen C. Non-visual effects of light on melatonin, alertness and cognitive performance: can blue-enriched light keep us alert? *PLoS One*. (2011) 266:e16429. doi: 10.1371/journal.pone.0016429
4. Lockley SW, Evans EE, Scheer FA, Brainard GC, Czeisler CA, Aeschbach D. Short-wavelength sensitivity for the direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans. *Sleep*. (2006) 29:161–8. doi: 10.1093/sleep/29.2.161
5. Hankins MW, Lucas RJ. The primary visual pathway in humans is regulated according to long-term light exposure through the action of a nonclassical photopigment. *Curr Biol*. (2002) 12:191–8. doi: 10.1016/S0960-9822(02)00659-0
6. Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res*. (2020) 69:e12655. doi: 10.1111/jpi.12655
7. Prayag AS, Najjar RP, Gronfier C. Melatonin suppression is exquisitely sensitive to light and primarily driven by melanopsin in humans. *J Pineal Res*. (2019) 66:e12562. doi: 10.1111/jpi.12562
8. Souman JL, Tinga AM, Te Pas SF, van Ee R, Vlaskamp BNS. Acute alerting effects of light: a systematic literature review. *Behav Brain Res*. (2018) 337:228–39. doi: 10.1016/j.bbr.2017.09.016
9. Cajochen C, Frey S, Anders D, Späti J, Bues M, Pross A, et al. Evening exposure to a light-emitting diodes (LED)-backlit computer screen affects circadian physiology and cognitive performance. *J Appl Physiol (1985)*. (2011) 110:1432–8. doi: 10.1152/japplphysiol.00165.2011
10. Chellappa SL, Viola AU, Schmidt C, Bachmann V, Gabel V, Maire M, et al. Human melatonin and alerting response to blue-enriched light depend on a polymorphism in the clock gene PER3. *J Clin Endocrinol Metab*. (2012) 97:E433–437. doi: 10.1210/jc.2011-2391
11. Phipps-Nelson J, Redman JR, Schlangen LJ, Rajaratnam SM. Blue light exposure reduces objective measures of sleepiness during prolonged nighttime performance testing. *Chronobiol Int*. (2009) 26:891–912. doi: 10.1080/07420520903044364
12. Rahman SA, St. Hilaire MA, Lockley SW. The effects of spectral tuning of evening ambient light on melatonin suppression, alertness and sleep. *Physiol Behav*. (2017) 177:221–9. doi: 10.1016/j.physbeh.2017.05.002
13. Santhi N, Thorne HC, van der Veen DR, Johnsen S, Mills SL, Hommes V, et al. The spectral composition of evening light and individual differences in the suppression of melatonin and delay of sleep in humans. *J Pineal Res*. (2012) 53:47–59. doi: 10.1111/j.1600-079X.2011.00970.x
14. Rahman SA, Flynn-Evans EE, Aeschbach D, Brainard GC, Czeisler CA, Lockley SW. Diurnal spectral sensitivity of the acute alerting effects of light. *Sleep*. (2014) 37:271–81. doi: 10.5665/sleep.3396
15. Revell VL, Arendt J, Fogg LF, Skene DJ. Alerting effects of light are sensitive to very short wavelengths. *Neurosci Lett*. (2006) 399:96–100. doi: 10.1016/j.neulet.2006.01.032
16. Šmotek M, Vlček P, Saifutdinova E, Koprivová J. Objective and subjective characteristics of vigilance under different narrow-bandwidth light conditions: do shorter wavelengths have an alertness-enhancing effect? *Neuropsychobiology*. (2019) 78:238–48. doi: 10.1159/000502962
17. Cajochen C, Freyburger M, Basishvili T, Garbazza C, Rudzik F, Renz C, et al. Effect of daylight LED on visual comfort, melatonin, mood, waking performance and sleep. *Light Res Technol*. (2019) 51:1044–62. doi: 10.1177/1477153519828419
18. Te Kulve M, Schlangen L, Schellen L, Souman JL, van Marken Lichtenbelt W. Correlated colour temperature of morning light influences alertness and body temperature. *Physiol Behav*. (2018) 185:1–13. doi: 10.1016/j.physbeh.2017.12.004
19. Studer P, Brucker JM, Haag C, Van Doren J, Moll GH, Heinrich H, et al. Effects of blue- and red-enriched light on attention and sleep in typically developing adolescents. *Physiol Behav*. (2019) 199:11–9. doi: 10.1016/j.physbeh.2018.10.015
20. Alkozei A, Smith R, Dailey NS, Bajaj S, Killgore WDS. Acute exposure to blue wavelength light during memory consolidation improves verbal memory performance. *PLoS One*. (2017) 12:e0184884. doi: 10.1371/journal.pone.0184884
21. Chellappa SL, Bromundt V, Frey S, Schlote T, Goldblum D, Cajochen C, et al. Intraocular cataract lens replacement and light exposure potentially impact procedural learning in older adults. *J Sleep Res*. (2020) e13043. doi: 10.1111/jsr.13043
22. Lund HG, Reider BD, Whiting AB, Richard JR. Sleep patterns and predictors of disturbed sleep in a large population of college students. *J Adolesc Health*. (2010) 46:124–32. doi: 10.1016/j.jadohealth.2009.06.016
23. Phillips AJK, Clerx WM, O'Brien CS, Sano A, Barger LK, Picard RW, et al. Irregular sleep/wake patterns are associated with poorer academic performance and delayed circadian and sleep/wake timing. *Sci Rep*. (2017) 7:3216. doi: 10.1038/s41598-017-03171-4
24. CIE. (2018). *CIE S 026:2018. System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light*. Vienna: CIE.
25. Wyatt JK, Ritz-De Ceccp A, Czeisler CA, Dijk D-J. Circadian temperature and melatonin rhythms, sleep and neurobehavioral function in humans living on a 20-h day. *Am J Physiol*. (1999) 277:R1152–63. doi: 10.1152/ajpregu.1999.277.4.R1152
26. Wright KP Jr, Hull JT, Czeisler CA. Relationship between alertness, performance, and body temperature in humans. *Am J Physiol Regul Integr Comp Physiol*. (2002) 283:R1370–7. doi: 10.1152/ajpregu.00205.2002

27. Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. *Int J Neurosci*. (1990) 52:29–37. doi: 10.3109/00207459008994241
28. Keis O, Helbig H, Streb J, Hille K. Influence of blue-enriched classroom lighting on students? cognitive performance. *Trends Neurosci Educ*. (2014) 3:86–92. doi: 10.1016/j.tine.2014.09.001
29. Ellenbogen JM, Hulbert JC, Stickgold R, Dinges DF, Thompson-Schill SL. Interfering with theories of sleep and memory: sleep, declarative memory, and associative interference. *Curr Biol*. (2006) 16:1290–4. doi: 10.1016/j.cub.2006.05.024
30. Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R. Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron*. (2002) 35:205–11. doi: 10.1016/S0896-6273(02)00746-8
31. Viola AU, James LM, Schlangen LJ, Dijk DJ. Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. *Scand J Work Environ Health*. (2008) 34:297–306. doi: 10.5271/sjweh.1268
32. Bakker M, Wicherts JM. Outlier removal, sum scores, and the inflation of the Type I error rate in independent samples t tests: The power of alternatives and recommendations. *Psychol Methods*. (2014) 19:409–27. doi: 10.1037/met0000014
33. Alkozei A, Smith R, Pisner DA, Vanuk JR, Berryhill SM, Fridman A, et al. Exposure to blue light increases subsequent functional activation of the Prefrontal Cortex during performance of a Working Memory Task. *Sleep*. (2016) 39:1671–80. doi: 10.5665/sleep.6090
34. Lehrl S, Gerstmeyer K, Jacob JH, Frieling H, Henkel AW, Meyrer R, et al. Blue light improves cognitive performance. *J Neural Transm*. (2007) 114:457–60. doi: 10.1007/s00702-006-0621-4
35. Vandewalle G, Gais S, Schabus M, Balteau E, Carrier J, Darsaud A, et al. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure. *Cereb Cortex*. (2007) 17:2788–95. doi: 10.1093/cercor/bhm007
36. Münch M, Nowozin C, Regente J, Bes F, De Zeeuw J, Hädel S, et al. Blue-enriched morning light as a countermeasure to light at the wrong time: effects on cognition, sleepiness, sleep, and circadian phase. *Neuropsychobiology*. (2016) 74:207–18. doi: 10.1159/000477093
37. Ru T, de Kort YAW, Smolders KCHJ, Chen Q, Zhou G. Non-image forming effects of illuminance and correlated color temperature of office light on alertness, mood, and performance across cognitive domains. *Build Environ*. (2019) 149:253–63. doi: 10.1016/j.buildenv.2018.12.002
38. Santhi N, Groeger JA, Archer SN, Gimenez M, Schlangen LJ, Dijk DJ. Morning sleep inertia in alertness and performance: effect of cognitive domain and white light conditions. *PLoS One*. (2013) 8:e79688. doi: 10.1371/journal.pone.0079688
39. Segal AY, Sletten TL, Flynn-Evans EE, Lockley SW, Rajaratnam SM. Daytime exposure to short- and medium-wavelength light did not improve alertness and neurobehavioral performance. *J Biol Rhythms*. (2016) 31:470–82. doi: 10.1177/0748730416659953
40. St Hilaire MA, Rüger M, Fratelli F, Hull JT, Phillips AJ, Lockley SW. Modeling neurocognitive decline and recovery during repeated cycles of extended sleep and chronic sleep deficiency. *Sleep*. (2017) 40:zsw009. doi: 10.1093/sleep/zsw009
41. Van Dongen HPA, Maislin G, Mullington JM, Dinges DF. The cumulative cost of additional wakefulness: Dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*. (2003) 26:117–26. doi: 10.1093/sleep/26.2.117
42. Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, Ott GE, et al. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep*. (1997) 20:267–77.
43. Belenky G, Wesensten NJ, Thorne DR, Thomas ML, Sing HC, Redmond DP, et al. Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study. *J Sleep Res*. (2003) 12:1–12. doi: 10.1046/j.1365-2869.2003.00337.x
44. Hershner SD, Chervin RD. Causes and consequences of sleepiness among college students. *Nat Sci Sleep*. (2014) 6:73–84. doi: 10.2147/NSS.S62907
45. Rajaratnam SM, Middleton B, Stone BM, Arendt J, Dijk DJ. Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. *J Physiol*. (2004) 561:339–51. doi: 10.1113/jphysiol.2004.073742
46. Barkmann C, Wessolowski N, Schulte-Markwort M. Applicability and efficacy of variable light in schools. *Physiol Behav*. (2012) 105:621–7. doi: 10.1016/j.physbeh.2011.09.020
47. Sleegers PJC, Mollenaar NM, Galetzka M, Pruyn A, Sarroukh BE, van der Zande B. Lighting effects students' concentration positively: findings from three Dutch studies. *Lighting Res Technol*. (2013) 45:159–75. doi: 10.1177/1477153512446099
48. Mills PR, Tomkins SC, Schlangen LJM. The effects of high correlated color temperature office lighting on employee wellbeing and work performance. *J Circadian Rhythms*. (2007) 5:2. doi: 10.1186/1740-3391-5-2

Conflict of Interest: SL reports commercial interests from the last 3 years (2017–2020) unrelated to the study reported herein but are reported in the interests of full disclosure. SL has received consulting fees from the BHP Billiton, EyeJust Inc., Noble Insights, and Team C Racing; honoraria and/or paid travel from BHP Billiton, DIN, Emory University, IES, Ineos, MIT, Roxbury Latin School, SLTBR, Solemma and Teague; has current consulting contracts with Akili Interactive; Apex 2100 Ltd.; Consumer Sleep Solutions; Headwaters Inc.; Hintsa Performance AG; Light Cognitive; Lighting Science Group Corporation; Mental Workout; PlanLED; Six Senses; Stantec; and Wyle Integrated Science and Engineering; has received unrestricted equipment gifts from Bionetics Corporation and F. Lux Software LLC; royalties from Oxford University Press; and has served as a paid expert in legal proceedings related to light, sleep, and health. He is an unpaid Board Member of the Midwest Lighting Institute (non-profit) and was the Program Leader for "Safety and Productivity Improvements" in the CRC for Alertness, Safety, and Productivity from 2015 to 2019. Dr. Lockley's interests were reviewed and managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies. SR holds patents for Prevention of Circadian Rhythm Disruption by Using Optical Filters and Improving sleep performance in subject exposed to light at night; SR owns equity in Melcort Inc.; has provided paid consulting services to Sultan and Knight Limited, Bambu Vault LLC, Lucidity Lighting Inc.; and has received honoraria as an invited speaker and travel funds from Starry Skies Lake Superior, University of Minnesota Medical School, PennWell Corp., and Seoul Semiconductor Co., Ltd. These interests were reviewed and managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies.

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 Neurology

METHODS
published: 04 March 2021
doi: 10.3389/fneur.2021.624861

The Lighting Environment, Its Metrology, and Non-visual Responses

Luc J. M. Schlangen[1*†] *and Luke L. A. Price*[2†]

[1] Department Human-Technology Interaction, Intelligent Lighting Institute, Eindhoven University of Technology, Eindhoven, Netherlands, [2] Centre for Radiation, Chemical and Environmental Hazards, Public Health England, Didcot, United Kingdom

International standard CIE S 026:2018 provides lighting professionals and field researchers in chronobiology with a method to characterize light exposures with respect to non-visual photoreception and responses. This standard defines five spectral sensitivity functions that describe optical radiation for its ability to stimulate each of the five α-opic retinal photoreceptor classes that contribute to the non-visual effects of light in humans via intrinsically-photosensitive retinal ganglion cells (ipRGCs). The CIE also recently published an open-access α-opic toolbox that calculates all the quantities and ratios of the α-opic metrology in the photometric, radiometric and photon systems, based on either a measured (user-defined) spectrum or selected illuminants (A, D65, E, FL11, LED-B3) built into the toolbox. For a wide variety of ecologically-valid conditions, the melanopsin-based photoreception of ipRGCs has been shown to account for the spectral sensitivity of non-visual responses, from shifting the timing of nocturnal sleep and melatonin secretion to regulating steady-state pupil diameter. Recent findings continue to confirm that the photopigment melanopsin also plays a role in visual responses, and that melanopsin-based photoreception may have a significant influence on brightness perception and aspects of spatial vision. Although knowledge concerning the extent to which rods and cones interact with ipRGCs in driving non-visual effects is still growing, a CIE position statement recently used melanopic equivalent daylight (D65) illuminance in preliminary guidance on applying "proper light at the proper time" to manipulate non-visual responses. Further guidance on this approach is awaited from the participants of the 2nd International Workshop on Circadian and Neurophysiological Photometry (in Manchester, August 2019). The new α-opic metrology of CIE S 026 enables traceable measurements and a formal, quantitative specification of personal light exposures, photic interventions and lighting designs. Here, we apply this metrology to everyday light sources including a natural daylight time series, a range of LED lighting products and, using the toobox, to a smartphone display screen. This collection of examples suggests ways in which variations in the melanopic content of light over the day can be adopted in strategies that use light to support human health and well-being.

Keywords: melanopsin, intrinsically-photosensitive retinal ganglion cells, circadian rhtyms, melatonin, visual perception, non-image forming effects of light, sleep, light therapy

OPEN ACCESS

Edited by:
Manuel Spitschan, University of Oxford, United Kingdom

Reviewed by:
Pablo A. Barrionuevo, Instituto de Investigación en Luz, Ambiente y Visión (CONICET), Argentina
Kevin William Houser, Oregon State University, United States

****Correspondence:***
Luc J. M. Schlangen
l.j.m.schlangen@tue.nl

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *01 November 2020*
Accepted: *04 February 2021*
Published: *04 March 2021*

Citation:
Schlangen LJM and Price LLA (2021) The Lighting Environment, Its Metrology, and Non-visual Responses. Front. Neurol. 12:624861. doi: 10.3389/fneur.2021.624861

INTRODUCTION

Light is essential for vision, but starting from the earliest weeks of life (1–5) it also drives important non-image-forming (NIF) effects that are powerful determinants of sleep (6), circadian rhythms (7), alertness (8, 9), mood (10) and hormone secretion (11). This paper is intended for lighting professionals, policy makers and researchers with a practical interest in lights' eye-mediated NIF effects, chronobiology and health. It explains and discusses a standardized light metrology (12) that is based on five retinal photoreceptor types, each of which has a distinct spectral sensitivity and may contribute to non-visual or NIF responses (13). Significantly, melanopsin is the functional photopigment for one of these five photoreceptor types.

Accumulating evidence (6, 14–21) suggests that the spectral sensitivity of melanopsin is the most successful and parsimonious model to predict responses to medium and long duration exposures to ambient light like circadian phase shifting, or modulations in pupil-size, alertness, and melatonin secretion. However, no single action spectrum or proxy will ever provide the complete picture (13, 22) for all the testable variations in intensity, timing, duration, and patterns of light exposure that can be created in laboratory settings (23, 24). Moreover, the effects of light in field settings are often confounded by various uncertainties which may be due to non-photic effects, interindividual variations in sensitivity to light (25), differences in the populations studied and the reduced environmental and behavioral control in real-life environments. Whilst acknowledging these limitations, some examples will be presented to suggest ways in which the melanopsin-based quantities from the standardized light metrology (12) can already be applied in practice.

The pineal hormone melatonin is an important, commonly used marker of circadian rhythms and the effects of light on its nocturnal secretion are well-established (11, 14, 15, 26, 27). In humans, melatonin facilitates sleep initiation and sleep consolidation (28), and is only secreted (resulting in detectable levels) during the period that we habitually sleep. Nocturnal light exposure acutely suppresses circulating melatonin levels (11), but being awake, or asleep, by itself has no direct effect on urinary melatonin (29). Under constant dim light conditions, melatonin levels start rising in the evening and peak at night about 2 h before the core body temperature reaches its nadir (denoted as CBTmin), with this nadir typically occurring a further 2 h before (habitual) wake-up time (30, 31).

The sleep-wake cycle closely follows the 24 h melatonin cycle: habitual bedtime is about 2 h after the melatonin onset (in dim light), while habitual wake-up typically occurs about 10 h after melatonin onset (in dim light), with melatonin onset being defined as the time point at which the salivary melatonin concentration increased to and stayed above either 4 pg/ml or 25% of its fitted amplitude (32, 33). Around the habitual wake-up time, melatonin concentrations are decreasing and drop to undetectable levels, even in dim light conditions. When living outdoors for a week in summer, camping under natural light and without any electric light exposure, average melatonin onset occurs near sunset, while average melatonin offset occurs before wake time, just after sunrise (34). An abrupt change of the sleep-wake cycle leaves the melatonin 24 h profile (virtually) unaffected (35), whilst a single laboratory light exposure with the appropriate timing and duration can shift the phase of the melatonin rhythm by up to 3 h (27, 36). However, negative feedback in the genetic clock mechanism, regulated by *Sik1*, limits the phase-shifting effects of light (37) and in jet-lagged humans and most other mammals behavioral phase shifts remain restricted to about 1 h per day (one time zone) (38).

The effects of light on the 24 h melatonin profile are shown schematically in **Figures 1A–D**. Morning light exposure advances the timing of melatonin secretion, facilitating earlier bedtimes and sleep onset, while evening light exposure postpones melatonin secretion, thus delaying the drive to go to bed (27). The circadian system considers light exposure that occurs before the CBTmin to be evening light, whereas light exposure that occurs during the hours after the CBTmin is considered to be morning light (27). Daytime light exposure can enhance nocturnal melatonin secretion (39), strengthen the body clock and reduce sensitivity to late evening/nighttime light exposures (40–45). Even a single 2.5 h bright light exposure in the early evening is sufficient to reduce the acute sleep-disruptive effects of late evening light exposure (46).

Figures 1E,F show the effect of morning and evening light on the sleep-wake cycle within a double-plotted actogram. When the light-dark cycle has a low amplitude, i.e., insufficient contrast between day and night, the circadian rhythm is free-running. A person that lives in constant dim light, has a sleep-wake cycle that shifts slowly to a later time every next day. This is depicted in **Figure 1G**, and is due to the fact that under dim light the circadian rhythm is free running at its endogenous period, which is on average about 24.2 h for humans (35, 47–50). The right combination of morning and evening light exposure entrains the circadian rhythm, so that it remains in sync with the 24 h light-dark cycle, see **Figure 1H**.

Evidence from the US suggests people in modern society may spend around 90% of their time indoors (51–53). The typical human indoor environment provides relatively little light during daytime, especially compared to the natural light outdoors, where illuminances may be 1, 2, or even 3 orders of magnitude higher. For instance, the European standard for lighting of work places (54) specifies minimum values for maintained horizontal illuminance in offices between 200 and 750 lx, depending on the specific task, whereas the horizontal illuminance outdoors can be as high as 150 klx (55). In the late-evening hours and at night, the widespread use of electrical light and luminous display devices results in extended exposures to light (56). Through their impact on circadian rhythms, these unnatural lighting conditions enhance eveningness (34). Moreover, modern lifestyles and (unnatural) light exposures are known to result in more "social jet-lag," and this has negative consequences for sleep, performance, well-being and health (57, 58). Evolution shaped us to live in much brighter daytime conditions than present in our modern indoor life. For a healthy lit environment, people with a normal diurnal activity pattern (i.e., day-oriented, and usually in bed at night) need bright white light during the day, and especially in the morning, while

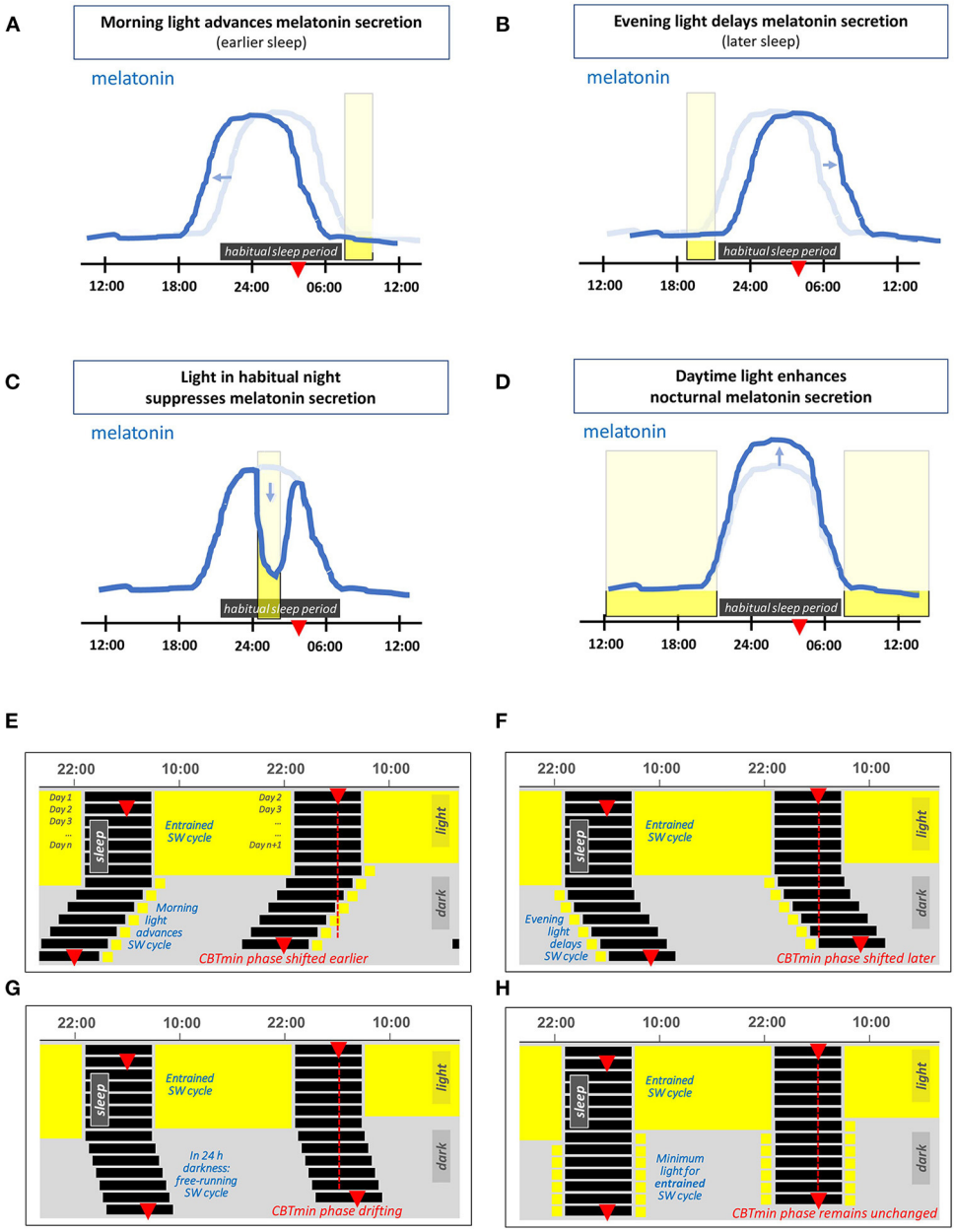


FIGURE 1 | (A–D) Schematic representation of the effects of light on the 24 h melatonin profile. This profile marks the circadian rhythm and the habitual sleep period. The latter is indicated by the horizontal dark rectangle, the light blue line represents the corresponding melatonin profile for an individual in 24 h dim light conditions. The red triangle indicates the time at which the core body temperature reaches its nadir at about 2 h before (habitual) wake-up time. The vertical rectangles denote a particular light exposure. **(A)** Light exposure in the morning advances the timing of melatonin secretion (i.e., supports earlier bedtime and awakening). **(B)** Light exposure in the evening delays the timing of melatonin secretion. **(C)** Light exposure during the habitual sleep period acutely suppresses melatonin secretion. **(D)** Daytime light exposure strengthens subsequent nocturnal melatonin secretion. **(E–H)** Double-plotted actograms schematically showing patterns of the human sleep-wake (SW) cycle resulting from different light exposures, each starting with several days in 16L:8D and with light restricted on subsequent days. **(E)** Light restricted to 1 h in the morning on waking, **(F)** light restricted to 1 h in the late evening light. **(G)** In complete darkness (a D:D cycle), since the intrinsic period of the circadian rhythm in humans slightly exceeds 24 h, the timing of the SW cycle drifts later and later across days. **(H)** A theoretical example with sufficient light each morning and evening to entrain the SW cycle.

they should reduce prolonged exposures in the late evening and avoid light as much as possible at night [see also CIE position statement (59)].

Although the introduction concentrates on chronobiology, it should be noted that chronobiological responses are just a subset of non-visual responses to light. The non-visual metrology tools

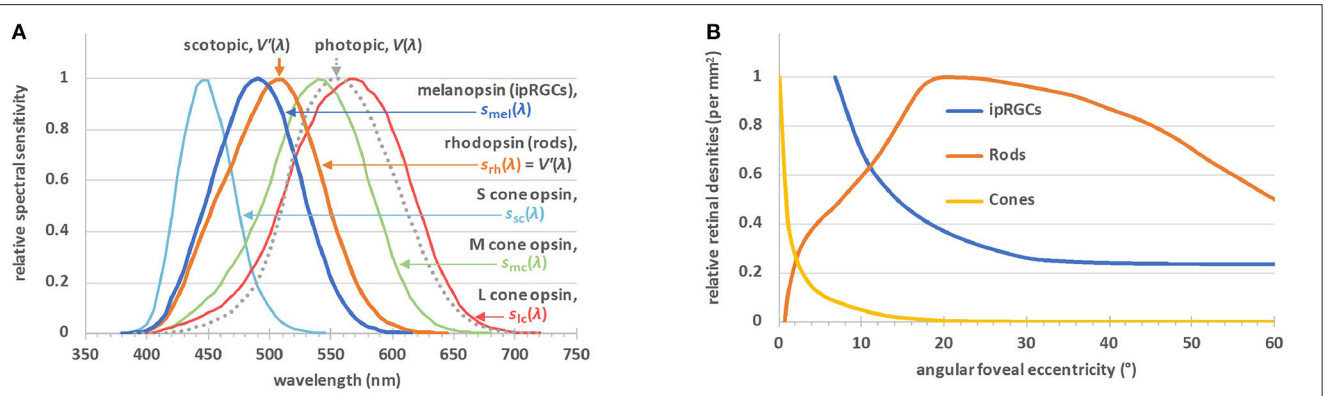


FIGURE 2 | (A) The α-opic action spectra for non-visual metrology (12), $s_\alpha(\lambda)$; S-cone opic (α = sc), M-cone-opic (α = mc), L-cone opic (α = lc), rhodopic (α = rh), or melanopic (α = mel), where $s_{rh}(\lambda)$ is defined to be equal to the spectral luminous efficiency function for scotopic vision, $V'(\lambda)$. The spectral luminous efficiency function for photopic vision, $V(\lambda)$, is also plotted. **(B)** The relative densities of the rods, cones and ipRGCs by angular eccentricity from the central fovea (61, 62). There are no ipRGCs in the central visual field, but outside this field their density falls off to a steady value. The maximum density of the ipRGCs is ∼20–25 cells·mm^{-2}, 4 orders of magnitude lower than the maximum densities of the rods or cones.

described in this paper, and the information presented below, can also be applied to other retinal responses to ambient light.

RETINAL PHOTORECEPTORS

Early this century a new class of retinal photoreceptor, the intrinsically-photosensitive retinal ganglion cell (ipRGC), was discovered (60). In addition to receiving extrinsic input signals from rods and cones, this class of photoreceptor expresses melanopsin which gives rise to the intrinsic light sensitivity after which it is named (13). **Figure 2A** shows the spectral sensitivities of the five classes of photoreceptors involved in non-visual photoreception (12), together with the well-known $V(\lambda)$ function officially denoted as *the spectral luminous efficiency function for photopic vision*. In humans, melanopsin photoreception occurs efficiently across the short wavelength range of the visible spectrum between 420 and 560 nm, with a peak sensitivity *in vivo* at ∼490 nm (13). Melanopsin-based signaling is more sluggish in onset and more sustained than rod or cone signaling (63–65). At least six subtypes of ipRGCs, M1–M6, have been identified in the mammalian retina (M1–M5 to date in humans) (66–69). Unlike rods and cones, ipRGCs have photosensitive dendrites that extend transversely across the retina. **Figure 2B** shows the relative densities of the rods, cones and ipRGCs as a function of retinal eccentricity. Melanopsin-based photoreception predicts both clock-mediated and acute non-visual responses under a range of everyday light exposures (21). The clock-mediated effects include regulation of the sleep-wake cycle and circadian phase shifting, whereas melatonin suppression, control of alertness and the steady state pupil diameter are examples of acute responses to light (17, 18, 20, 21).

During the first 5 years after birth, the crystalline lens in the human eye is still transmissive for short wavelength visible light and even for ultraviolet radiation (UVR) close to 320 nm (70). It becomes opaque to UVR at about an age of 5, and as age increases, the lens transmittance in the short wavelength range (i.e., violet and blue) of the visible spectrum decreases. Consequently, retinal photoreceptors receive less light input at older ages, particularly the short-wavelength sensitive photoreceptors (rods, S-cones and ipRGCs). Although adaptation mechanisms and neural plasticity may compensate for the age-induced decline in short-wavelength light that actually reaches the retina, the number of ipRGCs drops with age advancing beyond 50 (71). This loss of ipRGCs is accompanied by changes in cell morphology and an observable increase in randomness of the ipRGC distribution pattern.

It has been suggested that a decline in melanopsin photoreception with age could play a significant, deteriorating role in sleep and neuro-cognitive effects of aging (71), including those related to dementia as well as general senescence. It is plausible that these effects may be partly mediated by the negative effects on sleep due to a compromised non-visual circadian regulation with increasing age (72–74). Partly corroborating this hypothesis, it has also been observed that more fragmented and less stable sleep-activity patterns are associated with a higher all-cause mortality (up to ∼20%) in the middle-aged and the elderly, independently of age (75).

QUANTIFYING LIGHT FOR LUMINOUS PERCEPTION

Traditional lighting practice primarily targets visual performance, comfort and other aspects of the visual domain, quantifying lighting designs and installations and light exposures using luminous flux (in lumens), illuminance (in lux) and other visually related quantities. These quantities describe the luminous sensation of a light source under photopic conditions [i.e., for luminances above 5 cd/m^2 (76)], where cones drive human visual responses. Scotopic vision occurs while the eye is adapted to very low luminances (below 0.001 cd/m^2). Under scotopic conditions, visual responses are driven by rods. The conversion between luminance and illuminance depends on the

apparent source size measured in steradians, so general scotopic and photopic thresholds cannot be expressed in lux.

Individually, photoreceptors follow the principle of univariance, meaning they cannot discriminate between a change in intensity and a change in wavelength (77). As such, the spectral sensitivities of the human luminous sensation for photopic and scotopic vision can be described by the spectral luminous efficiency functions $V(\lambda)$ and $V'(\lambda)$, respectively, see **Figure 2A**. The spectral power of light, for instance, can be photopically-weighted or scotopically-weighted by multiplying each wavelength by $V(\lambda)$ or $V'(\lambda)$, respectively. Photometric units (such as the lumen, lux or candela) are obtained after summing the result (which is now a photopically- or scotopically-weighted spectrum) over all wavelengths and multiplying the result by the corresponding efficacy constants (K_m and K'_m, respectively), as described below.

By definition, monochromatic radiation with a frequency of 540×10^{12} Hz, (which corresponds to the wavelength 555 nm in standard air[1]) has a luminous efficacy of 683 lm/W (78). Since the $V(\lambda)$ function reaches its peak value at 555 nm, this is where the maximum luminous efficiency for photopic vision (denoted by constant K_m) equals 683 lm/W. The maximum luminous efficiency for scotopic vision (denoted by constant K'_m) equals 1,700 lm/W, which follows from the relationship $K_m \cdot V(555\,\text{nm}) = K'_m \cdot V'(555\,\text{nm})$.

The ratio of the luminous output (of a source) as evaluated using the scotopic efficiency function to the luminous output evaluated using the photopic efficiency function is known as the S/P ratio. The S/P ratio is a characteristic of the spectral distribution of the light, and by definition, equals 1 for monochromatic radiation with a frequency of 540×10^{12} Hz, or a wavelength of 555 nm (in air). An S/P ratio above 1 denotes that a light source is more activating to rods per (photopic) lumen than 1 lumen of monochromatic light at 555 nm.

Mesopic vision occurs while the eye is adapted to light levels that are in between photopic and scotopic conditions. In this range, i.e., in the mesopic regime, the combined action of rods and cones defines the human visual response. However, ipRGCs are implicated in retinal adaptation (79) and may be involved in the regulation of mesopic and photopic visual sensitivity (80).

Do and Yau (81) provided an extensive review of ipRGCs and their functions, including their roles in visual responses. Already in 2002, Hankins and Lucas had demonstrated that adaptations of the human primary cone visual pathway according to time of day are driven by a non-rod, non-cone photopigment with a spectral sensitivity profile that matches the standard profile of an opsin:vitamin A-based pigment with a peak at ∼483 nm (79). The resulting curve is now widely accepted as the prototype action spectrum of the photopigment melanopsin and describes the intrinsic light sensitivity in ipRGCs. Another demonstration that melanopsin can drive visual perception comes from a case study of a blind individual lacking functional rods and cones who could report whether a monochromatic light stimulus of 480 nm was on or off, but failed to do so for other wavelengths (82).

Recent studies suggest the possibility of further melanopic influences on visual responses. Human brightness perception can be greater when the light stimulus has a larger melanopic content while being isoluminant for rods and cones (83), and further experiments have quantified the effect of melanopsin on brightness perception in more detail (84, 85). Melanopsin effects can increase brightness perception by up to 10%, especially for brightness discrimination tasks that involve little or no differences in luminance and hue (86). Finally, it is worth noting that melanopsin photoreception can also improve the detectability of coarse patterns (80). Together these results indicate that melanopsin is not only implicated in non-visual responses and visual adaptation, but may also contribute meaningfully to further visual responses like brightness perception and pattern recognition. However, proper demonstration of melanopic influences to vision is methodologically complex and still faces many challenges (87). At present, the relevance of melanopsin-based photoreception for brightness perception beyond laboratory settings is not yet settled and merits further investigation.

QUANTIFYING LIGHT FOR NON-VISUAL RESPONSES: α-OPIC METROLOGY

As detailed above, the melanopsin-based photoreception of ipRGCs constitutes an important driver of non-visual responses. In their work, many lighting designers already draw on a wide understanding of the visual, architectural and psychological aspects of light and lighting. Awareness amongst lighting professionals is increasing that next to cone-dominated metrics such as correlated color temperature (CCT), illuminance and luminance, there is a need to consider melanopsin-based photoreception in specifications, codes, recommendations and research. All these metrics are useful tools for quantifying or comparing individual aspects within a lighting scheme, but they cannot replace an experienced designer's overall appreciation of the interplay between the diverse effects of light. In addition, NIF photoreception relates to the light arriving at the eyes from all directions. This requires recommendations framed in terms of light arriving at eye level—e.g., measured normal to the visual axis in the vertical plane—rather than with reference to the light falling on the horizontal plane, walls or object surfaces.

No single action spectrum or proxy can describe all eye-mediated non-visual responses to light (13, 22). All five known receptor types can contribute to these responses, and the relative contribution of each individual photoreceptor type can vary depending on the specific response and upon light exposure properties such as intensity, spectrum, duration, timing (external and internal/circadian), prior light history and sleep deprivation state of the individual. Based on the Lucas et al. review paper (13), the International Commission on Illumination (CIE)—the worldwide body responsible for developing international standards and reports on light and lighting—has published CIE S 026:2018 "*CIE System for Metrology of Optical Radiation*

[1]For readability, 555 nm will be written instead of $\lambda_d \approx 555.016$ nm for the wavelength of light corresponding to a frequency of 540×10^{12} Hz for light in standard air.

TABLE 1 | Glossary of α-opic metrology (12), where $s_\alpha(\lambda)$ refer to the α-opic action spectra shown in **Figure 2A**, $K_{\alpha,v}$ is the "α-opic stimulus per lumen," $K_{\alpha,v}$ calculated for D65 (i.e., the α-opic ELR for D65, $K_{\alpha,v}^{D65}$) is a normalization constant. There are two ways to calculate the α-opic DER: α-opic DER = α-opic ELR / α-opic ELR for D65 = α-opic EDI / illuminance.

Quantity	Abbreviation	Formula	Meaning	Unit
α-opic irradiance *see note 1*	–	$E_\alpha = \int E_{e,\lambda}(\lambda)s_\alpha(\lambda)d\lambda$	Weighted spectral irradiance, $E_{e,\lambda}$, integrated over wavelength	$W{\cdot}m^{-2}$
α-opic efficacy of luminous radiation	α-opic ELR	$K_{\alpha,v} = E_\alpha / E_v$	Quotient of α-opic irradiance, E_α, and illuminance, E_v	$W{\cdot}lm^{-1}$
α-opic equivalent daylight (D65) illuminance	α-opic EDI	$E_{v,\alpha}^{D65} = E_\alpha / K_{\alpha,v}^{D65}$ *see notes 2 & 3*	Illuminance level of daylight (D65), producing an equal α-opic irradiance, E_α, as the test light	lx
α-opic daylight (D65) efficacy ratio	α-opic DER	$\gamma_{\alpha,v}^{D65} = K_{\alpha,v} / K_{\alpha,v}^{D65}$ *see note 2*	Ratio of the α-opic ELR of the test light, $K_{\alpha,v}$, to the α-opic ELR of daylight (D65), $K_{\alpha,v}^{D65}$	–

Note 1. The α-opic photoreceptor types are denoted by five subscripts: S-cone opic (α = sc), M-cone-opic (α = mc), L-cone opic (α = lc), rhodopic (α = rh) and melanopic (α = mel), and the spectral weighting functions s_α (λ) are shown in ***Figure 2A****.*

Note 2. The five normalization constants, $K_{\alpha,v}^{D65}$, are $K_{sc,v}^{D65}$ = 0.8173 mW·lm^{-1}, $K_{mc,v}^{D65}$ = 1.4558 mW·lm^{-1}, $K_{lc,v}^{D65}$ = 1.6289 mW·lm^{-1}, $K_{rh,v}^{D65}$ = 1.4497 mW·lm^{-1}, and $K_{mel,v}^{D65}$ = 1.3262 mW·lm^{-1}.

Note 3. The non-standard quantity "melanopic equivalent illuminance" (often referred to as EML) can be converted into the melanopic EDI by a multiplication with 0.9058 (i.e., the α-opic DER of illuminant E). The other "α-opic equivalent illuminances" have no such linear relationship with their α-opic EDI analogs, as CIE S 026 and Lucas et al. (13) use slightly different action spectra for the rods and cones.

for ipRGC-Influenced Responses to Light" (12). This new International Standard defines spectral sensitivity functions, quantities and metrics to describe optical radiation for its ability to stimulate each of the five retinal photoreceptor classes that, via ipRGCs, can contribute to the non-visual effects and functions of light in humans.

The Lucas et al. (13) authors used an opsin template and a lens transmittance function to establish five action spectra that describe the spectral sensitivity of all five known retinal photoreceptors that can contribute to non-visual responses. The CIE standard (12) adopts the same melanopsin action spectrum as the Lucas et al. (13) authors. However, for consistency with existing standards and psychophysical data, CIE S 026 adopts the 10-degree cone fundamentals (88) and the spectral luminous efficiency function for scotopic vision, $V'(\lambda)$, to describe the cone and rod action spectra, respectively.

Figure 2A shows the five spectral weighting functions or action spectra, $s_\alpha(\lambda)$, for the five retinal photoreceptor classes: S cone, M cone, L cone, rhodopsin and melanopsin-encoded photoreception of ipRGCs as defined in CIE S 026. For each of these five (α-opic) photoreceptors, an α-opic irradiance can be calculated from the spectral irradiance, $E_{e,\lambda}$, of a (test) light source, see **Table 1**. The α-opic irradiance of a test light divided by its illuminance, E_v, defines its α-opic efficacy of luminous radiation (α-opic ELR). The ratio of this α-opic ELR to the α-opic ELR of standard daylight (D65) defines the α-opic daylight (D65) efficacy ratio (α-opic DER) of the test light.

REFERENCE ILLUMINANTS, EQUIVALENT ILLUMINANCES, S/P, AND M/P RATIOS

Since daylight is a naturally occurring stimulus under which we evolved, it is an interesting and relevant point of reference to evaluate and express the properties of human light conditions within the built environment. The CIE standard illuminant D65 is adopted as the reference illuminant in CIE S 026 (2018) to express each of the five α-opic irradiances as a photometric equivalent quantity[2]. These quantities are the five α-opic equivalent daylight (D65) illuminances (α-opic EDIs). Each α-opic EDI is expressed in lx and corresponds to the illuminance of D65 radiation that is required to provide an equal α-opic irradiance as the test light, for a given α-opic photoreceptor. The term "test light" used here refers to the light being considered, to differentiate it from the reference illuminant.

The photometric equivalent concept adopted in S 026 is not restricted to illuminance (unit lx), and luminance (unit cd/m^2). It can also be applied to other quantities such as light exposure (unit lx·h), luminous energy (unit lm·s), and luminous intensity (unit cd) [3].

Returning to CIE S 026, when describing the spectral properties of a test light, the ratio of the α-opic EDI of a test light to its illuminance defines the α-opic DER of the test light, see **Table 1**. In other words, the melanopic DER represents the ratio of the melanopic flux ("M") per photopic luminous flux ("P") of a test light, and this dimensionless quantity can usefully be thought of as the new "M/P ratio." By definition, this ratio is normalized to 1 for the reference illuminant D65. The S/P ratio is an established lighting metric. It equals 1 for monochromatic radiation of 555 nm, as the S/P ratio effectively uses radiation of 555 nm as its normalizing reference illuminant. In case the melanopic EDI is 30 lx, the test light has the same activating effect on ipRGCs as 30 lx of radiation conforming to the spectrum of

[2]D65 represents daylight with a color temperature of ∼6,500 K. Other reference illuminants (like standard illuminant A or equi-energy illuminant E) could be used instead of D65 to define equivalent illuminances, but such non-standard quantities should be avoided as much as possible. Lucas et al. (13) adopted the equi-energy illuminant (E) as the reference illuminant when introducing the "α-opic equivalent illuminance" concept for non-visual metrology (13, 89), but without explicit mention of the reference illuminant selected.

[3]In the same order, the photometric quantities that correspond to this list are α-opic equivalent daylight (D65) light exposure [lx·h], α-opic equivalent daylight (D65) luminous energy [lm·s], and α-opic equivalent daylight (D65) luminous intensity [cd].

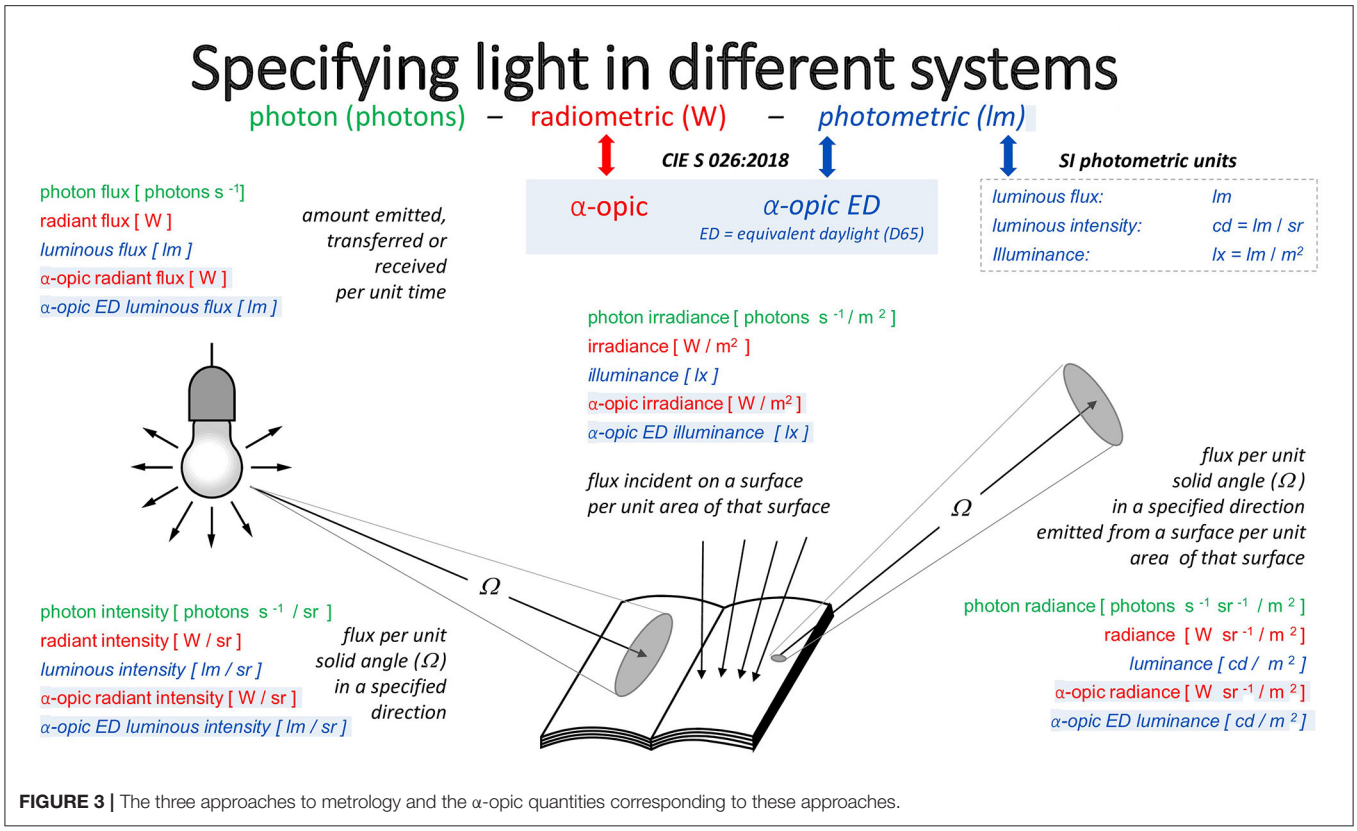


FIGURE 3 | The three approaches to metrology and the α-opic quantities corresponding to these approaches.

D65 daylight. In the same way, a scotopic illuminance of 30 lx indicates that the test light has the same effect on rods as 30 lx of radiation at 555 nm.

PHOTOMETRIC AND RADIOMETRIC α-OPIC QUANTITIES

There are three different mainstream metrological approaches for quantifying visible optical radiation:

- radiometry based on spectral energy,
- radiometry based on spectral count of photons, and
- photometry based on spectral luminous efficiency function for photopic vision, $V(\lambda)$, and the efficacy constant, K_m (or $V'(\lambda)$ and K'_m for scotopic vision).

In the SI system, radiometry is described as "the field of metrology related to the physical measurement of the properties of electromagnetic radiation, including visible light." Radiometric quantities can be unweighted, but photobiological quantities are typically weighted according to a suitable action spectrum that describes the relative efficiency of radiation as a function of wavelength in producing an effect.

Energy-based radiometry is often used by physicists, whereas photobiologists and photochemists often use the photon system, and the light and lighting professions have a strong preference for photometry. Photometry uses special SI units like cd, lm and lx. Radiometry and photometry and their units are closely related through the current definition of the SI base constant K_{cd} ($K_{cd} \approx K_m$, see earlier) and the corresponding SI base unit for the photometric quantity luminous intensity, namely the candela. Of the seven SI base units (and their defining constants) the candela and its defining constant K_{cd} are unique in relating to human vision, rather than a fundamental physical phenomenon. The photon system is very similar to the radiometric system with energy units replaced by number of photons (requiring an adjustment[4] to spectral weighting functions and quantities), and is often expressed after taking logs, due to the very large numbers involved.

Figure 3 illustrates the deep connections between these three metrological approaches. The set of quantities (illuminance, luminous flux, luminance, etc.) in the photometric system has the analogs *photopically-weighted* (irradiance, radiant flux, radiance) in the radiometric system and the analogs *photopically-weighted photon* (irradiance, flux, radiance) in the photon system. These analogs have units (lx, lm, cd/m^2), (W/m^2, W, W/sr/m^2), and ($m^{-2}\cdot s^{-1}$, s^{-1}, $sr^{-1}\cdot m^{-2}\cdot s^{-1}$), respectively. For melanopic quantities—with exactly the same units—the respective quantities are [melanopic EDI, melanopic equivalent

[4]The energy, E, of a single photon depends on Planck's constant, h, the speed of light, c, its wavelength, λ, and the refractive index of the medium (n_{air}, say), so that for N_p photons (as it is expressed in the photon system) at a given wavelength, the corresponding radiometric energy is $E_e = N_p \cdot h \cdot c/\lambda \cdot n_{air}$. When converting action spectra from the photon system to the radiometric energy system, or vice versa, once the adjustment has been applied at every wavelength, the entire action spectrum must also be renormalized so that its new maximum value is equal to 1.

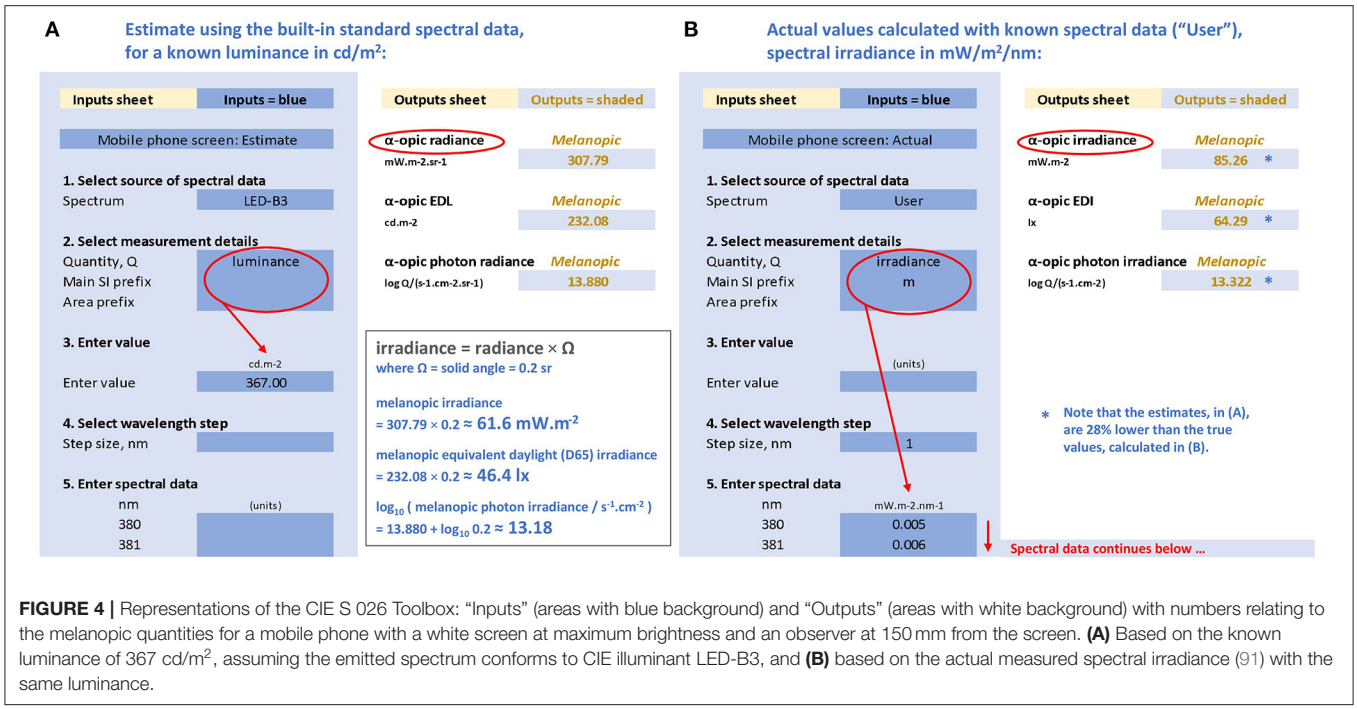


FIGURE 4 | Representations of the CIE S 026 Toolbox: "Inputs" (areas with blue background) and "Outputs" (areas with white background) with numbers relating to the melanopic quantities for a mobile phone with a white screen at maximum brightness and an observer at 150 mm from the screen. **(A)** Based on the known luminance of 367 cd/m^2, assuming the emitted spectrum conforms to CIE illuminant LED-B3, and **(B)** based on the actual measured spectral irradiance (91) with the same luminance.

daylight (D65) luminous flux, melanopic equivalent daylight (D65) luminance], *melanopic* (irradiance, radiant flux, radiance) and *melanopic photon* (irradiance, flux, radiance). Equally, for the other four α-opic quantities, the same relationships hold. Under CIE S 026 definitions, melanopic equivalent daylight (D65) luminance can be abbreviated to melanopic EDL.

α-OPIC TOOLBOX

To calculate α-opic quantities in the radiometric, photon and photometric systems, and convert from one system to another, CIE has published an interactive Excel^{TM} spreadsheet, the "CIE S 026 Toolbox" (90). Access to the toolbox is free on the CIE website [doi: 10.25039/S026.2018.TB], and also an introductory video and a user guide are provided. The toolbox features include weighting functions, spectral weighting charts and a concise glossary.

Toolbox users can enter a spectral measurement and calculate all the quantities that are the geometric analogs of irradiance and radiance, including the illuminance and α-opic EDIs for this spectrum (**Figure 4A**). Alternatively, even without spectral data, users can familiarize themselves with the links between the three systems using one of the five built-in spectral distributions selected from the CIE standard illuminants (A, D65, E, FL11, LED-B3; **Figure 4B**).

EVERYDAY EXAMPLES

The CIE has proposed "integrative lighting" to be the official term for lighting that is specifically intended to integrate visual and non-visual effects, producing physiological and psychological effects on humans that are reflected in scientific evidence (59, 92). In the context of this promising new approach, we reconsider the light that people are exposed to in their daily lives. To investigate and characterize potential light exposures in relation to non-visual responses, a number of measurements of familiar sources of light were made, where possible re-using information from previous investigations.

The α-opic toolbox was used to evaluate the absolute and relative melanopic content of these sources in more detail. Taken together, subject to the potential limitations of the melanopic model for predicting NIF responses to light (see Introduction), the information provides useful context and further evidence for advice relating to light and health.

Experimental Methods

All the spectral data were measured using equipment sets subject to secondary calibrations, and traceable to national standards performed, and maintained in-house (Public Health England, Didcot, Oxfordshire, UK). The data were checked against comparable alternative measurements of the same sources. Spectral equipment sets consisted of TE-cooled spectroradiometers (BW Tek, Newark, USA), coupled via optical fibers (Newport Spectra-Physics Ltd., Didcot, UK) to optical diffusers (Bentham, Reading, UK).

Daylight characteristics analyzed relate to a clear day (29 May 2020) and a cloudy day (18 June 2020), and are based on global spectral irradiance data from a solar monitoring laboratory at (51.575° N, 1.318° W, altitude 125 m), measured in the horizontal plane at 5-min intervals using in-house acquisition software (Public Health England, Didcot, Oxfordshire, UK).

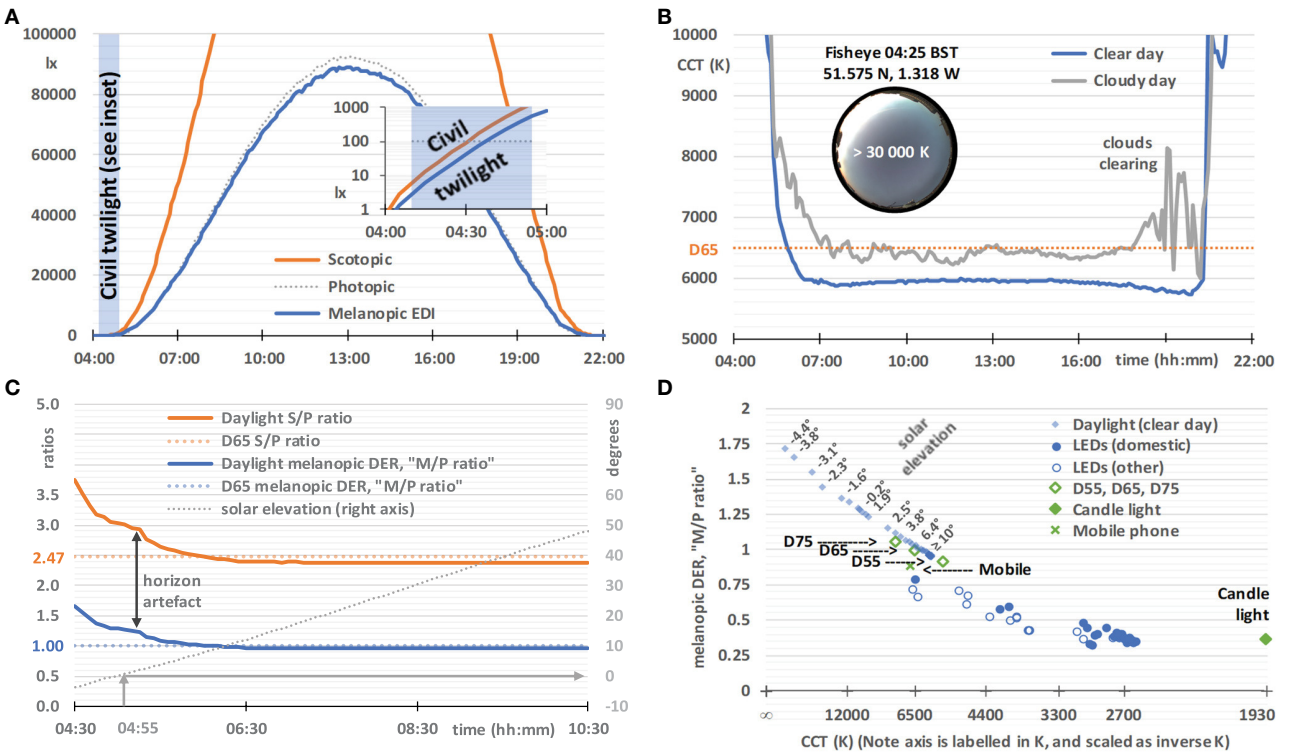


FIGURE 5 | Daylight characteristics on a clear day (29 May 2020, sunrise 04:55, sunset 21:10, times in BST), based on horizontal global spectral irradiance data from a solar monitoring laboratory in Didcot, Oxfordshire, UK. **(A)** Illuminance (dotted gray line), scotopic illuminance (orange line), and melanopic EDI (blue line), with an inset semi-log graph spanning the end of twilight (04:10) and sunrise. **(B)** The correlated color temperature (CCT) on a clear day (blue line, as above from 29 May 2020) and a cloudy day (gray line, 18 June 2020, sunrise 04:50, sunset 21:25), with an inset 2π-fisheye image of the sky at 04:25. **(C)** The melanopic daylight (D65) efficacy ratio (melanopic DER, or M/P ratio, blue line), S/P ratio (orange line), and solar elevation (dotted gray line), with the melanopic DER and S/P ratio for standard daylight illuminant D65 for comparison (colored dotted lines). The vertical arrow indicates artifacts likely to be due to the slightly elevated local horizon. **(D)** Melanopic DER, or M/P ratio, plotted against an inverse-CCT axis (labeled by CCT), with solar elevation labels next to selected pairs of data points. Also plotted are CIE standard daylight illuminants (D55, D65, and D75; open diamonds), candle light (1,930 K blackbody; filled diamonds), a mobile phone (plus symbol, details given in Experimental Methods), domestic LEDs (filled circles, $n = 25$, details given in Experimental Methods), selected office LEDs and selected street light LEDs [combined as open circles, $n = 16$, also taken from (93)].

The photographic fisheye image taken at 04:25 on 29 May 2020 in **Figure 5B** is part of a parallel series, also taken at 5-min intervals, using Q24 hemispheric outdoor camera (Mobotix AG, Hauptsitz, Germany), at the same location.

LED spectral irradiance data were measured in temperature-controlled laboratory conditions in two earlier studies (91, 93): firstly, a modern mobile phone model (from 2016 but still in widespread use in 2021) displaying a white screen at full power at a distance of 150 mm [ID 13, (91)] and, secondly, an LED lighting sample which included any 40W-equivalent GU10 (spots) and any 60W-equivalent BC22 (bayonet light bulbs) general service lighting product types available to a UK retail consumer in 2015 over a 10-day period either online or through local and national stores (within an area bounded by Aylesbury, High Wycombe and Oxford). The latter sample included a number of comparator LED lighting products with different fittings, but excluded color-tunable products (93).

The simplified spectral emissions of a candle were modeled as arising from a Planckian radiator with a color temperature of ~1,930 K (94).

Results: Daylight

On an ideal clear day, horizontal illuminance, scotopic illuminance and melanopic EDI follow smooth bell-shaped curves, and melanopic EDI values are similar to illuminance values (**Figure 5A**). This close agreement results from the melanopic EDI-normalization using standard daylight illuminant D65. The daylight characteristics in **Figure 5** may not correspond exactly to daylight at high altitude, in different atmospheric conditions, and when measured with different fields-of-view. During the hour preceding dawn (see **Figure 5A** inset), and after sunset, the melanopic EDI increases, but decreases relative to the visual measure of illuminance, and vice versa after sunset. Other characteristics derived from the spectral daylight data also progress smoothly on a clear day, but **Figure 5B** illustrates how a cloudy day introduces volatility, exemplified here using the visual metric correlated color temperature (CCT). In contrast, on the clear day (verified with fisheye photographs such as the one shown in the **Figure 5B** inset), the CCT falls rapidly in the hours either side of dawn. The minimum CCT

occurs ∼1 h either after dawn or before sunset, with a small increase in CCT to a local maximum at approximately solar noon. Atmospheric conditions may give rise to asymmetry in the spectral characteristics on either side of solar noon.

Earlier studies have analyzed spectral and/or melanopic daylight time-series data averaged over a number of days (21, 95, 96). However, we are particularly interested in the results on a clear day and the melanopic daylight (D65) efficacy ratio, that, as explained earlier, can be thought of as an M/P ratio with similarities to the S/P ratio (see **Figure 5C**), both being ratios of the quantities shown in **Figure 5A**. In common with CCT, these ratios are highly dependent on solar elevation, and hence solar time on any given day. For solar elevations above 10° the ratios remained stable (i.e., for the main part of the day). For D65, with a CCT of ∼6,500 K, the melanopic DER or M/P ratio equals 1 by definition and the S/P ratio equals 2.47. For solar elevations above 10°, the M/P and S/P ratios observed were slightly below 1 (see **Figure 5C**), which reflected the difference between the observed CCTs and that of D65 (see **Figure 5B**). When the sun is down or low in the sky, an elevated horizon can obscure the brightest part of the sky or the sun. In this way trees, buildings and the landscape can cause deviations from the smooth curve that would otherwise be observed. **Figure 5D** shows the CCT dependence of the melanopic DER for daylight on a clear day. In the next section we will compare this to white LED lighting.

Results: White LED Lighting

Figure 5D shows the CCT dependence of the melanopic DER for the non-color-tunable white LED lighting (2015 retail products), all of which were based on a blue LED plus yellow phosphor, with the GU10 and BC22 domestic LEDs shown as a separate series. For the domestic LEDs ($n = 25$), CCT explained 87% of the variance in melanopic DER, and CCT plus CRI (Color Rendering Index, R_a) explained 95% (multiple linear regression). This chart shows that this CCT dependence of the melanopic DER for the LED technology common to this white LED lighting sample does not match the CCT dependence of the melanopic DER for daylight on a clear day. Further, all the LED lighting in **Figure 5D** has a significantly lower melanopic DER than daylight on a clear day, typically by around 25% for a CCT of 6,500 K. At other CCT values the deficit in melanopic DER relative to daylight is higher, and it remains significant, even after adjusting for the CCT-dependencies within the daylight and LED melanopic DER series. In other words, this supports the viewpoint that all the LED lights in this sample were relatively inefficient at producing melanopic light for a given combination of CCT and luminous flux. The lower melanopic efficiency of white LED lighting with respect to natural daylight has also been reported previously (97, 98). In addition to a reduced illuminance, a lower melanopic DER may be appropriate at night and within spaces designed to be restful, whereas in active workplaces a higher melanopic DER and an elevated illuminance may engender a healthier daytime environment.

Results: Mobile Phone Screen—Toolbox Example

To further illustrate the α-opic metrology and the S 026 Toolbox, we will consider the melanopic EDI (in lx) produced by a typical modern mobile phone (plotted as a green cross in **Figure 5D**). There is some concern about the effects on sleep of using display screen equipment before bedtime, including the use of mobile phones and tablets in bed, because of the light they emit (45, 99, 100), so the data we present here will provide a relevant and helpful example to place the α-opic quantities in context. Indeed, a number of groups have directly studied the effects that different light exposures can have on sleep (25, 26, 100).

There are two approaches for performing calculations available in the toolbox. The first is a simplified approach using the spectra from the five built-in standard illuminants (A, D65, E, FL11, LED-B3). The second approach requires the user to enter the actual spectral data of the test light in consideration. These two approaches are chosen to illustrate why using the simplified approach (i.e., generalizing results from standardized spectral distributions) will not always be appropriate, and may cause errors.

Simplified Approach

For a white mobile phone screen at full power backlit with an LED, the luminance is 367 cd/m^2 (91). If the spectral data are not known, the toolbox might still be used if it can be assumed that the light emission of this phone conforms to the CIE illuminant LED-B3 built-in into the toolbox (however, as will be shown, this assumption is not tenable). On this tentative basis, the melanopic radiance, the melanopic equivalent daylight (D65) luminance (melanopic EDL) and the melanopic photon irradiance can be calculated with the toolbox (see **Figure 4A**). As the screen subtends an angle of approximately a 5th of a steradian at a viewing distance of 150 mm, the melanopic irradiance, melanopic EDI and melanopic photon irradiance can be obtained as follows:

$$\begin{aligned}&\text{melanopic irradiance} = \text{melanopic radiance} \times \text{solid angle}\\&\quad \approx 308\text{mW/sr/m}^2 \times 0.2\,\text{sr} = 61.6\,\text{mW/m}^2\\&\text{melanopic EDI} = \text{melanopic EDL} \times \text{solid angle}\\&\quad \approx 232\text{cd/m}^2 \times 0.2\,\text{sr} = 46.4\,\text{lx}\\&\log_{10}\text{melanopic photon irradiance}/(\text{cm}^{-2}\cdot\text{s}^{-1})\\&\quad \approx 13.88 + \log_{10}(0.2) \approx 13.18\end{aligned}$$

However, we may not be able to rely on the above estimates. We assumed that the spectrum of the mobile phone conforms to LED-B3. This is likely to cause problems, as the spectrum from mobile phones may have a higher blue content and, unlike LED-B3, is produced by three or more single color LEDs rather than by using a blue LED in combination with a yellow phosphor. In order to replace the above estimates with accurate figures, we need to use the actual spectral data.

Spectral Data Approach

When using the toolbox with the spectral irradiance data collected for the selected LED screen [ID 13, (91)], the toolbox

output sheet (see **Figure 4B**) gives the following results:

$$\text{melanopic irradiance} \approx 85\ \text{mW/m}^2$$
$$\text{melanopic EDI} \approx 64.3\ \text{lx}$$
$$\log_{10}\text{melanopic photon irradiance}/(\text{cm}^{-2}\cdot\text{s}^{-1}) \approx 13.32$$

This spectral analysis shows that the simplified approach with the assumption that the phone's light emission conforms to LED-B3 resulted in underestimating the melanopic irradiance and EDI by almost 30%.

Exposure at 150 mm distance from a phone screen (at full white power) is a plausible worst-case scenario for mobile screen use in children and young adults, but it is unlikely that the screen would be used in its brightest setting only. The mix of light and dark within the images displayed on the screen will reduce the spatially-averaged screen brightness as well as the time-averaged melanopic EDI measured at the user's eye. The brightness and the blue emissions may also be reduced in power in the evening using a suitable app. Finally, holding the phone at a further distance reduces the average melanopic EDI incident at the eye, by reducing the "visual" field occupied by the screen.

In preliminary guidance on applying "proper light at the proper time," and in the absence of a formal consensus, a CIE position statement (59) recently recommended using melanopic EDI as an interim approach to manipulate non-visual responses. Further guidance on this approach is awaited from the participants of the 2nd International Workshop on Circadian and Neurophysiological Photometry (held in Manchester, August 2019), and this is expected to take the form of a scientific publication with melanopic-EDI centered recommendations for healthy indoor light exposures. Further research may be needed to investigate the potential limitations of using melanopic EDI in such recommendations and to explore the correlations between the α-opic quantities and non-visual responses in more detail. While this knowledge develops, and acknowledging the considerations set out in the introduction, the melanopic action spectrum can be considered a good model for predicting melatonin suppression responses: a melanopic EDI below 4 lx results in minimal responses (<25% of maximum melatonin suppression) and a melanopic EDI above 300 lx strongly suppresses salivary melatonin (>75% of the maximum), depending on the exposure duration and experimental context (21). Furthermore, dose-response relationships are subject to a large interindividual variability, for instance the human sensitivity to light for melatonin suppression (i.e., the melanopic EDI needed to produce 50% of maximum melatonin suppression) is reported to vary between individuals by more than one order of magnitude, based on the 95% confidence interval (25). Together with the melanopic EDI values in **Table 2**, these findings provide inconclusive evidence whether the melatonin suppression induced by mobile phone light emissions in the evening are at levels that raise practical concerns. However, the possibility still remains that prolonged evening use of indoor electric lighting may result in light exposures that are relevant for melatonin suppression.

Furthermore, whilst the studies mentioned above suggested that mobile phone screens can have statistically significant effects on sleep, a more representative comparison (99) demonstrated that a 4-h exposure to an e-reader compared to a printed book (when repeated on five consecutive nights with a scheduled 06:00 am morning wake-up time) only resulted in an average reduction of 5 min in total nightly sleep duration and 12 min in REM sleep duration, so these effects of light may be less significant in a practical sense. Insufficient exposure to light during the day in modern (indoor) lifestyles may be of greater concern, and, as set out earlier, daytime light exposures increase the robustness of circadian rhythms and reduce the disruption caused by light exposures in the evening, see the Introduction section and **Figure 1D**.

TABLE 2 | Color, RGB, illuminance, scotopic illuminance, and melanopic EDI of a modern mobile phone the screen set to a uniform color at its maximum brightness, and as viewed at a distance of 150 mm [phone ID 13 from (91)].

Screen color	RGB values	(Photopic) illuminance, lx	Scotopic illuminance, lx	Melanopic EDI, lx
1. White	255, 255, 255	73.3	164.6	64.3
2. Purple	48, 0, 179	3.1	30.2	15.5
3. Blue	0, 0, 210	3.6	42.6	22.0
4. Green	0, 100, 100	7.8	21.8	8.6
5. Lilac	102, 0, 255	7.8	65.9	34.0
6. Cyan	142, 201, 230	40.4	110.8	45.0

CONCLUDING REMARKS

Daily variations in the light environment are important for sleep, well-being and long-term health. The knowledge base concerning the contributions and interactions of retinal photoreceptors in driving non-visual effects is becoming more mature. Although the science is by no means complete, measures of the environment expressed in terms of melanopic EDI are now thought to have ecological validity. New recommendations for future building and lighting standards are therefore expected to incorporate both minimum thresholds for daytime melanopic EDI and maximum thresholds for evening melanopic EDI. These recommendations should be carefully integrated with the visual components within existing lighting codes. One way of limiting evening melanopic EDI would be by recommending dimmer lighting, and this is more effective when simultaneously lowering melanopic DERs (i.e., reducing M/P ratios). Another recommendation could be to strive for near darkness wherever people are expected to sleep at night. The CIE S 026 Toolbox has been introduced, partly to support this expected shift in lighting practice, and partly to enable researchers to expand the evidence base for future lighting standards, guidance and health advice.

Figure 5D shows that the melanopic DER for daylight on a clear day is significantly greater than the melanopic DER within a recent sample of white LED lighting with a range of CCTs. This supports the viewpoint that the LEDs sampled are relatively inefficient at producing melanopic light for a given combination of CCT and luminous flux, in agreement with others (97, 98). New lighting products, including those with tunable M/P ratios, may help to address this. Higher M/P ratios, similar

to daylight, might be considered a beneficial characteristic for the daytime indoor environment. Daylight entry within the built environment is a good way to achieve this.

If the aim is to minimize melanopic light exposures, the lighting used at night for navigation and perceptions of safety should be restricted to lower M/P ratios. Increased daytime light exposures can reduce the adverse effects of evening light (39–46), and daytime light exposure may be as important as avoiding bright light before bedtime. During the day, indoor electric lighting could reproduce the melanopic light exposures (and other facets) of the outdoor environment, although this entails greatly increased indoor illuminances. Nevertheless, daylight is an excellent, natural, energy-efficient source of melanopic-rich light, and public health policies should encourage a daytime (natural) light-seeking lifestyle, especially during the first morning hours after bed and starting from the very first days after birth.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are subject to UK Crown copyright and will usually be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. Both authors have contributed extensively to the gray literature on this topic in unpaid voluntary roles, including (12, 59, 89, 90).

ACKNOWLEDGMENTS

Marina Khazova (PHE) is thanked for her generous help with the measurements.

REFERENCES

1. Price DA, Close GC, Fielding BA. Age of appearance of circadian rhythm in salivary cortisol values in infancy. *Arch Dis Childh.* (1983) 58:454–6. doi: 10.1136/adc.58.6.454
2. Rivkees SA, Hofman PL, Fortman J. Newborn primate infants are entrained by low intensity lighting. *Proc Natl Acad Sci USA.* (1997) 94:292–7. doi: 10.1073/pnas.94.1.292
3. Morag I, Ohlsson A. Cycled light in the intensive care unit for preterm and low birth weight infants. *Cochrane Database Syst Rev.* (2013) 8:CD006982. doi: 10.1002/14651858.CD006982.pub3
4. Vasquez-Ruiz S, Maya-Barrios JA, Torres-Narvaez P, Vega-Martinez BR, Rojas-Granados A, Escobar C, et al. A light/dark cycle in the NICU accelerates body weight gain and shortens time to discharge in preterm infants. *Early Hum Dev.* (2014) 90:535–40. doi: 10.1016/j.earlhumdev.2014.04.015
5. Fernandez DC, Komal R, Langel J, Ma J, Duy PQ, Penzo MA, et al. Retinal innervation tunes circuits that drive nonphotic entrainment to food. *Nature.* (2020) 581:194–8. doi: 10.1038/s41586-020-2204-1
6. Santhi N, Thorne HC, Van Der Veen DR, Johnsen S, Mills SL, Hommes V, et al. The spectral composition of evening light and individual differences in the suppression of melatonin and delay of sleep in humans. *J Pineal Res.* (2011) 53:47–59. doi: 10.1111/j.1600-079X.2011.00970.x
7. Duffy JF, Czeisler CA. Effect of light on human circadian physiology. *Sleep Med Clin.* (2009) 4:165–77. doi: 10.1016/j.jsmc.2009.01.004
8. Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. *Behav Brain Res.* (2000) 115:75–83. doi: 10.1016/S0166-4328(00)00236-9
9. Souman JL, Tinga AM, Te Pas SF, Van Ee R, Vlaskamp BNS. Acute alerting effects of light: a systematic literature review. *Behav Brain Res.* (2018) 337:228–39. doi: 10.1016/j.bbr.2017.09.016
10. Wirz-Justice A, Terman M, Oren DA, Goodwin FK, Kripke DF, Whybrow PC, et al. Brightening depression. *Science.* (2004) 303:467–9. doi: 10.1126/science.303.5657.467c
11. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. *Science.* (1980) 210:1267–9. doi: 10.1126/science.7434030
12. CIE. CIE S 026:2018 CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light. *International Standard.* (2018). doi: 10.25039/S026.2018. Available online at: http://www.cie.co.at/publications/cie-system-metrology-optical-radiation-iprgc-influenced-responses-light-0 (accessed October 31, 2020).
13. Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, et al. Measuring and using light in the melanopsin age. *Trends Neurosci.* (2014) 37:1–9. doi: 10.1016/j.tins.2013.10.004
14. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci.* (2001) 21:6405–12. doi: 10.1523/JNEUROSCI.21-16-06405.2001
15. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J Physiol.* (2001) 535:261–7. doi: 10.1111/j.1469-7793.2001.t01-1-00261.x
16. Hommes V, Gimenez MC. A revision of existing karolinska sleepiness scale responses to light: a melanopic perspective. *Chronobiol Int.* (2015) 32:750–6. doi: 10.3109/07420528.2015.1043012
17. Nowozin C, Wahnschaffe A, Rodenbeck A, De Zeeuw J, Hadel S, Kozakov R, et al. Applying melanopic lux to measure biological light effects on melatonin suppression and subjective sleepiness. *Curr Alzheimer Res.* (2017) 14:1042–52. doi: 10.2174/1567205014666170523094526
18. Souman JL, Borra T, De Goijer I, Schlangen LJM, Vlaskamp BNS, Lucassen MP. Spectral tuning of white light allows for strong reduction in melatonin suppression without changing illumination level or color temperature. *J Biol Rhythms*. (2018) 33:420–31. doi: 10.1177/0748730418784041
19. Prayag AS, Najjar RP, Gronfier C. Melatonin suppression is exquisitely sensitive to light and primarily driven by melanopsin in humans. *J Pineal Res.* (2019) 66:e12562. doi: 10.1111/jpi.12562
20. Spitschan M. Photoreceptor inputs to pupil control. *J Vis*. (2019) 19:5–5. doi: 10.1167/19.9.5
21. Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res.* (2020) 69:e12655. doi: 10.1111/jpi.12655
22. Zeitzer JM. When is a proxy not a proxy? The foibles of studying non-image forming light. *J Physiol.* (2018) 596:2029–30. doi: 10.1113/JP276076
23. Rimmer DW, Boivin DB, Shanahan TL, Kronauer RE, Duffy JF, Czeisler CA. Dynamic resetting of the human circadian pacemaker by intermittent bright light. *Am J Physiol Regul Integr Comp Physiol.* (2000) 279:R1574–9. doi: 10.1152/ajpregu.2000.279.5.R1574
24. Gooley JJ, Rajaratnam SM, Brainard GC, Kronauer RE, Czeisler CA, Lockley SW. Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Sci Transl Med.* (2010). 2:31ra33. doi: 10.1126/scitranslmed.3000741
25. Phillips AJK, Vidafar P, Burns AC, Mcglashan EM, Anderson C, Rajaratnam SMW, et al. High sensitivity and interindividual variability in the response of the human circadian system to evening light. *Proc Natl Acad Sci USA*. (2019) 116:20190–24. doi: 10.1073/pnas.1901824116

26. Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J Physiol.* (2000) 526:695–702. doi: 10.1111/j.1469-7793.2000.00695.x
27. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. A phase response curve to single bright light pulses in human subjects. *J Physiol.* (2003) 549:945–52. doi: 10.1113/jphysiol.2003.040477
28. Dijk DJ, Shanahan TL, Duffy JF, Ronda JM, Czeisler CA. Variation of electroencephalographic activity during non-rapid eye movement and rapid eye movement sleep with phase of circadian melatonin rhythm in humans. *J Physiol.* (1997) 505:851–8. doi: 10.1111/j.1469-7793.1997.851ba.x
29. Morris M, Lack L, Barrett J. The effect of sleep/wake state on nocturnal melatonin excretion. *J Pineal Res.* (1990) 9:133–8. doi: 10.1111/j.1600-079X.1990.tb00701.x
30. Shanahan TL, Czeisler CA. Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *J Clin Endocrinol Metab.* (1991) 73:227–35. doi: 10.1210/jcem-73-2-227
31. Barrett J, Lack L, Morris M. The sleep-evoked decrease of body temperature. *Sleep*. (1993) 16:93–9.
32. Duffy JF, Wright KP Jr. Entrainment of the human circadian system by light. *J Biol Rhythms.* (2005) 20:326–38. doi: 10.1177/0748730405277983
33. Crowley SJ, Van Reen E, Lebourgeois MK, Acebo C, Tarokh L, Seifer R, et al. A longitudinal assessment of sleep timing, circadian phase, and phase angle of entrainment across human adolescence. *PLoS ONE.* (2014) 9:e112199. doi: 10.1371/journal.pone.0112199
34. Wright KP Jr, Mchill AW, Birks BR, Griffin BR, Rusterholz T, Chinoy ED. Entrainment of the human circadian clock to the natural light-dark cycle. *Curr Biol*. (2013) 23:1554–8. doi: 10.1016/j.cub.2013.06.039
35. Van De Werken M, Booij SH, Van Der Zwan JE, Simons MJ, Gordijn MC, Beersma DG. The biological clock modulates the human cortisol response in a multiplicative fashion. *Chronobiol Int.* (2014) 31:572–80. doi: 10.3109/07420528.2013.868472
36. Chang AM, Santhi N, St Hilaire M, Gronfier C, Bradstreet DS, Duffy JF, et al. Human responses to bright light of different durations. *J Physiol.* (2012) 590:3103–12. doi: 10.1113/jphysiol.2011.226555
37. Jagannath A, Butler R, Godinho S, Couch Y, Brown L, Vasudevan S, et al. The CRTC1-SIK1 pathway regulates entrainment of the circadian clock. *Cell.* (2013) 154:1100–11. doi: 10.1016/j.cell.2013.08.004
38. Aschoff J. (1981). *Biological Rhythms*. New York, NY: Springer US.
39. Mishima K, Okawa M, Shimizu T, Hishikawa Y. Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. *J Clin Endocrinol Metab.* (2001) 86:129–34. doi: 10.1210/jc.86.1.129
40. Hebert M, Martin SK, Lee C, Eastman CI. The effects of prior light history on the suppression of melatonin by light in humans. *J Pineal Res.* (2002) 33:198–203. doi: 10.1034/j.1600-079X.2002.01885.x
41. Smith KA, Schoen MW, Czeisler CA. Adaptation of human pineal melatonin suppression by recent photic history. *J Clin Endocrinol Metab.* (2004) 89:3610–4. doi: 10.1210/jc.2003-032100
42. Chang AM, Scheer FA, Czeisler CA. The human circadian system adapts to prior photic history. *J Physiol.* (2011) 589:1095–102. doi: 10.1113/jphysiol.2010.201194
43. Zeitzer JM, Friedman L, Yesavage JA. Effectiveness of evening phototherapy for insomnia is reduced by bright daytime light exposure. *Sleep Med.* (2011) 12:805–7. doi: 10.1016/j.sleep.2011.02.005
44. Chang AM, Scheer FA, Czeisler CA, Aeschbach D. Direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans depend on prior light history. *Sleep.* (2013) 36:1239–46. doi: 10.5665/sleep.2894
45. Rangtell FH, Ekstrand E, Rapp L, Lagermalm A, Liethof L, Bucaro MO, et al. Two hours of evening reading on a self-luminous tablet vs. reading a physical book does not alter sleep after daytime bright light exposure. *Sleep Med*. (2016) 23:111–8. doi: 10.1016/j.sleep.2016.06.016
46. Te Kulve M, Schlangen LJM, Van Marken Lichtenbelt WD. Early evening light mitigates sleep compromising physiological and alerting responses to subsequent late evening light. *Sci Rep.* (2019) 9:16064. doi: 10.1038/s41598-019-52352-w
47. Carskadon MA, Labyak SE, Acebo C, Seifer R. Intrinsic circadian period of adolescent humans measured in conditions of forced desynchrony. *Neurosci Lett.* (1999) 260:129–32. doi: 10.1016/S0304-3940(98)00971-9
48. Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science.* (1999) 284:2177–81. doi: 10.1126/science.284.5423.2177
49. Kelly TL, Neri DF, Grill JT, Ryman D, Hunt PD, Dijk DJ, et al. Nonentrained circadian rhythms of melatonin in submariners scheduled to an 18-hour day. *J Biol Rhythms.* (1999) 14:190–6. doi: 10.1177/074873099129000597
50. Brown SA, Fleury-Olela F, Nagoshi E, Hauser C, Juge C, Meier CA, et al. The period length of fibroblast circadian gene expression varies widely among human individuals. *PLoS Biol.* (2005) 3:e338. doi: 10.1371/journal.pbio.0030338
51. Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, et al. The national human activity pattern survey (NHAPS): a resource for assessing exposure to environmental pollutants. *J Expo Anal Environ Epidemiol.* (2001) 11:231–52. doi: 10.1038/sj.jea.7500165
52. Murray K, Godbole S, Natarajan L, Full K, Hipp JA, Glanz K, et al. The relations between sleep, time of physical activity, and time outdoors among adult women. *PLoS ONE.* (2017) 12:e0182013. doi: 10.1371/journal.pone.0182013
53. Beyer KMM, Szabo A, Hoormann K, Stolley M. Time spent outdoors, activity levels, and chronic disease among American adults. *J Behav Med.* (2018) 41:494–503. doi: 10.1007/s10865-018-9911-1
54. CEN. *European Standard EN12464-1:2011:E "Light and lighting - Lighting for Work Places:- Part : Indoor Work places"* (2011).
55. IESNA. *The IESNA Lighting Handbook: Reference & Application*, 9th ed. New York, NY (2000).
56. Czeisler CA. Perspective: casting light on sleep deficiency. *Nature.* (2013) 497:S13. doi: 10.1038/497S13a
57. Roenneberg T, Allebrandt KV, Merrow M, Vetter C. Social jetlag and obesity. *Curr Biol.* (2012) 22:939–43. doi: 10.1016/j.cub.2012.03.038
58. Giuntella O, Mazzonna F. Sunset time and the economic effects of social jetlag: evidence from US time zone borders. *J Health Econ.* (2019) 65:210–26. doi: 10.1016/j.jhealeco.2019.03.007
59. CIE. *CIE Position Statement on Non-Visual Effects of Light: Reccommending Proper Light at the Proper Time*, 2nd ed. (2019). Available online at: https://cie.co.at/publications/position-statement-non-visual-effects-light-recommending-proper-light-proper-time
60. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* (2002) 295:1070–3. doi: 10.1126/science.1067262
61. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol.* (1990) 292:497–523. doi: 10.1002/cne.902920402
62. Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature.* (2005) 433:749–54. doi: 10.1038/nature03387
63. Gooley JJ, Ho Mein I, St Hilaire MA, Yeo SC, Chua EC, Van RE, et al. Melanopsin and rod-cone photoreceptors play different roles in mediating pupillary light responses during exposure to continuous light in humans. *J Neurosci.* (2012) 32:14242–53. doi: 10.1523/JNEUROSCI.1321-12.2012
64. Keenan WT, Rupp AC, Ross RA, Somasundaram P, Hiriyanna S, Wu Z, et al. A visual circuit uses complementary mechanisms to support transient and sustained pupil constriction. *eLife.* (2016) 5:e15392. doi: 10.7554/eLife.15392.029
65. Charng J, Jacobson SG, Heon E, Roman AJ, Mcguigan DB, III, Sheplock R, et al. Pupillary light reflexes in severe photoreceptor blindness isolate the melanopic component of intrinsically photosensitive retinal ganglion cells. *Invest Ophthalmol Vis Sci.* (2017) 58:3215–24. doi: 10.1167/iovs.17-21909
66. Schmidt TM, Chen SK, Hattar S. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends Neurosci.* (2011) 34:572–80. doi: 10.1016/j.tins.2011.07.001
67. Zhao X, Stafford BK, Godin AL, King WM, Wong KY. Photoresponse diversity among the five types of intrinsically photosensitive retinal ganglion cells. *J Physiol.* (2014) 592:1619–36. doi: 10.1113/jphysiol.2013.262782

68. Mure LS, Vinberg F, Hanneken A, Panda S. Functional diversity of human intrinsically photosensitive retinal ganglion cells. *Science.* (2019) 366:1251–5. doi: 10.1126/science.aaz0898
69. Quattrochi LE, Stabio ME, Kim I, Ilardi MC, Michelle Fogerson P, Leyrer ML, et al. The M6 cell: a small-field bistratified photosensitive retinal ganglion cell. *J Comp Neurol.* (2019) 527:297–311. doi: 10.1002/cne.24556
70. Boettner EA, Wolter JR. Transmission of the ocular media. *Invest Ophthalmol Vis Sci.* (1962) 1:776–83.
71. Esquiva G, Lax P, Pérez-Santonja JJ, García-Fernández JM, Cuenca N. Loss of melanopsin-expressing ganglion cell subtypes and dendritic degeneration in the aging human retina. *Front Aging Neurosci.* (2017) 9:79. doi: 10.3389/fnagi.2017.00079
72. Van Someren EJ. Circadian and sleep disturbances in the elderly. *Exp Gerontol.* (2000) 35:1229–37. doi: 10.1016/S0531-5565(00)00191-1
73. Van Someren EJ, Riemersma RF, Swaab DF. Functional plasticity of the circadian timing system in od age: light exposure. In: Hofman MA, Boer GJ, Holtmaat AJGD, Someren E, Verhagen J, Swaab DF. *Progress in Brain Research*, Vol. 138. Elsevier Science (2002). p. 205–31. doi: 10.1016/S0079-6123(02)38080-4
74. Riemersma-Van Der Lek R, Swaab DF, Twisk J, Hol EM, Hoogendijk WJG, Van Someren EJW. Effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities: a randomized controlled trial. *JAMA.* (2008) 299:2642–55. doi: 10.1001/jama.299.22.2642
75. Zuurbier LA, Luik AI, Hofman A, Franco OH, Van Someren EJ, Tiemeier H. Fragmentation and stability of circadian activity rhythms predict mortality: the Rotterdam study. *Am J Epidemiol.* (2015) 181:54–63. doi: 10.1093/aje/kwu245
76. CIE. *CIE 191:2010 Recommended System for Mesopic Photometry Based on Visual Performance* (2010).
77. Rushton WA. Pigments and signals in colour vision. *J Physiol.* (1972) 220:1P–31P. doi: 10.1113/jphysiol.1972.sp009719
78. BIPM. *The International System of Units (SI), 9th edition of the SI Brochure, Bureau International des Poids et Mesures (ISBN 978-92-822-2272-0).* (2019). Avialabe online at: https://www.bipm.org/en/publications/si-brochure/ (accessed October 31, 2020).
79. Hankins MW, Lucas RJ. The primary visual pathway in humans is regulated according to long-term light exposure through the action of a nonclassical photopigment. *Curr Biol.* (2002) 12:191–8. doi: 10.1016/S0960-9822(02)00659-0
80. Allen AE, Martial FP, Lucas RJ. Form vision from melanopsin in humans. *Nat Commun.* (2019) 10:2274. doi: 10.1038/s41467-019-10113-3
81. Do MT, Yau KW. Intrinsically photosensitive retinal ganglion cells. *Physiol Rev.* (2010) 90:1547–81. doi: 10.1152/physrev.00013.2010
82. Zaidi FH, Hull JT, Peirson SN, Wulff K, Aeschbach D, Gooley JJ, et al. Short-wavelength light sensitivity of circadian, pupillary, and visual awareness in humans lacking an outer retina. *Curr Biol.* (2007) 17:2122–8. doi: 10.1016/j.cub.2007.11.034
83. Brown TM, Tsujimura S, Allen AE, Wynne J, Bedford R, Vickery G, et al. Melanopsin-based brightness discrimination in mice and humans. *Curr Biol.* (2012) 22:1134–41. doi: 10.1016/j.cub.2012.04.039
84. Zele AJ, Adhikari P, Feigl B, Cao D. Cone and melanopsin contributions to human brightness estimation. *J Opt Soc Am A.* (2018) 35:B19–B25. doi: 10.1364/JOSAA.35.001783
85. Yamakawa M, Tsujimura SI, Okajima K. A quantitative analysis of the contribution of melanopsin to brightness perception. *Sci Rep.* (2019) 9:7568. doi: 10.1038/s41598-019-44035-3
86. Delawyer T, Tsujimura SI, Shinomori K. Relative contributions of melanopsin to brightness discrimination when hue and luminance also vary. *J Opt Soc Am A Opt Image Sci Vis.* (2020) 37:A81–8. doi: 10.1364/JOSAA.382349
87. Spitschan M. Melanopsin contributions to non-visual and visual function. *Curr Opin Behav Sci.* (2019) 30:67–72. doi: 10.1016/j.cobeha.2019.06.004
88. CIE. *CIE 170-1:2006: Fundamental Chromaticity Diagram With Physiological Axes - Part 1* (2006).
89. CIE. *CIE TN 003:2015: Report on the First International Workshop on Circadian and Neurophysiological Photometry, 2013.* (2015). Available online at: http://files.cie.co.at/785_CIE_TN_003-2015.pdf (accessed October 31, 2020).
90. CIE. *CIE S 026 a-opic Toolbox.* (2020). doi: 10.25039/S026.2018.TB. Available online at: http://cie.co.at/news/launch-cie-s-026-toolbox-and-user-guide (accessed October 31, 2020).
91. O'hagan JB, Khazova M, Price LL. Low-energy light bulbs, computers, tablets and the blue light hazard. *Eye.* (2016) 30:230–3. doi: 10.1038/eye.2015.261
92. CIE. *CIE S 017/E:2020 ILV: International Lighting Vocabulary*, 2nd ed (2020). Available online at: https://cie.co.at/publications/ilv-international-lighting-vocabulary-2nd-edition-0
93. CIBSE. *Human Responses to Lighting Based on Led Lighting Solutions. Commissioned by the Chartered Institution of Building Services Engineers and the Society of Light and Lighting. CRCE-RDD 01-2016.* Balham, London, UK (2016).
94. Walsh JWT. *Photometry.* Constable & Co. Ltd, London (1926).
95. Walmsley L, Hanna L, Mouland J, Martial F, West A, Smedley AR, et al. Colour as a signal for entraining the mammalian circadian clock. *PLoS Biol.* (2015) 13:e1002127. doi: 10.1371/journal.pbio.1002127
96. Spitschan M, Aguirre GK, Brainard DH, Sweeney AM. Variation of outdoor illumination as a function of solar elevation and light pollution. *Sci Rep.* (2016) 6:26756. doi: 10.1038/srep26756
97. Cao D, Barrionuevo PA. The importance of intrinsically photosensitive retinal ganglion cells and implications for lighting design. *J Solid State Light.* (2015) 2:10. doi: 10.1186/s40539-015-0030-0
98. Preciado OU, Issolio LA, Manzano E, Colombo E, Barrionuevo PA. Melanopsin excitation in conditions of natural and artificial lighting. *ANALES AFA.* (2018) 25–30:1168. doi: 10.31527/analesafa.2018.inVisionT.25
99. Chang AM, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. *Proc Natl Acad Sci USA.* (2015) 112:1232–7. doi: 10.1073/pnas.1418490112
100. Van Der Lely S, Frey S, Garbazza C, Wirz-Justice A, Jenni OG, Steiner R, et al. Blue blocker glasses as a countermeasure for alerting effects of evening light-emitting diode screen exposure in male teenagers. *J Adolesc Health.* (2015) 56:113–9. doi: 10.1016/j.jadohealth.2014.08.002

Conflict of Interest: LS's full time position at Eindhoven University of Technology is partially funded by Signify.

The remaining 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 Neurology

MINI REVIEW
published: 09 March 2021
doi: 10.3389/fneur.2021.625698

Intermittent Light Exposures in Humans: A Case for Dual Entrainment in the Treatment of Alzheimer's Disease

Mariana G. Figueiro[1,2]* *and Sagan Leggett*[2,3]

[1] Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2] Lighting Research Center, Rensselaer Polytechnic Institute, Troy, NY, United States, [3] Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY, United States

Circadian sleep disorders are common among American adults and can become especially acute among older adults, especially those living with Alzheimer's disease (AD) and mild cognitive impairment (MCI), leading to the exacerbation of symptoms and contributing to the development and advancement of the diseases. This review explores the connections between circadian sleep disorders, cognition, and neurodegenerative disease, offering insights on rapidly developing therapeutic interventions employing intermittent light stimuli for improving sleep and cognition in persons with AD and MCI. Light therapy has the potential to affect sleep and cognition via at least two pathways: (1) a regular and robust light-dark pattern reaching the retina that promotes circadian phase shifting, which can promote entrainment and (2) 40 Hz flickering light that promotes gamma-wave entrainment. While this is a new area of research, preliminary evidence shows the potential of dual circadian and gamma-wave entrainment as an important therapy not only for those with AD, but for others with cognitive impairment.

Keywords: Alzheimer's disease, circadian entrainment, flashing light, gamma entrainment, memory, sleep

OPEN ACCESS

Edited by:
Shadab Rahman,
Harvard Medical School,
United States

Reviewed by:
Axel Steiger,
Ludwig Maximilian University of Munich, Germany
Marilyn J. Duncan,
University of Kentucky, United States

***Correspondence:**
Mariana G. Figueiro
mariana.figueiro@mountsinai.org

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *03 November 2020*
Accepted: *15 February 2021*
Published: *09 March 2021*

Citation:
Figueiro MG and Leggett S (2021) Intermittent Light Exposures in Humans: A Case for Dual Entrainment in the Treatment of Alzheimer's Disease. Front. Neurol. 12:625698. doi: 10.3389/fneur.2021.625698

INTRODUCTION

Forty-five percent of Americans report sleep problems that affect their daily activities at least once per week, with 35% reporting poor or fair sleep quality and 20% reporting that they did not feel refreshed by sleep on any day of the past week (1, 2). Asynchrony between normal work and social schedules and the timing of the internal clock (3) can lead to sleep disorders and sleep deprivation, particularly if the asynchrony is prolonged for an extended period, which in turn can negatively affect task performance, cognition, and general health (4–6). Sleep disorders and their attendant decrements can become especially acute among older adults, especially those living with Alzheimer's disease (AD) and mild cognitive impairment (MCI), leading to the exacerbation of symptoms and contributing to the development and advancement of the diseases (7–9). In fact, of the estimated 5.8 million people in the United States living with AD and related dementias (ADRD) (10), at least one-third experience difficulty sleeping (11, 12) and approximately two-thirds of their estimated 18.5 million unpaid caregivers report sleep disturbances themselves (10, 13, 14).

This review explores the connections between circadian sleep disorders, cognition, and neurodegenerative disease, offering insights on rapidly developing therapeutic interventions employing entraining light stimuli (both continuous and intermittent) for the treatment of sleep disorders, including in those with AD and MCI. Light therapy has the potential to affect sleep and cognition via at least two pathways: (1) a regular and robust light-dark pattern reaching the retina that promotes circadian phase shifting and thus, entrainment and (2) 40 Hz flickering light that promotes gamma wave entrainment. Both are discussed below.

CIRCADIAN RHYTHMS

Circadian rhythms are endogenously driven biological rhythms that have a period close to 24 h and that can be entrained by exogenous time cues. Circadian rhythms are generated and regulated by a biological clock located in the suprachiasmatic nuclei (SCN) in the hypothalamus in the brain. In the absence of external cues, these rhythms free-run with a period close to, but not exactly 24 h (15). In humans, circadian rhythms free-run with an average period of 24.2 h. Light-dark patterns reaching the retina are the major synchronizers of circadian rhythms to the local position on earth and to the 24-h solar day, a process referred to as entrainment (16). Given that the circadian system in humans free-run with a period slightly longer than 24 h, the human circadian system needs to receive light after minimum core body temperature (usually in the morning hours) to maintain daily entrainment. This is because morning light will advance the timing of the clock while evening light (prior to minimum core body temperature) will delay the timing of the clock. It should be noted, however, that in general, entrainment is assessed in controlled laboratory conditions, while the studies performed in the field measure phase shifting, such as phase advance or phase delay using downstream outcome measures, such as sleep-wake cycle. As befits a diurnal species, the human biological clock interacts with the sleep-wake cycle to maintain waking during the day and sleep at night. The sleep-wake cycle is regulated by two systems, the circadian system and the homeostatic system (17, 18). Sleep consolidation and quality are reported to be best when the circadian and homeostatic systems are aligned (19). With greater time awake, homeostatic mechanisms increase sleep pressure as bedtime approaches. The circadian system sends an alerting signal to the body to counteract sleep pressure during the day and a sleeping signal during the night, promoting a consolidated night of sleep.

SLEEP, BRAIN ACTIVITY, AND BRAIN PATHOLOGY

Studies have shown that non-rapid eye movement (NREM) sleep, rapid-eye movement (REM) sleep, and slow oscillations (SOs, 0.3–1 Hz, detected in the cerebral cortex during NREM sleep, when neuronal activity is synchronized) are associated with improvement in cognition (20), attention (21), and memory (22). Specifically, an increase in NREM sleep has been associated with improved long-term memory formation (23) and SOs have been shown to be independently associated with improved cognitive performance (24–28).

With respect to AD, sleep disturbance has been investigated as both a symptom of and a risk factor for the disease (4, 29, 30). Research has shown a correlation between sleep disruption and subjective cognitive decline, before MCI or AD manifest (31); less sleep fragmentation has been linked to lower risk for AD in older adults (32); and treating apnea-related sleep disturbance can delay the onset of MCI (33). Research relates sleep problems with AD pathology (Aβ and tau), showing a bidirectional relationship between sleep disruption and Aβ and tau accumulation in rodents and drosophila (30, 34–37). Consistently, increasing cortical Aβ accumulation is also associated with sleep fragmentation (34, 35).

Poor sleep correlates with Aβ and tau pathology severity among people with AD and MCI (38–42), and recent studies indicate associations between AD pathology and NREM sleep (40, 43). Recently, Fultz et al. (44) simultaneously measured functional magnetic resonance imaging (fMRI) studies measuring blood-oxygenation-level-dependent (BOLD) signals, electroencephalogram (EEG) and cerebrospinal fluid (CSF) and observed that at 0.05 Hz (SO that occurs during NREM sleep), there was a large-amplitude pulsatile flow of CSF.

Given that the glymphatic system has been shown to clear Aβ during sleep (36), along with the corollary that sleep disturbance permits the accumulation of Aβ, it is reasonable to conclude that techniques for improving sleep could be employed to counteract Aβ accumulation, and thus, memory decline and progression from MCI to AD.

GAMMA BAND OSCILLATIONS AND BRAIN HEALTH

Gamma activity is composed of rhythmic oscillations that reflect underlying neural synchronizations. Although there is no agreed-upon frequency band that corresponds to gamma oscillations, the accepted lower and upper limits are usually in the range of 20–30 Hz and 80–120 Hz, respectively.

For the most part, gamma oscillations reflect the excitatory and inhibitory activity of interneurons. The cycle starts when excitatory neurons fire, triggering a synchronized discharge of inhibitory interneurons that impede the original excitatory neurons, briefly silencing them. The cycle restarts when the inhibitory signal wears off and allows the excitatory neurons to resume firing (45). Gamma oscillations can be observed throughout the cerebral cortex and correspond to the activation of the cerebral cortex. In the sensory cortex, gamma oscillations can be modulated by presence of sensory stimulation. For example, rhythmic visual stimuli at a certain frequency (i.e., flickering light) will elicit a brain response in synchrony with the frequency of those stimuli. In the visual cortex, exposure to visual stimuli (e.g., light bars) increases power in the 35–50 Hz range (46). In their study with cats, Gray and Singer (46) showed that the probability of neurons to fire in response to the presentation of optimally aligned light bars within their receptive

field is greater when the stimulus has a peak frequency near 40 Hz. They also observed that this was a cortical response (i.e., a response of local neurons in the visual cortex) rather than a thalamic response.

Gamma-band oscillations have been associated with attention, working memory, and associative learning (47). During information-processing events, gamma oscillations allow for selective transmission of sensory information across distributed neurocircuits. Enhanced gamma activity is associated with the enhanced coherence between brain areas (48–51). For example, working memory processes have been associated with the coupling between the phase of theta (4–8 Hz) and the amplitude of gamma (52). In fact, Tort et al. (53) demonstrated that theta–gamma coupling strength directly correlates with increased performance during learning sessions in rats, suggesting theta-gamma coupling plays a role in memory recall (53).

Interestingly, human AD patients (54–56) and AD mouse models (57–59) show reduced power of oscillatory activity in the gamma range (30–100 Hz), which mediates essential neural functions including cortical arousal, sensory processing, working memory, attention-dependent stimuli, and higher order cognition (60–64). This deficit provides a valuable avenue to explore potential treatments for humans with AD or other cognitive impairments.

THERAPEUTIC LIGHTING TECHNIQUES

Light for Entrainment and Phase Shifting of the Circadian System

It is well known that the light-dark cycle is the primary stimulus for synchronizing the circadian system, whose rhythms (e.g., the sleep-wake cycle) repeat approximately every 24 h. Ideally, light of the appropriate amount, spectrum, distribution, duration, and timing synchronizes the internal human circadian clock with solar day-night cycle to help maintain synchrony with work demand times (65). In humans, this synchronization occurs when the circadian system phase advances daily and outcome measures are generally downstream measures.

Lighting characteristics affecting the circadian system, as measured by acute melatonin suppression and phase shifting of dim light melatonin onset (DLMO), a marker of the timing of the biological clock, differ from those affecting our ability to read black font on a white paper. While less light than was originally demonstrated in the 1980s is needed to suppress nocturnal melatonin production, significantly higher amounts of light are needed to affect melatonin than those needed to activate the human visual system (66, 67). Indoor daytime workers, however, may spend their days in "biological darkness" because typical exposures to electrical lighting in indoor environments can be insufficient for entraining the circadian system (68). The lack of a strong light-dark stimulus to the circadian system can lead to sleep disturbances, such as those experienced by older adults, including AD patients, living in more controlled environments. Indeed, it has been shown that middle-aged adults receive ∼58 min of bright light per day (69) while older adults in assisted-living facilities receive bright light for only 35 min per day (70). Adults in nursing homes see as little as 2 min per day.

Following the discovery of the intrinsically photosensitive retinal ganglion cell (ipRGC), a series of animal-model studies showed that circadian phase shifting can occur via input from the ipRGCs and/or the rods and cones, either alone or in combination (71, 72). These studies clearly suggest that, although the ipRGCs are instrumental to transduce the signal from the retina to the SCN (73, 74), melanopsin alone, the opsin that provides the ipRGCs with its intrinsic photosensitivity, is not needed for circadian phase shifting, and neither are rods and cones alone (74).

The peak spectral sensitivity for acute melatonin suppression and phase shifting of DLMO is close to 460 nanometers (nm) (75–77). Timing of exposure is also important for affecting the biological clock. The same stimulus presented in the morning—or after the minimum core body temperature, CBT_{min}, that typically occurs in the second half of the night—will advance the timing of the clock in the following cycle (i.e., bedtimes and waketimes will be earlier the following day). Light given in the evening and early part of the night (before CBT_{min}) will delay bedtimes and waketimes (78).

The duration of light exposure required for melatonin suppression depends on the light stimulus's magnitude (79). Continuous exposure to 74 $\mu W\ cm^{-2}$ of a narrowband, short-wavelength light stimulus (peak close to 470 nm), for example, will elicit measurable melatonin suppression after 5–10 min. Continuous exposure to 2 $\mu W\ cm^{-2}$ of the same blue light source, on the other hand, will elicit measurable melatonin suppression only after 90 min (79). Consistently, a 12-min exposure to 4,100 K fluorescent light (>6,000 lx at the cornea) more effectively phase-shifts circadian rhythms than exposures to lower light levels for longer durations (e.g., 6.4 h), as demonstrated by Chang et al. (80).

Finally, research shows that it is important to accurately measure light exposures over the 24-h day, as opposed to taking just a "snapshot" measurement of light exposure at one certain place and time (81, 82). Given that the circadian system appears to keep track of light exposure, knowing an individual's light exposure history over the past 24 h can help determine the best light prescription for the next 24 h (83). Therefore, a light treatment designed to promote earlier bedtimes should not be limited to reduced exposure to blue light in the morning but should instead control the total circadian light exposure during waking hours.

Amid ongoing investigation into the retinal mechanisms involved in photic stimulation of the circadian system, Rea et al. (77, 84) have proposed and continued to develop a model of human circadian phototransduction that is consistent with known retinal neuroanatomy and neurophysiology (85). The human circadian phototransduction model is based on the response of the ipRGCs (86), but it also includes responses from rods and cones, which have also been shown to provide input to the ipRGCs (71, 72). The ipRGCs, through the retinohypothalamic tract (RHT), transduce the combined photic signal to the master pacemaker, located in the suprachiasmatic nuclei (SCN). According to the Rea et al. model (77, 85), the

cones provide indirect input to the SCN via synapses in the retina, one of which includes the spectrally opponent (blue vs. yellow) S-cone bipolar neurons that combine input from all three cone types to provide depolarizing-only (S-ON) input to the ipRGCs. The ipRGCs convey the combined photic information to the SCN. Following the model, while the intrinsically photosensitive response from the ipRGCs combines its signal with depolarizing "blue" response from the S-ON bipolar, the hyperpolarizing "yellow" response will not be received by the ipRGCs. In the case of light spectra that evoke a "yellow" response from the S-ON bipolar, the ipRGCs' response alone determines the photic information conveyed to the SCN.

Continuous Light for Circadian Phase Shifting and Entrainment

Light therapy for improving sleep, mood, and behavior in AD patients has been the subject of investigation since the 1990s. A comprehensive review of the impact of light on circadian phase shifting and thus, entrainment has been published elsewhere (87), but a summary of some of these studies is presented below.

One of the first studies showing the positive impact of light on circadian rhythms of AD patients was published by Van Someren et al. (88). They studied the effects of increased levels of bright light during the day on 22 institutionalized older adults with severe ADRD. Ambient, unattended high light levels (>1,000 lux at the eye) delivered from ceiling luminaires and windows were used to deliver the intervention in spaces where most of the patients stayed during the day. The results showed that 4 weeks of bright light exposure over the course of a day improved disturbed circadian activity/rest rhythms in older adults with severe dementia.

Yamadera et al. (89) evaluated the effect of bright light exposure (3,000 lux for 2 h each morning) on cognitive functions and circadian rhythms in individuals with AD. The 27 participants experienced the intervention over four consecutive weeks. There was also a significant decrease in daytime napping, awakenings during sleep, and overall percentage of time of sleep increased for all participants. The authors speculated that the bright light exposure improved circadian rhythms and cognitive functions for participants in the early stages of the disease.

Not all of the studies, however, showed positive impacts of light therapy on sleep. Dowling et al. (90) investigated the effect of 1 h of bright light (≥2,500 lux) in the morning for 10 consecutive weeks on nighttime sleep, wake time during the day, and circadian rhythms in a study of 46 ADRD patients. No significant changes were observed in sleep efficiency, sleep time, wake time, or number of awakenings between the control group and the intervention group. However, the authors noted that the greatest improvements in the activity/rest rhythms occurred in those who experienced their 10 most active hours during typical hours of sleep at baseline.

In terms of long-term light therapy, Riemersma-van der Lek et al. (91) were the first to investigate the effect of long-term (maximum of 3.5 years) light exposure and oral melatonin in a study of 189 ADRD patients. Ceiling-mounted fixtures with fluorescent tubes were installed in a common living room. Results showed the light exposure attenuated cognitive decline, ameliorated depressive symptoms, and attenuated the increase in functional limitations by over half. Melatonin increased negative mood and withdrawn behavior, but shortened sleep latency and increased sleep duration.

Although studies to date have shown that light can be a powerful therapy for mitigating sleep disturbances and increasing memory consolidation in older adults with AD, not all of the studies showed consistent positive results (92, 93). This lack of consistency in the results is likely due to the fact that most of the studies to date lack a formal specification of the stimulus. The amount, spectrum, timing, distribution, and duration of the light exposures are not always described in the studies, and in many cases, not even taken into account when delivering the intervention in the field. In fact, a 2014 Cochrane review (94) of eight studies found insufficient evidence to recommend the use of light therapy for improving sleep and behavior in AD patients. The reviewed studies included different lighting interventions, however, and none of them controlled or measured the actual light dose that participants received during the interventions (94). The lack of control for the light delivery methods may have affected the outcomes of the analyses.

With the goal of addressing the issue of lack of control of the stimulus delivery and measurement, Figueiro and colleagues developed a tailored lighting intervention (TLI) designed to effectively affect circadian phase, and, thus, promote entrainment of the circadian system. Although circadian entrainment was not directly measured in the field, it was operationally defined that better circadian entrainment was associated with better sleep, mood and behavior. Figueiro and colleagues used the Rea et al. model to develop the TLI, which was used to deliver a robust light-dark pattern to persons with AD living at home (95) and in more controlled environments (96–98). In their studies, circadian-effective light was delivered from waking to 6 p.m. and circadian ineffective light was delivered during evening hours. Treatment duration varied from 4 weeks to 6 months. Results showed that the lighting intervention significantly reduced subjective sleep disturbances, increased objective sleep measures, an improved behavior, as observed by a reduction in depression and agitation scores (98). These findings were consistent with earlier studies showing that all-day or morning light exposures for short (4 weeks) and long (3.5 years) periods consolidated activity/rest patterns and improved subjective measures of depression (88, 91, 92, 99).

Intermittent Light for Circadian Phase Shifting and Entrainment

In an experiment by Zeitzer et al. (100), 2-ms light pulses presented to the open eyes of six subjects every 1 min for a duration of 60 min during the early part of the night shifted (delayed) circadian phase by an average of 45 min, based on DLMO measurements. They also observed improvements in objective (electroencephalography) and subjective (Stanford Sleepiness Scale) measures of alertness. Although there was a significant phase shift of DLMO, the 2-ms light pulse intervention did not significantly reduce melatonin

concentrations in the intervention night compared to the dark control night. While this was the first study showing the impact of pulsed light intervention in humans, its impact on animal circadian phase had been demonstrated before (101, 102).

At first, these findings appear to contradict predictions from models of human circadian phototransduction and the circadian pacemaker's response to light (103, 104), and suggest that melatonin suppression and phase shifting do not exhibit similar spectral and absolute sensitivities to light (67, 80, 105). Indeed, until the above-cited publication by Zeitzer et al. (100), most studies usually showed that prolonged exposure to light stimuli (i.e., longer than several milliseconds) would reliably phase shift DLMO and acutely suppress melatonin (79, 80).

The responses of the photoreceptor classes vary widely; cones respond very quickly to light stimuli (<50 ms) (106) while the ipRGC's response is much slower (>10 s) (86), and cones display a rapid, heightened response to pulses of bright light that is followed by a slow decay, even after the stimulus is extinguished (107, 108). One hypothesis tested by Figueiro et al. (82) was that, based on this slow decay of cone responses, flashes of bright, short-wavelength light could stimulate the SCN via the depolarizing, S-ON bipolar synapse without necessarily inducing a direct response from the ipRGCs. The Rea et al. model is therefore consistent with the hypothesis that a series of brief, short-wavelength light flashes stimulating the S-cones (a "blue" response) could convey photic information to the SCN. Conversely, a series of brief, longer wavelength green-, yellow-, or red-light flashes (i.e., a "yellow" response) would not elicit a response because it could not circumvent ipRGC sensitivity's high threshold and slow response.

First made possible by determining the human eyelid's spectral transmittance (109), subsequent laboratory research confirmed the efficacy of a light mask worn during sleep that delivered 60 min of continuous green ($\lambda_{max} \approx 527$ nm) light through the closed eyelids of sleeping subjects for suppressing nocturnal melatonin and phase shifting DLMO (110). In a second publication, Figueiro et al. (82) tested the intermittent light hypothesis by comparing the effectiveness of flashing green and flashing blue lights on phase shifting of DLMO and on acute melatonin suppression in humans. They tested the hypothesis that brief pulses of short-wavelength light (blue) delivered in the early part of the night would delay DLMO and suppress nocturnal melatonin, while similarly delivered longer wavelength (green) light would not elicit the same circadian system responses. Three light-stimulus conditions were delivered to 16 subjects during sleep: (1) 111 W m^{-2} of blue ($\lambda_{max} \approx$ 480 nm) light presented as 2-s flashes at 1-min intervals for 1 h, (2) 131 W m^{-2} of green ($\lambda_{max} \approx 527$ nm) light presented continuously for 1 h, and (3) the same green light presented as 2-s flashes at 1-min intervals for 1 h. After correcting for mean eyelid transmittance, the corneal irradiance levels of the flashing blue and the flashing and continuous green lights were set to values previously shown to be of approximately equal effectiveness for stimulating the human circadian system over a continuous exposure of 1 h (circadian stimulus or CS = 0.31 for blue and CS = 0.38 for green). Results showed that, compared to a dark control night and corrected for the natural drift of circadian phase, the flashing blue light evoked a statistically significant phase delay as measured by the incremental change in DLMO (**Figure 1**) and reliably suppressed melatonin. The flashing green light, conversely, did not reliably shift DLMO compared to the natural drift in phase observed for the dark control, nor did it reliably suppress nocturnal melatonin.

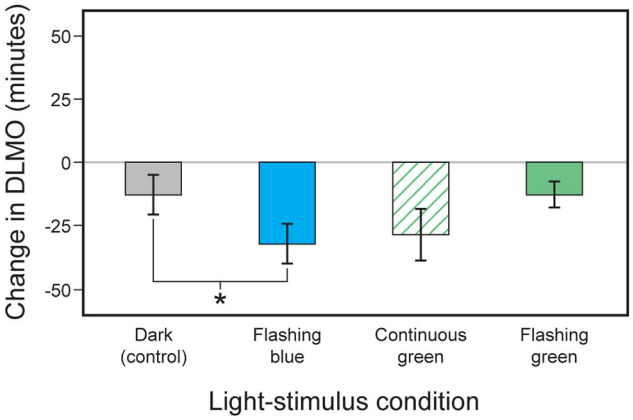


FIGURE 1 | Changes in the timing of DLMO observed for the four light-stimulus conditions employed by Figueiro et al. (82). The error bars represent standard error of the mean. *$p < 0.05$.

This study was followed by another study where the impact of the flashing blue lights was tested in a laboratory setting and a follow-up study conducted in the field, where exposures to light outside the intervention were measured using a calibrated personal device, but not controlled (111). In the laboratory study, they exposed 11 subjects to the flashing blue light delivered to close eyelids for 1 h in the early part of the night. The goal of this laboratory study was to determine the effectiveness of the light to suppress nocturnal melatonin. Melatonin levels during the intervention night, when the flashing lights were energized for 1 h, were significantly reduced compared to the control night, when the mask was worn but not energized. For the field portion of the study, 10 subjects completed a 2-week protocol. During the first week (baseline), they lived their normal lives while wearing light meters and actigraphs. At the end of the week, they came to the laboratory to collect saliva samples for DLMO measurements. During the second week (intervention), light masks delivering flashing blue lights were programmed to turn on 1 h after their bedtimes and remain energized for 2 h, turning off at least 1 h before predicted CBT_{min}; therefore, the intervention was designed to delay their DLMO times. DLMO was significantly delayed ($p = 0.003$) after intervention compared to the control (average delay was 24.5 min), extending the results observed by Figueiro et al. (82) to field settings.

Following the laboratory and field studies confirming their hypothesis, Figueiro (112) tested the effectiveness of the flashing blue light on delaying the circadian system of those with early sleep onset living in their homes. They recruited 28 subjects (9 early awakening insomniacs) to participate in an 8-week, placebo-controlled, within subjects, crossover study. Two

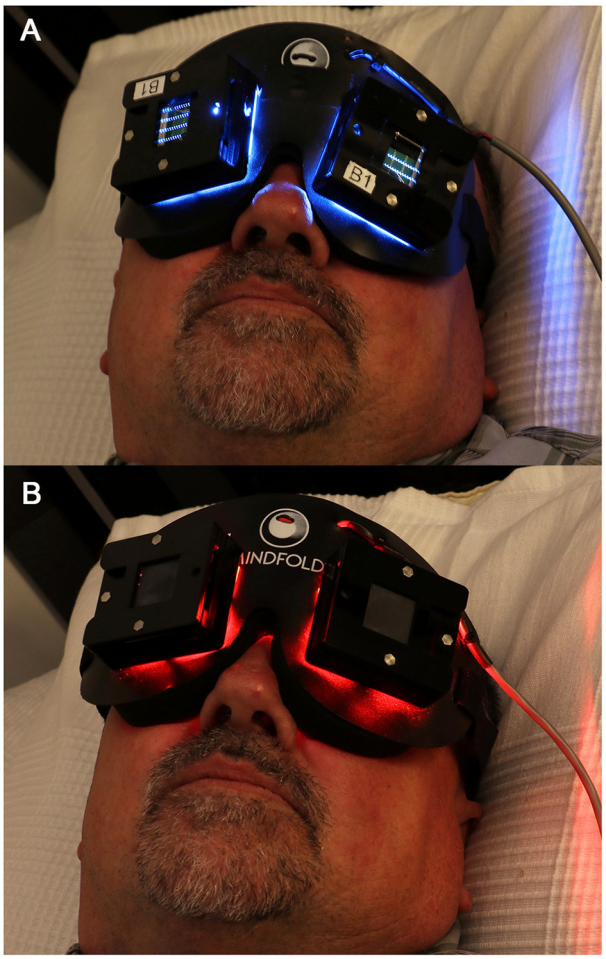


FIGURE 2 | The light mask used to deliver light pulses through closed eyelids during sleep. The light mask contained 2 LED arrays for each eyelid: **(A)** active blue (λ_{max} = 480 nm, FWHM = 24 nm) or **(B)** placebo red (λ_{max} = 640 nm, FWHM = 25 nm). Written informed consent was obtained from the individual shown in this figure for the publication of this image.

lighting conditions were tested, an active (flashing blue light delivered during early part of the night) and a control (flashing red light also delivered during the early part of the night). For each lighting condition, the subjects collected data during 2 baseline weeks and 1 intervention week. Saliva samples for DLMO measurements were collected at the end of each baseline and intervention week. Actigraphs and a calibrated light meter were used during the entire study.

After 1 week of the lighting intervention, results showed that exposure to the flashing (2-s pulses, every 30 s) blue light for durations ≤3 h, starting at least 1 h after bedtime, delayed DLMO by an average of 34 min. A control intervention, delivering a flashing red light (λ_{max} = 640 nm) exposure of the same timing and duration, however, delayed DLMO only minimally (6 min). Sleep start times were significantly delayed (by ∼46 min) at day 7 compared to day 1 after the flashing blue light, and sleep efficiency was not affected by the intervention. Although DLMO and sleep start times were successfully delayed among subjects reporting a history of early awakening insomnia, it remained unknown whether the use of the light mask over longer periods would delay circadian phase and the timing of sleep among those with early sleep onset.

In order to investigate the effectiveness of the flashing light mask in a larger group in real-life conditions, using a crossover, placebo-controlled design, Figueiro et al. (113) exposed 32 subjects to either an active blue (λ_{max} = 480 nm) lighting intervention or a placebo red (λ_{max} = 640 nm) control through closed eyelids during sleep. The light stimulus was presented 1 h after bedtime for consecutive 8 weeks. The light was administered via custom-built light masks that delivered a series of 2-s light pulses at 30-s intervals for ≤2 h (∼240 pulses/night). Subjective measures of sleep and depression (questionnaires) and objective measures of sleep (wrist actigraphy) served as dependent variables. Statistically significant ($p < 0.05$) improvement in seven of the eight subjective sleep measures were reported within both conditions, but no differences were observed between the two conditions. It should be noted that in this long-term study, subjects' daytime light exposures—particularly in the morning, which would have counteracted the effect of the flashing blue light in the evening—were not controlled. The authors hypothesized that if orange-tinted glasses had been worn by subjects in the morning, the impact of the intervention would have been more pronounced.

This method of delivering flashing light through closed eyelids while people sleep (**Figure 2**) nonetheless holds considerable promise for the clinical correction of circadian misalignment, given that light applied close to the CBT_{min} has been shown to maximally shift the circadian pacemaker's timing (78, 114). The CBT_{min} occurs in the second half of the night, ∼2 h prior to natural waking. For the success of this application, however, light exposures during wakefulness should be monitored at all times, perhaps aided by smartphone applications that provide timing recommendations for receiving and removing light stimuli. Future studies should examine how effectively such closed-loop systems might phase-shift the biological clock in real-life situations.

The effects of temporally modulated light stimuli that are delivered in the morning during wakefulness on circadian entrainment, rather than on circadian phase shifting, remains unexplored even though, as shown by the studies discussed above, the Rea et al. model (77, 85) permits qualitative predictions of the impact of temporally modulated light pulses for stimulating the human circadian system, as measured by acute melatonin suppression and shifting of the DLMO times.

Intermittent Light at 40 Hz for Gamma Power Entrainment

Intermittent light stimuli permits gamma oscillations in the brain to resynchronize with the frequency of a flickering light; therefore, the administration of a visual stimulus flickering at 40 Hz will induce gamma oscillations at exactly 40 Hz (59). Such resynchronization has been shown to improve both learning and memory skills in a murine model (115). Because hippocampal

gamma oscillations are linked to cognitive function, it is also assumed that these resynchronized oscillations are responsible for the increase in cognitive performance (116). The research in this area is still new and more work needs to be performed to determine the benefits of 40 Hz flashing lights on cognition.

Recent research has demonstrated that in hippocampal region CA1, as little as 1 h of optogenetic stimulation of parvalbumin (PV) interneurons, which are known to induce gamma oscillations (117, 118), could reduce Aβ peptides levels by ∼50% in 5XFAD mice (59). A similar reduction was also observed in the visual cortex after mice were exposed to an external flickering light at 40 Hz. When 1-h stimulation was repeated daily over the course of 7 days, 40 Hz flickering light also reduced plaque pathology in the visual cortex of 6-month-old 5XFAD mice, and exposures of longer duration (i.e., 1 h per day for either 22 or 42 days) similarly reduced the loss of neuronal and synaptic density. The same stimuli also modified microglia morphology, consistent with increased phagocytic activity responsible for neuronal corpse removal in the brain (59, 119). Long-term (>6 weeks) daily exposure to 40 Hz flickering lights decreased microglia-mediated inflammation in P301S and CK-p25 mice, improved behavioral performance, and reduced loss of neurons in various parts of the brain (59, 120). This was confirmed by Garza et al. (121), who found that 40 Hz flickering light administered to healthy mice increased the expression of cytokines, which plays a central role in microglial recruitment. In a healthy brain, microglia exercise a protective function that restrains the accumulation of Aβ and may prevent neurodegeneration (122). It has been hypothesized that resynchronization of gamma oscillations stimulates recruitment of microglia (59, 123) which take part in ridding the central nervous system of undesirable features such as dysfunctional neurons or amyloid plaques (124). If proven to be viable, harnessing the maintenance role of microglia would be revolutionary, as it would permit the reduction of plaque accumulation using endogenous processes and thereby avoid the introduction of foreign substances to the body. It should be noted that the protective role of microglia in the brain may be lost in later stages of the disease. As toxic amyloid types accumulate in the brain, tau pathology builds up in stressed or damaged neurons, microglia transform into a destructive or inflammatory state that destroys synapses, secretes neurotoxic cytokines that harm neurons and may make matters worse, by spreading tau pathology (122).

It should also be stressed that this therapeutic approach appears to be effective for removing plaques via microglia activation/recruitment in murine models, but it remains unknown whether these findings will translate well to humans. If beneficial in humans, it will be important to determine at which stages of the disease this microglia activation by gamma entrainment switches from being beneficial to detrimental (122).

The first step to initiate this new line of research will be to show that 40 Hz flickering lights leads to an increase in gamma power in the brain. While we are a long way away from proving the efficacy of this intervention for improving cognition in those diagnosed with MCI and AD, a recent small pilot study by Sahin and Figueiro (125) demonstrated that 11 healthy young adults receiving a 40 Hz flickering red light stimulus induced a significant increase in 40 Hz power as well as an overall increase in low gamma power (30–55 Hz). Red light was used as the intervention stimulus because it does not affect the circadian system. There was also a significant correlation between the increase in 40 Hz power and a reduction in subjective sleepiness, as measured by the Karolinska Sleepiness Scale scores. The intervention did not have a significant impact on short-term performance and subjective sleepiness, as measured by the Karolinska Sleepiness Scale, compared to the dark control. According to authors, this may have been due to a "ceiling effect," given that these were normal, healthy young adults. Moving forward, it will be useful to perform similar experiments in those with MCI for a longer period (weeks or months) to determine whether this increase in gamma power resulting from exposure to 40 Hz flickering light can improve cognition and delay transition to AD.

Intermittent Light at 40 Hz: A Case for Dual Entrainment

In the only publication investigating the impact of an intermittent light at 40 Hz on both circadian clock genes and gamma entrainment, Yao et al. (123) compared mRNA levels of clock genes (BMAL1, Per2, and Clock) before and after exposure to 40 Hz flickering light using an AD mouse model (APP/PS1). They showed that 40 Hz flicker increased gamma in the visual cortex, decreased Aβ deposition, and decreased protein expressions of APP and phosphorylated tau in the hippocampus. They also showed that 40 Hz flicker lights partly restored CLOCK, BMAL1, and PER3 gene expression in the APP/PS1 mice, which was shown to be reduced compared to their controls. As noted by the authors, it was not possible from their studies to determine whether it was the light itself or the flicker that drove the observed changes. The wavelength of the light they used for their experiments, 462.8 nm (blue light), is close to the peak sensitivity of the circadian system, thus making it difficult to distinguish the cause of the expression level change. In order to confirm that the restoration of expression levels of genes was due to the flickering light, and not the short-wavelength light, it would be advised to conduct an identical experiment with either a random flicker or with a 40 Hz circadian-ineffective light. Ideally, these studies should be tested in humans and results will help develop new light therapies designed to promote both circadian and gamma entrainment.

DISCUSSION

Light-dark exposures reaching the retina are the major synchronizer of circadian rhythms to the local position on Earth. In general, this synchronization results from small phase advances that occur daily, give that the human circadian clock free runs with a period slight longer than 24 h. Therefore, to promote entrainment would be to advance the clock daily. In studies where light stimulus was carefully specified and measured, the effects on sleep, mood and behavior of various populations, in particular AD patients, were generally

positive (95–97). For the most part, however, light therapy for circadian phase shifting resulting in better entrainment has been continuous, not intermittent. Only a few studies to date have investigated the effectiveness of intermittent light to phase shift the biological clock. This is perhaps due to the limited number of lab and field trials as well as to the fact that there is no clear justification for the use of intermittent light for promoting circadian phase shifting and entrainment alone.

One emerging line of research using phototherapy is the use of intermittent light delivering 40 Hz to promote entrainment of gamma oscillations in the brain. This line of research is still in its infancy and unlike with circadian entrainment, dose response curves to determine optimum light level, spectrum, timing and duration of exposure have not been developed for gamma entrainment therapy. The efficacy of light or sound alone and the combined use of light and sound therapy to promote gamma entrainment, while promising, is still under investigation (115, 120) and there are limited studies performed in human subjects.

Future research, and perhaps more-novel approaches, should investigate the development of a light therapy device that promotes phase shifting of the timing of the clock, and thus, entrainment of circadian rhythms while also promoting entrainment of gamma activity in humans. This would be a good justification for the use of intermittent light to promote circadian entrainment. For that purpose, the use of bright white light or narrow-band short-wavelength (blue) light would be needed, instead of the red light used in the Sahin and Figueiro (125) study. This dual entrainment therapy would likely have additive benefit because it has the potential to improve cognition directly, via entrainment of gamma waves, and indirectly, via improving circadian entrainment and sleep, which in itself is associated with better cognition. One issue that should be considered is the comfort and acceptance of the 40 Hz flickering light and its effectiveness when used in combination with ambient light. Moreover, although the 40 Hz flicker is outside the 0.1–30 Hz flicker range that is better known to induce epileptic seizures, at least one study (126) showed the onset of localized seizures at higher frequencies (60–100 Hz), and therefore, those who are known to suffer from epileptic seizures should not use the flickering lights. It has been suggested that AD patients are more prone to seizures (127), and it is not known whether the flickering lights would have a different effect in those with neurodegenerative diseases compared to healthy people. Nevertheless, intermittent flickering light therapy shows a lot of promise due to its affordable price and easy administration. Initially, testing these interventions in MCI and mild AD patients would likely be the most beneficial, given that this population suffers from *both* sleep disturbances and cognitive deficit.

In summary, this dual entrainment intervention may be very beneficial to those with neurodegenerative diseases. If the same lighting device can be used to target both, gamma and circadian entrainment, this could be easily incorporated in the homes of those with early stages of neurodegeneration. While the impact of light for promoting entrainment in those with early and late stages of AD has been shown in the field (95–97), it is yet to the determined whether the flickering light will also have a positive effect in those who are in early, and more importantly, at later stages of the disease. This is a new area of research, but given that preliminary evidence shows its potential as an important therapy for those suffering from sleep disturbances or cognitive impairment, new research should investigate the additive effect of these two therapies.

AUTHOR CONTRIBUTIONS

MF conceptualized and wrote the manuscript. SL wrote parts of the manuscript. Both authors reviewed and approved the final version.

FUNDING

The research was funded by a NIH Institutional Training Grant (T32 AG057464), the National Institute on Aging (R01 AG034157, R01 AG042602, and R01 AG062288), and the Michael Richter Family.

ACKNOWLEDGMENTS

The authors acknowledge the editorial and technical assistance of David Pedler, Barbara Plitnick, Levent Sahin, Bridget Bradley, Sharon Lesage, Andrew Bierman, and Mark S. Rea.

REFERENCES

1. National Sleep Foundation. *Lack of Sleep is Affecting Americans, Finds the National Sleep Foundation.* (2014). Washington, DC: National Sleep Foundation. Available online at: https://www.sleepfoundation.org/press-release/lack-sleep-affecting-americans-finds-national-sleep-foundation#:~:text=Image-,Lack%20of%20Sleep%20is%20Affecting%20Americans%2C%20Finds%20the%20National%20Sleep,inaugural%20Sleep%20Health%20Index%E2%84%A2 (accessed October 26, 2020).
2. National Sleep Foundation. *Sleep in America Poll.* (2014). Washington, DC: National Sleep Foundation. Available online at: https://sleepfoundation.org/sites/default/files/2014-NSF-Sleep-in-America-poll-summary-of-findings---FINAL-Updated-3-26-14-.pdf (accessed October 26, 2020).
3. Turek FW, Zee PC. Introduction to sleep and circadian rhythms. In: Turek FW, Zee PC, editors. *Regulation of Sleep and Circadian Rhythm*. Boca Raton, FL: CRC Press (1999). p. 1–17. doi: 10.1201/9781420001211
4. Yaffe K, Falvey CM, Hoang T. Connections between sleep and cognition in older adults. *Lancet Neurol.* (2014) 13:1017–28. doi: 10.1016/S1474-4422(14)70172-3
5. De Almondes KM, Costa MV, Malloy-Diniz LF, Diniz BS. Insomnia and risk of dementia in older adults: systematic review and meta-analysis. *J. Psychiatr. Res.* (2016) 77:109–15. doi: 10.1016/j.jpsychires.2016.02.021
6. Wardle-Pinkston S, Slavish DC, Taylor DJ. Insomnia and cognitive performance: a systematic review and meta-analysis. *Sleep Med Rev.* (2019) 48:101205. doi: 10.1016/j.smrv.2019.07.008
7. Guarnieri B, Sorbi S. Sleep and cognitive decline: a strong bidirectional relationship. It is time for specific recommendations on routine assessment and the management of sleep disorders in patients with mild cognitive impairment and dementia. *Eur. Neurol.* (2015) 74:43–8. doi: 10.1159/000434629
8. Bubu OM, Brannick M, Mortimer J, Umasabor-Bubu O, Sebastião YV, Wen Y, et al. Sleep, cognitive impairment, and Alzheimer's disease: a systematic

review and meta-analysis. *Sleep.* (2017) 40:zsw032. doi: 10.1093/sleep/zsw032
9. Shi L, Chen S-J, Ma M-Y, Bao Y-P, Han Y, Wang Y-M, et al. Sleep disturbances increase the risk of dementia: a systematic review and meta-analysis. *Sleep Med. Rev.* (2018) 40:4–16. doi: 10.1016/j.smrv.2017.06.010
10. Alzheimer's Association. 2019 Alzheimer's disease facts and figures. *Alzheimers Dement.* (2019) 15:321–87. doi: 10.1016/j.jalz.2019.01.010
11. Vitiello MV, Bliwise DL, Prinz PN. Sleep in Alzheimer's disease and the sundown syndrome. *Neurology.* (1992) 42:89–93.
12. Vitiello MV, Borson S. Sleep disturbances in patients with Alzheimer's disease: epidemiology, pathophysiology and treatment. *CNS Drugs.* (2001) 15:777–96. doi: 10.2165/00023210-200115100-00004
13. Mccurry SM, Teri L. Sleep disturbance in elderly caregivers of dementia patients. *Clin. Gerontol.* (1996) 16:51–66. doi: 10.1300/J018v16n02_05
14. Mccurry SM, Gibbons LE, Logsdon RG, Vitiello MV, Teri L. Insomnia in caregivers of persons with dementia: who is at risk and what can be done about it? *Sleep Med. Clin.* (2009) 4:519–26. doi: 10.1016/j.jsmc.2009.07.005
15. Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science.* (1999) 284:2177–81. doi: 10.1126/science.284.5423.2177
16. Duffy JF, Wright KP. Entrainment of the human circadian system by light. *J. Biol. Rhythms.* (2005) 20:326–38. doi: 10.1177/0748730405277983
17. Borbély AA, Achermann P. Sleep homeostasis and models of sleep regulation. *J. Biol. Rhythms.* (1999) 14:557–68. doi: 10.1177/074873099129000894
18. Borbély AA, Daan S, Wirz-Justice A, Deboer T. The two-process model of sleep regulation: a reappraisal. *J. Sleep Res.* (2016) 25:131–43. doi: 10.1111/jsr.12371
19. Deboer T. Sleep homeostasis and the circadian clock: do the circadian pacemaker and the sleep homeostat influence each other's functioning? *Neurobiol. Sleep Circadian Rhythm.* (2018) 5:68–77. doi: 10.1016/j.nbscr.2018.02.003
20. Diekelmann S, Born J. The memory function of sleep. *Nat. Rev. Neurosci.* (2010) 11:114–26. doi: 10.1038/nrn2762
21. Lim J, Dinges DF. Sleep deprivation and vigilant attention. *Ann. N.Y. Acad. Sci.* (2008) 1129:305–22. doi: 10.1196/annals.1417.002
22. Stickgold R. Sleep-dependent memory consolidation. *Nature.* (2005) 437:1272–8. doi: 10.1038/nature04286
23. Backhaus J, Born J, Hoeckesfeld R, Fokuhl S, Hohagen F, Junghanns K. Midlife decline in declarative memory consolidation is correlated with a decline in slow wave sleep. *Learn Mem.* (2007) 14:336–41. doi: 10.1101/lm.470507
24. Gais S, Mölle M, Helms K, Born J. Learning-dependent increases in sleep spindle density. *J. Neurosci.* (2002) 22:6830–4. doi: 10.1523/JNEUROSCI.22-15-06830.2002
25. Schabus M, Gruber G, Parapatics S, Sauter C, Klösch G, Anderer P, et al. Sleep spindles and their significance for declarative memory consolidation. *Sleep.* (2004) 27:1479–85. doi: 10.1093/sleep/27.7.1479
26. Clemens Z, Fabó D, Halász P. Overnight verbal memory retention correlates with the number of sleep spindles. *Neuroscience.* (2005) 132:529–35. doi: 10.1016/j.neuroscience.2005.01.011
27. Mednick SC, Mcdevitt EA, Walsh JK, Wamsley E, Paulus M, Kanady JC, et al. The critical role of sleep spindles in hippocampal-dependent memory: a pharmacology study. *J. Neurosci.* (2013) 33:4494–504. doi: 10.1523/JNEUROSCI.3127-12.2013
28. Oyanedel CN, Binder S, Kelemen E, Petersen K, Born J, Inostroza M. Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats. *Behav. Brain Res.* (2014) 275:126–30. doi: 10.1016/j.bbr.2014.09.008
29. Mander BA, Winer JR, Jagust WJ, Walker MP. Sleep: a novel mechanistic pathway, biomarker, and treatment target in the pathology of Alzheimer's disease? *Trends Neurosci.* (2016) 39:552–66. doi: 10.1016/j.tins.2016.05.002
30. Cedernaes J, Osorio RS, Varga AW, Kam K, Schioöth HB, Benedict C. Candidate mechanisms underlying the association between sleep-wake disruptions and Alzheimer's disease. *Sleep Med. Rev.* (2017) 31:102–11. doi: 10.1016/j.smrv.2016.02.002
31. Lauriola M, Esposito R, Delli Pizzi S, De Zambotti M, Londrillo F, Kramer JH, et al. Sleep changes without medial temporal lobe or brain cortical changes in community-dwelling individuals with subjective cognitive decline. *Alzheimers Dement.* (2016) 13:783–91. doi: 10.1016/j.jalz.2016.11.006
32. Lim AS, Kowgier M, Yu L, Buchman AS, Bennett DA. Sleep fragmentation and the risk of incident Alzheimer's disease and cognitive decline in older persons. *Sleep.* (2013) 36:1027–32. doi: 10.5665/sleep.2802
33. Osorio RS, Gumb T, Pirraglia E, Varga AW, Lu SE, Lim J, et al. Sleep-disordered breathing advances cognitive decline in the elderly. *Neurology.* (2015) 84:1964–71. doi: 10.1212/WNL.0000000000001566
34. Kang J-E, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-β dynamics are regulated by orexin and the sleep-wake cycle. *Science.* (2009) 326:1005–7. doi: 10.1126/science.1180962
35. Roh JH, Huang Y, Bero AW, Kasten T, Stewart FR, Bateman RJ, et al. Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology. *Sci. Transl. Med.* (2012) 4:150ra122. doi: 10.1126/scitranslmed.3004291
36. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, et al. Sleep drives metabolite clearance from the adult brain. *Science.* (2013) 342:373–7. doi: 10.1126/science.1241224
37. Tabuchi M, Lone SR, Liu S, Liu Q, Zhang J, Spira AP, et al. Sleep interacts with abeta to modulate intrinsic neuronal excitability. *Curr. Biol.* (2015) 25:702–12. doi: 10.1016/j.cub.2015.01.016
38. Spira AP, Gamaldo AA, An Y, Wu MN, Simonsick EM, Bilgel M, et al. Self-reported sleep and beta-amyloid deposition in community-dwelling older adults. *JAMA Neurol.* (2013) 70:1537–43. doi: 10.1001/jamaneurol.2013.4258
39. Liguori C, Romigi A, Nuccetelli M, Zannino S, Sancesario G, Martorana A, et al. Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease. *JAMA Neurol.* (2014) 71:1498–505. doi: 10.1001/jamaneurol.2014.2510
40. Mander BA, Marks SM, Vogel JW, Rao V, Lu B, Saletin JM, et al. β-amyloid disrupts human NREM slow waves and related hippocampus-dependent memory consolidation. *Nat. Neurosci.* (2015) 18:1051–7. doi: 10.1038/nn.4035
41. Sprecher KE, Bendlin BB, Racine AM, Okonkwo OC, Christian BT, Koscik RL, et al. Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. *Neurobiol. Aging.* (2015) 36:2568–76. doi: 10.1016/j.neurobiolaging.2015.05.004
42. Branger P, Arenaza-Urquijo EM, Tomadesso C, Mezenge F, Andre C, De Flores R, et al. Relationships between sleep quality and brain volume, metabolism, and amyloid deposition in late adulthood. *Neurobiol. Aging.* (2016) 41:107–14. doi: 10.1016/j.neurobiolaging.2016.02.009
43. Sharma RA, Kam K, Parekh A, Uribe-Cano S, Tweardy S, Bubu OM, et al. Reduced spindle frequency and density in stage 2 NREM sleep is associated with increased CSF *p-Tau* in cognitively normal elderly. *Sleep.* (2017) 40:A281. doi: 10.1093/sleepj/zsx050.757
44. Fultz NE, Bonmassar G, Setsompop K, Stickgold RA, Rosen BR, Polimeni JR, et al. Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. *Science.* (2019) 366:628–31. doi: 10.1126/science.aax5440
45. Sohal VS. How close are we to understanding what (if anything) γ oscillations do in cortical circuits? *J. Neurosci.* (2016) 36:10489–95. doi: 10.1523/JNEUROSCI.0990-16.2016
46. Gray CM, Singer W. Stimulus-specific neuronal oscillations in orientation columns of cat visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* (1989) 86:1698–702. doi: 10.1073/pnas.86.5.1698
47. Başar E, Güntekin B. A review of brain oscillations in cognitive disorders and the role of neurotransmitters. *Brain Res.* (2008) 1235:172–93. doi: 10.1016/j.brainres.2008.06.103
48. Gregoriou GG, Gotts SJ, Zhou H, Desimone R. High-frequency, long-range coupling between prefrontal and visual cortex during attention. *Science.* (2009) 324:1207–10. doi: 10.1126/science.1171402
49. Bosman CA, Schoffelen J-M, Brunet N, Oostenveld R, Bastos AM, Womelsdorf T, et al. Attentional stimulus selection through selective synchronization between monkey visual areas. *Neuron.* (2012) 75:875–88. doi: 10.1016/j.neuron.2012.06.037
50. Yamamoto J, Suh J, Takeuchi D, Tonegawa S. Successful execution of working memory linked to synchronized high-frequency gamma oscillations. *Cell.* (2014) 157:845–57. doi: 10.1016/j.cell.2014.04.009

51. Rohenkohl G, Bosman CA, Fries P. Gamma synchronization between V1 and V4 improves behavioral performance. *Neuron.* (2018) 100:953–963.e953. doi: 10.1016/j.neuron.2018.09.019
52. Canolty RT, Knight RT. The functional role of cross-frequency coupling. *Trends Cogn. Sci.* (2010) 14:506–15. doi: 10.1016/j.tics.2010.09.001
53. Tort ABL, Komorowski RW, Manns JR, Kopell NJ, Eichenbaum H. Theta–gamma coupling increases during the learning of item–context associations. *Proc. Natl. Acad. Sci. U.S.A.* (2009) 106:20942–7. doi: 10.1073/pnas.0911331106
54. Herrmann CS, Demiralp T. Human EEG gamma oscillations in neuropsychiatric disorders. *Clin. Neurophysiol.* (2005) 116:2719–33. doi: 10.1016/j.clinph.2005.07.007
55. Koenig T, Prichep L, Dierks T, Hubl D, Wahlund LO, John ER, et al. Decreased EEG synchronization in Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging.* (2005) 26:165–71. doi: 10.1016/j.neurobiolaging.2004.03.008
56. Guillon J, Attal Y, Colliot O, La Corte V, Dubois B, Schwartz D, et al. Loss of brain inter-frequency hubs in Alzheimer's disease. *Sci. Rep.* (2017) 7:10879. doi: 10.1038/s41598-017-07846-w
57. Verret L, Mann EO, Hang GB, Barth AMI, Cobos I, Ho K, et al. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell.* (2012) 149:708–21. doi: 10.1016/j.cell.2012.02.046
58. Gillespie AK, Jones EA, Lin Y-H, Karlsson MP, Kay K, Yoon SY, et al. Apolipoprotein E4 causes age-dependent disruption of slow gamma oscillations during hippocampal sharp-wave ripples. *Neuron.* (2016) 90:740–51. doi: 10.1016/j.neuron.2016.04.009
59. Iaccarino HF, Singer AC, Martorell AJ, Rudenko A, Gao F, Gillingham TZ, et al. Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature.* (2016) 540:230–5. doi: 10.1038/nature20587
60. Uhlhaas PJ, Singer W. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. *Neuron.* (2006) 52:155–68. doi: 10.1016/j.neuron.2006.09.020
61. Tallon-Baudry C. The roles of gamma-band oscillatory synchrony in human visual cognition. *Front. Biosci.* (2009) 14:321–32. doi: 10.2741/3246
62. Jia X, Kohn A. Gamma rhythms in the brain. *PLoS Biol.* (2011) 9:e1001045. doi: 10.1371/journal.pbio.1001045
63. Buzsáki G, Wang X-J. Mechanisms of gamma oscillations. *Annu. Rev. Neurosci.* (2012) 35:203–25. doi: 10.1146/annurev-neuro-062111-150444
64. Mcdermott B, Porter E, Hughes D, Mcginley B, Lang, M., O'Halloran, M., et al. Gamma band neural stimulation in humans and the promise of a new modality to prevent and treat Alzheimer's disease. *J. Alzheimers Dis.* (2018) 65:363–92. doi: 10.3233/JAD-180391
65. Roenneberg T, Pilz LK, Zerbini G, Winnebeck EC. Chronotype and social jetlag: a (self-) critical review. *Biology.* (2019) 8:54. doi: 10.3390/biology8030054
66. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA. Light suppresses melatonin secretion in humans. *Science.* (1980) 210:1267–9. doi: 10.1126/science.7434030
67. Zeitzer JM, Dijk DJ, Kronauer RE, Brown EN, Czeisler CA. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J. Physiol.* (2000) 526:695–702. doi: 10.1111/j.1469-7793.2000.00695.x
68. Rea MS, Figueiro MG, Bullough JD. Circadian photobiology: an emerging framework for lighting practice and research. *Lighting Res. Technol.* (2002) 34:177–87. doi: 10.1191/1365782802lt057oa
69. Espiritu RC, Kripke DF, Ancoli-Israel S, Mowen MA, Mason WJ, Fell RL, et al. Low illumination experienced by San Diego adults: association with atypical depressive symptoms. *Biol. Psychiatry.* (1994) 35:403–7. doi: 10.1016/0006-3223(94)90007-8
70. Sanchez R, Ge Y, Zee P. A comparison of the strength of external zeitgeber in young and older adults. *Sleep Res.* (1993) 22:416–22.
71. Belenky MA, Smeraski CA, Provencio I, Sollars PJ, Pickard GE. Melanopsin ganglion cells receive bipolar and amacrine cell synapse. *J. Comp. Neurol.* (2003) 460:380–93. doi: 10.1002/cne.10652
72. Hattar S, Lucas RJ, Mrosovsky N, Thompson SH, Douglas RH, Hankins MW, et al. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature.* (2003) 424:75–81. doi: 10.1038/nature01761
73. Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, et al. Melanopsin cells are the principal conduits for rod–cone input to non-image-forming vision. *Nature.* (2008) 453:102–5. doi: 10.1038/nature06829
74. Panda S. Multiple photopigments entrain the mammalian circadian oscillator. *Neuron.* (2007) 53:619–21. doi: 10.1016/j.neuron.2007.02.017
75. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J. Neurosci.* (2001) 21:6405–12. doi: 10.1523/JNEUROSCI.21-16-06405.2001
76. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J. Physiol.* (2001) 535:261–7. doi: 10.1111/j.1469-7793.2001.t01-1-00261.x
77. Rea MS, Figueiro MG, Bullough JD, Bierman A. A model of phototransduction by the human circadian system. *Brain Res. Rev.* (2005) 50:213–28. doi: 10.1016/j.brainresrev.2005.07.002
78. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. A phase response curve to single bright light pulses in human subjects. *J. Physiol.* (2003) 549:945–52. doi: 10.1113/jphysiol.2003.040477
79. Figueiro MG, Lesniak NZ, Rea MS. Implications of controlled short-wavelength light exposure for sleep in older adults. *BMC Res. Notes.* (2011) 4:334. doi: 10.1186/1756-0500-4-334
80. Chang AM, Santhi N, St Hilaire M, Gronfier C, Bradstreet DS, Duffy JF, et al. Human responses to bright light of different durations. *J. Physiol.* (2012) 590:3103–12. doi: 10.1113/jphysiol.2011.226555
81. Rea MS, Bierman A, Figueiro MG, Bullough JD. A new approach to understanding the impact of circadian disruption on human health. *J. Circadian Rhythms.* (2008) 6:7. doi: 10.1186/1740-3391-6-7
82. Figueiro MG, Bierman A, Rea MS. A train of blue light pulses delivered through closed eyelids suppresses melatonin and phase shifts the human circadian system. *Nat. Sci. Sleep.* (2013) 5:133–41. doi: 10.2147/NSS.S52203
83. Figueiro MG, Nonaka S, Rea MS. Daylight exposure has a positive carryover effect on nighttime performance and subjective sleepiness. *Lighting Res. Technol.* (2014) 46:506–19. doi: 10.1177/1477153513494956
84. Rea MS, Figueiro MG, Bierman A, Hamner R. Modelling the spectral sensitivity of the human circadian system. *Lighting Res. Technol.* (2012) 44:386–96. doi: 10.1177/1477153511430474
85. Rea MS, Nagare R, Figueiro MG. Modeling circadian phototransduction: retinal neurophysiology and neuroanatomy. *Front. Neurosci.* (2021) 14:615305. doi: 10.3389/fnins.2020.615305
86. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* (2002) 295:1070–3. doi: 10.1126/science.1067262
87. Figueiro MG, Nagare R, Price LLA. Non-visual effects of light: how to use light to promote circadian entrainment and elicit alertness. *Lighting Res. Technol.* (2018) 50:38–62. doi: 10.1177/14771535177721598
88. Van Someren EJW, Kessler A, Mirmiran M, Swaab DF. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. *Biol. Psychiatry.* (1997) 41:955–63. doi: 10.1016/S0006-3223(97)89928-3
89. Yamadera H, Ito T, Suzuki H, Asayama K, Ito R, Endo S. Effects of bright light on cognitive and sleep–wake (circadian) rhythm disturbances in Alzheimer-type dementia. *Psychiatry Clin. Neurosci.* (2000) 54:352–3. doi: 10.1046/j.1440-1819.2000.00711.x
90. Dowling GA, Hubbard EM, Mastick J, Luxenberg JS, Burr RL, Van Someren EJ. Effect of morning bright light treatment for rest-activity disruption in institutionalized patients with severe Alzheimer's disease. *Int. Psychogeriatr.* (2005) 17:221–36. doi: 10.1017/S1041610205001584
91. Riemersma-Van Der Lek RF, Swaab DF, Twisk J, Hol EM, Hoogendijk WJ, Van Someren EJW. Effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities: a randomized controlled trial. *J. Am. Med. Assoc.* (2008) 299:2642–55. doi: 10.1001/jama.299.22.2642
92. Ancoli-Israel S, Martin JL, Kripke DF, Marler M, Klauber MR. Effect of light treatment on sleep and circadian rhythms in demented nursing home patients. *J. Am. Geriatr. Soc.* (2002) 50:282–9. doi: 10.1046/j.1532-5415.2002.50060.x

93. Dowling GA, Burr RL, Van Someren EJ, Hubbard EM, Luxenberg JS, Mastick J, et al. Melatonin and bright-light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. *J. Am. Geriatr. Soc.* (2008) 56:239–46. doi: 10.1111/j.1532-5415.2007.01543.x
94. Forbes D, Blake CM, Thiessen EJ, Peacock S, Hawranik P. Light therapy for improving cognition, activities of daily living, sleep, challenging behaviour, and psychiatric disturbances in dementia. *Cochrane Database Syst. Rev.* (2014) 2:CD003946. doi: 10.1002/14651858.CD003946.pub4
95. Figueiro MG, Hunter CM, Higgins PA, Hornick TR, Jones GE, Plitnick B, et al. Tailored lighting intervention for persons with dementia and caregivers living at home. *Sleep Health.* (2015) 1:322–30. doi: 10.1016/j.sleh.2015.09.003
96. Figueiro MG, Plitnick BA, Lok A, Jones GE, Higgins P, Hornick TR, et al. Tailored lighting intervention improves measures of sleep, depression, and agitation in persons with Alzheimer's disease and related dementia living in long-term care facilities. *Clin. Interv. Aging.* (2014) 9:1527–37. doi: 10.2147/CIA.S68557
97. Figueiro MG, Plitnick B, Roohan C, Sahin L, Kalsher M, Rea MS. Effects of a tailored lighting intervention on sleep quality, rest–activity, mood, and behavior in older adults with Alzheimer's disease and related dementias: a randomized clinical trial. *J. Clin. Sleep Med.* (2019) 15:1757–67. doi: 10.5664/jcsm.8078
98. Figueiro MG, Sahin L, Kalsher M, Plitnick B, Rea MS. Long-term, all-day exposure to circadian-effective light improves sleep, mood, and behavior in persons with dementia. *J. Alzheimers Dis. Rep.* (2020) 4:297–312. doi: 10.3233/ADR-200212
99. Sloane PD, Williams CS, Mitchell CM, Preisser JS, Wood W, Barrick AL, et al. High-intensity environmental light in dementia: effect on sleep and activity. *J. Am. Geriatr. Soc.* (2007) 55:1524–33. doi: 10.1111/j.1532-5415.2007.01358.x
100. Zeitzer JM, Ruby NF, Fisicaro RA, Heller HC. Response of the human circadian system to millisecond flashes of light. *PLoS ONE.* (2011) 6:e22078. doi: 10.1371/journal.pone.0022078
101. Arvanitogiannis A, Amir S. Resetting the rat circadian clock by ultra-short light flashes. *Neurosci. Lett.* (1999) 261:159–62. doi: 10.1016/S0304-3940(99)00021-X
102. Vidal L, Morin LP. Absence of normal photic integration in the circadian visual system: response to millisecond light flashes. *J. Neurosci.* (2007) 27:3375–82. doi: 10.1523/JNEUROSCI.5496-06.2007
103. Kronauer RE, Forger DB, Jewett ME. Quantifying human circadian pacemaker response to brief, extended, and repeated light stimuli over the phototopic range. *J. Biol. Rhythms.* (1999) 14:500–16. doi: 10.1177/074873099129001073
104. Kronauer RE, Forger DB, Jewett ME. Erratum for: Quantifying human circadian pacemaker response to brief, extended, and repeated light stimuli over the phopic range. *J. Biol. Rhythms.* (2000) 15:184–6.
105. Wright HR, Lack LC. Effect of light wavelength on suppression and phase delay of the melatonin rhythm. *Chronobiol. Int.* (2001) 18:801–8. doi: 10.1081/CBI-100107515
106. Cao D, Zele AJ, Pokorny J. Linking impulse response functions to reaction time: rod and cone reaction time data and a computational model. *Vision Res.* (2007) 47:1060–74. doi: 10.1016/j.visres.2006.11.027
107. Baylor DA, Hodgkin AL, Lamb TD. The electrical response of turtle cones to flashes and steps of light. *J. Physiol.* (1974) 242:685–727. doi: 10.1113/jphysiol.1974.sp010731
108. Hestrin S, Korenbrot JI. Activation kinetics of retinal cones and rods: response to intense flashes of light. *J Neurosci.* (1990) 10:1967–73. doi: 10.1523/JNEUROSCI.10-06-01967.1990
109. Bierman A, Figueiro MG, Rea MS. Measuring and predicting eyelid spectral transmittance. *J. Biomed. Opt.* (2011) 16:067011. doi: 10.1117/1.3593151
110. Figueiro MG, Rea MS. Preliminary evidence that light through the eyelids can suppress melatonin and phase shift dim light melatonin onset. *BMC Res. Notes.* (2012) 5:221. doi: 10.1186/1756-0500-5-221
111. Figueiro MG, Plitnick B, Rea MS. Pulsing blue light through closed eyelids: effects on phase shifting of dim light melatonin onset in older adults living in a home setting. *Nat. Sci. Sleep.* (2014) 6:149–56. doi: 10.2147/NSS.S73856
112. Figueiro MG. Individually tailored light intervention through closed eyelids to promote circadian alignment and sleep health. *Sleep Health.* (2015) 1:75–82. doi: 10.1016/j.sleh.2014.12.009
113. Figueiro MG, Sloane PD, Ward K, Reed D, Zimmerman S, Preisser JS, et al. Impact of an individually tailored light mask on sleep parameters in older adults with advanced phase sleep disorder. *Behav. Sleep Med.* (2020) 18:226–40. doi: 10.1080/15402002.2018.1557189
114. Jewett ME, Rimmer DW, Duffy JF, Klerman EB, Kronauer RE, Czeisler CA. Human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients. *Am. J. Physiol.* (1997) 273:R1800–9. doi: 10.1152/ajpregu.1997.273.5.R1800
115. Martorell AJ, Paulson AL, Suk H-J, Abdurrob F, Drummond GT, Guan W, et al. Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. *Cell.* (2019) 177:256–71.e222. doi: 10.1016/j.cell.2019.02.014
116. Zheng L, Yu M, Lin R, Wang Y, Zhuo Z, Cheng N, et al. Rhythmic light flicker rescues hippocampal low gamma and protects ischemic neurons by enhancing presynaptic plasticity. *Nat. Commun.* (2020) 11:3012. doi: 10.1038/s41467-020-16826-0
117. Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature.* (2009) 459:663–7. doi: 10.1038/nature08002
118. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature.* (2009) 459:698–702. doi: 10.1038/nature07991
119. Damisah EC, Hill RA, Rai A, Chen F, Rothlin CV, Ghosh S, et al. Astrocytes and microglia play orchestrated roles and respect phagocytic territories during neuronal corpse removal *in vivo*. *Sci. Adv.* (2020) 6:eaba3239. doi: 10.1126/sciadv.aba3239
120. Adaikkan C, Middleton SJ, Marco A, Pao P-C, Mathys H, Kim DN-W, et al. Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron.* (2019) 102:929–43.e928. doi: 10.1016/j.neuron.2019.04.011
121. Garza KM, Zhang L, Borron B, Wood LB, Singer AC. Gamma visual stimulation induces a neuroimmune signaling profile distinct from acute neuroinflammation. *J. Neurosci.* (2020) 40:1211–25. doi: 10.1523/JNEUROSCI.1511-19.2019
122. Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *J. Cell Biol.* (2018) 217:459–72. doi: 10.1083/jcb.201709069
123. Yao Y, Ying Y, Deng Q, Zhang W, Zhu H, Lin Z, et al. Non-invasive 40-Hz light flicker ameliorates Alzheimer's-associated rhythm disorder via regulating central circadian clock in mice. *Front. Physiol.* (2020) 11:294. doi: 10.3389/fphys.2020.00294
124. Chew G, Petretto E. Transcriptional networks of microglia in Alzheimer's disease and insights into pathogenesis. *Genes.* (2019) 10:798. doi: 10.3390/genes10100798
125. Sahin L, Figueiro MG. Flickering red-light stimulus for promoting coherent 40 Hz neural oscillation: a feasibility study. *J. Alzheimers Dis.* (2020) 75:911–21. doi: 10.3233/JAD-200179
126. Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain.* (2004) 127:1496–506. doi: 10.1093/brain/awh149
127. Powell G, Ziso B, Larner AJ. The overlap between epilepsy and Alzheimer's disease and the consequences for treatment. *Expert Rev. Neurother.* (2019) 19:653–61. doi: 10.1080/14737175.2019.1629289

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

REVIEW
published: 25 March 2021
doi: 10.3389/fneur.2021.636330

Intrinsically Photosensitive Retinal Ganglion Cells of the Human Retina

Ludovic S. Mure [1,2]*

[1] Institute of Physiology, University of Bern, Bern, Switzerland, [2] Department of Neurology, Zentrum für Experimentelle Neurologie, Inselspital University Hospital Bern, Bern, Switzerland

Light profoundly affects our mental and physical health. In particular, light, when not delivered at the appropriate time, may have detrimental effects. In mammals, light is perceived not only by rods and cones but also by a subset of retinal ganglion cells that express the photopigment melanopsin that renders them intrinsically photosensitive (ipRGCs). ipRGCs participate in contrast detection and play critical roles in non-image-forming vision, a set of light responses that include circadian entrainment, pupillary light reflex (PLR), and the modulation of sleep/alertness, and mood. ipRGCs are also found in the human retina, and their response to light has been characterized indirectly through the suppression of nocturnal melatonin and PLR. However, until recently, human ipRGCs had rarely been investigated directly. This gap is progressively being filled as, over the last years, an increasing number of studies provided descriptions of their morphology, responses to light, and gene expression. Here, I review the progress in our knowledge of human ipRGCs, in particular, the different morphological and functional subtypes described so far and how they match the murine subtypes. I also highlight questions that remain to be addressed. Investigating ipRGCs is critical as these few cells play a major role in our well-being. Additionally, as ipRGCs display increased vulnerability or resilience to certain disorders compared to conventional RGCs, a deeper knowledge of their function could help identify therapeutic approaches or develop diagnostic tools. Overall, a better understanding of how light is perceived by the human eye will help deliver precise light usage recommendations and implement light-based therapeutic interventions to improve cognitive performance, mood, and life quality.

Keywords: retina, retinal ganglion cell, intrinsically photosensitive ganglion cell, melanopsin (OPN4), non-visual responses to light

OPEN ACCESS

Edited by:
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***Correspondence:**
Ludovic S. Mure
ludovic.mure@unibe.ch

Specialty section:
This article was submitted to Neuro-Ophthalmology, a section of the journal Frontiers in Neurology

Received: *01 December 2020*
Accepted: *15 February 2021*
Published: *25 March 2021*

Citation:
Mure LS (2021) Intrinsically Photosensitive Retinal Ganglion Cells of the Human Retina. Front. Neurol. 12:636330. doi: 10.3389/fneur.2021.636330

INTRODUCTION

The last years have seen an increased awareness of the impact of light on health, particularly of its detrimental effects when light is not delivered at the appropriate time. Light at night, also called "light pollution," is becoming a major environmental and health concern (1–4). Even low-level light exposure from light-emitting devices, smartphones, or tablets may disrupt sleep (5, 6). As inappropriate illumination can be detrimental to health, optimal lighting can be a simple, cost-efficient population-level intervention to improve health: if light is delivered at the right time and in the right amount, it can ameliorate the quality of life in the nursing home and improve cognitive performances at school and at work (7–9).

Both beneficial and detrimental effects of light are mediated not only by rods and cones, the well-known photoreceptors that serve vision but also by a third class of cells in our retina. These cells are a subset of retinal ganglion cells (RGCs) expressing the photopigment melanopsin that renders them sensitive to light. They have been referred to as either photosensitive, intrinsically photosensitive retinal ganglion cells (pRGCs, ipRGCs), or melanopsin-expressing retinal ganglion cells (mRGCs) according to the context, i.e., when the studies focus on their response to light or on the presence of melanopsin respectively. Here, for simplicity, I will use the acronym ipRGCs. ipRGCs play a major role in what is called "non-visual" or "non-image-forming" responses to light. These responses include the alignment of our internal clock to the environmental day/night cycle, the regulation of the sleep-wake cycles, of the pupillary reflex to light (PLR), and the modulation of mood (10–12). More recently, it has been shown that melanopsin-driven response of ipRGCs also participates in some aspects of vision (13–16).

Twenty years after their discovery (17, 18), ipRGCs are well-documented in rodents and have been reviewed in depth elsewhere (19–21). Although there are only a few thousand ipRGCs per retina, they exhibit remarkable heterogeneity. They differ regarding dendritic arborization, expression levels of melanopsin, brain targets, and light response properties. In the mouse retina, six different morphological subtypes (M1 through M6) have been characterized and at least five functional subtypes are described. While the M1 subtype expresses high levels of melanopsin, the M2–M6 subtypes express lower amounts of melanopsin and also exhibit reduced intrinsic photosensitivity. Accordingly, each ipRGC subtype is thought to execute distinct light-regulated functions at specific levels of light intensity or time constants. For example, a fraction of M1 ipRGCs mediates the photoentrainment of the circadian clock while M4 ipRGCs are involved in the effect of light on mood. In contrast, all ipRGC subtypes seem to project to visual structures [dLGN, superior colliculus (SC)], and it is believed that they all participate in some aspects of vision. Finally, while ipRGCs are the principal conduits for all light input to the non-image-forming visual responses, anatomical and electrophysiological evidence suggests that ipRGCs also receive input from rod/cone photoreceptors.

In stark contrast to rodent ipRGCs, the exploration of ipRGCs in primates and in human, in particular, was, until recently, extremely limited. There is, however, a strong rationale to study them. Human and mouse are respectively diurnal and nocturnal animals. Human retina differs from the rodent retina on several levels, from the regional specialization of the retina to photoreceptor types and distribution (**Figure 1**). Human retina is adapted for high definition, color vision. This is achieved thanks to the fovea, a central zone of the retina (∼1.2 mm of diameter), where three types of cones are densely packed. These cones (S, M, and L for short-, middle-, and long-wavelength cones) mostly express a unique photopigment with absorption peaks at 430, 531, and 561 nm, respectively (26, 27). In contrast, laboratory mice are nocturnal and their retina, devoid of fovea, is largely dominated by rods and expresses only two types of cone opsins [S- and M-opsin, with peak sensitivities at 360 nm and 508 nm, respectively (28, 29) often co-expressed in the same cone (30). As a consequence, there is a lack of appropriate murine models for some humane ocular disorders, such as age-related macular degeneration (31). Apart from anatomical discrepancies, there is also the genetic gap between the two species, which may result in different phenotypes in some cases of genetically inherited diseases (32). Another caveat is human modern lifestyle that results in a number of disorders such as diabetic retinopathy, which does not naturally occur in rodents.

Fortunately, the gap of knowledge in human ipRGCs is progressively being filled. New approaches and techniques have allowed characterizing morphological and functional human ipRGC subtypes, their transcriptome, and realizing that, in several disorders, they are either more resilient or vulnerable than conventional RGCs. The present paper reviews this recent progress in our knowledge of human ipRGCs, briefly compares their characteristics with those of the most studied model, the laboratory mouse, and highlights some outstanding questions and future challenges.

HUMAN ipRGCs COMPRISE SEVERAL MORPHOLOGICAL SUBTYPES

Shortly after its discovery in the mouse, melanopsin was also found in the human inner retina (33). Melanopsin expression was detected in a subpopulation of RGCs located in the ganglion cell layer but also sometimes displaced in the inner nuclear cell layer. Melanopsin-expressing cells have a particular morphology with two to four dendritic processes constituting an extensive network throughout the retina. Melanopsin immunoreactivity is present in the soma and neuronal processes membranes and, to some extent, in the cytoplasm (33–35). Rare melanopsin-positive cones were also described in the human retina (36).

The morphological characterization of ipRGCs in the human retina has now advanced substantially; several recent studies provided a detailed morphological description of ipRGCs in the retina of human donors (**Figure 1**) (22–25, 37). In humans, the reported number of ipRGCs varies from ∼4,000 to more than 7,000, but it remains extremely marginal (0.4–1.5%) compared to the 1.07 million ganglion cells in the human retina (22–24, 35, 38, 39). Two distinct morphological types roughly correspond to the M1 type of the mice, with dendrites that are primarily or exclusively in the outer sublamina of the inner plexiform layer (IPL), and the M2 type of the mice with dendrites that are primarily or exclusively in the inner sublamina of the IPL (40). The fovea is devoid of ipRGCs. The ipRGCs are most abundant in the peri-foveal region (∼15–40 cells/mm^2) and their number declines to <5 cells/mm^2 at 10 mm eccentricity and beyond (23–25); in that, they parallel the decrease of density of RGCs from the center to periphery of the retina. Additional morphological subtypes of ipRGCs have been reported in specific studies including M3, M4, and types that further subdivide M1 type into standard M1, gigantic M1, displaced M1 (dM1), and gigantic dM1 (22–25) (**Figure 2**). Of note, in human, but not in the mouse, dM1 constitute the majority of M1. Importantly, these morphological studies relied on immunostaining of melanopsin,

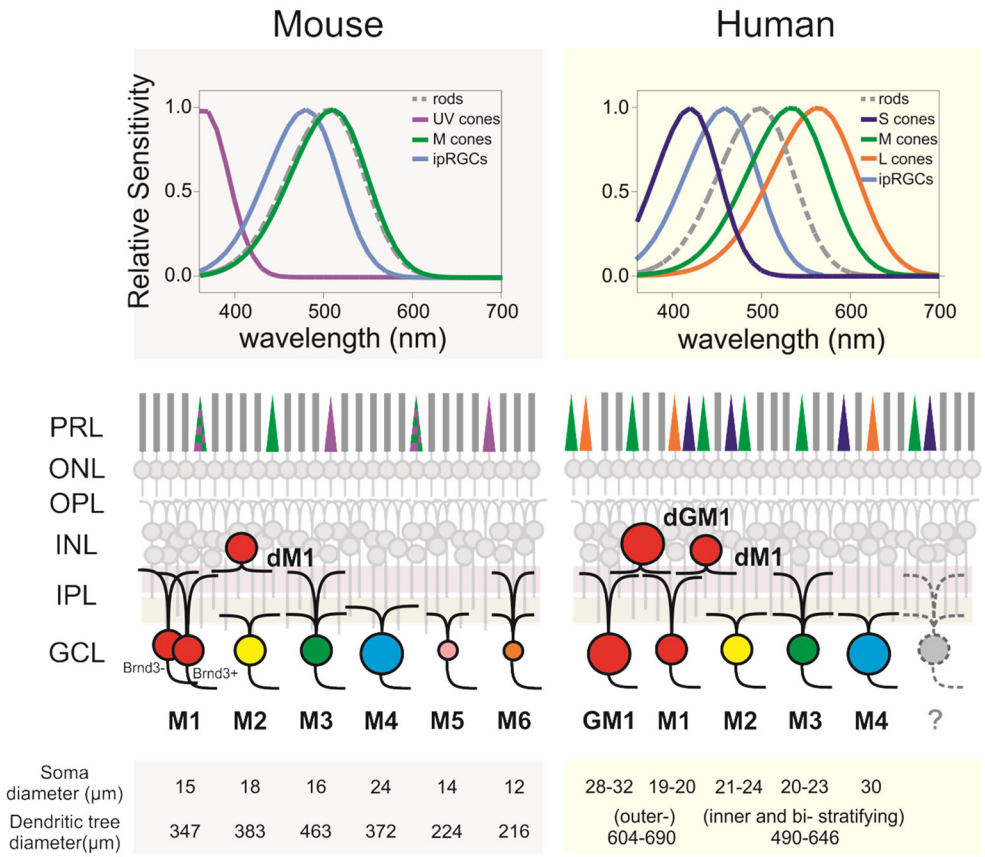

FIGURE 1 | ipRGCs in the mouse and human retinas. (Upper panels) Relative spectral sensitivity of the rods, cones, and ipRGCs. (Middle panels) Diagram of murine and human retinas displaying the differences regarding the morphological subtypes of ipRGCs, their IPL dendritic stratification, and outer retina photoreceptors. (Lower panels) Morphological comparison between subtypes and species. Soma and dendritic tree measurements are rounded to the closest integer. Mouse data are from Sondereker et al. (21) that compiled them from literature. Human data are from Esquiva et al. (22), Hannibal et al. (23), Liao et al. (24), and Nasir-Ahmad et al. (25). GM1, gigantic M1; dM1, displaced M1; dGM1, displaced gigantic M1; PRL, photoreceptors layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner plexiform layer; IPL, inner plexiform layer; GCL, ganglion cells layer.

a method that, in mice, has been shown to fail to detect all ipRGCs [see Aranda and Schmidt (19)]. This suggests a probable underestimation of the total number of ipRGCs and potential bias in the reported subtype distribution.

ipRGCs BRAIN TARGETS

Mapping the projections of ipRGCs in the brain has been instrumental to discover their multiple functions. In the mouse, ipRGCs convey light information to more than a dozen brain regions, including several nuclei implicated in circadian rhythms [suprachiasmatic nucleus (SCN), intergeniculate leaflet (IGL)], sleep and wake regulation [in the hypothalamus, the ventrolateral preoptic area (VLPO) and lateral hypothalamus (LH), and the centro-medial nucleus in the thalamus], PLR control [olivary pretectal nucleus (OPN)], and mood (peri Habenula) (41–44). Visual structures such as the dorsal lateral geniculate nucleus (dLGN) and the superior colliculus (SC) are also targeted.

In human, the exploration of ipRGC projections is limited by the impossibility to use the appropriate techniques, e.g., injection of tracers or genetically encoded labels. However, Hannibal and colleagues (35) took advantage of the fact that the pituitary adenylate-cyclase-activating polypeptide (PACAP) is a marker for retinohypothalamic tract (RHT) projections to the SCN in rodents and human (45) and that PACAP is found in virtually all ipRGCs in the retina of human to describe ipRGC putative projections on the SCN. They found a dense terminal field of PACAP-positive nerve fibers in the retinorecipient zone (ventral part) of the SCN in two human donors (while no PACAP-immunoreactive cell bodies were found in the SCN). The fibers mainly arose from the optic chiasma and were found in close apposition to VIP-containing neurons in the ventral SCN.

Given the impossibility to use tracers in humans, studies in non-human primates remain essential for completing the mapping of ipRGC central projections in the primate. Classical retrograde tracing from the lateral geniculate complex and the pretectum in macaque identified these areas as targets for the ipRGCs (34). Using immunohistochemical staining of

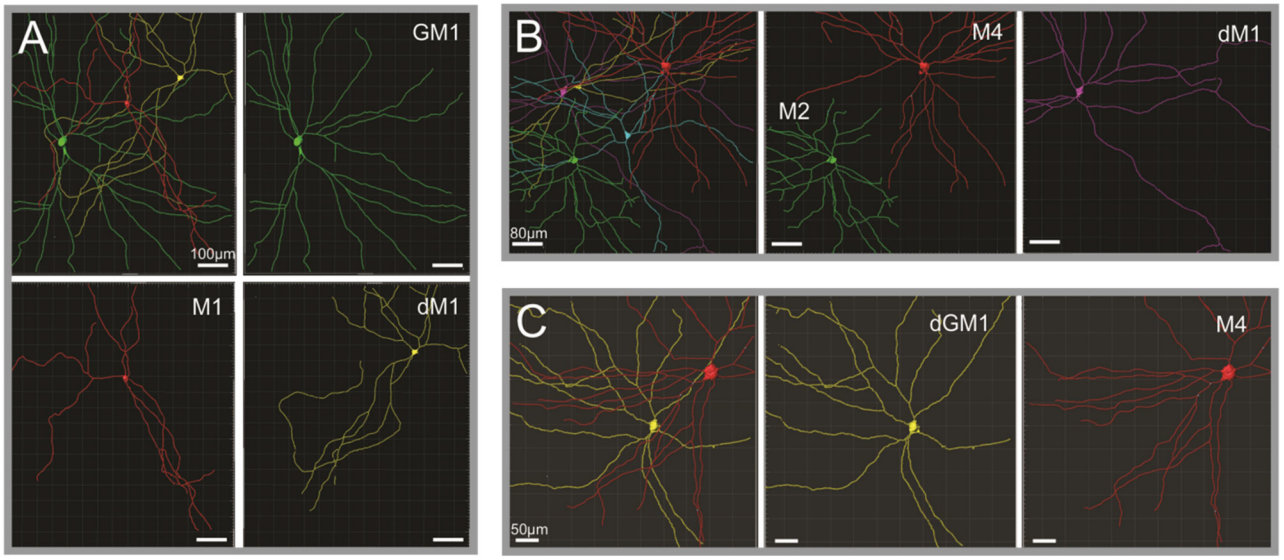

FIGURE 2 | Human ipRGCs morphological subtypes. **(A–C)** Reconstruction and pseudocoloring of ipRGCs from three separate human retina volumes based on melanopsin immunoreactivity. Upper left subpanels illustrate the different ipRGCs detected in the volumes, their relative size, and arrangement toward each other. In the other subpanels, each ipRGC is then identified and represented separately to appreciate the details of their dendritic arborization. dM1, displaced M1; GM1, gigantic M1; dGM1, displaced gigantic M1. Scale bars: A, 100 µm; B, 80 µm; C, 50 µm [Figure adapted from Hannibal et al. (23); courtesy of Dr. J. Hannibal and Journal of Comparative Neurology].

PACAP in combination with staining for the anterograde tracer (Cholera Toxin Fragment B) delivered by intraocular injection, ipRGC projections to the SCN were confirmed in macaque (46). Additionally, projections to the LGN including the pregeniculate nucleus [which is thought to correspond to the rodents IGL (47)], the OPN, the nucleus of the optic tract, the brachium of the SC, and the SC were identified (46). Interestingly, in the macaque, ipRGC projections to the dLGN emerge from both inner and outer stratifying melanopsin cells (hence potentially from all ipRGC subtypes), while in the mouse, the majority of melanopsin ganglion cell innervation of the dLGN appears to be provided only by inner stratifying cells [non-M1 cells (41, 44, 48)]. Whether this discrepancy reflects an extended role of ipRGCs in vision in the primate remains to be clarified. Finally, in the mouse, ipRGC terminals are found in numerous hypothalamic nuclei in addition to the SCN, including the VLPO, LH, anterior hypothalamic nucleus, ventral subparaventricular zone, and peri-supraoptic nucleus (42, 44). Retinal projections to these hypothalamic nuclei also exist in the primate (49, 50). However, whether these projections include ipRGCs remains to be verified. It is not a trivial question as these nuclei often heavily influence physiology through the control they exert on sleep, appetite, and thermoregulation to name a few.

FUNCTIONAL PROPERTIES AND DIVERSITY OF HUMAN ipRGCs

The first report of human RGCs direct electrophysiological recording was published by Weinstein et al. (51). This study measured the spectral sensitivity of two RGCs around the photopic peak (555 nm). However, such recordings in the human retina would then remain anecdotal until recently. There have been as many studies, peer-reviewed articles and non-peer-reviewed, preprint manuscripts, on the human retina physiology over the last 2 years as in the previous 50 years (52–57).

So far, only one study has been specifically designed to capture human RGCs' intrinsic sensitivity and to describe ipRGC responses to light and functional diversity (55). Overall, the characteristic features of pharmacologically isolated human ipRGC responses, i.e., when their response is solely driven by melanopsin, seem similar to that of rodents and macaque (17, 34, 58, 59). Human ipRGCs' intrinsic responses to light are slow, sustained over the entire stimulation, and do not extinguish immediately after light OFF. These kinetic properties make ipRGC responses very different from rod- and cone-driven responses that are extremely fast (<100 ms). Intrinsic photoresponses of human ipRGCs are reversibly inhibited by opsinamide, a drug that specifically blocks melanopsin (60). Mure et al. also found that ipRGCs' intrinsic sensitivity was low; ipRGCs did not seem to respond to light intensities below photopic level, even following dark adaptation. Their spectral sensitivity peaked in the blue region of the spectrum (∼460 nm), different from the peaks of human rods and cones but close to mouse and macaque melanopsin peaks (17, 34) and to the human melanopsin expressed in HEK293 cells (61). This result is also consistent with ipRGCs' role in human non-visual responses to light such as nocturnal melatonin peak suppression (62, 63), PLR (64, 65), non-cone/non-rod visual awareness (13, 66), cognition

(67), and heart rate modulation (68) that are also maximally sensitive to blue light.

Human ipRGCs' response parameters and time courses suggest that they consist of several functional groups. Mure et al. described three ipRGC subtypes, each one displaying unique response kinetics and sensitivity to light (**Figure 3**). Type 1 ipRGCs are more sensitive to light and sustain response long after the light is turned off. Type 2 ipRGCs are less sensitive and turn OFF faster. At low irradiance levels, type 2 ipRGCs exhibit longer response latency to the test light pulse. Type 1 responses are recorded 50% more frequently than Type 2 responses. A third type of ipRGCs responded only in the presence of exogenous chromophore (11-cis retinal) in the medium. These Type 3 cells responded more strongly, but only to the high irradiance levels, and extinguished faster after light OFF. Altogether, the features of Type 1, Type 2, and Type 3 ipRGCs suggest that they could correspond to mouse ipRGC subtypes that have been labeled M1, M2, and M4 ipRGCs, respectively (69–71). However, the link between the human physiological and morphological ipRGC subtypes, and their correspondence with the murine subtypes, remains to be established. Also, Mure et al.'s study was performed on a limited number of donors; these findings need to be independently replicated. The effort must be pursued to refine the results and to increase the number and diversity of donors. Recently, light-induced melatonin suppression in the evening, a process under ipRGCs control, has been shown to vary up to 50 times between subjects (72). It would be interesting to determine to which extent ipRGCs contribute to such variability in light sensitivity (73).

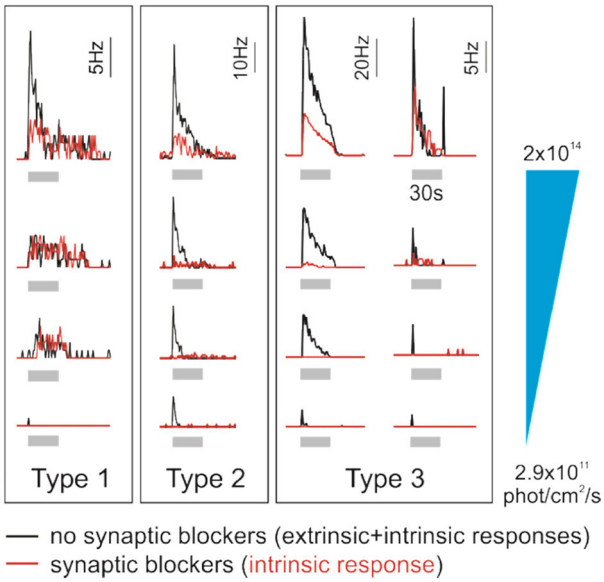

FIGURE 3 | Human ipRGCs integrate extrinsic signals. Individual examples of type 1, 2, and 3 ipRGCs' responses to increasing irradiance light pulses (gray bars, 30 s, 470 nm; from bottom to top, irradiance is 2.9×10^{11}, 3.5×10^{12}, 2×10^{13}, and 2×10^{14} photons/cm^2 per second). Red traces represent the responses of pharmacologically isolated ipRGCs, which reflect their intrinsic photosensitivity. In contrast, black traces report the responses from the same cells in the absence of synaptic blockers and thus integrating input from outer retina photoreceptors. Time course, sensitivity, and intrinsic properties of the response differ between the ipRGC subtypes. The contribution from rods/cones to the overall ipRGC responses to light also seems to be subtype-specific. Interestingly, ipRGC subtypes may receive different inputs from photoreceptors. Of note, in human, morphological and functional ipRGC subtypes are not yet fully consolidated; here, ipRGC subtypes are labeled as in the original study from which this figure is adapted (55).

TRANSCRIPTOME DIVERSITY OF HUMAN ipRGCs

Underlying the morphological and functional diversity are the different gene expression profiles of ipRGCs. In mice, the first indication of the molecular heterogeneity of ipRGCs came with the observation that all ipRGCs express the transcription factor Brn3b except for the fraction of M1 cells that project to the SCN (74). Thus, while all M1 ipRGCs are morphologically and electrophysiologically similar, two molecularly different subpopulations co-exist and innervate different brain regions (SCN for M1 Brn3b– and OPN for M1 Brn3b+). This additional dimension of identity is now easily approachable. High-throughput methods [single-cell RNA sequencing (scRNAseq) or RNAseq applied on RGCs-enriched samples] allowed distinguishing several ipRGC subpopulations in both mouse and primate (75–79).

In macaque and human retina, scRNAseq performed on CD90+ cells to enrich the samples with RGCs (CD90 or Thy1 is a cell surface protein marker of RGC class) allowed differentiating up to 18 RGCs subpopulations (77, 78). The four most abundant RGC clusters were easily identified as ON and OFF midget RGCs and ON and OFF parasol RGCs that account for respectively >80% and ∼10% of all RGCs in the primate retina. The remaining RGC clusters each consists of ∼1% or less of all RGCs. Melanopsin was expressed at detectable levels in a few of these RGC clusters in the peripheral retina, three in the macaque (77) and two in human (78). In human, the authors noted a sensible difference in expression levels of melanopsin and hypothesized a correspondence between the cluster expressing the highest level of melanopsin and M1 ipRGCs, which express the highest levels of melanopsin in mice (20), while other subtypes (M2–M6) would constitute the remaining cluster or be too rare to be detected.

Interestingly, the comparative study of murine and macaque retina cell transcriptomes indicates that the ganglion cells are the less conserved retinal cell type between the two species. However, while conventional RGCs only show weak correspondence in terms of both diversity and distribution, ipRGCs seem to be among the most conserved features (77, 79). This may reflect the differences in the visual signal tracked by nocturnal and diurnal animals and thus in the organization of their respective visual systems. In contrast, the features of the light signal relevant to non-visual responses such as the ambient level of light for the circadian system are similar for most organisms and may rely on similar cell types.

INTEGRATION OF EXTERNAL INPUT FROM PHOTORECEPTORS

In a similar way to conventional RGCs, ipRGCs convey rod- and cone-initiated photoresponses and integrate these extrinsic signals and their intrinsic photosensitivity (80, 81). The contribution of outer retina photoreceptors to human ipRGC signaling can be studied by comparing ipRGC responses before and after application of synaptic blockers that isolate RGCs from extrinsic input (55) (**Figure 3**). It is important to keep in mind, however, that the photoreceptor responses may be differentially affected by the preparation itself. For example, in the absence of RPE *in vitro*, the input from rods and cones may be diminished and their contribution may be underestimated. In the absence of synaptic blockers, a large number of RGCs respond to light. Most of them become silent after incubation with blockers as conventional RGCs do not receive rod and cone signals anymore. ipRGC responses persist; however, their response is generally altered. More specifically, the response threshold is higher and the latency is longer while the amplitude is decreased. Of note, the part of rod and cone responses in the overall response seems to be specific to the ipRGC subtypes. For all subtypes, extrinsic input to ipRGCs shortens the response latencies and lowers the response thresholds. However, only for Type 2 and 3 ipRGCs did the extrinsic input account for a significant portion of the sustained response and increase their sensitivity. A similar observation was made in the mouse where the contribution of rods and cones to ipRGC responses seems inversely proportional to melanopsin photosensitivity; while mouse M1 ipRGC responses are moderately influenced, the M2–M5 subtype responses rely more heavily on extrinsic inputs (82). The response of Type 3 ipRGCs, in particular, seems to rely the most on input from rods/cones, which is in line with the description of M4 ipRGCs (83, 84). Type 1 ipRGCs receive only minimal extrinsic inputs compared to other subtypes. M1 ipRGCs, which may be the mouse orthologous of human type 1 ipRGCs, are sufficient to photoentrain the clock (74). This is consistent with the finding that cones, while they may contribute to the entrainment of the clock in humans (85), are not required for it (86). As mentioned above, human and mouse cones differ in number and peak wavelength sensitivity, which suggests different weights of their input to ipRGCs in response to the same light stimulus. There may also be important functional divergences. For example, short-wavelength cones and melanopsin are antagonistic in controlling the primate PLR but additive in the murine PLR (87, 88). This illustrates the importance of elucidating the subtype-specific contribution of rods and cones as they can dramatically alter ipRGC spectral sensitivity; i.e., they can shift their action spectra from blue toward shorter or longer wavelengths.

Overall, the rod/cone input to ipRGCs expands the dynamic range of irradiance and temporal frequencies over which the ipRGCs signal (17, 34, 55). The diversity in ipRGC subtypes combined with the way they specifically integrate rod and cone signals could explain their ability to regulate such a variety of responses to light functioning at various time constants and light levels.

ipRGCs IN AGING AND DISEASE

Several recent studies have highlighted the progressive loss of ipRGCs with aging, which is aggravated in neurodegenerative diseases (22, 89–92). A decrease in the total number of ipRGCs and the size of dendritic arborization occurs progressively with aging [31% loss in healthy subjects older than 70 years (22)]. However, there are conflicting reports about the functional significance of such decline. Some reports suggest that ipRGC response properties might show a functional compensation by increasing their sensitivity and/or firing rate so that no significant change in ipRGC-dependent response such as PLR is observed in older individuals (93, 94). However, there are also reports of reduced amplitude of circadian rhythm in body temperature and increasing prevalence of sleep fragmentation among the elderly (95, 96), which can be improved by bright light (8). ipRGC responses measured directly in an old donor (>70 years) display longer latency (i.e., it responds slower to a light pulse) and overall shorter duration (55). While this observation needs to be confirmed, it suggests that not only ipRGCs' number but also their function may be altered in aging.

The specific loss of ipRGCs observed with aging is accelerated in Alzheimer's and Parkinson's diseases (AD and PD). AD and PD patients have 25–30% fewer ipRGCs compared to healthy age-matched controls (37, 90), and surviving ipRGCs display dendritic processes. Protein aggregates have been observed in and around ipRGCs of AD patients and may be the cause of altered neuronal physiology (97). These results suggest that ipRGC degeneration may lead to circadian rhythm and sleep dysfunction in neurodegenerative disorders (89, 98). In glaucoma, ipRGCs, while initially more resilient than conventional RGCs, are lost at advanced stages (91). Finally, a dramatic loss of ipRGCs is observed in diabetic retinopathy; however, it correlates with the overall loss of RGCs (92). In summary, histological assessments show a decline in the number of ipRGCs in old age and neurodegenerative diseases. Although some evidence suggests that ipRGCs' function is also altered in old age, whether the ipRGCs' intrinsic light response, the input of rod and cones, and/or the abundancy of ipRGCs subtypes are affected during aging and neurodegeneration remains to be investigated.

Of note, ipRGCs are not always more vulnerable than conventional RGCs; they possess a higher ability to survive certain pathological and experimental conditions. In the mouse, ipRGCs appear more resistant than other RGCs to various insults, including optic nerve injury, glutamate-induced excitotoxicity, and early-stage glaucoma (99, 100). In human patients, ipRGCs resist neurodegeneration in two inherited mitochondrial disorders that cause blindness: Leber hereditary optic neuropathy and dominant optic atrophy (101). This ability seems to be independent from melanopsin expression *per se* as ipRGCs' resilience is preserved in a mouse model bearing the mutation causing dominant optic atrophy and lacking melanopsin (102). Specific metabolic properties, such as higher mitochondrial activity or content, have been hypothesized as potential neuroprotective mechanisms. However, the reason why ipRGCs are relatively spared is still not well=understood.

The peculiar behavior of ipRGCs (i.e., increased vulnerability or resilience to certain disorders) compared to conventional RGCs has important implications. First, a better molecular characterization of each ipRGC subtype across aging and diseases will allow identifying the expression programs associated with differential cell survival and will provide therapeutic targets to diminish the loss of vision following optic nerve injury or ocular disease (100). Then, ipRGCs could be a promising marker to assess CNS disorders, corroborating the old saying that the eyes are a window to the soul (103, 104). The idea is appealing when one considers that PLR is a cost-efficient, fast, non-invasive readout of ipRGCs' function (64, 65). The PLR assay is now considered an emerging method to assess retinal and CNS disorders (105, 106) and has been suggested in the context of neurodegeneration as potential diagnostic or follow-up tools (107, 108). This translation has been unsuccessful with AD so far (109, 110), but this may just emphasize the need for direct measurements of ipRGCs' function in patient donors. These data would allow precisely pointing out the part of the response that is altered and designing more suited stimulation protocols that target it. A limitation might be that PLR relies on, and consequently will inform only on, specific ipRGC subtypes (part of M1 and M2 ipRGCs); it cannot be generalized as a proxy for all ipRGCs and thus will not be predictive of all ipRGC-dependent disorders.

CONCLUDING REMARKS

Knowledge of human ipRGCs is now catching up with what we know of these cells in the mouse. To date, these results emerge from a still limited number of labs; they would need to be replicated. Some points also remain to be clarified; for example, regarding the existing ipRGC's populations. Does the M3 subtype detected in some studies constitute a real ipRGC's subpopulation in human (22) or are the few resembling cells just marginal between M1 and M2 (24)? M4 are only described by one group (23) while M5 and M6 ipRGCs have not been described yet in the human retina. Does it mean that these ipRGC subtypes do not exist, are not morphologically distinct or too rare, and may be discovered later as in the mouse? Then, how do the projection maps compare? ipRGCs seem to target the same visual structures in both mouse and human while the subtypes of cells are not necessarily the same. Whether the numerous hypothalamic projections observed in the mouse translate in human (other than the SCN) need to be confirmed. This is particularly important given the control exerted by the hypothalamus over the body homeostasis and behaviors. Finally, a challenge that applies not only to human ipRGCs but also to the field, in general, is to consolidate ipRGC subtype classification by reconciling morphological, functional, and transcriptional identities. New approaches like patch-seq that combines scRNA-seq profiling with electrophysiological and morphological characterization of individual neurons may be an approach to consider (111, 112). This would constitute the first step toward completing the assignment of a specific function (and potential role in disorders) to each ipRGC subtype and fully elucidating both the circuits up- and downstream of each ipRGC subtype.

The differences that emerged between mouse and primate highlight the compelling need to include human donor retina in the standard models. Non-human primates remain necessary for some studies like mapping the projections. However, they are not advantageous ethically or economically over human preparations and consequently do not allow for a larger sample size. Furthermore, the tissue collection can be planned and operated within similar delays in monkey and human, at least for the surgical samples. The parameters affecting the fitness of the preparation may thus be controlled (hypoxia delay, pH, or nutrients) (52, 53). Human ipRGC exploration may also include the development of additional human *ex vivo* and *in vitro* models such as long-term culture of retina or retina organoids. Some results are very encouraging as retina organoids are photosensitive, organized in layers, and display a cellular diversity that partly recapitulates the diversity of functional peripheral retina (53).

There is a strong incentive to pursue these efforts as this handful of cells plays a major role in our physiology, cognitive performances, and overall well-being. Also, as progress in lighting science now allows for precise manipulation of quality, quantity, and timing of light, understanding how ipRGCs operate in the human eye in health and diseases will enable new applications. For example, the insights could be used to design indoor lights that offer better day–night synchronization or which improve our moods. It will offer a framework for improving the "spectral diet" of human at home, at work, or in public spaces (113).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This work was supported by the Department of Physiology of the University of Bern.

ACKNOWLEDGMENTS

The author would like to thank Dr. G. Benegiamo and J. Kralik for critical reading of the manuscript.

REFERENCES

1. Fleury G, Masís-Vargas A., Kalsbeek A. Metabolic implications of exposure to light at night: lessons from animal and human studies. *Obesity*. (2020) 28:S18–28. doi: 10.1002/oby.22807
2. Gaston KJ. Lighting up the nighttime. *Science*. (2018) 362:744–6. doi: 10.1126/science.aau8226
3. Kyba CCM, Kuester T, Sánchez de Miguel A, Baugh K, Jechow A, Hölker F, et al. Artificially lit surface of Earth at night increasing in radiance and extent. *Sci Adv*. (2017) 3:e1701528. doi: 10.1126/sciadv.1701528

4. Paksarian D, Rudolph KE, Stapp EK, Dunster GP, He J, Mennitt D, et al. Association of outdoor artificial light at night with mental disorders and sleep patterns among US adolescents. *JAMA Psychiatry*. (2020) 77:1266–75. doi: 10.1001/jamapsychiatry.2020.1935
5. Chang A-M, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. *Proc Natl Acad Sci USA*. (2015) 112:1232–7. doi: 10.1073/pnas.1418490112
6. Touitou Y, Touitou D, Reinberg A. Disruption of adolescents' circadian clock: the vicious circle of media use, exposure to light at night, sleep loss and risk behaviors. *J Physiol*. (2016) 110:467–79. doi: 10.1016/j.jphysparis.2017.05.001
7. Figueiro MG, Plitnick BA, Lok A, Jones GE, Higgins P, Hornick TR, et al. Tailored lighting intervention improves measures of sleep, depression, and agitation in persons with Alzheimer's disease and related dementia living in long-term care facilities. *Clin Interv Aging*. (2014) 9:1527–37. doi: 10.2147/CIA.S68557
8. Riemersma-van der Lek RF, Swaab DF, Twisk J, Hol EM, Hoogendijk WJ, Van Someren EJ. Effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities: a randomized controlled trial. *JAMA*. (2008) 299:2642–55. doi: 10.1001/jama.299.22.2642
9. Viola AU, James LM, Schlangen LJM, Dijk D-J. Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. *Scand J Work Environ Health*. (2008) 34:297–306. doi: 10.5271/sjweh.1268
10. Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range conditions. *J Pineal Res*. (2020) 69:e12655. doi: 10.1111/jpi.12655
11. Foster RG, Hughes S, Peirson SN. Circadian photoentrainment in mice and humans. *Biology*. (2020) 9:180. doi: 10.3390/biology9070180
12. Spitschan M. Photoreceptor inputs to pupil control. *J Vision*. (2019) 19:5. doi: 10.1167/19.9.5
13. Allen AE, Martial FP, Lucas RJ. Form vision from melanopsin in humans. *Nat Commun*. (2019) 10:2274. doi: 10.1038/s41467-019-10113-3
14. Brown TM, Tsujimura S, Allen AE, Wynne J, Bedford R, Vickery G, et al. Melanopsin-based brightness discrimination in mice and humans. *Curr Biol*. (2012) 22:1134–41. doi: 10.1016/j.cub.2012.04.039
15. Lucas RJ, Allen AE, Milosavljevic N, Storchi R, Woelders T. Can we see with melanopsin? *Annu Rev Vis Sci*. (2020) 6:453–68. doi: 10.1146/annurev-vision-030320-041239
16. Spitschan M, Bock AS, Ryan J, Frazzetta G, Brainard DH, Aguirre GK. The human visual cortex response to melanopsin-directed stimulation is accompanied by a distinct perceptual experience. *Proc Natl Acad Sci USA*. (2017) 114:12291–6. doi: 10.1073/pnas.1711522114
17. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. (2002) 295:1070–3. doi: 10.1126/science.1067262
18. Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: an opsin in melanophores, brain, and eye. *Proc Natl Acad Sci USA*. (1998) 95:340–5. doi: 10.1073/pnas.95.1.340
19. Aranda ML, Schmidt TM. Diversity of intrinsically photosensitive retinal ganglion cells: circuits and functions. *Cell Mol Life Sci*. (2020) 78:889–907. doi: 10.1007/s00018-020-03641-5
20. Do MTH. Melanopsin and the intrinsically photosensitive retinal ganglion cells: biophysics to behavior. *Neuron*. (2019) 104:205–26. doi: 10.1016/j.neuron.2019.07.016
21. Sondereker KB, Stabio ME, Renna JM. Crosstalk: the diversity of melanopsin ganglion cell types has begun to challenge the canonical divide between image-forming and non-image-forming vision. *J Comp Neurol*. (2020) 528:2044–67. doi: 10.1002/cne.24873
22. Esquiva G, Lax P, Pérez-Santonja JJ, García-Fernández JM, Cuenca N. Loss of melanopsin-expressing ganglion cell subtypes and dendritic degeneration in the aging human retina. *Front Aging Neurosci*. (2017) 9:79. doi: 10.3389/fnagi.2017.00079
23. Hannibal J, Christiansen AT, Heegaard S, Fahrenkrug J, Kiilgaard JF. Melanopsin expressing human retinal ganglion cells: subtypes, distribution, and intraretinal connectivity. *J Comp Neurol*. (2017) 525:1934–61. doi: 10.1002/cne.24181
24. Liao H-W, Ren X, Peterson BB, Marshak DW, Yau K-W, Gamlin PD, et al. Melanopsin-expressing ganglion cells on macaque and human retinas form two morphologically distinct populations. *J Comp Neurol*. (2016) 524:2845–72. doi: 10.1002/cne.23995
25. Nasir-Ahmad S, Lee SCS, Martin PR, Grünert U. Melanopsin-expressing ganglion cells in human retina: morphology, distribution, and synaptic connections. *J Comp Neurol*. (2017) 527:312–27. doi: 10.1002/cne.24176
26. Kraft TW, Schneeweis DM, Schnapf JL. Visual transduction in human rod photoreceptors. *J Physiol*. (1993) 464:747–65. doi: 10.1113/jphysiol.1993.sp019661
27. Schnapf JL, Kraft TW, Baylor DA. Spectral sensitivity of human cone photoreceptors. *Nature*. (1987) 325:439–41. doi: 10.1038/325439a0
28. Jacobs GH, Neitz J, Deegan JF. Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature*. (1991) 353:655–6. doi: 10.1038/353655a0
29. Nikonov SS, Kholodenko R, Lem J, Pugh EN. Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. *J Gen Physiol*. (2006) 127:359–74. doi: 10.1085/jgp.200609490
30. Applebury ML, Antoch MP, Baxter LC, Chun LLY, Falk JD, Farhangfar F, et al. The Murine Cone Photoreceptor: A Single Cone Type Expresses Both S and M Opsins with Retinal Spatial Patterning. *Neuron*. (2000) 27:513–23. doi: 10.1016/S0896-6273(00)00062-3
31. Pennesi ME, Neuringer M, Courtney RJ. Animal models of age related macular degeneration. *Mol Aspects Med*. (2012) 33:487–509. doi: 10.1016/j.mam.2012.06.003
32. Veleri S, Lazar CH, Chang B, Sieving PA, Banin E, Swaroop A. Biology and therapy of inherited retinal degenerative disease: insights from mouse models. *Dis Models Mech*. (2015) 8:109–29. doi: 10.1242/dmm.017913
33. Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. *J Neurosci*. (2000) 20:600–5. doi: 10.1523/JNEUROSCI.20-02-00600.2000
34. Dacey DM, Liao H-W, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. (2005) 433:749–54. doi: 10.1038/nature03387
35. Hannibal J, Hindersson P, Ostergaard J, Georg B, Heegaard S, Larsen PJ, et al. Melanopsin is expressed in PACAP-containing retinal ganglion cells of the human retinohypothalamic tract. *Invest Ophthalmol Vis Sci*. (2004) 45:4202–9. doi: 10.1167/iovs.04-0313
36. Dkhissi-Benyahya O, Rieux C, Hut RA, Cooper HM. Immunohistochemical evidence of a melanopsin cone in human retina. *Invest Ophthalmol Vis Sci*. (2006) 47:1636–41. doi: 10.1167/iovs.05-1459
37. Ortuño-Lizarán I, Esquiva G, Beach TG, Serrano GE, Adler CH, Lax P, et al. Degeneration of human photosensitive retinal ganglion cells may explain sleep and circadian rhythms disorders in Parkinson's disease. *Acta Neuropathologica Commun*. (2018) 6:90. doi: 10.1186/s40478-018-0596-z
38. Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol*. (1990) 300:5–25. doi: 10.1002/cne.903000103
39. La Morgia CL, Ross-Cisneros FN, Hannibal J, Montagna P, Sadun AA, Carelli V. Melanopsin expressing retinal ganglion cells: implications for human diseases. *Vision Res*. (2010) 51:296–302. doi: 10.1016/j.visres.2010.07.023
40. Schmidt TM, Kofuji P. Functional and morphological differences among intrinsically photosensitive retinal ganglion cells. *J Neurosci*. (2009) 29:476–82. doi: 10.1523/JNEUROSCI.4117-08.2009
41. Brown TM, Gias C, Hatori M, Keding SR, Semo M, Coffey PJ, et al. Melanopsin contributions to irradiance coding in the thalamo-cortical visual system. *PLoS Biol*. (2010) 8:e1000558. doi: 10.1371/journal.pbio.1000558
42. Delwig A, Larsen DD, Yasumura D, Yang CF, Shah NM, Copenhagen DR. Retinofugal projections from melanopsin-expressing retinal ganglion cells revealed by intraocular injections of cre-dependent virus. *PLoS ONE*. (2016) 11:e0149501. doi: 10.1371/journal.pone.0149501
43. Fernandez DC, Fogerson PM, Ospri LL, Thomsen MB, Layne RM, Severin D, et al. Light affects mood and learning through distinct retina-brain pathways. *Cell*. (2018) 175:71–84. doi: 10.1016/j.cell.2018.08.004

44. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau K-W, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. (2006) 497:326–49. doi: 10.1002/cne.20970
45. Dai J, Van der Vliet J, Swaab DF, Buijs RM. Human retinohypothalamic tract as revealed by in vitro postmortem tracing. *J Comp Neurol*. (1998) 397:357–70. doi: 10.1002/(SICI)1096-9861(19980803)397:3<357::AID-CNE4>3.0.CO;2-1
46. Hannibal J, Kankipati L, Strang CE, Peterson BB, Dacey D, Gamlin PD. Central projections of intrinsically photosensitive retinal ganglion cells in the macaque monkey. *J Comp Neurol*. (2014) 522:2231–48. doi: 10.1002/cne.23555
47. Lima RRM, Pinato L, Nascimento RBS, Engelberth RCGJ, Nascimento ESJ, Cavalcante JC, et al. Retinal projections and neurochemical characterization of the pregeniculate nucleus of the common marmoset (Callithrix jacchus). *J Chem Neuroanat*. (2012) 44:34–44. doi: 10.1016/j.jchemneu.2012.04.001
48. Ecker JL, Dumitrescu ON, Wong KY, Alam NM, Chen S-K, LeGates T, et al. Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. *Neuron*. (2010) 67:49–60. doi: 10.1016/j.neuron.2010.05.023
49. Abizaid A, Horvath B, Keefe DL, Leranth C, Horvath TL. Direct visual and circadian pathways target neuroendocrine cells in primates. *Eur J Neurosci*. (2004) 20:2767–76. doi: 10.1111/j.1460-9568.2004.03737.x
50. Costa MS, Santee UR, Cavalcante JS, Moraes PR, Santos NP, Britto LR. Retinohypothalamic projections in the common marmoset (Callithrix jacchus): a study using cholera toxin subunit B. *J Comp Neurol*. (1999) 415:393–403. doi: 10.1002/(sici)1096-9861(19991220)415:3<393::aid-cne5>3.0.co;2-r
51. Weinstein GW, Hobson RR, Baker FH. Extracellular recordings from human retinal ganglion cells. *Science*. (1971) 171:1021–2. doi: 10.1126/science.171.3975.1021
52. Abbas F, Becker S, Jones BW, Mure LS, Panda S, Hanneken A, et al. Revival of light-evoked neuronal signals in the post-mortem mouse and human retina. *bioRxiv*. (2020). doi: 10.1101/2020.06.30.180497
53. Cowan CS, Renner M, Gennaro MD, Gross-Scherf B, Goldblum D, Hou Y, et al. Cell types of the human retina and its organoids at single-cell resolution. *Cell*. (2020) 182:1623–40. doi: 10.1016/j.cell.2020.08.013
54. Kling A, Gogliettino AR, Shah NP, Wu EG, Brackbill N, Sher A, et al. Functional organization of midget and parasol ganglion cells in the human retina. *bioRxiv*. (2020). doi: 10.1101/2020.08.07.240762
55. Mure LS, Vinberg F, Hanneken A, Panda S. Functional diversity of human intrinsically photosensitive retinal ganglion cells. *Science*. (2019) 366:1251–5. doi: 10.1126/science.aaz0898
56. Reinhard K, Münch TA. Visual properties of human retinal ganglion cells. *PLOS ONE*. (2021) 16:e0246952. doi: 10.1371/journal.pone.0246952
57. Soto F, Hsiang J-C, Rajagopal R, Piggott K, Harocopos GJ, Couch SM, et al. Efficient coding by midget and parasol ganglion cells in the human retina. *Neuron*. (2020) 107:656–6.e5. doi: 10.1016/j.neuron.2020.05.030
58. Karnas D, Hicks D, Mordel J, Pévet P, Meissl H. Intrinsic photosensitive retinal ganglion cells in the diurnal rodent, Arvicanthis ansorgei. *PLoS ONE*. (2013) 8:e73343. doi: 10.1371/journal.pone.0073343
59. Reifler AN, Chervenak AP, Dolikian ME, Benenati BA, Meyers BS, Demertzis ZD, et al. The rat retina has five types of ganglion-cell photoreceptors. *Exp Eye Res*. (2015) 130:17–28. doi: 10.1016/j.exer.2014.11.010
60. Jones KA, Hatori M, Mure LS, Bramley JR, Artymyshyn R, Hong S-P, et al. Small-molecule antagonists of melanopsin-mediated phototransduction. *Nat Chem Biol*. (2013) 9:630–5. doi: 10.1038/nchembio.1333
61. Bailes HJ, Lucas RJ. Human melanopsin forms a pigment maximally sensitive to blue light (λmax $\approx$ 479 nm) supporting activation of G(q/11) and G(i/o) signalling cascades. *Proc Biol Sci*. (2013) 280:20122987. doi: 10.1098/rspb.2012.2987
62. Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci*. (2001) 21:6405–12. doi: 10.1523/JNEUROSCI.21-16-06405.2001
63. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J Physiol*. (2001) 535:261–7. doi: 10.1111/j.1469-7793.2001.t01-1-00261.x
64. Gamlin PDR, McDougal DH, Pokorny J, Smith VC, Yau K-W, Dacey DM. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Res*. (2007) 47:946–54. doi: 10.1016/j.visres.2006.12.015
65. Mure LS, Cornut P-L, Rieux C, Drouyer E, Denis P, Gronfier C, et al. Melanopsin bistability: a fly's eye technology in the human retina. *PLoS ONE*. (2009) 4:e5991. doi: 10.1371/journal.pone.0005991
66. Zaidi FH, Hull JT, Peirson SN, Wulff K, Aeschbach D, Gooley JJ, et al. Short-wavelength light sensitivity of circadian, pupillary, and visual awareness in humans lacking an outer retina. *Curr Biol*. (2007) 17:2122–8. doi: 10.1016/j.cub.2007.11.034
67. Vandewalle G, Gais S, Schabus M, Balteau E, Carrier J, Darsaud A, et al. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure. *Cereb Cortex*. (2007) 17:2788–95. doi: 10.1093/cercor/bhm007
68. Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, et al. High Sensitivity of Human Melatonin, Alertness, Thermoregulation, and Heart Rate to Short Wavelength Light. *J Clin Endocrinol Metab*. (2005) 90:1311–6. doi: 10.1210/jc.2004-0957
69. Schmidt TM, Kofuji P. Structure and function of bistratified intrinsically photosensitive retinal ganglion cells in the mouse. *J Comp Neurol*. (2011) 519:1492–504. doi: 10.1002/cne.22579
70. Sexton TJ, Bleckert A, Turner MH, Van Gelder RN. Type I intrinsically photosensitive retinal ganglion cells of early post-natal development correspond to the M4 subtype. *Neural Dev*. (2015) 10:17. doi: 10.1186/s13064-015-0042-x
71. Tu DC, Zhang D, Demas J, Slutsky EB, Provencio I, Holy TE, et al. Physiologic diversity and development of intrinsically photosensitive retinal ganglion cells. *Neuron*. (2005) 48:987–99. doi: 10.1016/j.neuron.2005.09.031
72. Phillips AJK, Vidafar P, Burns AC, McGlashan EM, Anderson C, Rajaratnam SMW, et al. High sensitivity and interindividual variability in the response of the human circadian system to evening light. *PNAS*. (2019) 116:12019–24. doi: 10.1073/pnas.1901824116
73. Chellappa SL. Individual differences in light sensitivity affect sleep and circadian rhythms. *Sleep*. (2020) 13:zsaa214. doi: 10.1093/sleep/zsaa214
74. Chen S-K, Badea TC, Hattar S. Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. *Nature*. (2011) 476:92–5. doi: 10.1038/nature10206
75. Berg DJ, Kartheiser K, Leyrer M, Saali A, Berson DM. Transcriptomic signatures of postnatal and adult intrinsically photosensitive ganglion cells. *ENeuro*. (2019) 6:ENEURO.0022-19.2019. doi: 10.1523/ENEURO.0022-19.2019
76. Laboissonniere LA, Goetz JJ, Martin GM, Bi R, Lund TJS, Ellson L, et al. Molecular signatures of retinal ganglion cells revealed through single cell profiling. *Sci Rep*. (2019) 9:1–15. doi: 10.1038/s41598-019-52215-4
77. Peng Y-R, Shekhar K, Yan W, Herrmann D, Sappington A, Bryman GS, et al. Molecular classification and comparative taxonomics of foveal and peripheral cells in primate retina. *Cell*. (2019) 176:1222–37. doi: 10.1016/j.cell.2019.01.004
78. Yan W, Peng Y-R, van Zyl T, Regev A, Shekhar K, Juric D, et al. Cell atlas of the human fovea and peripheral retina. *Sci Rep*. (2020) 10:9802. doi: 10.1101/2020.02.11.943779
79. Rheaume BA, Jereen A, Bolisetty M, Sajid MS, Yang Y, Renna K, et al. Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nat Commun*. (2018) 9:2759. doi: 10.1038/s41467-018-05792-3
80. Lee SK, Sonoda T, Schmidt TM. M1 intrinsically photosensitive retinal ganglion cells integrate rod and melanopsin inputs to signal in low light. *Cell Rep*. (2019) 29:3349–55. doi: 10.1016/j.celrep.2019.11.024
81. Wong KY, Dunn FA, Graham DM, Berson DM. Synaptic influences on rat ganglion-cell photoreceptors. *J Physiol*. (2007) 582:279–96. doi: 10.1113/jphysiol.2007.133751
82. Zhao X, Stafford BK, Godin AL, King WM, Wong KY. Photoresponse diversity among the five types of intrinsically photosensitive retinal ganglion cells. *J Physiol (Lond)*. (2014) 592:1619–36. doi: 10.1113/jphysiol.2013.262782
83. Estevez ME, Fogerson PM, Ilardi MC, Borghuis BG, Chan E, Weng S, et al. Form and function of the M4 cell, an intrinsically photosensitive retinal

ganglion cell type contributing to geniculocortical vision. *J Neurosci*. (2012) 32:13608–20. doi: 10.1523/JNEUROSCI.1422-12.2012
84. Schroeder MM, Harrison KR, Jaeckel ER, Berger HN, Zhao X, Flannery MP, et al. The roles of rods, cones, and melanopsin in photoresponses of M4 intrinsically photosensitive retinal ganglion cells (ipRGCs) and optokinetic visual behavior. *Front Cell Neurosci*. (2018) 12:203. doi: 10.3389/fncel.2018.00203
85. Gooley JJ, Rajaratnam SM, Brainard GC, Kronauer RE, Czeisler CA, Lockley SW. Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Sci Transl Med*. (2010) 2:31ra33. doi: 10.1126/scitranslmed.3000741
86. Spitschan M, Garbazza C, Kohl S, Cajochen C. Rest-activity cycles and melatonin phase angle of circadian entrainment in people without cone-mediated vision. *bioRxiv*. (2020). doi: 10.1101/2020.06.02.129502
87. Hayter EA, Brown TM. Additive contributions of melanopsin and both cone types provide broadband sensitivity to mouse pupil control. *BMC Biol*. (2018) 16:83. doi: 10.1186/s12915-018-0552-1
88. Spitschan M, Jain S, Brainard DH, Aguirre GK. Opponent melanopsin and S-cone signals in the human pupillary light response. *PNAS*. (2014) 111:15568–72. doi: 10.1073/pnas.1400942111
89. La Morgia C, Ross-Cisneros FN, Sadun AA, Carelli V. Retinal ganglion cells and circadian rhythms in Alzheimer's disease, Parkinson's disease, and beyond. *Front Neurol*. (2017) 8:162. doi: 10.3389/fneur.2017.00162
90. La Morgia C, Ross-Cisneros FN, Koronyo Y, Hannibal J, Gallassi R, Cantalupo G, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease. *Ann Neurol*. (2016) 79:90–109. doi: 10.1002/ana.24548
91. Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of melanopsin-expressing retinal ganglion cells in severely staged glaucoma patients. *Invest Ophthalmol Vis Sci*. (2016) 57:4661–7. doi: 10.1167/iovs.16-19997
92. Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of melanopsin-expressing retinal ganglion cells in patients with diabetic retinopathy. *Invest Ophthalmol Vis Sci*. (2017) 58:2187–92. doi: 10.1167/iovs.16-21168
93. Adhikari P, Pearson CA, Anderson AM, Zele AJ, Feigl B. Effect of age and refractive error on the melanopsin mediated post-illumination pupil response (PIPR). *Sci Rep*. (2015) 5:17610. doi: 10.1038/srep17610
94. Daneault V, Vandewalle G, Hébert M, Teikari P, Mure LS, Doyon J, et al. Does pupil constriction under blue and green monochromatic light exposure change with age? *J Biol Rhythms*. (2012) 27:257–64. doi: 10.1177/0748730412441172
95. Ancoli-Israel S, Parker L, Sinaee R, Fell RL, Kripke DF. Sleep fragmentation in patients from a nursing home. *J Gerontol*. (1989) 44:M18–21. doi: 10.1093/geronj/44.1.M18
96. Cajochen C, Münch M, Knoblauch V, Blatter K, Wirz-Justice A. Age-related changes in the circadian and homeostatic regulation of human sleep. *Chronobiol Int*. (2006) 23:461–74. doi: 10.1080/07420520500545813
97. Bae JR, Kim SH. Synapses in neurodegenerative diseases. *BMB Rep*. (2017) 50:237–46. doi: 10.5483/BMBRep.2017.50.5.038
98. Feigl B, Dumpala S, Kerr GK, Zele AJ. Melanopsin cell dysfunction is involved in sleep disruption in Parkinson's disease. *J Parkinsons Dis*. (2020) 10:1467–76. doi: 10.3233/JPD-202178
99. Cui Q, Ren C, Sollars PJ, Pickard GE, So K-F. The injury resistant ability of melanopsin-expressing intrinsically photosensitive retinal ganglion cells. *Neuroscience*. (2015) 284:845–53. doi: 10.1016/j.neuroscience.2014.11.002
100. Tran NM, Shekhar K, Whitney IE, Jacobi A, Benhar I, Hong G, et al. Single-cell profiles of retinal ganglion cells differing in resilience to injury reveal neuroprotective genes. *Neuron*. (2019) 104:1039–55. doi: 10.1016/j.neuron.2019.11.006
101. La Morgia C, Ross-Cisneros FN, Sadun AA, Hannibal J, Munarini A, Mantovani V, et al. Melanopsin retinal ganglion cells are resistant to neurodegeneration in mitochondrial optic neuropathies. *Brain*. (2010) 133:2426–38. doi: 10.1093/brain/awq155
102. González-Menéndez I, Reinhard K, Tolivia J, Wissinger B, Münch TA. Influence of Opa1 mutation on survival and function of retinal ganglion cells. *Invest Ophthalmol Vis Sci*. (2015) 56:4835–45. doi: 10.1167/iovs.15-16743
103. La Morgia C, Carelli V, Carbonelli M. Melanopsin retinal ganglion cells and pupil: clinical implications for neuro-ophthalmology. *Front Neurol*. (2018) 9:1047. doi: 10.3389/fneur.2018.01047
104. White OB, Costello F. Melanopsin effects on pupil responses: is the eye the window to the weary soul? *JAMA Neurol*. (2017) 74:506–8. doi: 10.1001/jamaneurol.2016.5385
105. Meltzer E, Sguigna PV, Subei A, Beh S, Kildebeck E, Conger D, et al. Retinal architecture and melanopsin-mediated pupillary response characteristics: a putative pathophysiologic signature for the retino-hypothalamic tract in multiple sclerosis. *JAMA Neurol*. (2017) 74:574–82. doi: 10.1001/jamaneurol.2016.5131
106. Najjar RP, Sharma S, Atalay E, Rukmini AV, Sun C, Lock JZ, et al. Pupillary responses to full-field chromatic stimuli are reduced in patients with early-stage primary open-angle glaucoma. *Ophthalmology*. (2018) 125:1362–71. doi: 10.1016/j.ophtha.2018.02.024
107. Chougule PS, Najjar RP, Finkelstein MT, Kandiah N, Milea D. Light-induced pupillary responses in Alzheimer's disease. *Front Neurol*. (2019) 10:360. doi: 10.3389/fneur.2019.00360
108. Joyce DS, Feigl B, Kerr G, Roeder L, Zele AJ. Melanopsin-mediated pupil function is impaired in Parkinson's disease. *Sci Rep*. (2018) 8:7796. doi: 10.1038/s41598-018-26078-0
109. Kawasaki A, Ouanes S, Crippa SV, Popp J. Early-stage Alzheimer's Disease does not alter pupil responses to colored light stimuli. *J Alzheimers Dis*. (2020) 75:1273–82. doi: 10.3233/JAD-200120
110. Oh AJ, Amore G, Sultan W, Asanad S, Park JC, Romagnoli M, et al. Pupillometry evaluation of melanopsin retinal ganglion cell function and sleep-wake activity in pre-symptomatic Alzheimer's disease. *PLoS ONE*. (2019) 14:e0226197. doi: 10.1371/journal.pone.0226197
111. Bardy C, van den Hurk M, Kakaradov B, Erwin JA, Jaeger BN, Hernandez RV, et al. Predicting the functional states of human iPSC-derived neurons with single-cell RNA-seq and electrophysiology. *Mol Psychiatry*. (2016) 21:1573–88. doi: 10.1038/mp.2016.158
112. Cadwell CR, Palasantza A, Jiang X, Berens P, Deng Q, Yilmaz M, et al. Electrophysiological, transcriptomic and morphologic profiling of single neurons using Patch-seq. *Nat Biotechnol*. (2016) 34:199–203. doi: 10.1038/nbt.3445
113. Webler FS, Spitschan M, Foster RG, Andersen M, Peirson SN. What is the "spectral diet" of humans? *Curr Opin Behav Sci*. (2019) 30:80–6. doi: 10.31219/osf.io/dqkv3

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 Neurology

ORIGINAL RESEARCH
published: 26 March 2021
doi: 10.3389/fneur.2021.625443

Exposure to Blue Wavelength Light Is Associated With Increases in Bidirectional Amygdala-DLPFC Connectivity at Rest

Anna Alkozei[1], Natalie S. Dailey[1†], Sahil Bajaj[2†], John R. Vanuk[1], Adam C. Raikes[1] and William D. S. Killgore[1]*

[1] Social, Cognitive, and Affective Neuroscience Laboratory, Department of Psychiatry, University of Arizona, Tucson, AZ, United States, [2] Multimodal Clinical Neuroimaging Laboratory (MCNL), Center for Neurobehavioral Research, Boys Town National Research Hospital, Boys Town, NE, United States

Blue wavelength light has been used successfully as a treatment method for certain mood disorders, but, the underlying mechanisms behind the mood enhancing effects of light remain poorly understood. We investigated the effects of a single dose of 30 min of blue wavelength light ($n = 17$) vs. amber wavelength light ($n = 12$) exposure in a sample of healthy adults on subsequent resting-state functional and directed connectivity, and associations with changes in state affect. Individuals who received blue vs. amber wavelength light showed greater positive connectivity between the right amygdala and a region within the left dorsolateral prefrontal cortex (DLPFC). In addition, using granger causality, the findings showed that individuals who received blue wavelength light displayed greater bidirectional information flow between these two regions relative to amber light. Furthermore, the strength of amygdala-DLPFC functional connectivity was associated with greater decreases in negative mood for the blue, but not the amber light condition. Blue light exposure may positively influence mood by modulating greater information flow between the amygdala and the DLPFC, which may result in greater engagement of cognitive control strategies that are needed to perceive and regulate arousal and mood.

Keywords: fMRI, depression, light therapy, neuroimaging, amygdala, PFC

OPEN ACCESS

Edited by:
Manuel Spitschan, University of Oxford, United Kingdom

Reviewed by:
Yu-Shiuan Lin, University of Basel, Switzerland
Gilles Vandewalle, University of Liège, Belgium

***Correspondence:**
William D. S. Killgore
killgore@psychiatry.arizona.edu

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *03 November 2020*
Accepted: *03 March 2021*
Published: *26 March 2021*

Citation:
Alkozei A, Dailey NS, Bajaj S, Vanuk JR, Raikes AC and Killgore WDS (2021) Exposure to Blue Wavelength Light Is Associated With Increases in Bidirectional Amygdala-DLPFC Connectivity at Rest. Front. Neurol. 12:625443. doi: 10.3389/fneur.2021.625443

INTRODUCTION

Light has a powerful effect on mood. A sunny day often seems to enhance positive outlook and helping behavior (1, 2), and it is well-established that bright light can be an effective treatment for mood disorders, with effects as potent as some pharmacologic treatments (3, 4). Even a single half-hour exposure to bright light seems to improve mood (5). Moreover, the wavelength of light also seems to be important to these effects, with considerable evidence now pointing to the importance of blue wavelengths (460–480 nm) on mood and cognition. Specifically, studies have shown that acute exposure to blue wavelength or bright broad-spectrum light leads to immediate increases in simple alertness and attention (6, 7) as well as more complex cognitive functions, such as improved working memory performance (8) and short-term verbal memory retention (9). Daily morning blue wavelength light exposure over several weeks has also been used as an effective treatment for

seasonal and non-seasonal depression (10–13), certain sleep disorders, such as delayed sleep phase syndrome (14), and to improve symptoms of fatigue in individuals with neurological conditions, such as acquired brain injury (15–18). The exact mechanisms underlying these beneficial effects of blue light on emotion and cognition, however, are not well-understood.

Growing evidence suggests that many of these mood and cognitive effects of light are likely mediated by the non-image forming pathways of the visual system. It is well-documented that bright light, especially within the blue wavelengths, stimulates intrinsically photosensitive retinal ganglion cells (ipRGCs) which transmit signals to several sub-cortical nuclei. These include the suprachiasmatic nucleus (SCN) in the hypothalamus, which has a strong influence on the circadian rhythm of sleep and wake (19, 20). Extensive research has shown that exposure to bright light, particularly in the blue wavelengths, leads to activation of the SCN, which in turn sends signals to the pineal gland to suppress the production of the hormone melatonin (21, 22). Melatonin is released and suppressed daily in accordance with the circadian rhythm, and its release precedes sleep onset (23). Consequently, exposure to blue light at times that are out of phase with the normal circadian rhythm can lead to disruptions in the sleep-wake schedule. For instance, blue light exposure during the night, when melatonin levels are high, appears to lead to increases in alertness by suppressing the immediate production of melatonin (6) and phase delaying the circadian onset of sleep. Conversely, light in the morning hours will produce a phase advance in the circadian rhythm. Notably, by targeting light exposure to specific times in the day, the rhythm of melatonin release can be shifted to treat certain sleep disorders (e.g., to treat delayed sleep phase syndrome, daily morning bright light exposure over several days leads to a phase advance of melatonin release and earlier sleep onset) (24). It has been proposed that seasonal depression is associated with a disturbance in the circadian rhythm during the darker winter months, and that among affected individuals, daily bright light treatment may produce its mood-enhancing effect, in part, by "resynchronizing" the biological clock (25).

While circadian factors have been suggested for the outcomes mentioned above, they do not appear to account for all of the mood and cognitive enhancing effects of blue light. For example, suppression of melatonin cannot effectively account for the alerting effects of light during the middle of the day, when melatonin levels are already naturally low, or for the effects of light on non-seasonal depression (i.e., during times of year when sunlight is more prevalent). Direct projections from the ipRGCs, as well as indirect projections from the SCN, to other brain regions involved in emotion and cognition, are proposed to explain some of those non-circadian effects (26).

For example, there is evidence from several animal studies to suggest that ipRGCs have direct projections to the medial amygdala (27–30) which may therefore also be modulated by blue light exposure. In fact, studies in human subjects have shown that exposure to blue vs. green light in the MRI scanner led to immediate increases in activity within the right amygdala that declined over time, suggesting a habituation process (31, 32), and enhanced connectivity between the left amygdala and the voice sensitive area of the superior temporal gyrus and the hypothalamus during an auditory emotional processing task during blue vs. green light (32). Further, one study in healthy males demonstrated that 3 weeks of bright light therapy reduced bilateral amygdala and prefrontal reactivity to threat (i.e., angry and fearful faces) in a dose-dependent manner; and that left amygdala and medial prefrontal connectivity also increased in a dose-dependent manner (33). The role of blue light exposure in modulating amygdala activation potentially as a result of direct projections from ipRGCs may be particularly relevant for individuals with mood disorders, such as depression. Compared to healthy controls, individuals with depression show increased amygdala reactivity to negative stimuli, and disrupted resting-state functional connectivity, such as hypoconnectivity between the amygdala and several areas of the prefrontal cortex, which may underlie difficulties with emotion regulation (34–36).

Considering that blue wavelength light appears to lead to immediate increases in activation in the right amygdala (31), and that 3 weeks of bright light therapy led to increases in connectivity between the amygdala and PFC (33), it is possible that exposure to blue wavelength light may also lead to more *immediate* changes in functional connectivity between the amygdala and PFC. Because negative mood states are often associated with reduced prefrontal regulation and increased amygdala activation, alterations in the strength of connectivity via light exposure may contribute to its well-established antidepressant or mood-enhancing effects.

The aim of this study was to answer these research questions by investigating the effects of a single, 30-min controlled exposure to blue vs. non-blue (amber) light on subsequent (i) positive and negative affect, (ii) functional and directed brain connectivity of the amygdala as a seed with other brain regions, and (iii) the association between changes in affect and observed changes in functional brain connectivity.

Considering the scarcity of previous findings of the effects of blue wavelength light on amygdala responsiveness and connectivity, we have deliberately kept our hypotheses broad (e.g., have not made specific hypotheses regarding laterality or connectivity patterns). The following hypotheses were postulated:

1. Blue light exposure would increase positive affect and decrease negative affect in comparison to amber light exposure.
2. Blue light exposure would lead to increased functional and directed functional connectivity between the amygdala and the PFC.
3. Connectivity patterns would be associated with more positive and less negative affect.

METHODS

Participants

Twenty-nine healthy adults between 18 and 32 years of age ($M = 21.52$, $SD = 2.82$; 16 men, 13 women) took part in the study and provided useable functional and structural magnetic resonance imaging (MRI) data for analysis (out of a total of 35 participants). Six participants had to be excluded due to excessive head movement in the scanner. Participants completed an average of

14.1 years of education (SD = 1.95). According to self-report, all were right handed, primary English speaking, and reported a regular sleep schedule of going to bed between 10:00 p.m. and 1:00 a.m. and waking between 6:00 and 9:00 a.m. Further, participants were screened for a history of major psychiatric disorders, including major depressive disorder, anxiety disorders, obsessive-compulsive disorders, post-traumatic stress disorder and substance use disorders. In addition, participants were asked whether they had ever been in psychiatric treatment and for what reason. Participants were asked to keep their regular sleep schedule and were asked to consume their normal levels of caffeine on the day of the study. Participants were randomly assigned to the blue ($n = 17$) or amber light condition ($n = 12$) (see below). The two groups did not differ on age, sex, number of hours slept the night before the assessment, or number of reported hours slept on weeknights (see **Table 1**). In addition, four participants in the blue light group and four participants in the amber light group reported having had one caffeinated product on the day of the assessment. Separate data from this same study have been reported elsewhere (8, 9, 37, 38), but the functional connectivity and mood findings reported here are novel and have not been previously published. This project was approved by the Institutional Review Board at the University of Arizona and the U.S. Army Human Research Protections Office, and all participants provided written informed consent prior to study participation.

TABLE 1 | Descriptive statistics.

	Blue group ($n = 17$) Mean (SD)	Amber group ($n = 12$) Mean (SD)	Statistic
Age	21.47 (2.85)	21.58 (2.91)	$t_{(27)} = -0.10$. $p = 0.92$
Sex	47.05% female	41.66% female	$\chi^2 = 0.08$, $p = 0.77$
Number of hours slept on weeknights	7.25 (0.97)	7.29 (1.015)	$t_{(27)} = -0.11$, $p = 0.91$
Number of hours slept the night prior to the assessment	6.88 (0.54)	6.79 (.62)	$t_{(27)} = 0.42$, $p = 0.68$
BDI-II	1.53 (1.94)	2.58 (3.32)	$t_{(27)} = -1.08$, $p = 0.29$
PANAS-P pre-light	28.94 (8.97)	28.00 (5.46)	$t_{(27)} = 0.32$, $p = 0.75$
PANAS-P post-light	27.18 (11.40)	24.25 (8.35)	$t_{(27)} = 0.75$, $p = 0.45$
PANAS-N pre-light	12.29 (1.44)	12.67 (2.46)	$t_{(27)} = -0.51$, $p = 0.61$
PANAS-N post-light	11.00 (2.42)	10.42 (.80)	$t_{(27)} = 0.80$, $p = 0.43$

BDI-II, Beck Depression Inventory; PANAS, Positive and Negative Affective Schedule (PANAS-P: positive affect, PANAS-N: negative affect).

Materials

Positive and Negative Affect Schedule (PANAS)

The PANAS (39) was used to measure positive and negative affect. Participants were asked to indicate, on a 5-point scale, the extent to which (from "very slightly to not at all" to "extremely") they were feeling a number of positive (e.g., interested, excited, proud) and negative feelings (e.g., nervous, determined, irritable) at that very moment. Total scores for positive affect (PANAS-P) and negative affect (PANAS-N) were calculated separately and each had a possible range from 10 to 50. The PANAS is a widely used measure of state affect that has shown good internal consistency, reliability, and validity (40).

Light Exposure

The light protocol is described in detail in the Procedure section. The blue light exposure devices were commercially available Philips goLITE BLU® Energy Light devices (Model HF3321/60; Philips Electronics, Stamford, CT). The amber light devices were provided by the manufacturer for research purposes and were virtually identical to the goLITE BLU devices, with the exception of being fit with a different color LED. Each device consisted of a plastic table-mounted chassis with a 10 × 6 array of light emitting diodes (LEDs), encased in 1 × 1 cm cubical projection elements and a translucent plastic window cover. The light devices were set up in such a way that they were centered at 45° to each side of the participant with a distance of ∼80 cm from the participant's nasion which means that only part of the field of view was covered. The goLITE BLU Energy Light is commercially available and has a narrow bandwidth [peaking at $\lambda = 469$ nm, at 214 Lux at eye level, and panel irradiance (mW/cm^2) = 1.23]. The amber devices were otherwise identical to the goLITE BLU devices, with the exception that they were fitted with amber LEDs [peaking at $\lambda = 578$ nm, at 188 Lux at eye level, and total irradiance (mW/cm^2) = 0.35]. The alpha-opic irradiances of the blue and amber conditions are summarized in **Table 2**.

Procedure

All participants completed the study procedures at the same time of day to control for circadian effects. Specifics of the procedure are detailed in our previous paper (8). In brief, at 7:45 a.m., participants arrived for the study and completed informed consent as well as basic demographic questionnaires and cognitive tasks. Participants completed light exposure, cognitive tests, and mood assessments in a room located next to the magnetic resonance imaging (MRI) scanner. At ∼8:30 a.m., participants completed the first PANAS. Participants also completed mood questionnaires, several cognitive assessments, such as general intelligence tests, and memory tests, and questionnaires about their daily habits. At 9:45 a.m., participants underwent a "blue light washout" period in an otherwise darkened room, with only two amber light devices placed on the table in front of them for 30 min. The light devices were adjusted until the pair of amber devices used during the initial washout period resulted in a 20-lux reading as measured by a light meter (Digital Lux Meter LX1330B) on each side of the participant's nose (i.e., at eye level). Participants were instructed not to look directly at the light devices, and to relax with their eyes open and maintain a generally forward gaze. This washout period was completed to ensure residual effects of outdoor and ambient lighting had dissipated before beginning the experimental light exposure manipulation while at the same time allowing participants to see their surroundings and continue engaging with the experimenter. At 10:15 a.m., the two Washout Period light devices were replaced with the *four* Exposure Period devices (see **Figure 1B**). Specifically, during the Exposure Period, participants were randomized to receive either 30 min of blue

TABLE 2 | Alpha-opic irradiances of the light conditions.

	S-cone-opic	M-cone-opic	L-cone-opic	Rhodopic	Melanopic
BLUE WAVELENGTH LIGHT					
α-opic irradiance, $W \cdot m^{-2}$	1.36	0.77	0.46	1.74	2.09
α-opic efficacy of luminous radiation, $mW \cdot lm^{-1}$	6.30	3.57	2.13	8.09	9.69
α-opic equivalent daylight (D65) illuminance, lx	1660.04	528.75	282.19	1202.59	1573.80
AMBER WAVELENGTH LIGHT					
α-opic irradiance, $W \cdot m^{-2}$	0.01	0.20	0.29	0.05	0.02
α-opic efficacy of luminous radiation, $mW \cdot lm^{-1}$	0.03	1.10	1.59	0.30	0.11
α-opic equivalent daylight (D65) illuminance, lx	7.27	136.11	175.24	36.90	14.53

($n = 17$) or amber ($n = 12$) light exposure. The 30-min Exposure Period was initiated by illuminating the two pairs of light devices (either blue or amber, depending on condition), with each pair mounted side by side on the desk in front of the participant, centered at the same location as the Washout Period amber lights at a distance of ∼80 cm from the participant's eyes. During the 30-min Exposure and Washout Periods, participants maintained a forward gaze and completed two computerized practice tasks (a working memory and a multi-source interference task) to prepare them for their time in the scanner. To minimize blue light from the computer screen, an amber tinted plexiglass shield was placed in front of the laptop screen. Participants were asked to sit relatively still with their legs uncrossed and to maintain a forward gaze. Apart from completing the tasks on the computer using their fingers, participants did not move, stretch, stand up, drink, eat, or close their eyes during the light exposure sessions. Immediately after the light exposure period, at ∼10:50 a.m., participants completed the second PANAS. At 11:00 a.m., participants were escorted to the MRI scanner, where the resting state scan was initiated at ∼11:15 a.m. There are no measurements of the lux level inside the MRI scanner, but it was ∼50 lux and the ambient light level was held constant across participants. The resting state scan was the first functional scan participants completed and was followed by longer versions of the working memory and multi-source interference tasks.

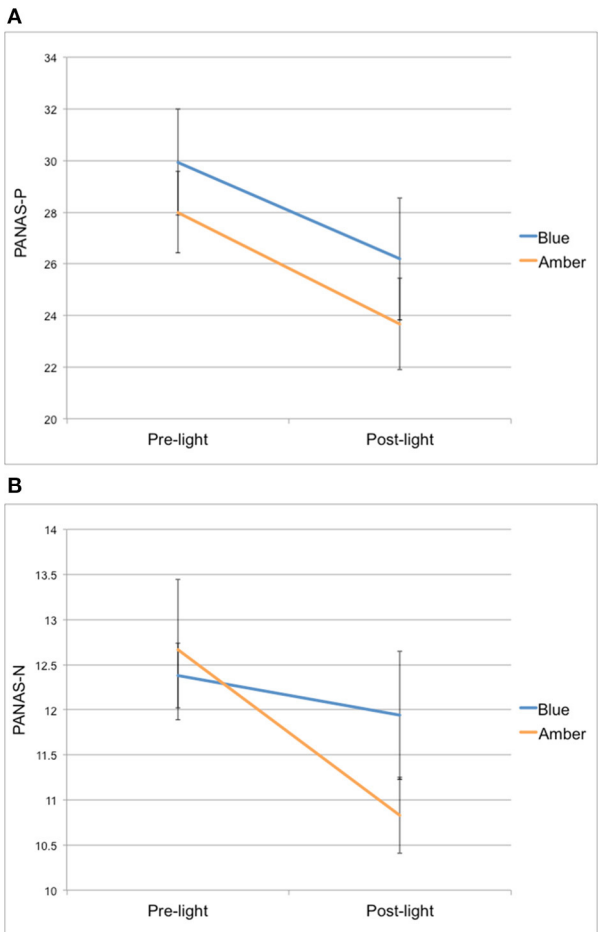

FIGURE 1 | (A) PANAS positive affect (PANAS-P) and **(B)** negative affect (PANAS-N) scores from pre-light and post-light exposure for both groups.

Neuroimaging Methods

Neuroimaging scans were collected on Skyra 3T scanner (Siemens, Erlangen, Germany) using a 32-channel head coil. High resolution structural images were first acquired using a T1-weighted 3D MPRAGE sequence (TR/TE/flip angle = 2.1 s/2.33 ms/12°) over 176 sagittal slices (256 × 256) and a slice thickness of 1 mm (voxel size = 1 × 1 × 1 mm^3). For the resting state functional scan, participants were instructed to let their mind wander while keeping their eyes open. Functional scans were acquired over 32 transverse slices (2.5 mm thickness; matrix: 88 × 84). Each volume was collected with an interleaved sequence (TR/TE/flip angle = 2 s/25 ms/90°). The voxel size of the T2* sequence was 2.5 × 2.5 (i.e., with a 40% slice gap, allowing collection of 180 volumes within a 6-min acquisition time). The field of view (FOV) was 220 mm.

Resting-State Pre-processing

Neuroimaging data were analyzed using the publicly available CONN functional connectivity toolbox (version 16.a; www.nitrc.org/projects/conn, RRID:SCR_009550), in conjunction with SPM12 (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm).

Raw functional images were realigned (motion corrected) and unwarped, slice-time corrected using the middle slice as the reference, and coregistered to each subject's high-resolution structural image in accordance with standard algorithms. The Artifact Detection Tool (ART; http://www.nitrc.org/projects/artifact_detect/) was used to regress out scans as nuisance covariates in the first-level analysis exceeding 3 SD in mean global signal intensity and scan-to-scan motion exceeding 0.5 mm. Six participants were excluded for an excessive number of outlier images (i.e., >36 scans flagged as outliers). These were included in addition to covariates for the six ridged-body parameters that characterize estimated subject motion, and used to regress out residual movement-related effects. Images were then normalized to Montreal Neurological Institute (MNI) coordinate space, spatially smoothed (8 mm full-width at half maximum), and resliced to a voxel size of 2 × 2 × 2 mm.

Functional Connectivity Analysis

Using a standard seed-to-voxel approach, functional connectivity analyses were performed using the default functional connectivity processing pipeline in the CONN toolbox [for details, see (41)]. In this processing pipeline, physiological and other spurious sources of noise were estimated with the aCompCor method (42, 43) and subsequently removed together with the movement- and artifact-related covariates mentioned above. The residual blood oxygen level dependent (BOLD) time-series was then band-pass filtered (0.01–0.1 Hz). Every participant's structural image was segmented into gray matter, white matter, and cerebral spinal fluid using SPM12. Confounding effects of white matter and cerebral spinal fluid were removed through linear regression. Two seed regions of interest (ROIs) were placed corresponding to the left and right amygdala as defined by the Automated Anatomical Labeling (AAL) atlas (44). After the removal of confounds, the residual BOLD time-series from the bilateral amygdala seed ROIs were averaged to generate a mean time-series. Bivariate correlation maps (Fisher-transformed) were then computed with all other voxels in the brain to derive whole-brain connectivity maps. A group-level approach was used to compare differences in connectivity between the blue and amber groups controlling for the effects of age and sex. False positive control in seed to voxel analysis is implemented through a combination of a voxel height threshold ($p < 0.001$, two-sided; uncorrected) and a cluster level extent threshold ($p < 0.05$, cluster-size FDR-correction) (41).

Directed Functional Connectivity Analysis: Granger Causality

In a second step, we investigated the strength of directionality of information flow [i.e., granger causality (GC)] between the amygdala and significant clusters from the functional connectivity analysis, using parametric GC (45, 46). Granger causality is a method for investigating causality between two time series, i.e., the extent to which one time series can predict the other. The strength of GC was estimated by quantifying the inter-relationships between their corresponding oscillatory mechanisms as a function of frequency (f) of oscillations. For that, the raw time-series data were first band pass filtered using the Butterworth filter design with a higher cutoff frequency of 0.0028 Hz (f_1) and a lower cutoff frequency of 0.1 Hz (f_2). Next, the time-series for the bilateral amygdala and significant clusters from the functional connectivity analysis was zero-mean corrected in order to remove slow trends and physiological noise. Furthermore, the optimal model order for the parametric approach was calculated by comparing power spectra from the parametric and non-parametric approaches (45). Different model orders from 1 to 10 were tested, and the model order that yielded the lowest power difference was selected.

The threshold level for statistically significant GC strength, corrected for multiple comparisons, was estimated from surrogated data using permutation tests (45, 46) ($n = 2{,}000$) and a gamma function under a null hypothesis of no interdependence at the significance level of $p = 0.0025$.

RESULTS

Affect Change From Pre- to Post-light Exposure

For PANAS-P scores, there was a significant main effect of time [$F_{(1,26)} = 25{,}67$, $p < 0.001$], but no time × group interaction [$F_{(1,26)} = 0.13$, $p = 0.72$] from pre- to post-light exposure. Overall, both light groups showed a decrease in their PANAS-P scores from pre- to post-light exposure (see **Figure 1A**).

For PANAS-N scores, there was also a significant main effect of time [$F_{(1,26)} = 6.15$, $p = 0.02$], but no time × group interaction [$F_{(1,26)} = 2.32$, $p = 0.14$] from pre- to post-light exposure. Overall, both light groups showed a decrease in their PANAS-N scores from pre- to post-light exposure (see **Figure 1B**).

Strength of Functional Connectivity

Compared to participants in the amber light group, participants in the blue light group showed significantly greater positive intrinsic functional connectivity between the right amygdala and a cluster of voxels in the left dorsolateral prefrontal cortex (DLPFC) (x = −24, y = 46, z = 18, k = 90, volume p-FDR corrected, $p < 0.001$) (see **Figure 2**). No effect for the left amygdala was found. In summary, individuals who received blue light exposure showed significantly greater positive functional connectivity between the right amygdala and the left DLPFC compared to participants who received amber light.

Strength of Granger Causality

To determine the directionality of the connectivity between the right amygdala and left DLPFC, we employed GC and found that it was bidirectional. In other words, both the strength of the feed-forward [right amygdala (R. AMG) to left DLPFC (L. DLPFC)] (**Figure 3A**) and feed-backward (L. DLPFC to R. AMG) (**Figure 3B**) connectivity was significant for the blue-light group, but not the amber-light group. In **Figure 3**, the dotted line corresponds to a GC value of 0.0422, which represents a significance level at $p < 0.0025$ ($p = 0.01/4$, corrected for multiple comparisons).

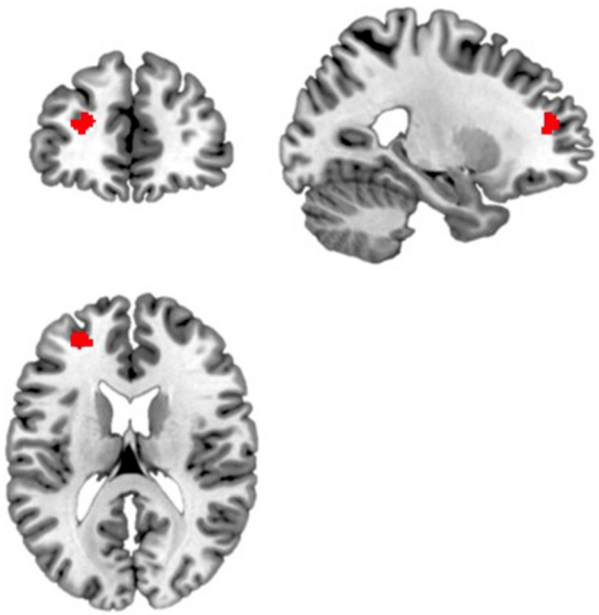

FIGURE 2 | Blue light led to increased functional connectivity between the right amygdala and a region within the left dorsolateral prefrontal cortex. Displayed is the significant cluster of voxels (x = −24, y = 46, z = 18, k = 90, volume p-FDR corrected, $p < 0.001$) from the seed-to-voxel analysis using the right amygdala as the seed region.

The Relationship Between Connectivity Patterns and Changes in Affect

To investigate whether functional and directed connectivity was associated with changes in affect, we ran non-parametric Spearman's correlations between connectivity values and PANAS change scores. Spearman's correlations were used due to the small sample sizes and because the PANAS data violated assumptions of normality. For the blue light group, there was a statistically significant moderate negative relationship with PANAS-N change scores and functional connectivity ($\rho = -0.55$, $p = 0.03$), indicating that greater functional connectivity between the amygdala and DLPFC was monotonically associated with reduced negative mood following light exposure (see **Figure 4**). This relationship was not present for the amber light group ($\rho = -0.18$, $p = 0.55$). The strength of the monotonic relationship for directed connectivity was much smaller and non-significant for the blue light group (feed forward connectivity: $\rho = -0.26$, $p = 0.33$; feed backward connectivity $\rho = -0.27$, $p = 0.29$) and for the amber light group (feed forward connectivity: $\rho = -0.24$, $p = 0.45$; feed-backwards: $\rho = -0.30$, $p = 0.34$). There were no significant correlations between connectivity patterns and PANAS-P change scores.

Potential Effects of Caffeine Consumption on Changes in Mood and Functional Connectivity

We compared the connectivity values for individuals who stated that they consumed caffeine prior to the assessments and those who stated that they did not consume caffeine (4 participants in each group consumed 1 caffeinated beverage prior to the assessment), and did not find a significant difference [total group: $t_{(27)} = -1.30$, $p = 0.20$; blue: $t_{(15)} = -0.31$, $p = 0.76$; amber: $t_{(10)} = -2.03$, $p = 0.07$]. There was also no difference between individuals who drank caffeine and those who did not on PANAS-N or PANAS-P change scores [PANAS-N: total group: $t_{(27)} = 0.33$, $p = 0.74$; blue: $t_{(15)} = -0.26$, $p = 0.80$; amber: $t_{(10)} = 0.91$, $p = 0.38$; PANAS-P: total group: $t_{(27)} = -0.83$, $p = 0.42$; blue: $t_{(15)} = 0.33$, $p = 0.75$; amber: $t_{(10)} = -1.50$, $p = 0.17$].

DISCUSSION

This study investigated the effects of exposure to 30-min of blue wavelength light on subsequent functional brain connectivity at rest and associations with changes in affect. The results showed that individuals exposed to a single 30-min pulse of blue wavelength light showed greater positive functional connectivity between the right amygdala and the left DLPFC than individuals exposed to an equivalent period of amber light, and that greater functional connectivity between these two areas was associated with greater decreases in negative mood. These findings build upon results from previous studies that have demonstrated that even after 50 s of blue light vs. green light exposure, there are transient increases in activation within the right amygdala (31). In line with this, there is evidence to suggest that the amygdala receives direct projections from ipRGCs (29) and that swift modulations of amygdala activation may contribute to the antidepressant effect of blue wavelength light (47). Specifically, previous work also showed enhanced functional connectivity between the left amygdala, the voice area of the temporal cortex and the hypothalamus during an auditory emotional task in the context of blue vs. green light exposure (32). The authors proposed that their results suggest that blue light exposure leads to increased limbic reactivity to emotional stimuli which may lead to quicker behavioral adaptations to emotional challenges, which could ultimately enhance emotional regulation. Our results support those previous findings that it is likely that blue wavelength light exposure leads to immediate amygdala activation and associated increases in arousal via the direct amygdalar projections from the ipRGCs. Our study also builds upon previous functional connectivity findings by showing that blue light exposure was associated with greater bi-directional information flow between the amygdala and DLPFC. We speculate that in order to respond to these increases in arousal, the DLPFC may, in turn, respond by engaging in cognitive control strategies, such as selectively directing attention to stimuli in the environment, recruiting emotion regulation strategies by modulating amygdala activation, and influencing social decision-making. Our interpretation is similar to Vandewalle et al.'s (32) interpretation of their functional connectivity findings and may explain why blue wavelength light exposure is an effective treatment method for individuals with seasonal and non-seasonal depression (10–13), but future research will be necessary to explore these possibilities further.

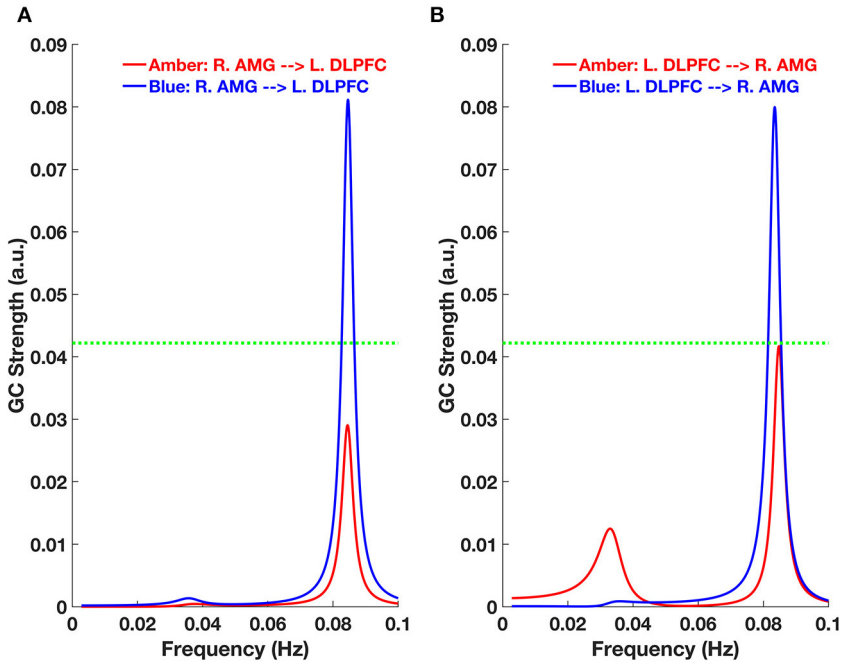

FIGURE 3 | Granger Causality (GC)-frequency spectra for **(A)** feed-forward [right amygdala (R. AMG) to left DLPFC (L. DLPFC)] and **(B)** feed-backward (L. DLPFC to R. AMG) connections for the amber and blue light groups. The green dotted line here represents the threshold chosen for significant GC strength (~0.0422 at $p <$ 0.0025, permutation test).

A growing body of work demonstrates that depression is characterized by impaired cognitive and emotional processing which may reflect increased activation within areas such as the amygdala and decreased activity within areas involved in the effortful regulation of emotional behavior, such as the DLPFC and other frontal regions (48). A number of studies demonstrate that during active emotion regulation, individuals with depression (compared to healthy control subjects) show less negative (i.e., inverse or anti-correlated) connectivity between the amygdala and the PFC and that this reverses when symptom severity decreases to levels no longer considered clinically significant (49). Functional resting-state studies, however, suggest that individuals with depression show reduced positive connectivity between the amygdala and several prefrontal regions, in comparison with healthy controls (50–52). It should be noted that the majority of these studies have not reported a direct link between the *right* amygdala and the *left* DLPFC, specifically. One study, however, showed that after successful transcutaneous vagus nerve stimulation (tVNS) individuals with depression showed increased resting state functional connectivity between the right amygdala and the left DLPFC which were associated with decreased depression symptoms (53). The results from the present study suggest that blue light exposure may have an immediate beneficial effect on functional connectivity between the amygdala and PFC similar to that of tVNS by increasing information flow between these regions at rest. This is further supported by our finding that greater amygdala–DLPFC connectivity following blue light exposure was associated with greater decreases in negative affect from pre- to post-light exposure, just as tVNS was associated with decreases in depression scores. The expression of depression can vary greatly between individuals and the circuits that may be impacted by depressed mood are equally manifold and can include dysregulated reward processing, heightened reactivity to negative environmental cues, increased default mode activity, and more (54). Different treatments for depression can therefore influence depressive symptoms in various ways by targeting distinct neuronal circuits. It is possible that blue wavelength light may have a unique impact on modulating amygdala and DLPFC reactivity, similar to tVNS, which may be particularly useful for certain individuals, but future research will be necessary to replicate and expand on these findings. In addition, future research should consider that light exposure may be even more beneficial when used in conjunction with emotional learning/therapy. For example, we speculate that blue light exposure while practicing emotional regulation skills might have an even stronger effect than blue light exposure without affective content. As previous studies have proposed that exposure to blue light may facilitate learning through increased norepinephrine release from the brainstem (9), using light therapy in conjunction with therapeutic approaches that enable better emotion regulation abilities, such as cognitive reappraisal, should be the focus of future research. Some evidence already suggests that such interventions may be effective, as

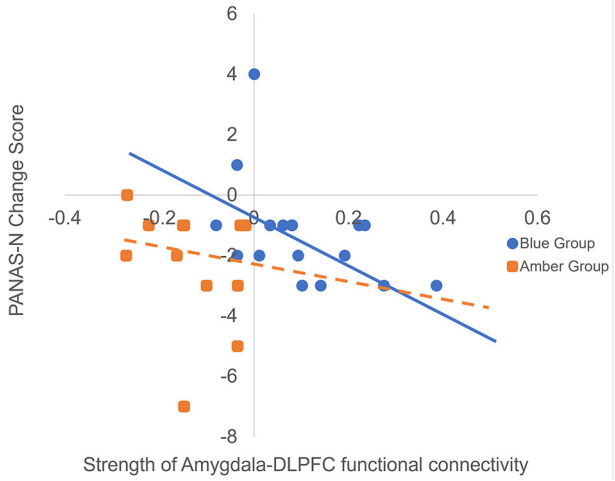

FIGURE 4 | Here we are presenting the correlation between raw amygdala-DLPFC connectivity values and raw changes in PANAS negative affect (PANAS-N) scores from pre- to post-light exposure for the blue vs. amber groups (Greater negative values on the PANAS-N change scores indicate greater reduction in negative affect over time). We decided to present the raw scores rather than the Spearman rank order correlation for ease of interpretation, however we have included the trendlines for each group from the original analysis. The figure shows that increased functional connectivity between the amygdala and DLPFC was monotonically associated with reduced PANAS-N scores for the blue light group ($\rho = -0.55$, $p = 0.03$) but not the amber light group ($\rho = -0.18$, $p = 0.55$).

individuals who underwent exposure therapy for panic and post-traumatic stress disorder showed greater reduction in anxiety and depression scores if the therapy was accompanied by concurrent bright light (vs. dim light) exposure (55). Another study showed that bright light exposure during fear extinction learning in healthy individuals suppressed fear acquisition and enhanced fear extinction (56).

It is important to highlight, that while we interpret our results as being the result of blue wavelength light exposure, it is also possible that amber wavelength light had a unique effect on dampening the strength of amygdala—DLPFC connectivity (e.g., amber light could have a "relaxing effect") rather than that blue light exposure led to an increase in connectivity between these two areas, although the potential mechanism for such an effect is unclear. The cross-sectional design also leaves the possibility open that a third variable not under study investigation contributed to the findings, such as that individuals in the blue light group had stronger amygdala-PFC connectivity at baseline and that the observed correlations with changes in mood are explained by baseline differences in brain function.

It should be noted that participants in our sample were free from known psychological disorders, making the detection of subtle changes in mood/affect more challenging. The fact that we did observe a moderate to strong association between the strength of functional connectivity and changes in negative affect ($\rho = 0.55$) makes these results notable. However, replication of this study in a clinical sample of depressed individuals would shed additional light onto the immediate effects of blue light on mood and allow extension to of these findings to clinical depression. While our stimulation period lasted only 30 min on a single occasion, it is conceivable that exposure to blue wavelength light for longer durations, perhaps over several weeks, may positively impact the coupling of the amygdala-DLPFC connection for individuals with clinical depression, which in turn may improve effective emotion regulation skills and executive function. There is some evidence for this, as Fisher et al. (33) demonstrated that 3 weeks of bright light therapy in healthy males reduced bilateral amygdala and prefrontal reactivity to threat (i.e., angry and fearful faces) in a dose-dependent manner; and that left amygdala and prefrontal connectivity also increased in a dose-dependent manner (33). However, these changes did not correspond with changes in mood or stress.

As participants in this study were not clinically depressed, it is unclear how these neurobiological changes would correspond to changes in mood in a sample of individuals with clinical psychopathology. Further research is necessary to understand better how bright light, particularly in the blue wavelengths, affects functional brain responses in individuals with depression and how those may be associated with improved mood.

Of note, the effects of blue wavelength light on amygdala-DLPFC functional connectivity at rest may represent just one mechanism by which light affects emotion and cognition. The ipRGCs, which are believed to be the primary system by which blue light affects brain activation and circadian physiology, also project to other brain areas important in mood disorders, such as the habenula and hypothalamus. While we focused on the amygdala and prefrontal cortex here, these other mood-relevant brain structures may also be influenced by blue light exposure (57–60). Future studies should therefore expand their analyses to other brain areas that may contribute to some of the mood enhancing effects of blue wavelength light. Other lines of work have focused on the effects of light exposure on the influence of serotonin signaling, which may interact with the effects of blue light on cortico-limbic reactivity. For example, individuals with seasonal affective disorder (SAD) display enhanced serotonin transporter signaling during the winter months, which normalizes with successful treatment or during natural summer remission (61). Another study showed that after a bright-light intervention in healthy males, differences in the 5-HTTLPR genotype moderated changes in functional brain responses between the amygdala and PFC toward threat (33). Future research would likely benefit from investigating the potential interplay between serotonin signaling and amygdala-PFC connectivity on emotional functioning and mood after blue light therapy.

It should be pointed out that our results did not support the notion that a single exposure to blue wavelength light during the day would lead to increased positive and decreased negative affect. Both groups showed a decrease in the *intensity* of emotion for positive and negative affect over time, raising the possibility that the effects of increased amygdala-PFC connectivity following blue light exposure resulted in a global moderating effect on mood. In other words, it is possible that blue light exposure may simply shift mood away from the extremes and toward

the middle ranges of intensity. Of course, it is also possible that group differences in mood changes were not detected due to other confounding study effects, such as intense positive and negative feelings at the beginning of the study, which may have dissipated toward the end for both groups (e.g., feeling excited, but apprehensive at the beginning of the day because of the novelty of being in a research study and/or the MRI acquisition). Future research would benefit from investigating the immediate changes in affect in response to light exposure in greater detail, including using more precise forms of self-report measures, investigating differences between healthy controls and those with depression, as well as the potential effects of time of day of exposure.

The results of this study should be interpreted with several limitations in mind. This study employed a between-subjects design and it is therefore possible that the observed results could be explained by individual differences between the groups rather than the light condition. Additionally, the sample sizes of the two groups were relatively small and not equivalent in size across groups which means that the larger sample in the blue light group could have yielded a different signal to noise ratio than in the amber light group. It is also important to mention that our results were considered as statistically significant using a cluster-level threshold. Eklund et al. (62) have raised the possibility that this can potentially result in inflated false-positive rates which should be kept in mind when interpreting our findings. This study also relied on participants' self-report regarding their sleep-wake history rather than more objective measurements, such as actigraphy data, potentially affecting brain responses to light. Additionally, while we attempted to control for circadian phase effects by performing the experiment at the same clock time for each participant, control would have been enhanced by regulating and monitoring their sleep in the sleep lab to control for circadian effects. These methodological limitations may have influenced the results of this study and it should therefore be replicated using a within-subjects design with a larger sample size. Further, in this study, we aimed to investigate one potential mechanism through which blue wavelength light might influence affect and chose to study a group of healthy individuals without psychopathology. However, it is unclear how these results apply to individuals with clinically significant levels of depression. For example, it is possible that blue light exposure has a different effect on depressed individuals, and a similar study incorporating a sample of currently depressed individuals is necessary to help elucidate this. In addition, to better understand how light therapy may alter brain function in depressed individuals, subsequent research would benefit from investigating changes in brain function at rest, as well as in response to emotional tasks, before and after light therapy. Finally, while this research contributes to our understanding of the mechanism by which blue wavelength light leads to improved mood, additional research is necessary to determine the precise pathways through which light influences functional brain responses and how those immediate modulations in functional connections lead to long-term behavioral and emotional changes in mood and well-being.

CONCLUSION

The present findings suggest that a single 30-min exposure to blue wavelength light during the day increased the bi-directional strength of positive functional connectivity between the right amygdala and the left dorsolateral prefrontal cortex and that this was associated with improvements in affect, particularly a reduction in negative affect. These findings suggest a neurobiological mechanism that may underlie the often-observed association between light exposure and improved mood.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation upon request.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

AA contributed to the study design, data collection, data analysis, and manuscript preparation. ND and SB contributed to the data analysis and manuscript preparation. AR and JV contributed to the manuscript preparation. WK contributed to the study design, data analysis, and manuscript preparation. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by a U.S. Army US Army Medical Acquisition Activity (USAMRAA) grant (W81XWH-14-1-0571) (WK) as well as by an Arizona Health Education Centers (AHEC) Research Grant (AA).

REFERENCES

1. Beecher ME, Eggett D, Erekson D, Rees LB, Bingham J, Klundt J, et al. Sunshine on my shoulders: weather, pollution, and emotional distress. *J Affect Disord.* (2016) 205:234–8. doi: 10.1016/j.jad.2016.07.021
2. Guéguen N, Lamy L. Weather and helping: additional evidence of the effect of the sunshine Samaritan. *J Soc Psychol.* (2013) 153:123–6. doi: 10.1080/00224545.2012.720618
3. Maruani J, Geoffroy PA. Bright light as a personalized precision treatment of mood disorders. *Front Psychiatry.* (2019) 10:85. doi: 10.3389/fpsyt.2019.00085

4. Killgore WDS. Lightening the mood: evidence for blue light exposure in the treatment of post-concussion depression. *Expert Rev Neurother.* (2020) 20:1081–3. doi: 10.1080/14737175.2020.1814147
5. Aan Het Rot M, Miloserdov K, Buijze ALF, Meesters Y, Gordijn MCM. Premenstrual mood and empathy after a single light therapy session. *Psychiatry Res.* (2017) 256:212–8. doi: 10.1016/j.psychres.2017.06.052
6. Cajochen C, Munch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. *J Clin Endocrinol Metab.* (2005) 90:1311–6. doi: 10.1210/jc.2004-0957
7. Phipps-Nelson J, Redman JR, Schlangen LJ, Rajaratnam SM. Blue light exposure reduces objective measures of sleepiness during prolonged nighttime performance testing. *Chronobiol Int.* (2009) 26:891–912. doi: 10.1080/07420520903044364
8. Alkozei A, Smith R, Pisner D, Vanuk JR, Markowski SM, Fridman A, et al. Exposure to blue light increases subsequent functional activation of the prefrontal cortex during performance of a working memory task. *Sleep.* (2016) 39:1671–80. doi: 10.5665/sleep.6090
9. Alkozei A, Smith R, Dailey NS, Bajaj S, Killgore WDS. Acute exposure to blue wavelength light during memory consolidation improves verbal memory performance. *PLoS ONE.* (2017) 12:e0184884. doi: 10.1371/journal.pone.0184884
10. Even C, Schröder CM, Friedman S, Rouillon F. Efficacy of light therapy in nonseasonal depression: a systematic review. *J Affect Disord.* (2008) 108:11–23. doi: 10.1016/j.jad.2007.09.008
11. Glickman G, Byrne B, Pineda C, Hauck WW, Brainard GC. Light therapy for seasonal affective disorder with blue narrow-band light-emitting diodes (LEDs). *Biol Psychiatry.* (2006) 59:502–7. doi: 10.1016/j.biopsych.2005.07.006
12. Perera S, Eisen R, Bhatt M, Bhatnagar N, de Souza R, Thabane L, et al. Light therapy for non-seasonal depression: systematic review and meta-analysis. *Br J Psychiatry Open.* (2016) 2:116–26. doi: 10.1192/bjpo.bp.115.001610
13. Strong RE, Marchant BK, Reimherr FW, Williams E, Soni P, Mestas R. Narrow-band blue-light treatment of seasonal affective disorder in adults and the influence of additional nonseasonal symptoms. *Depress Anxiety.* (2009) 26:273–8. doi: 10.1002/da.20538
14. Lack L, Bramwell T, Wright H, Kemp K. Morning blue light can advance the melatonin rhythm in mild delayed sleep phase syndrome. *Sleep Biol Rhythms.* (2007) 5:78–80. doi: 10.1111/j.1479-8425.2006.00250.x
15. Killgore WDS, Vanuk JR, Shane BR, Weber M, Bajaj S. A randomized, double-blind, placebo-controlled trial of blue wavelength light exposure on sleep and recovery of brain structure, function, and cognition following mild traumatic brain injury. *Neurobiol Dis.* (2020) 134:104679. doi: 10.1016/j.nbd.2019.104679
16. Raikes AC, Dailey NS, Shane BR, Forbeck B, Alkozei A, Killgore WDS. Daily morning blue light therapy improves daytime sleepiness, sleep quality, and quality of life following a mild traumatic brain injury. *J Head Trauma Rehabil.* (2020) 35:E405–21. doi: 10.1097/HTR.0000000000000579
17. Raikes AC, Dailey NS, Forbeck B, Alkozei A, Killgore WDS. Daily morning blue light therapy for post-mTBI sleep disruption: effects on brain structure and function. *Front Neurol.* (2021) 12:625431. doi: 10.3389/fneur.2021.625431
18. Sinclair KL, Ponsford JL, Taffe J, Lockley SW, Rajaratnam SM. Randomized controlled trial of light therapy for fatigue following traumatic brain injury. *Neurorehabil Neural Repair.* (2014) 28:303–13. doi: 10.1177/1545968313508472
19. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* (2002) 295:1070–3. doi: 10.1126/science.1067262
20. Dijk DJ, Archer SN. Light, sleep, and circadian rhythms: together again. *PLoS Biol.* (2009) 7:1221. doi: 10.1371/journal.pbio.1000145
21. Cajochen C. Alerting effects of light. *Sleep Med Rev.* (2007) 11:453–64. doi: 10.1016/j.smrv.2007.07.009
22. Chellappa SL, Steiner R, Blattner P, Oelhafen P, Götz T, Cajochen C. Non-visual effects of light on melatonin, alertness and cognitive performance: can blue-enriched light keep us alert. *PLoS ONE.* (2011) 6:e16429. doi: 10.1371/journal.pone.0016429
23. Pandi-Perumal SR, Smits M, Spence W, Srinivasan V, Cardinali DP, Lowe AD, et al. Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* (2007) 31:1–11. doi: 10.1016/j.pnpbp.2006.06.020
24. Chesson AL Jr., Littner M, Davila D, Anderson WM, Grigg-Damberger M, et al. Practice parameters for the use of light therapy in the treatment of sleep disorders. Standards of Practice Committee, American Academy of Sleep Medicine. *Sleep.* (1999) 22:641–60. doi: 10.1093/sleep/22.5.641
25. Pail G, Huf W, Pjrek E, Winkler D, Willeit M, Praschak-Rieder N, et al. Bright-light therapy in the treatment of mood disorders. *Neuropsychobiology.* (2011) 64:152–62. doi: 10.1159/000328950
26. Rautkyä E, Puolakka M, Halonen L. Alerting effects of daytime light exposure–a proposed link between light exposure and brain mechanisms. *Light Res Technol.* (2011) 44:238–52. doi: 10.1177/1477153511409294
27. Mick G, Cooper H, Magnin M. Retinal projection to the olfactory tubercle and basal telencephalon in primates. *J Compar Neurol.* (1993) 327:205–19. doi: 10.1002/cne.903270204
28. Johnson RF, Morin LP, Moore RY. Retinohypothalamic projections in the hamster and rat demonstrated using cholera toxin. *Brain Res.* (1988) 462:301–12. doi: 10.1016/0006-8993(88)90558-6
29. Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol.* (2006) 497:326–49. doi: 10.1002/cne.20970
30. Morin LP, Studholme KM. Retinofugal projections in the mouse. *J Compar Neurol.* (2014) 522:3733–53. doi: 10.1002/cne.23635
31. Vandewalle G, Schmidt C, Albouy G, Sterpenich V, Darsaud A, Rauchs G, et al. Brain responses to violet, blue, and green monochromatic light exposures in humans: prominent role of blue light and the brainstem. *PLoS ONE.* (2007) 2:e1247. doi: 10.1371/journal.pone.0001247
32. Vandewalle G, Schwartz S, Grandjean D, Wuillaume C, Balteau E, Degueldre C, et al. Spectral quality of light modulates emotional brain responses in humans. *Proc Natl Acad Sci USA.* (2010) 107:19549–54. doi: 10.1073/pnas.1010180107
33. Fisher PM, Madsen MK, Mc Mahon B, Holst KK, Andersen SB, Laursen HR, et al. Three-week bright-light intervention has dose-related effects on threat-related corticolimbic reactivity and functional coupling. *Biol Psychiatry.* (2014) 76:332–9. doi: 10.1016/j.biopsych.2013.11.031
34. Tang Y, Kong L, Wu F, Womer F, Jiang W, Cao Y, et al. Decreased functional connectivity between the amygdala and the left ventral prefrontal cortex in treatment-naive patients with major depressive disorder: a resting-state functional magnetic resonance imaging study. *Psychol Med.* (2013) 43:1921–7. doi: 10.1017/S0033291712002759
35. Cullen KR, Westlund MK, Klimes-Dougan B, Mueller BA, Houri A, Eberly LE, et al. Abnormal amygdala resting-state functional connectivity in adolescent depression. *JAMA Psychiatry.* (2014) 71:1138–47. doi: 10.1001/jamapsychiatry.2014.1087
36. Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA. Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting-state functional connectivity. *JAMA Psychiatry.* (2015) 72:603–11. doi: 10.1001/jamapsychiatry.2015.0071
37. Alkozei A, Smith R, Killgore WD. Exposure to blue wavelength light modulates anterior cingulate cortex activation in response to 'uncertain' versus 'certain' anticipation of positive stimuli. *Neurosci Lett.* (2016) 616:5–10. doi: 10.1016/j.neulet.2016.01.034
38. Killgore WDS, Dailey NS, Raikes AC, Vanuk JR, Taylor E, Alkozei A. Blue light exposure enhances neural efficiency of the task positive network during a cognitive interference task. *Neurosci Lett.* (2020) 735:135242. doi: 10.1016/j.neulet.2020.135242
39. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol.* (1988) 54:1063. doi: 10.1037/0022-3514.54.6.1063
40. Crawford JR, Henry JD. The Positive and Negative Affect Schedule (PANAS): Construct validity, measurement properties and normative data in a large non-clinical sample. *Br J Clin Psychol.* (2004) 43:245–65. doi: 10.1348/0144665031752934
41. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect.* (2012) 2:125–41. doi: 10.1089/brain.2012.0073
42. Behzadi Y, Restom K, Liau J, Liu TT. A component based noise correction method (CompCor) for BOLD and perfusion based

fMRI. *Neuroimage.* (2007) 37:90–101. doi: 10.1016/j.neuroimage.2007.04.042
43. Chai XJ, Castañón AN, Öngür D, Whitfield-Gabrieli S. Anticorrelations in resting state networks without global signal regression. *Neuroimage.* (2012) 59:1420–8. doi: 10.1016/j.neuroimage.2011.08.048
44. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage.* (2002) 15:273–89. doi: 10.1006/nimg.2001.0978
45. Dhamala M, Rangarajan G, Ding M. Analyzing information flow in brain networks with nonparametric Granger causality. *Neuroimage.* (2008) 41:354–62. doi: 10.1016/j.neuroimage.2008.02.020
46. Dhamala M, Rangarajan G, Ding M. Estimating Granger causality from fourier and wavelet transforms of time series data. *Phys Rev Lett.* (2008) 100:018701. doi: 10.1103/PhysRevLett.100.018701
47. Vandewalle G, Maquet P, Dijk DJ. Light as a modulator of cognitive brain function. *Trends Cogn Sci.* (2009) 13:429–38. doi: 10.1016/j.tics.2009.07.004
48. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: implications for major psychiatric disorders. *Biol Psychiatry.* (2003) 54:515–28. doi: 10.1016/S0006-3223(03)00171-9
49. Johnstone T, van Reekum CM, Urry HL, Kalin NH, Davidson RJ. Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *J Neurosci.* (2007) 27:8877–84. doi: 10.1523/JNEUROSCI.2063-07.2007
50. Cheng W, Rolls ET, Qiu J, Xie X, Lyu W, Li Y, et al. Functional connectivity of the human amygdala in health and in depression. *Soc Cogn Affect Neurosci.* (2018) 13:557–68. doi: 10.1093/scan/nsy032
51. Connolly CG, Ho TC, Blom EH, LeWinn KZ, Sacchet MD, Tymofiyeva O, et al. Resting-state functional connectivity of the amygdala and longitudinal changes in depression severity in adolescent depression. *J Affect Disord.* (2017) 207:86–94. doi: 10.1016/j.jad.2016.09.026
52. Ramasubbu R, Konduru N, Cortese F, Bray S, Gaxiola I, Goodyear B. Reduced intrinsic connectivity of amygdala in adults with major depressive disorder. *Front Psychiatry.* (2014) 5:17. doi: 10.3389/fpsyt.2014.00017
53. Liu J, Fang J, Wang Z, Rong P, Hong Y, Fan Y, et al. Transcutaneous vagus nerve stimulation modulates amygdala functional connectivity in patients with depression. *J Affect Disord.* (2016) 205:319–26. doi: 10.1016/j.jad.2016.08.003
54. Woody ML, Gibb BE. Integrating NIMH research domain criteria (RDoC) into depression research. *Curr Opin Psychol.* (2015) 4:6–12. doi: 10.1016/j.copsyc.2015.01.004
55. Kawamura A, Yoshiike T, Yoshimura A, Koizumi H, Nagao K, Fujii Y, et al. Bright light exposure augments cognitive behavioral therapy for panic and posttraumatic stress disorders: a pilot randomized control trial. *Sleep Biol Rhythms.* (2019) 18:1–7. doi: 10.1007/s41105-019-00248-7
56. Yoshiike T, Honma M, Yamada N, Kim Y, Kuriyama K. Effects of bright light exposure on human fear conditioning, extinction, and associated prefrontal activation. *Physiol Behav.* (2018) 194:268–76. doi: 10.1016/j.physbeh.2018.06.015
57. Do MTH, Yau KW. Intrinsically photosensitive retinal ganglion cells. *Physiol Rev.* (2010) 162:59–90. doi: 10.1152/physrev.00013.2010
58. Lawson RP, Nord CL, Seymour B, Thomas DL, Dayan P, Pilling S, et al. Disrupted habenula function in major depression. *Mol Psychiatry.* (2017) 22:202–8. doi: 10.1038/mp.2016.81
59. Huang LU, Xi Y, Peng Y, Yang Y, Huang X, Fu Y, et al. A visual circuit related to habenula underlies the antidepressive effects of light therapy. *Neuron.* (2019) 102:128–42. doi: 10.1016/j.neuron.2019.01.037
60. Vandewalle G, Hébert M, Beaulieu C, Richard L, Daneault V, Garon ML, et al. Abnormal hypothalamic response to light in seasonal affective disorder. *Biol Psychiatry.* (2011) 70:954–61. doi: 10.1016/j.biopsych.2011.06.022
61. Willeit M, Sitte HH, Thierry N, Michalek K, Praschak-Rieder N, Zill P, et al. Enhanced serotonin transporter function during depression in seasonal affective disorder. *Neuropsychopharmacology.* (2008) 33:1503. doi: 10.1038/sj.npp.1301560
62. Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci USA.* (2016) 113:7900–5. doi: 10.1073/pnas.1602413113

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

REVIEW
published: 29 March 2021
doi: 10.3389/fneur.2021.625369

Light/Clock Influences Membrane Potential Dynamics to Regulate Sleep States

Masashi Tabuchi, Kaylynn E. Coates, Oscar B. Bautista and Lauren H. Zukowski*

Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, United States

The circadian rhythm is a fundamental process that regulates the sleep–wake cycle. This rhythm is regulated by core clock genes that oscillate to create a physiological rhythm of circadian neuronal activity. However, we do not know much about the mechanism by which circadian inputs influence neurons involved in sleep–wake architecture. One possible mechanism involves the photoreceptor cryptochrome (CRY). In *Drosophila*, CRY is receptive to blue light and resets the circadian rhythm. CRY also influences membrane potential dynamics that regulate neural activity of circadian clock neurons in *Drosophila*, including the temporal structure in sequences of spikes, by interacting with subunits of the voltage-dependent potassium channel. Moreover, several core clock molecules interact with voltage-dependent/independent channels, channel-binding protein, and subunits of the electrogenic ion pump. These components cooperatively regulate mechanisms that translate circadian photoreception and the timing of clock genes into changes in membrane excitability, such as neural firing activity and polarization sensitivity. In clock neurons expressing CRY, these mechanisms also influence synaptic plasticity. In this review, we propose that membrane potential dynamics created by circadian photoreception and core clock molecules are critical for generating the set point of synaptic plasticity that depend on neural coding. In this way, membrane potential dynamics drive formation of baseline sleep architecture, light-driven arousal, and memory processing. We also discuss the machinery that coordinates membrane excitability in circadian networks found in *Drosophila*, and we compare this machinery to that found in mammalian systems. Based on this body of work, we propose future studies that can better delineate how neural codes impact molecular/cellular signaling and contribute to sleep, memory processing, and neurological disorders.

Keywords: circadian clock, sleep, synaptic plasticity, membrane potential, neural coding

OPEN ACCESS

Edited by:
Fabian-Xosé Fernandez, University of Arizona, United States

Reviewed by:
Angelique Lamaze, University of Münster, Germany
Nara Ines Muraro, CONICET Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA), Argentina

****Correspondence:***
Masashi Tabuchi
masashi.tabuchi@case.edu

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *02 November 2020*
Accepted: *15 February 2021*
Published: *29 March 2021*

Citation:
Tabuchi M, Coates KE, Bautista OB and Zukowski LH (2021) Light/Clock Influences Membrane Potential Dynamics to Regulate Sleep States. Front. Neurol. 12:625369. doi: 10.3389/fneur.2021.625369

INTRODUCTION

Circadian rhythms regulate an endogenous biological clock that dictates a sleep–wake cycle running in ∼24-h intervals. These rhythms occur even in complete darkness, but they are reset by light. Over the past 50 years, researchers have elucidated many molecular genetic components that regulate the Light/Clock interactions. Many of these core clock genes were identified with forward mutagenesis screens in *Drosophila*. For example, period (PER) and timeless (TIM) oscillate to create transcription–translation feedback loops (1–3). In addition, cryptochrome (CRY) was identified as

a clock-related gene that is sensitive to light and modulates the circadian rhythm in *Drosophila* (4). However, CRY is a central part of the molecular clock in mammals but lacks light sensitivity (5). In *Drosophila*, CRY is an important element that communicates with the light/clock integrator (6), and it influences the neural activity of circadian clock neurons (7) by interacting with potassium ion channel β-subunit redox sensor (8).

In addition to CRY signaling, other core clock output molecules modulate the neural activity of circadian clock neurons by interacting with a number of proteins, such as ligand-gated channels (9, 10), voltage-dependent/independent channels (11), channel-binding protein (12), and subunits of the electrogenic ion pump (12). In *Drosophila*, synaptic plasticity that regulates sleep was induced by specific sequences of spikes that occur during spontaneous activity in clock neurons (12). Moreover, based on recent studies, the interaction of light and clock information influences memory learning, possibly mediated by sleep (13, 14). These studies made an interesting link between light and clock information in forming neural coding, which is based on "neural activity" of circadian clock networks.

When neurophysiological researchers wish to quantify "neural activity," they often assess action potential firing (or spiking), which is typically shown as mean firing rate (15, 16), first spike latency (17), relative spike timing (18), or regularity of interspike intervals (19–23). This definition is based on the concept, in which neurons represent information with sequences of spikes (24, 25). However, a number of "non-spiking neurons" do not generate action potentials (26–29). Instead, they can transmit information with a temporal structure of subthreshold membrane potential (30, 31). Researchers question whether neurons and spiking activity are the basic units of brain function. This model is based on the prediction that neural network signals flow along well-behaved axonal rails and pass the activity baton at synapses.

Regardless of whether spiking activity is used for information representation, the "membrane potential dynamics" of neurons is the essence of neuronal information representation (32, 33). In this review, we define membrane potential dynamics as the stochastically/deterministically shaped temporal structure of membrane potential (34, 35) (**Figure 1**). The significance of membrane potential dynamics can be shaped by changes in hierarchical biophysical interactions (33), from microscopic [e.g., thermal noise (36), single-channel dwell time variability (37–39)] to mesoscopic [e.g., synaptic conductance variability (40–45)] to macroscopic [e.g., the hierarchical interplay of multiple neurons (46–49)] changes.

In this review, we propose that membrane potential dynamics produced by circadian photoreception and core clock molecules are critical for generating synaptic plasticity based on the fixed point of neural coding. In this way, these dynamics drive the formation of architecture that support baseline sleep, light-driven arousal, and processing of memory. We will also discuss the machinery that regulates the circadian rhythm and organizes internal (e.g., intrinsic channel conductance) and external (e.g., synaptic conductance) membrane excitability in *Drosophila*, and we will compare this machinery to that found in mammalian systems.

THE NEURAL NETWORK THAT REGULATES THE CIRCADIAN CLOCK

In mammals, the master of circadian clock networks is the suprachiasmatic nucleus (SCN) (50). The SCN resides in the hypothalamus and is composed of the ventrolateral (VL) core and VL shell (51). In the VL core, SCN neurons are photosensitive (52, 53) and produce arginine vasopressin (AVP) (54) and enkephalin (ENK) (55) (**Figure 2B**). Conversely, in the VL shell, SCN neurons are not photosensitive (53), and they express vasoactive intestinal peptide (VIP), gastrin-releasing peptide (GRP), and calretinin (50, 56). Functionally, SCN neurons in the VL core attain visual information relayed from the retina through the retinohypothalamic tract. This relay occurs while SCN neurons in the VL shell gather information from many nuclei, including the hypothalamus and brainstem (56). Interestingly, Pennartz et al. suggest that SCN neurons belong to two classes, class I and class II, that each display spontaneous firing rates (57). In this way, these neurons, which are regulated by calcium-dependent potassium currents, are likely the pacemaker cells of the SCN (58–61). Thus, the varying firing frequencies between the two classes of SCN neurons may influence neuropeptide release to entrain the circadian rhythm and regulate oscillations in circadian-relevant genes.

Neurons within the SCN have expansive heterogeneity, making it challenging for researchers to decipher how SCN neurons structure the circadian rhythm in mammals. To study the circadian rhythm and the neurons that control it, many researchers have turned to flies. In *Drosophila*, specialized clock neurons are enriched with core clock genes that cooperatively drive circadian-dependent activity (62). Approximately 150 clock neurons (63) have been found in the central brain of these flies (**Figure 2A**). These neurons are categorized based on their location: small VL neurons (s-LNvs) and large VL neurons (l-LNvs), dorsal neurons (DN1, DN2, DN3), lateral posterior neurons, and dorsal lateral neurons (LNds) (64). Although *Drosophila* have fewer neurons than mammals, their circadian clock neurons similarly express many different neuropeptides. Importantly, only half of clock neurons express CRY, yet all clock neurons are synchronized with the light–dark cycle.

To induce daily behavioral rhythms, each subgroup of clock neurons has a specific role in the circadian clock network: the neuronal activity of each subgroup fluctuates in a daily rhythm, peaking at specific times of day and, collectively, their synchronized activity shapes the circadian locomotor behavior. In flies, certain subsets of neurons anticipate morning and evening locomotor activity (65). The s-LNvs, in particular, are the "master clock cells" that entrain the entire circadian rhythm and are responsible for driving morning anticipation in which flies increase their locomotor behavior activity before sunrise. Similarly, the LN_{ds} and l-LNvs, and 5th PDF^{-} s-LNv (with CRY and not PDF) anticipate evening activity (66). Interestingly, DN1s expressing CRY could also contribute to

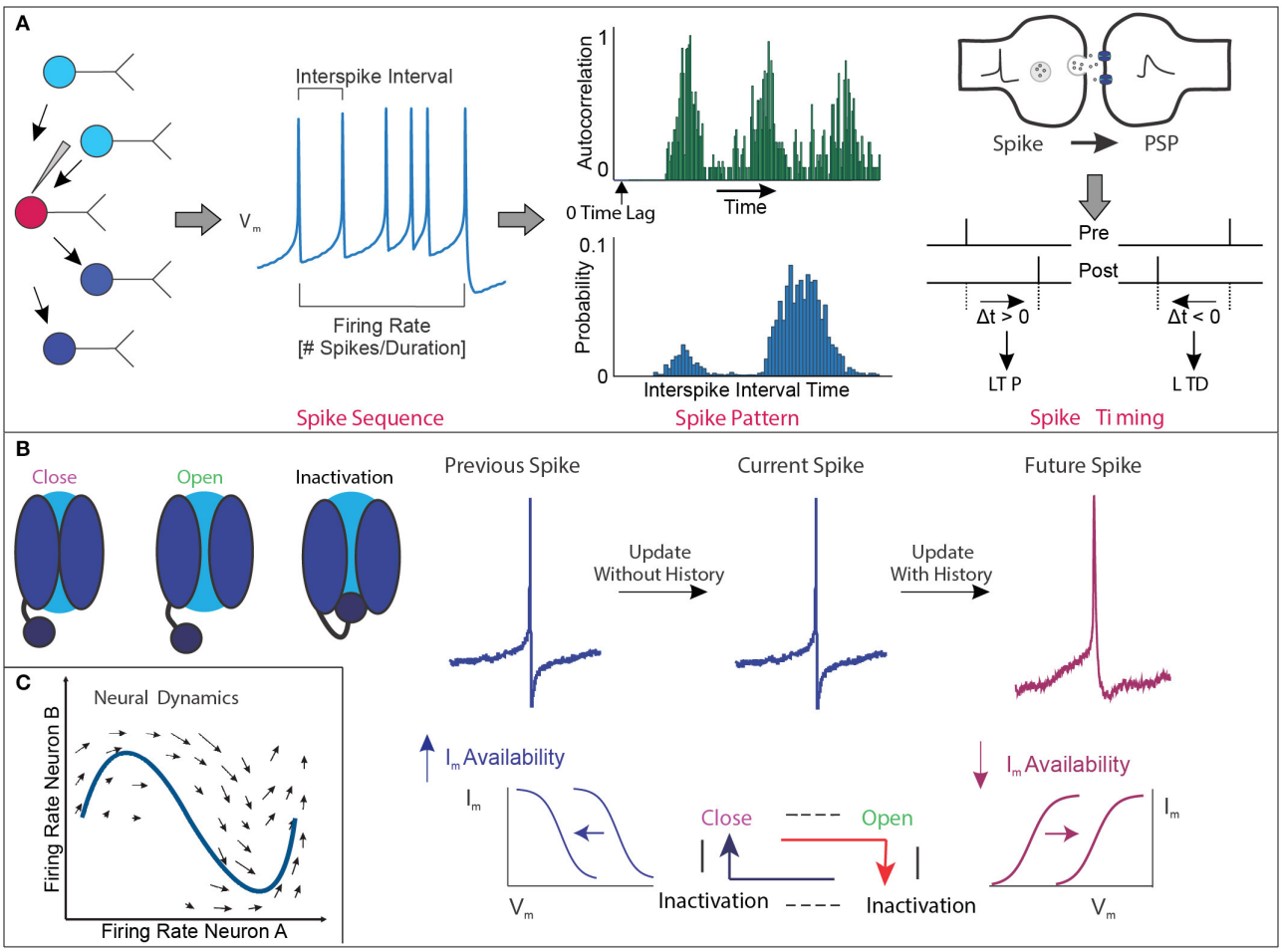

FIGURE 1 | Membrane potential dynamics. **(A)** Conceptual example of the quantification of membrane potential (Vm) dynamics representing the widely accepted temporal structure of the action potential spike sequence obtained during electrophysiology recordings. These analyses include the mean frequency of spikes within a given amount of time, regularity of spiking based on the probability distribution of the interspike interval, and autocorrelation function of spike trains over time to assess patterned neural timescales, as well as relative timing between presynaptic spike and postsynaptic spike to generate LTP/LTD. Note that the conceptual example focuses on the Vm of single cells and not a population of cells. **(B)** Conceptual example of hierarchical biophysical interactions between single channels and Vm state change to generate the hysteresis effect in time. In the presence of "perfect deactivation of inactivation" of voltage-gated ion channels following spike generation, availability of net ionic currents (Im) is increased, making set point shifting of Im–Vm relation (shown in blue). In this situation, there is no update of history in the next spike generation event, and thus each spike generation event is independent. In the presence of "imperfect deactivation of inactivation," availability of Im is decreased, making set point shifting of Im–Vm relation (shown in magenta). Decreased availability of Im makes "updated history" in the next spike generation event, and thus, the next spike generation event is significantly influenced by the previous event. These sequential chains shape hysteresis effect to generate the higher-order temporal structure of spike trains. **(C)** Conceptual example of neural activity in the activity space. As the firing frequency of the two neurons changes, the neural activity moves in this two-dimensional space. If we observe the activity of *n* neurons at the same time, the activity pattern can be described in an *n*-dimensional activity space.

this process (67). DN1s are an upstream target for s-LNvs and LNds, and they modulate sleep–wake patterns (68) by signaling through wake-promoting calcitonin gene-related peptide (a homolog of diuretic hormone 31 in flies) (69) and sleep-promoting glutamatergic connections to s-LNvs (70). Posterior DN1s (DN1ps) are temperature-sensitive neurons that integrate light intensity and temperature to drive evening anticipation (71). Inhibition of these neurons does not affect the delay of the siesta offset at warm temperatures or the decrease of night sleep, implying that these neurons do not promote sleep at warm temperatures (72). This suggests that, in addition to the endogenous clock mechanisms, multimodal sensory inputs can integrate and entrain the circadian rhythm [for DN1p review, see (73)].

In flies, sleep has been behaviorally characterized as consolidated immobility (74) [also reviewed in (75)]. After sleep deprivation, flies exhibit homeostatic recovery of lost sleep (76). Sleep homeostasis is facilitated by brain regions including the mushroom body (MB) (77, 78), dorsal fan-shaped body (FB) (79, 80), and ellipsoid body (EB) (81–83). These structures

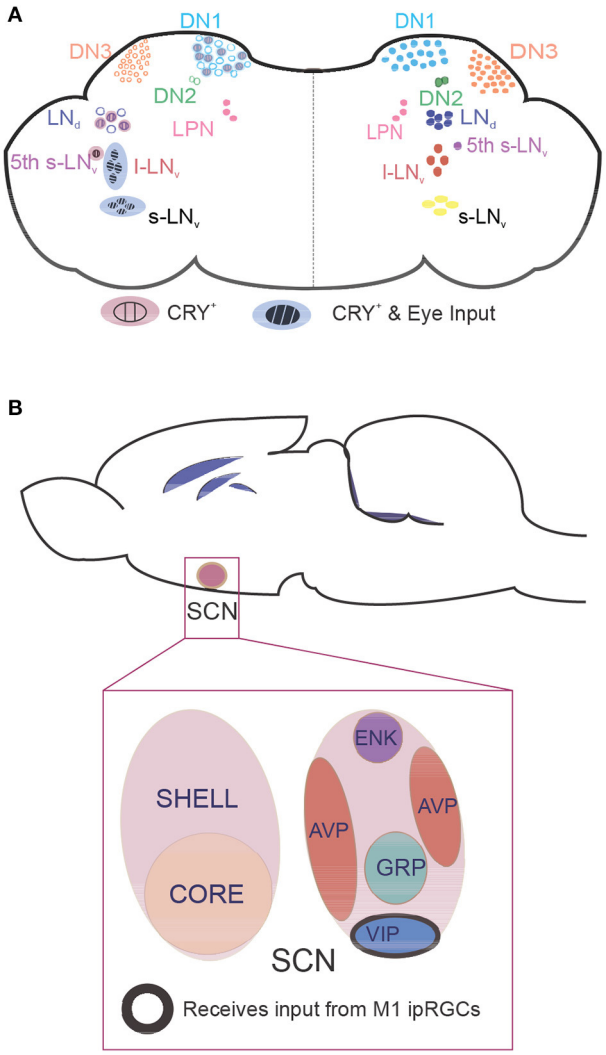

FIGURE 2 | Location of clock neurons in fly and mouse brains. Neurons that express core clock proteins localize in specific regions of the cortex. **(A)** In flies, ∼150 clock neurons can be generally categorized as dorsal neurons (DNs) or lateral neurons (LNs). The right hemisphere contains clock neuron populations, and the left hemisphere shows whether these neurons receive retinal input and/or are intrinsically photosensitive due to CRY expression. **(B)** In mammals, core clock neurons are found in the suprachiasmatic nucleus (SCN). Neurons in the photosensitive SCN core primarily express the neuropeptides vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP), whereas neurons in the SCN shell express arginine vasopressin (AVP) and enkephalin (ENK). M1 intrinsically photosensitive retinal ganglion cells innervate VIP neurons to pass photic information that synchronizes the clock. CRY, cryptochrome; l-LNv, large ventrolateral neuron; LNd, dorsal lateral neuron; LPN, lateral posterior neuron; s-LNv, small ventrolateral neuron.

are controlled by clock neurons and by each other through a variety of neurotransmitters (84). The MB comprises neuropil structures with Kenyon cells that promote wakefulness and sleep, depending on which lobes are affected (77). Serotonin (85) and GABA signaling (86) can inhibit wake-promoting MB neurons. On the other hand, MB output neurons can have a wake-promoting effect by using glutamate (87).

The FB comprises sleep-promoting ExFl2 neurons (88, 89). These neurons can be activated by clock neurons through glutamatergic inputs originating from Allatostatin A-expressing lateral posterior neurons (80). They can also be inhibited by clock neurons to promote sleep through dopamine signaling (79, 90) from the posterolateral cluster 1 and protocerebral posteromedial 3 neurons. To promote sleep, neurons in the FB integrate both types of inputs to signal wake or sleep activity (89).

Concerning sleep–wake regulation, the EB has been implicated to use a D1-like dopamine receptor (91). The EB comprises ring neurons that receive visual information from tubercular bulbar neurons in the anterior visual tract (92). One type of ring neuron, ring layer 5 (R5) neurons (92), may act as an integrator circuit for sleep homeostasis because of their high sensitivity to sleep and persistent upregulation of N-methyl-D-aspartate receptors (NMDARs) (81). R5 neurons exhibit state changes in the form of burst firing, which correlates with slow-wave oscillations (93), which synchronize the overall neuronal activity in this brain region. In addition to correlative activity, the R5 neuron network can use slow-wave oscillations (93) to directly increase the internal sleep drive through NMDARs (81).

The R5 neuron network also integrates information that supports sleep homeostasis by indirectly signaling to ExF12 neurons (81). In response to sleep need, ExF12 neurons induce a mechanism that augments calcium levels, upregulates NMDARs, increases the size and amount of active zones, and amplifies burst firing activity (81). These data support the idea that sleep information can be integrated in the sleep centers of the insect brain. Furthermore, these mechanistic networks may be highly conserved in mammalian circuitry (94–97).

Clock output molecules have been linked to circadian regulation of sleep through their interaction with neuronal excitability of circadian clock neurons (**Figure 3**) [reviewed in (98)]. Liu et al. (9) found that the circadian output molecule wide awake (WAKE) responds to CLK oscillations, reducing excitability in wake-promoting l-LN$_V$s. This pathway upregulates the GABA$_A$ receptor resistant to dieldrin (RDL). They also found that the mammalian homolog of WAKE (9), mWAKE, is expressed in both the SCN and dorsal medial hypothalamus (99). These data support the notion that mWAKE is conserved in driving wakefulness by modulating firing rates.

Conversely, the E3 ubiquitin ligase Fbxl4 downregulates GABA sensitivity in l-LNvs. This downregulation is influenced by RDL ubiquitination, as well as RDL degradation that occurs during the night and peaks at dawn (10). Another study assessed a homolog of the mammalian neuropeptide calcitonin gene-related peptide found in flies (69). This homolog, diuretic hormone 31, was secreted through PDF signaling, increasing neuronal activity to anticipate the morning (68). Additionally, the membrane excitability in DN1p neurons was modulated during the day by the narrow abdomen ion channel, the homolog of the sodium leak channel non-selective protein (NALCN) found in mammals (11). Also, the microRNA mir-92a, which depends on the core clock, was implicated to decrease neuronal excitability in PDF$^+$ cells during the night by inhibiting *sirt2* translation (100).

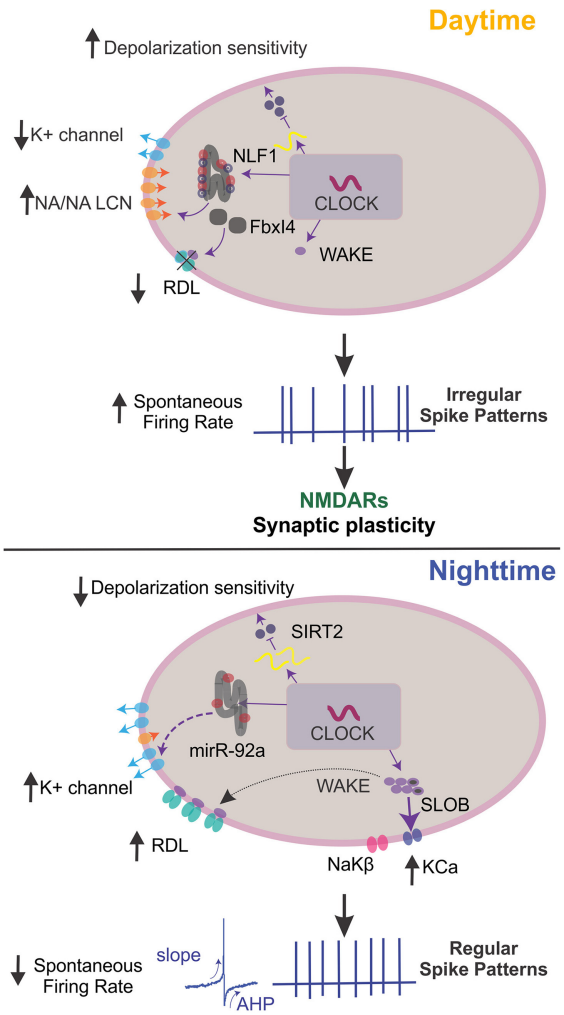

FIGURE 3 | Mechanisms of clock output genes that regulate neuronal excitability. In *Drosophila*, clock output genes modulate membrane potential dynamics to regulate sleep states. During the day, the clock drives high NCA localization factor 1 (NLF-1) activity. This activity, along with narrow abdomen (NA)/sodium leak channel non-selective protein (NALCN) in the lumen of the endoplasmic reticulum, enhances the activity of sodium leak channels and reduces potassium leak conductance to increase action potential (AP) firing rates, vice versa at night. During the night, microRNA-92a (miR-92a) regulates neuronal excitability by inhibiting SIRT2, a NAD-dependent deacetylase of the Sirtuin family and homolog of mammalian *sir2/3*. This inhibition drives a decrease in calcium influx and depolarization sensitivity. At night, wide-awake (WAKE) expression is upregulated and interacts with the $GABA_A$ channel Resistance to dieldrin (RDL) to decrease spontaneous AP firing rates and resting membrane potential hyperpolarization. The clock-dependent upregulation of WAKE at night also promotes increased slow-poke binding protein (SLOB) and NaKβ. This process then increases the K_{Ca} current and Na/K ATPase activity, altering spike biophysical dynamics to promote regular firing. When posterior DN1s fire regularly, pars intercerebralis (PI) neurons reduce firing rates, increasing sleep quality. During the day, FbxI4 is expressed and readily degrades RDL, which increases spontaneous AP rates and burst firing. Also, WAKE is minimally expressed, and clock neurons display irregular firing rates that drive NMDAR-dependent synaptic plasticity in the synapses between DN1p and PI neurons. This change in activity promotes and increases the firing rate in PI neurons, decreasing sleep quality. Note that not all described molecular mechanisms may function in all clock neurons. AHP, afterhyperpolarization.

THE ROLES OF CRY IN FLIES AND OPSINS IN MAMMALS

When the clock machinery in *Drosophila* is exposed to light, TIM degradation is the initial response (101), which is mediated by CRY (102). In light, the CRY–TIM complex is bound by JETLAG (JET), which facilitates the ubiquitination and degradation of TIM (103, 104). Once TIM is degraded, CRY binds to JET (105). JET degrades CRY in the presence of light, and the affinity balance between CRY/TIM depends on the two different TIM isoforms, which CRY has stronger affinity to short isoform of TIM (106). Therefore, as light periods progress, decreases in CRY protein reset the molecular clock (107) and augment PER and TIM expression (108, 109). Also, without light, the ubiquitin ligase Cullin-3 regulates circadian control of TIM oscillations (110).

In mammalian systems, light stimulates an electrical response, typically in photoreceptors (111), such as rods and cones. Intrinsically photosensitive retinal ganglion cells (ipRGCs) also participate in photoreception (112, 113). These ipRGCs readily express melanopsin, a blue light-sensitive photopigment encoded by the *Opn4* gene (114, 115). In mammalian models, the cell bodies of ipRGCs reside mostly in the outer nuclear layer (ONL) and 5–14% in the inner nuclear layer (INL) (116). A subset of ipRGCs can also be found in the area of the retinal edge called the ciliary marginal zone, inner plexiform layer (IPL) (116). Six classes of ipRGCs (M1–M6) have been characterized (117, 118), which are mainly dictated by where they stratify in the IPL and the synaptic input from bipolar cells [reviewed in (119)]. For example, M1 ipRGCs stratify in the outer sublamina of the IPL, where they form excitatory synapses with bipolar cells that detect light fluctuations; M2, M4, and M5 cells stratify solely in the inner sublamina; and M3 and M6 cells are bistratified in both layers (118, 120). Additionally, ipRGCs have distinct intrinsic membrane properties (118, 121), light responses (120, 122), and areas in the brain to which they send projections [reviewed in (123, 124)].

All classes of ipRGCs express melanopsin, to some capacity, and most express the Brn3b transcription factor. The only exception is a subclass of M1 ipRGCs (125). These Brn3b-negative neurons send projections to the SCN and the VL geniculate nucleus and intergeniculate leaflet in the thalamus (125, 126). Light processed by Brn3b-negative M1 ipRGCs in the retina project to the SCN via the retinohypothalamic tract, distinct from the canonical visual pathway, and synapse with neurons expressing VIP to mediate light-dependent circadian resetting (127, 128). Brn3b-negative M1 ipRGCs transduce light information to clock cells in the SCN through glutamatergic (129, 130) and GABAergic signaling (131). They also generate receptor potential through hyperpolarization-activated cyclic nucleotide–gated (132) and transient receptor potential cation (133) channels. These melanopsin-expressing ipRGCs are necessary for light processing as mammals cannot respond to external light conditions when melanopsin and rhodopsin in the retina are ablated (134). This effect occurs because circadian activity relies on light input information that the retinal ganglion cells receive

and relay through the retinohypothalamic tract to reach the SCN (135).

In *Drosophila*, CRY is expressed throughout the clock network, in photoreceptors in the retina, and peripheral tissues with clock activity (136). However, in mammals, CRY does not have light sensitivity. Instead, it works as a transcriptional regulator (5). In *Drosophila*, the function of CRY has been extensively studied. CRY is expressed in the eyes (137), along with six rhodopsins, and in various populations of clock neurons (138), including s-LNvs, l-LNvs, half of LNds, and some DN1s (**Figure 2A**). This localization allows CRY to integrate light information by modulating the neuronal firing rate through the redox sensor of the voltage-gated potassium-channel β-subunit hyperkinetic, at least in l-LNvs in *Drosophila* (7, 8). In addition, the light-induced electrical activity of certain clusters of clock neurons is regulated by visual structures (139), suggesting that both intrinsic and extrinsic light signals are processed in clock neurons. These data also suggest an interplay between CRY and rhodopsins, which could be observable in the specific light spectrum of wavelengths (140). However, only when CRY and photoreceptor cells (i.e., all six rhodopsins) are removed do clock neurons exhibit circadian blindness (141) in which light entrainment cannot be achieved. Flies express CRY in the rhabdomeres of the photoreceptor cells, which enhances light responses of the circadian clock (142, 143). Moreover, the role of the seventh rhodopsin (Rh7) in sensitizing the CRY-dependent circadian photoresponse has been recently suggested (144). These findings indicate multiple degrees by which photopigments and eyes cooperatively interpret light information.

Interestingly, CRY is expressed in the EB, in what seems to be R5 neurons (137, 145). Because the activity of R5 neurons cycles, the light/clock-generated coding in these cells may have a multilayered structure that influences the circadian clock network and sleep-drive circuits. This theory is supported by a recent paper showing how light-input controls night sleep at the circuit level, independent of the clock (146). Thus, light information could be an important state variable in the relationship between circadian regulation of sleep and sleep homeostasis.

SPIKE CODING IN THE *DROSOPHILA* CIRCADIAN CLOCK

The rate of action potential firing and changes in neuronal excitability have been observed in clock neurons in both mammals and flies (147). In mammals, SCN neurons exhibit spontaneous and signature firing patterns throughout the day, remain silent at night, increase action potential production at dawn, and maintain a steady firing pattern for the rest of the light period (148, 149). Many changes in SCN neuronal activity can be attributed to the intrinsic membrane currents of sodium and potassium (147). Sodium currents lead to increased excitability in the daytime, and potassium currents create a hyperpolarized membrane potential at night (150–152).

In mammals, voltage-gated potassium channels mediate changes in neuronal membrane dynamics in circadian clock neurons (151, 152). Recently, the *Drosophila* model showed that the interaction of CRY with voltage-gated potassium channels mediated changes in neuronal membrane dynamics (8). These channels help stabilize the membrane potential by maintaining it closer to the potassium equilibrium potential. This mechanism of stabilization occurs alongside terminating fast-acting action potentials and by controlling the interspike interval timing during recurrent neuronal firing (153). Stabilization is also augmented by lowering the membrane's sensitivity to excitatory inputs. Prompted by excitatory inputs, voltage-gated potassium channels undergo several inactivated conformational states (154, 155).

In addition to the steady-state transitions of potassium-channel kinetics, many researchers have proposed models for the non-steady-state kinetic activation and inactivation (156–158) of these voltage-gated potassium channels. They found that there are varying kinetic substates where there are more specific closed and inactivation states within these three simplified states. A variety of substates of voltage-gated channels differentially regulate membrane potential dynamics (159), and the proven interaction between CRY and potassium channels is related to beta-subunit (accessory) but not alpha-subunit (core) function (8). Thus, the overarching role of CRY-mediated signaling may be to modulate the timing of these state transitions, which could be a state variable of membrane potential dynamics. However, further studies are needed to support this speculation. In addition, Agrawal et al. showed an interaction between CRY and potassium channels in salivary glands (160). Although the association between CRY and potassium channels was found without the involvement of light, these data also support the idea that the signaling complex between CRY and voltage-gated potassium channels regulate membrane potential dynamics.

In response to upstream clock signaling, *Drosophila* show daily modulations of neuronal electrophysiological properties in clock neurons. Early work used local field potentials to detect changes in electrophysiological activity during dynamic sleep–wake states (161). Specifically, changes in potassium channels (162–164), sodium channels (39), and other modulatory molecules (165) regulate membrane potential-related changes. When exposed to light, flies use CRY signaling to increase action potential firing in l-LN_{VS} (7). In this process, CRY binds to the Shaker voltage-gated potassium-channel β-subunit channel subunit hyperkinetic (8), a protein implicated in sleep-dependent memory. This binding induces flavin redox-mediated regulation of potassium conductance (8). Additionally, Quasimodo (166), a light-responsive factor, modulates the firing rhythm of clock neurons via Shaw, a Kv3.1 potassium voltage-gated channel, and a sodium-potassium-chloride (Na^+ K^+ Cl^-) cotransporter. Because Quasimodo is involved in multiple components, further studies need to elucidate the interactive mechanism that modulates the membrane potential dynamics of l-LNvs. Shal/Kv4, a voltage-gated potassium channel, was sufficient to modulate sleep–wake transitions by suppressing time-specific rates of neuronal firing (167). Fernandez-Chiappe et al. performed a screen that identified hyperpolarization-activated cation current (168). They showed that this current is important for high-frequency bursting of l-LNvs (168). Apart

from molecular modulation of electrophysiological activity in neurons, slow-wave oscillations can confer increased sleep need in flies, further implicating the importance of electrical activity with behavioral states (93). These observations suggest that clock neurons readily manipulate ion conductance by modulating potassium activation–inactivation states to mediate changes in neuronal excitability and behavioral states.

Due to the growing body of literature on circadian clock neurons and changes in neuronal excitability, many new models attempt to explain how circadian oscillations are maintained in relation to changing excitability states. For example, output molecules that interact with core clock genes may reveal insights into the translation of clock information to behavior. Early models proposed that circadian-mediated oscillations in resting membrane potentials are driven by circadian cycles (169–171). Flourakis et al. found that membrane potential, regulated by sodium and potassium currents, is mediated by the circadian clock in *Drosophila* (11). *Drosophila* DN1p membrane potentials create rhythms throughout the day, as well as sodium and potassium conductance rhythms, indicating that resting membrane potentials may be mediated by circadian control. NALCN ion channels facilitate these rhythms in membrane conductance through the oscillatory expression of its localization ER protein Nlf-1 (11). Based on these data, Flourakis et al. propose a "bicycle" model in which two cycles of conductances work in opposed temporal phases. In the morning/day, potassium currents decrease, leading to increased sodium leak facilitated by NA/NALCN and more depolarized membrane potential in DN1p neurons that increases "resting" sodium conductance (172) and their firing rates. The opposite occurs in the evening/night period. This study supports a link between the circadian rhythm and membrane excitability in clock neurons, suggesting that the clock drives molecular rhythms and also physiological changes that maintain robust daily cycles.

Much of the literature deciphers the possible mechanisms through which the circadian clock can modulate neuronal membrane potential. However, fly researchers have started to study clock neurons and their changing excitability as they relate to sleep structure. Recently, Tabuchi et al. (12) questioned whether temporal codes (the timing and pattern of neuronal firing) can induce changes in neuronal firing rates or other related physiological behaviors. This study expanded previous work showing that sensory stimuli can induce temporal codes in target neurons (19). Tabuchi et al. found that DN1p neurons exhibited circadian-dependent spiking patterns with distinct characteristics based on daytime and nighttime settings (12). Specifically, in DN1p neurons, the daytime temporal code consisted of an irregular spike train with a second-order temporal structure, whereas the nighttime temporal code had a more regular pattern. The second-order temporal structure was defined by the probabilistic density of adjacent pairs of interspike intervals. They also found that these temporal spike patterns are generated by WAKE.

Oscillations in WAKE expression mediate the sleep quality by interacting with the calcium-dependent potassium channel (KCa) and a novel sodium/potassium ATPase β subunit, which are upregulated at night under clock and WAKE control. Upregulation of KCa activity leads to a deeper after hyperpolarization of DN1p spikes, which slows firing during periods of increased input. Conversely, sodium/potassium ATPase activity accelerates spike onset, which maintains spiking during periods of reduced input. The combination of increased KCa and sodium/potassium ATPase activity promotes spike morphologies with faster onset and deeper afterhyperpolarization, which leads to regular firing and greater sleep quality at night (**Figure 3**).

Tabuchi et al. also demonstrated a causal role for temporal coding in sleep behavior. They found that circadian-dependent changes in the spiking pattern of DN1p clock neurons encodes arousal and regulates sleep (12). However, we still do not know how DN1p clock neurons transmit the spiking pattern information to downstream neurons. DN1p clock neurons directly project to an arousal circuit in the pars intercerebralis (PI) to regulate sleep/wake behavior (173, 174). Tabuchi et al. examined how a cyclic spiking pattern in DN1p neurons affects downstream signaling. They discovered that only the irregular DN1p firing pattern increased the downstream PI neuron firing rate. This transmission of DN1p temporal codes to PI neuron rate codes was mediated by a novel form of synaptic plasticity driven solely by the temporal pattern of neural spiking. These observations implicate that temporal spike patterns may modulate behaviors, such as sleep, and induce synaptic plasticity in downstream targets (**Figure 3**).

These studies further our knowledge about how neurons can modulate output behaviors through changes in physiology. The circadian clock may modulate behavior through multiple mechanisms in which clock-dependent molecules influence membrane excitability and temporal spike codes. More circadian-driven behaviors could be modulated through temporal spiking patterns because clock neurons may use multiple distinct codes. However, further studies are needed to closely assess how environmental light can modulate membrane excitability and temporal spike codes.

CRY BRIDGES LIGHT INPUT AND SPIKE PATTERN

CRY is located in PDF^+ l-LNvs and s-LNvs, as well as LN_{ds} and DN1s (6, 138, 175), where the photoreceptor responds to light pulses by restarting the circadian oscillation of PER and TIM levels. This effect occurs through CRY binding to TIM (176, 177), although it has been reported that TIM degradation for phase delays (which CRY is required) within s-LNvs is neither necessary nor sufficient (178), suggesting a multiplex regulatory system. CRY also influences the rate of action potential firing (7). CRY can modulate membrane depolarization and the action potential firing rate by interacting with and using the Kvß redox sensor hyperkinetic (8). Wang et al. found evidence that mammalian systems have a non-transcriptional pathway for redox modulation under circadian control (179). They found that K^+ conductance oscillates in SCN neurons, suggesting that neuronal activity can be controlled by protein redox states (179).

As described above (see section The Neural Network that Regulates the Circadian Clock), distinct populations of clock neurons drive the circadian rhythm at different times of day.

As evening descends, the driving force of activity becomes the responsibility of LNds in the presence of light, illustrating the rearrangement of neuronal circuits according to the photoperiod (180). Thus, CRY-mediated signaling may control spike temporal coding to signal changes in behavior. Moreover, depending on the zeitgeber time, changes occur in global circuit switching (181), structural plasticity (182), and temporal spike coding in DN1p neurons (12). This drives neuronal patterning to arrhythmicity and PI neurons to activate through NMDAR-mediated synaptic plasticity, ultimately reducing sleep quality (12). Understanding the heterogeneous nature of DN1p could reveal the significance of differences between molecular signatures and activity.

PROPER MEMORY CONSOLIDATION REQUIRES A FUNCTIONAL CLOCK

The circadian clock may have strong implications in proper memory consolidation. In humans, chronic disruption in the circadian clock has been linked to mild cognitive impairment and even dementia and Alzheimer's disease (183, 184). The search for mechanisms that regulate memory consolidation affected by circadian desynchronization has been challenging in rodent models. Whether clock genes (CRY1 and 2) are genetically knocked out or the SCN is surgically ablated, rodent models seem to have no drastic changes in memory (185). This challenge could mean that for adult-onset memory phenotypes to occur in dysrhythmic animals, the SCN neural network must be intact, both genetically and physically. This theory has been supported by a study in Siberian hamsters. Indeed, Fernandez et al. found that the SCN circuitry must be preserved for an arrhythmic SCN to have deleterious effects on memory (186). They hypothesized that in arrhythmic SCN hamsters, daily GABA signaling from the SCN is disrupted and downstream target memory centers are inhibited (187). This mechanism could also mediate a clock-driven suppression of synaptic plasticity to prime learning centers for continued learning.

The hippocampus has long been implicated in long-term memory (LTM) and may modulate LTM through sleep. Recently, researchers proposed that sharp wave-ripples are important for the consolidation process of LTM that contributes to deficits in emotional memory (188), sequential memory (189), spatial memory (190–192), and synaptic plasticity (193, 194). Interestingly, sleep/wake states can further complicate the consolidation process by modulating sharp wave-ripples through hippocampal pathways (188, 194). These observations suggest that sleep may act upstream of important memory circuits and mechanisms in the hippocampus. Other studies used arrhythmic models to decipher how the circadian clock can further facilitate LTM. They found that the circadian clock modulates synaptic plasticity (195) and spatial memory (187). In addition, the role of astrocytes has recently been suggested. Brancaccio et al. demonstrated that NMDAR expressed in the dorsal SCN is responsible for neuron–astrocyte interactions to suppress SCN neurons during nighttime, suggesting astrocytes control extracellular glutamate circadian cycles to regulate the synchronization of the SCN neural network (196). McCauley et al. (197) also found that astrocytes specific to CA1 hippocampal pyramidal neurons oscillate near these neurons. These astrocytes also cycle NMDAR expression. Ultimately, these changes in receptor expression modify synaptic plasticity to mediate oscillations in hippocampal-dependent learning and implicate an astroglial cell type in modulating circadian-driven changes in behavior. A role of functional connections between astrocytes and l-LNv circadian clock neurons in modulating sleep drive EB in *Drosophila* also supports plastic mechanisms shaped by astrocytes and clock neurons (198).

In *Drosophila*, specific regions of the brain regulate learning and memory pathways. For example, MBs are located in the protocerebrum of the brain, where they are organized into five lobes that process olfactory learning and memory. Furthermore, MBs may also participate in the interplay between sleep regulation and memory centers (87). Due to the downscaled circuitry of the fly brain, a single pair of neurons that function as inputs to MBs, the dorsal paired medial neurons (DPMs), regulate the consolidation of odor memories. DPMs have a dual role in memory consolidation and GABAergic sleep promotion. Specifically, DPMs may inhibit mushroom bodies through GABA and 5-HT signaling to promote memory consolidation and increased sleep (86). Dorsal fan-shaped body neurons (dFBs) also exhibit dual roles in sleep and memory consolidation. In LTM formation, this form of memory consolidation would not occur without thermogenically activated dFB neurons and a courtship training paradigm. Thus, increasing sleep through DPMs and dFBs may simultaneously enhance the power of memory consolidation neurons (199).

Additionally, LTM is also controlled by clock genes, most notably PER. In *Drosophila*, the cAMP–MAPK–CREB pathway may be crucial to memory formation (200). CREB regulates PER expression by binding to an upstream domain, and PER null mutants cannot form LTM after courtship conditioning, indicating that in this context, PER is also crucial in LTM formation (201). In mammals, a similar mechanism may occur. Indeed, sleep deprivation decreases cAMP activity in the hippocampus of mammals, reducing memory consolidation (202). Overall, these studies in mammals and flies strongly suggest that there is a relationship between memory consolidation and circadian rhythms, a relationship that should be further explored.

LIGHT PLAYS A CRITICAL ROLE IN MAINTAINING LTM *VIA* CIRCADIAN CLOCK SIGNALING

To understand if sleep is necessary for memory consolidation, many studies have relied on methods involving sleep deprivation, as well as changes in proteome levels, stress levels, and neural activities. Alternatively, researchers have disrupted sleep continuity in a stress-free manner by optogenetically activating hypocretin (also known as orexin) neurons in mice (203). This approach revealed that only a minimal amount of continuous sleep is needed to properly consolidate memories.

Light, or the absence of light, could also maintain robust LTM. In human studies, certain light exposure can affect cortical areas that are important for cognition. For example,

differing wavelengths of light affected memory and attention (204–206). In rodent models, varying degrees of light exposure modulate tone-cued and contextual fear conditioning (207, 208). Light exposure was also found to modulate long-term potentiation through ipRGC mechanisms, which has been linked to modulating memory consolidation (209). Although these studies link light and cognitive processing, the tasks evaluated in these studies vary according to the type of memory processing. Also, we still do not know the molecular mechanisms that contribute to the connection between light photoreceptors and memory structures.

Recently, environmental light was implicated as necessary for maintaining LTM through PDF^+ l-LNvs. Inami et al. used a memory paradigm involving courtship conditioning to assess how consolidated memory is maintained and how environmental light participates in this process (13). In this study, they entrained flies to the paradigm and then placed them in either constant darkness or constant light. They found that flies in constant darkness exhibited impaired LTM, whereas flies in constant light had intact LTM (13). They also found that in flies exposed to constant darkness, LTM was rescued by activation of PDF^+ neurons. Moreover, in a recent paper, Flyer-Adams et al. showed that PDF is important for regulating olfactory associative memory in *Drosophila* (14). This finding suggests that light/clock-generated information in LTM that is mediated by l-LNvs could be associated with cognitive performance.

CONCLUSIONS

The circadian clock controls both molecular and behavioral rhythms that are needed for many organisms to survive. In this review, we propose that the balance of light (mediated by CRY in Drosophila and melanopsin in mammals) and core clock signaling regulate membrane potential dynamics to modulate synaptic plasticity that depends on neural coding, as well as circuitry, memory, and sleep architecture. Moreover, recent work in *Drosophila* and rodent models uncovered molecular pathways that underlie these changes. In addition, the non-circadian roles for light input and possible implications for brief light treatment therapy have been explored. However, we do not yet have a clear understanding of whether and how CRY can interact with these downstream clock effectors. Finding molecular links between these two mechanisms could reveal other pathways that are restricted to clock neurons and can facilitate circadian-dependent behavior. Future studies are needed to understand the mechanism through which CRY integrates light signals throughout the circadian clock. Importantly, mammalian CRY does not have photosensitivity, which is different from CRY found in *Drosophila*. However, in mammals, the light input system shifts the rhythm via melanopsin-positive ganglion cells in the retina, resulting in sleep–wake effects. Further investigation will help elucidate if the principles of the role of Light/Clock-generated neural coding can be said to be fundamentally conserved between *Drosophila* and mammals.

At the molecular level, computation in the brain starts with proteins, such as voltage-gated ion channels and receptors that can change their structural and functional state in response to environmental changes, such as light. These molecular mechanisms have been adapted into increasingly more complex computational frameworks. In synapses and neurons, local membrane potentials shaped by ion channels and receptors are temporally and spatially integrated. Furthermore, individual neurons are interconnected into networks and circuits, and the circuits are assembled into a brain capable of abstract thought. Not surprisingly, electronic computing has mirrored the same pattern in the engineering world, starting from transistors and integrated circuits to microprocessors and computers. When combined with voltage-gated sodium channels, voltage-gated potassium channels have a crucial function in generating action potentials at the molecular level. To generate action potentials with a specific shape and firing pattern, a neuron needs these voltage-gated ionic channels in appropriate subcellular locations.

How light-induced signaling impacts voltage-gated channels to regulate membrane potential dynamics is unclear. In this review, we propose that a decreased cooperativity of the voltage-gated potassium channel state change timing is vital to generating the higher-order temporal structure of membrane potential dynamics. Mathematically, this state change can be defined as an integrator/differentiator that converts the sum of input signals into an output signal over time, while the output decays steadily. This light-induced state change (mediated by CRY in *Drosophila* and melanopsin in mammals) could be a mechanism that functionally makes some voltage-gated channels unable to simultaneously participate in generating membrane potential dynamics. Therefore, such a decreased cooperativity of these channels must enhance the hysteresis effect and, thus, may lead to the higher-order temporal structure of membrane potential dynamics. Light signaling adds a powerful mechanism for sensing light and controlling the history-dependency of membrane potentials, which is critical for generating specific patterns of spike trains.

As we elucidate clearer connections between light, the circadian clock, and clock-driven behaviors, we can make meaningful efforts to use pharmaceuticals or simply light as a form of treatment against circadian pathologies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

MT was supported by a National Institutes of Health Grant (R00 NS101065) and the Tomizawa Jun-ichi and Keiko Fund of the Molecular Biology Society of Japan for Young Scientists.

ACKNOWLEDGMENTS

The authors thank Ian Blum, Keisuke Sakurai, and Kei Nakatani for insightful communications related to the manuscript.

REFERENCES

1. Hardin PE, Hall JC, Rosbash M. Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. *Nature*. (1990) 343:536–40. doi: 10.1038/343536a0
2. Sehgal A, Rothenfluh-Hilfiker A, Hunter-Ensor M, Chen Y, Myers MP, Young MW. Rhythmic expression of timeless: a basis for promoting circadian cycles in period gene autoregulation. *Science*. (1995) 270:808–10. doi: 10.1126/science.270.5237.808
3. Allada R, White NE, So WV, Hall JC, Rosbash M. A mutant *Drosophila* homolog of mammalian Clock disrupts circadian rhythms and transcription of period and timeless. *Cell*. (1998) 93:791–804. doi: 10.1016/S0092-8674(00)81440-3
4. Emery P, So WV, Kaneko M, Hall JC, Rosbash M. CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell*. (1998) 95:669–79. doi: 10.1016/S0092-8674(00)81637-2
5. Griffin EA, Jr., Staknis D, Weitz CJ. Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. *Science*. (1999) 286:768–71. doi: 10.1126/science.286.5440.768
6. Emery P, Stanewsky R, Helfrich-Förster C, Emery-Le M, Hall JC, Rosbash M. *Drosophila* CRY is a deep brain circadian photoreceptor. *Neuron*. (2000) 26:493–504. doi: 10.1016/S0896-6273(00)81181-2
7. Fogle KJ, Parson KG, Dahm NA, and Holmes TC. CRYPTOCHROME is a blue-light sensor that regulates neuronal firing rate. Science (2011) 331:1409-1413. doi: 10.1126/science.1199702
8. Fogle KJ, Baik LS, Houl JH, Tran TT, Roberts L, Dahm NA, et al. CRYPTOCHROME-mediated phototransduction by modulation of the potassium ion channel β-subunit redox sensor. *Proc Natl Acad Sci USA*. (2015) 112:2245–50. doi: 10.1073/pnas.1416586112
9. Liu S, Lamaze A, Liu Q, Tabuchi M, Yang Y, Fowler M, et al. WIDE AWAKE mediates the circadian timing of sleep onset. *Neuron*. (2014) 82:151–66. doi: 10.1016/j.neuron.2014.01.040
10. Li Q, Li Y, Wang X, Qi J, Jin X, Tong H, et al. Fbxl4 serves as a clock output molecule that regulates sleep through promotion of rhythmic degradation of the GABAA receptor. *Curr Biol*. (2017) 27:3616–25.e3615. doi: 10.1016/j.cub.2017.10.052
11. Flourakis M, Kula-Eversole E, Hutchison AL, Han TH, Aranda K, Moose DL, et al. A conserved bicycle model for circadian clock control of membrane excitability. *Cell*. (2015) 162:836–48. doi: 10.1016/j.cell.2015.07.036
12. Tabuchi M, Monaco JD, Duan G, Bell B, Liu S, Liu Q, et al. Clock-generated temporal codes determine synaptic plasticity to control sleep. *Cell*. (2018) 175:1213–27.e1218. doi: 10.1016/j.cell.2018.09.016
13. Inami S, Sato S, Kondo S, Tanimoto H, Kitamoto T, Sakai T. Environmental light is required for maintenance of long-term memory in *Drosophila*. *J Neurosci*. (2020) 40:1427–39. doi: 10.1523/JNEUROSCI.1282-19.2019
14. Flyer-Adams JG, Rivera-Rodriguez EJ, Yu J, Mardovin JD, Reed ML, Griffith LC. Regulation of olfactory associative memory by the circadian clock output signal pigment-dispersing factor (PDF). *J Neurosci*. (2020) 40:9066–77. doi: 10.1523/JNEUROSCI.0782-20.2020
15. Adrian ED, Zotterman Y. The impulses produced by sensory nerve-endings: part II. The response of a single end-organ. *J Physiol*. (1926) 61:151–71. doi: 10.1113/jphysiol.1926.sp002281
16. Adrian ED, Zotterman Y. The impulses produced by sensory nerve endings: part 3. Impulses set up by touch and pressure. *J Physiol*. (1926) 61:465–83. doi: 10.1113/jphysiol.1926.sp002308
17. Gollisch T, Meister M. Rapid neural coding in the retina with relative spike latencies. *Science*. (2008) 319:1108–111. doi: 10.1126/science.1149639
18. Cassenaer S, Laurent G. Hebbian STDP in mushroom bodies facilitates the synchronous flow of olfactory information in locusts. *Nature*. (2007) 448:709–13. doi: 10.1038/nature05973
19. Panzeri S, Brunel N, Logothetis NK, Kayser C. Sensory neural codes using multiplexed temporal scales. *Trends Neurosci*. (2010) 33:111–20. doi: 10.1016/j.tins.2009.12.001
20. Kostal L, Lansky P, Rospars JP. Neuronal coding and spiking randomness. *Eur J Neurosci*. (2007) 26:2693–701. doi: 10.1111/j.1460-9568.2007.05880.x
21. Shinomoto S, Shima K, Tanji J. Differences in spiking patterns among cortical neurons. *Neural Comput*. (2003) 15:2823–42. doi: 10.1162/089976603322518759
22. Holt GR, Softky WR, Koch C, Douglas RJ. Comparison of discharge variability *in vitro* and *in vivo* in cat visual cortex neurons. *J Neurophysiol*. (1996) 75:1806–14. doi: 10.1152/jn.1996.75.5.1806
23. Stiefel KM, Englitz B, Sejnowski TJ. Origin of intrinsic irregular firing in cortical interneurons. *Proc Natl Acad Sci USA*. (2013) 110:7886–91. doi: 10.1073/pnas.1305219110
24. Rieke F. *Spikes: Exploring the Neural Code*. Cambridge, MA: MIT Press (1997).
25. Izhikevich EM. Polychronization: computation with spikes. *Neural Comput*. (2006) 18:245–82. doi: 10.1162/089976606775093882
26. Takashima A, Hikosaka R, Takahata M. Functional significance of passive and active dendritic properties in the synaptic integration by an identified nonspiking interneuron of crayfish. *J Neurophysiol*. (2006) 96:3157–69. doi: 10.1152/jn.00680.2006
27. Tabuchi M, Dong L, Inoue S, Namiki S, Sakurai T, Nakatani K, et al. Two types of local interneurons are distinguished by morphology, intrinsic membrane properties, and functional connectivity in the moth antennal lobe. *J Neurophysiol*. (2015) 114:3002–13. doi: 10.1152/jn.00050.2015
28. Fusca D, Kloppenburg P. Odor processing in the cockroach antennal lobe-the network components. *Cell Tissue Res*. (2021) 383:59–73. doi: 10.1007/s00441-020-03387-3
29. Bufler J, Zufall F, Franke C, Hatt H. Patch-clamp recordings of spiking and nonspiking interneurons from rabbit olfactory bulb slices: GABA- and other transmitter receptors. *J Comp Physiol A*. (1992) 170:153–9. doi: 10.1007/BF00196897
30. Fujiwara T, Cruz TL, Bohnslav JP, Chiappe ME. A faithful internal representation of walking movements in the *Drosophila* visual system. *Nat Neurosci*. (2017) 20:72–81. doi: 10.1038/nn.4435
31. Papadopoulou M, Cassenaer S, Nowotny T, Laurent G. Normalization for sparse encoding of odors by a wide-field interneuron. *Science*. (2011) 332:721–5. doi: 10.1126/science.1201835
32. Tononi G, Edelman GM. Consciousness and complexity. *Science*. (1998) 282:1846–51. doi: 10.1126/science.282.5395.1846
33. Goldman JS, Tort-Colet N, di Volo M, Susin E, Boute J, Dali M, et al. Bridging single neuron dynamics to global brain states. *Front Syst Neurosci*. (2019) 13:75. doi: 10.3389/fnsys.2019.00075
34. Pospischil M, Piwkowska Z, Bal T, Destexhe A. Characterizing neuronal activity by describing the membrane potential as a stochastic process. *J Physiol Paris*. (2009) 103:98–106. doi: 10.1016/j.jphysparis.2009.05.010
35. van Vreeswijk C, Sompolinsky H. Chaos in neuronal networks with balanced excitatory and inhibitory activity. *Science*. (1996) 274:1724–6. doi: 10.1126/science.274.5293.1724
36. Poznanski RR. Thermal noise due to surface-charge effects within the Debye layer of endogenous structures in dendrites. *Phys Rev E Stat Nonlin Soft Matter Phys*. (2010) 81:021902. doi: 10.1103/PhysRevE.81.021902
37. Chakrapani S, Cordero-Morales JF, Perozo E. A quantitative description of KcsA gating II: single-channel currents. *J Gen Physiol*. (2007) 130:479–96. doi: 10.1085/jgp.200709844
38. Lin M, Hatcher JT, Wurster RD, Chen QH, Cheng ZJ. Characteristics of single large-conductance Ca2+-activated K+ channels and their regulation of action potentials and excitability in parasympathetic cardiac motoneurons in the nucleus ambiguus. American journal of physiology. *Cell Physiol*. (2014) 306:C152–66. doi: 10.1152/ajpcell.00423.2012
39. Ellerkmann RK, Riazanski V, Elger CE, Urban BW, Beck H. Slow recovery from inactivation regulates the availability of voltage-dependent Na(+) channels in hippocampal granule cells, hilar neurons and basket cells. *J Physiol*. (2001) 532:385–97. doi: 10.1111/j.1469-7793.2001.0385f.x
40. Brigham M, Destexhe A. Nonstationary filtered shot-noise processes and applications to neuronal membranes. *Phys Rev E Stat Nonlin Soft Matter Phys*. (2015) 91:062102. doi: 10.1103/PhysRevE.91.062102
41. Wolfart J, Debay D, Le Masson G, Destexhe A, Bal T. Synaptic background activity controls spike transfer from thalamus to cortex. *Nat Neurosci*. (2005) 8:1760–7. doi: 10.1038/nn1591
42. Rudolph M, Destexhe A. Inferring network activity from synaptic noise. *J Physiol Paris*. (2004) 98:452–66. doi: 10.1016/j.jphysparis.2005.09.015

43. Cafaro J, Rieke F. Noise correlations improve response fidelity and stimulus encoding. *Nature*. (2010) 468:964–7. doi: 10.1038/nature09570
44. Stemmler M, Usher M, Niebur E. Lateral interactions in primary visual cortex: a model bridging physiology and psychophysics. *Science*. (1995) 269:1877–80. doi: 10.1126/science.7569930
45. Shu Y, Hasenstaub A, McCormick DA. Turning on and off recurrent balanced cortical activity. *Nature*. (2003) 423:288–93. doi: 10.1038/nature01616
46. Siegle JH, Jia X, Durand S, Gale S, Bennett C, Graddis N, et al. Survey of spiking in the mouse visual system reveals functional hierarchy. *Nature*. (2021) doi: 10.1038/s41586-020-03171-x. [Epub ahead of print].
47. Cossart R, Aronov D, Yuste R. Attractor dynamics of network UP states in the neocortex. *Nature*. (2003) 423:283–8. doi: 10.1038/nature01614
48. Diesmann M, Gewaltig MO, Aertsen A. Stable propagation of synchronous spiking in cortical neural networks. *Nature*. (1999) 402:529–33. doi: 10.1038/990101
49. Beggs JM, Plenz D. Neuronal avalanches in neocortical circuits. *J Neurosci*. (2003) 23:11167–77. doi: 10.1523/JNEUROSCI.23-35-11167.2003
50. Welsh DK, Takahashi JS, Kay SA. Suprachiasmatic nucleus: cell autonomy and network properties. *Annu Rev Physiol*. (2010) 72:551–77. doi: 10.1146/annurev-physiol-021909-135919
51. Abrahamson EE, Moore RY. Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res*. (2001) 916:172–91. doi: 10.1016/S0006-8993(01)02890-6
52. Meijer JH, Watanabe K, Schaap J, Albus H, Detari L. Light responsiveness of the suprachiasmatic nucleus: long-term multiunit and single-unit recordings in freely moving rats. *J Neurosci*. (1998) 18:9078–87. doi: 10.1523/JNEUROSCI.18-21-09078.1998
53. Meijer JH, Groos GA, Rusak B. Luminance coding in a circadian pacemaker: the suprachiasmatic nucleus of the rat and the hamster. *Brain Res*. (1986) 382:109–18. doi: 10.1016/0006-8993(86)90117-4
54. van den Pol AN, Tsujimoto KL. Neurotransmitters of the hypothalamic suprachiasmatic nucleus: immunocytochemical analysis of 25 neuronal antigens. *Neuroscience*. (1985) 15:1049–86. doi: 10.1016/0306-4522(85)90254-4
55. Morin LP, Blanchard J, Moore RY. Intergeniculate leaflet and suprachiasmatic nucleus organization and connections in the golden hamster. *Visual Neurosci*. (1992) 8:219–30. doi: 10.1017/S095252380000287X
56. Moore RY, Speh JC, Leak RK. Suprachiasmatic nucleus organization. *Cell Tissue Res*. (2002) 309:89–98. doi: 10.1007/s00441-002-0575-2
57. Pennartz CM, De Jeu MT, Geurtsen AM, Sluiter AA, Hermes ML. Electrophysiological and morphological heterogeneity of neurons in slices of rat suprachiasmatic nucleus. *J Physiol*. (1998) 506:775–93. doi: 10.1111/j.1469-7793.1998.775bv.x
58. Schaap J, Albus H, VanderLeest HT, Eilers PH, Detari L, Meijer JH. Heterogeneity of rhythmic suprachiasmatic nucleus neurons: implications for circadian waveform and photoperiodic encoding. *Proc Natl Acad Sci USA*. (2003) 100:15994–9. doi: 10.1073/pnas.2436298100
59. Mazuski C, Abel JH, Chen SP, Hermanstyne TO, Jones JR, Simon T, et al. Entrainment of circadian rhythms depends on firing rates and neuropeptide release of VIP SCN neurons. *Neuron*. (2018) 99:555–63.e555. doi: 10.1016/j.neuron.2018.06.029
60. Colwell CS. Linking neural activity and molecular oscillations in the SCN. *Nat Rev Neurosci*. (2011) 12:553–69. doi: 10.1038/nrn3086
61. Pitts GR, Ohta H, McMahon DG. Daily rhythmicity of large-conductance Ca2+ -activated K+ currents in suprachiasmatic nucleus neurons. *Brain Res*. (2006) 1071:54–62. doi: 10.1016/j.brainres.2005.11.078
62. Ewer J, Frisch B, Hamblen-Coyle MJ, Rosbash M, Hall JC. Expression of the period clock gene within different cell types in the brain of *Drosophila* adults and mosaic analysis of these cells' influence on circadian behavioral rhythms. *J Neurosci*. (1992) 12:3321–49. doi: 10.1523/JNEUROSCI.12-09-03321.1992
63. Schubert FK, Hagedorn N, Yoshii T, Helfrich-Forster C, Rieger D. Neuroanatomical details of the lateral neurons of *Drosophila* melanogaster support their functional role in the circadian system. *J Comp Neurol*. (2018) 526:1209–31. doi: 10.1002/cne.24406
64. Allada R, Chung BY. Circadian organization of behavior and physiology in *Drosophila*. *Annu Rev Physiol*. (2010) 72:605–24. doi: 10.1146/annurev-physiol-021909-135815
65. Stoleru D, Peng Y, Nawathean P, Rosbash M. A resetting signal between *Drosophila* pacemakers synchronizes morning and evening activity. *Nature*. (2005) 438:238–42. doi: 10.1038/nature04192
66. Grima B, Chelot E, Xia R, Rouyer F. Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. *Nature*. (2004) 431:869–73. doi: 10.1038/nature02935
67. Alpert MH, Frank DD, Kaspi E, Flourakis M, Zaharieva EE, Allada R, et al. A circuit encoding absolute cold temperature in *Drosophila*. *Curr Biol*. (2020) 30:2275–88.e2275. doi: 10.1016/j.cub.2020.04.038
68. Kunst M, Hughes ME, Raccuglia D, Felix M, Li M, Barnett G, et al. Calcitonin gene-related peptide neurons mediate sleep-specific circadian output in *Drosophila*. *Curr Biol*. (2014) 24:2652–64. doi: 10.1016/j.cub.2014.09.077
69. Johnson EC, Shafer OT, Trigg JS, Park J, Schooley DA, Dow JA, et al. A novel diuretic hormone receptor in *Drosophila*: evidence for conservation of CGRP signaling. *J Exp Biol*. (2005) 208:1239–46. doi: 10.1242/jeb.01529
70. Guo F, Yu J, Jung HJ, Abruzzi KC, Luo W, Griffith LC, et al. Circadian neuron feedback controls the *Drosophila* sleep–activity profile. *Nature*. (2016) 536:292–7. doi: 10.1038/nature19097
71. Zhang Y, Liu Y, Bilodeau-Wentworth D, Hardin PE, Emery P. Light and temperature control the contribution of specific DN1 neurons to *Drosophila* circadian behavior. *Curr Biol*. (2010) 20:600–5. doi: 10.1016/j.cub.2010.02.044
72. Lamaze A, Ozturk-Colak A, Fischer R, Peschel N, Koh K, Jepson JE. Regulation of sleep plasticity by a thermo-sensitive circuit in *Drosophila*. *Sci Rep*. (2017) 7:40304. doi: 10.1038/srep40304
73. Lamaze A, Stanewsky R. DN1p or the "Fluffy" cerberus of clock outputs. *Front Physiol*. (2019) 10:1540. doi: 10.3389/fphys.2019.01540
74. Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A, et al. Rest in *Drosophila* is a sleep-like state. *Neuron*. (2000) 25:129–38. doi: 10.1016/S0896-6273(00)80877-6
75. Shafer OT, Keene AC. The regulation of *Drosophila* sleep. *Curr Biol*. (2021) 31:R38–49. doi: 10.1016/j.cub.2020.10.082
76. Huber R, Hill SL, Holladay C, Biesiadecki M, Tononi G, Cirelli C. Sleep homeostasis in *Drosophila* melanogaster. *Sleep*. (2004) 27:628–39. doi: 10.1093/sleep/27.4.628
77. Joiner WJ, Crocker A, White BH, Sehgal A. Sleep in *Drosophila* is regulated by adult mushroom bodies. *Nature*. (2006) 441:757–60. doi: 10.1038/nature04811
78. Pitman JL, McGill JJ, Keegan KP, Allada R. A dynamic role for the mushroom bodies in promoting sleep in *Drosophila*. *Nature*. (2006) 441:753–6. doi: 10.1038/nature04739
79. Liu Q, Liu S, Kodama L, Driscoll MR, Wu MN. Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in *Drosophila*. *Curr Biol*. (2012) 22:2114–23. doi: 10.1016/j.cub.2012.09.008
80. Ni JD, Gurav AS, Liu W, Ogunmowo TH, Hackbart H, Elsheikh A, et al. Differential regulation of the *Drosophila* sleep homeostat by circadian and arousal inputs. *Elife*. (2019) 8:e40487. doi: 10.7554/eLife.40487
81. Liu S, Liu Q, Tabuchi M, Wu MN. Sleep drive is encoded by neural plastic changes in a dedicated circuit. *Cell*. (2016) 165:1347–60. doi: 10.1016/j.cell.2016.04.013
82. Lamaze A, Kratschmer P, Chen KF, Lowe S, Jepson JEC. A wake-promoting circadian output circuit in *Drosophila*. *Curr Biol*. (2018) 28:3098–105.e3093. doi: 10.1016/j.cub.2018.07.024
83. Guo F, Holla M, Diaz MM, Rosbash M. A circadian output circuit controls sleep-wake arousal in *Drosophila*. *Neuron*. (2018) 100:624–35.e624. doi: 10.1016/j.neuron.2018.09.002
84. Artiushin G, Sehgal A. The *Drosophila* circuitry of sleep-wake regulation. *Curr Opin Neurobiol*. (2017) 44:243–50. doi: 10.1016/j.conb.2017.03.004
85. Yuan Q, Joiner WJ, Sehgal A. A sleep-promoting role for the *Drosophila* serotonin receptor 1A. *Curr Biol*. (2006) 16:1051–62. doi: 10.1016/j.cub.2006.04.032
86. Haynes PR, Christmann BL, Griffith LC. A single pair of neurons links sleep to memory consolidation in *Drosophila* melanogaster. *Elife*. (2015) 4:e0368. doi: 10.7554/eLife.03868
87. Sitaraman D, Aso Y, Jin X, Chen N, Felix M, Rubin GM, et al. Propagation of homeostatic sleep signals by segregated synaptic microcircuits of the *Drosophila* mushroom body. *Curr Biol*. (2015) 25:2915–27. doi: 10.1016/j.cub.2015.09.017

88. Donlea JM, Pimentel D, Miesenbock G. Neuronal machinery of sleep homeostasis in *Drosophila*. *Neuron*. (2014) 81:1442. doi: 10.1016/j.neuron.2014.03.008
89. Donlea JM, Pimentel D, Talbot CB, Kempf A, Omoto JJ, Hartenstein V, et al. Recurrent circuitry for balancing sleep need and sleep. *Neuron*. (2018) 97:378–89.e374. doi: 10.1016/j.neuron.2017.12.016
90. Ueno T, Tomita J, Tanimoto H, Endo K, Ito K, Kume S, et al. Identification of a dopamine pathway that regulates sleep and arousal in *Drosophila*. *Nat Neurosci*. (2012) 15:1516–23. doi: 10.1038/nn.3238
91. Lebestky T, Chang JS, Dankert H, Zelnik L, Kim YC, Han KA, et al. Two different forms of arousal in *Drosophila* are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. *Neuron*. (2009) 64:522–36. doi: 10.1016/j.neuron.2009.09.031
92. Omoto JJ, Keles MF, Nguyen BM, Bolanos C, Lovick JK, Frye MA, et al. Visual Input to the *Drosophila* central complex by developmentally and functionally distinct neuronal populations. *Curr Biol*. (2017) 27:1098–110. doi: 10.1016/j.cub.2017.02.063
93. Raccuglia D, Huang S, Ender A, Heim MM, Laber D, Suarez-Grimalt R, et al. Network-specific synchronization of electrical slow-wave oscillations regulates sleep drive in *Drosophila*. *Curr Biol*. (2019) 29:3611–21.e3613. doi: 10.1016/j.cub.2019.08.070
94. Scammell TE, Arrigoni E, Lipton JO. Neural circuitry of wakefulness and sleep. *Neuron*. (2017) 93:747–65. doi: 10.1016/j.neuron.2017.01.014
95. Branch AF, Navidi W, Tabuchi S, Terao A, Yamanaka A, Scammell TE, et al. Progressive loss of the orexin neurons reveals dual effects on wakefulness. *Sleep*. (2016) 39:369–77. doi: 10.5665/sleep.5446
96. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron*. (2003) 38:701–13. doi: 10.1016/S0896-6273(03)00331-3
97. Eban-Rothschild A, Appelbaum L, de Lecea L. Neuronal mechanisms for sleep/wake regulation and modulatory drive. *Neuropsychopharmacology*. (2018) 43:937–52. doi: 10.1038/npp.2017.294
98. Blum ID, Bell B, Wu MN. Time for bed: genetic mechanisms mediating the circadian regulation of sleep. *Trends Genet*. (2018) 34:379–88. doi: 10.1016/j.tig.2018.01.001
99. Bell BJ, Wang AA, Kim DW, Xiong J, Blackshaw S, Wu MN. Characterization of mWake expression in the murine brain. *J Comp Neurol*. (2020) 1–34. doi: 10.1101/2020.05.25.114363
100. Chen X, Rosbash M. MicroRNA-92a is a circadian modulator of neuronal excitability in *Drosophila*. *Nat Commun*. (2017) 8:14707. doi: 10.1038/ncomms14707
101. Zeng H, Qian Z, Myers MP, Rosbash M. A light-entrainment mechanism for the *Drosophila* circadian clock. *Nature*. (1996) 380:129–35. doi: 10.1038/380129a0
102. Ceriani MF, Darlington TK, Staknis D, Mas P, Petti AA, Weitz CJ, et al. Light-dependent sequestration of TIMELESS by CRYPTOCHROME. *Science*. (1999) 285:553–6. doi: 10.1126/science.285.5427.553
103. Myers MP, Wager-Smith K, Rothenfluh-Hilfiker A, Young MW. Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science*. (1996) 271:1736–40. doi: 10.1126/science.271.5256.1736
104. Koh K, Zheng X, Sehgal A. JETLAG resets the *Drosophila* circadian clock by promoting light-induced degradation of TIMELESS. *Science*. (2006) 312:1809–12. doi: 10.1126/science.1124951
105. Sathyanarayanan S, Zheng X, Kumar S, Chen CH, Chen D, Hay B, et al. Identification of novel genes involved in light-dependent CRY degradation through a genome-wide RNAi screen. *Genes Dev*. (2008) 22:1522–33. doi: 10.1101/gad.1652308
106. Peschel N, Chen KF, Szabo G, Stanewsky R. Light-dependent interactions between the *Drosophila* circadian clock factors cryptochrome, jetlag, and timeless. *Curr Biol.* (2009) 19:241–7. doi: 10.1016/j.cub.2008.12.042
107. Crosthwaite SK, Loros JJ, Dunlap JC. Light-induced resetting of a circadian clock is mediated by a rapid increase in frequency transcript. *Cell.* (1995) 81:1003–12. doi: 10.1016/S0092-8674(05)80005-4
108. Collins B, Mazzoni EO, Stanewsky R, Blau J. *Drosophila* CRYPTOCHROME is a circadian transcriptional repressor. *Curr Biol*. (2006) 16:441–9. doi: 10.1016/j.cub.2006.01.034
109. Kim EY, Bae K, Ng FS, Glossop NR, Hardin PE, Edery I. *Drosophila* CLOCK protein is under posttranscriptional control and influences light-induced activity. *Neuron*. (2002) 34:69–81. doi: 10.1016/S0896-6273(02)00639-6
110. Grima B, Dognon A, Lamouroux A, Chelot E, Rouyer F. CULLIN-3 controls TIMELESS oscillations in the *Drosophila* circadian clock. *PLoS Biol*. (2012) 10:e1001367. doi: 10.1371/journal.pbio.1001367
111. Nakatani K, Chen C, Yau KW, Koutalos Y. Calcium and phototransduction. *Adv Exp Med Biol*. (2002) 514:1–20. doi: 10.1007/978-1-4615-0121-3_1
112. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. (2002) 295:1070–3. doi: 10.1126/science.1067262
113. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. (2002) 295:1065–70. doi: 10.1126/science.1069609
114. Fu Y, Zhong H, Wang MH, Luo DG, Liao HW, Maeda H, et al. Intrinsically photosensitive retinal ganglion cells detect light with a vitamin A-based photopigment, melanopsin. *Proc Natl Acad Sci USA*. (2005) 102:10339–44. doi: 10.1073/pnas.0501866102
115. Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: an opsin in melanophores, brain, and eye. *Proc Natl Acad Sci USA*. (1998) 95:340–5. doi: 10.1073/pnas.95.1.340
116. Valiente-Soriano FJ, Garcia-Ayuso D, Ortin-Martinez A, Jimenez-Lopez M, Galindo-Romero C, Villegas-Perez MP, et al. Distribution of melanopsin positive neurons in pigmented and albino mice: evidence for melanopsin interneurons in the mouse retina. *Front Neuroanat*. (2014) 8:131. doi: 10.3389/fnana.2014.00131
117. Ecker JL, Dumitrescu ON, Wong KY, Alam NM, Chen SK, LeGates T, et al. Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. *Neuron*. (2010) 67:49–60. doi: 10.1016/j.neuron.2010.05.023
118. Schmidt TM, Kofuji P. Functional and morphological differences among intrinsically photosensitive retinal ganglion cells. *J Neurosci*. (2009) 29:476–82. doi: 10.1523/JNEUROSCI.4117-08.2009
119. Sondereker KB, Stabio ME, Renna JM. Crosstalk: the diversity of melanopsin ganglion cell types has begun to challenge the canonical divide between image-forming and non-image-forming vision. *J Comp Neurol*. (2020) 528:2044–67. doi: 10.1002/cne.24873
120. Schmidt TM, Taniguchi K, Kofuji P. Intrinsic and extrinsic light responses in melanopsin-expressing ganglion cells during mouse development. *J Neurophysiol*. (2008) 100:371–84. doi: 10.1152/jn.00062.2008
121. Tu DC, Zhang D, Demas J, Slutsky EB, Provencio I, Holy TE, et al. Physiologic diversity and development of intrinsically photosensitive retinal ganglion cells. *Neuron*. (2005) 48:987–99. doi: 10.1016/j.neuron.2005.09.031
122. Sekaran S, Foster RG, Lucas RJ, Hankins MW. Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Curr Biol*. (2003) 13:1290–8. doi: 10.1016/S0960-9822(03)00510-4
123. Schmidt TM, Chen SK, Hattar S. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends Neurosci*. (2011) 34:572–80. doi: 10.1016/j.tins.2011.07.001
124. Sonoda T, Schmidt TM. Re-evaluating the role of intrinsically photosensitive retinal ganglion cells: new roles in image-forming functions. *Integr Comp Biol*. (2016) 56:834–41. doi: 10.1093/icb/icw066
125. Chen SK, Badea TC, Hattar S. Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. *Nature*. (2011) 476:92–5. doi: 10.1038/nature10206
126. Li JY, Schmidt TM. Divergent projection patterns of M1 ipRGC subtypes. *J Comp Neurol*. (2018) 526:2010–8. doi: 10.1002/cne.24469
127. Vosko A, van Diepen HC, Kuljis D, Chiu AM, Heyer D, Terra H, et al. Role of vasoactive intestinal peptide in the light input to the circadian system. *Eur J Neurosci*. (2015) 42:1839–48. doi: 10.1111/ejn.12919
128. Aton SJ, Colwell CS, Harmar AJ, Waschek J, Herzog ED. Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nat Neurosci*. (2005) 8:476–83. doi: 10.1038/nn1419
129. Purrier N, Engeland WC, Kofuji P. Mice deficient of glutamatergic signaling from intrinsically photosensitive retinal ganglion cells exhibit abnormal circadian photoentrainment. *PLoS ONE*. (2014) 9:e111449. doi: 10.1371/journal.pone.0111449
130. Moldavan MG, Sollars PJ, Lasarev MR, Allen CN, Pickard GE. Circadian behavioral responses to light and optic chiasm-evoked glutamatergic EPSCs in the suprachiasmatic nucleus of ipRGC conditional vGlut2 knock-out mice. *eNeuro*. (2018) 5:ENEURO.0411-17.2018. doi: 10.1523/ENEURO.0411-17.2018

131. Sonoda T, Li JY, Hayes NW, Chan JC, Okabe Y, Belin S, et al. A noncanonical inhibitory circuit dampens behavioral sensitivity to light. *Science*. (2020) 368:527–31. doi: 10.1126/science.aay3152
132. Jiang Z, Yue WWS, Chen L, Sheng Y, Yau KW. Cyclic-nucleotide- and HCN-channel-mediated phototransduction in intrinsically photosensitive retinal ganglion cells. *Cell*. (2018) 175:652–64.e612. doi: 10.1016/j.cell.2018.08.055
133. Warren EJ, Allen CN, Brown RL, Robinson DW. The light-activated signaling pathway in SCN-projecting rat retinal ganglion cells. *Eur J Neurosci*. (2006) 23:2477–87. doi: 10.1111/j.1460-9568.2006.04777.x
134. Guler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature*. (2008) 453:102–5. doi: 10.1038/nature06829
135. McNeill DS, Altimus CM, Hattar S. Retina-clock relations dictate nocturnal to diurnal behaviors. *Proc Natl Acad Sci USA*. (2008) 105:12645–6. doi: 10.1073/pnas.0806878105
136. Peirson SN, Butler JN, Duffield GE, Takher S, Sharma P, Foster RG. Comparison of clock gene expression in SCN, retina, heart, and liver of mice. *Biochem Biophys Res Commun*. (2006) 351:800–7. doi: 10.1016/j.bbrc.2006.10.118
137. Yoshii T, Todo T, Wulbeck C, Stanewsky R, Helfrich-Forster C. Cryptochrome is present in the compound eyes and a subset of *Drosophila's* clock neurons. *J Comp Neurol*. (2008) 508:952–66. doi: 10.1002/cne.21702
138. Benito J, Houl JH, Roman GW, Hardin PE. The blue-light photoreceptor CRYPTOCHROME is expressed in a subset of circadian oscillator neurons in the *Drosophila* CNS. *J Biol Rhythms*. (2008) 23:296–307. doi: 10.1177/0748730408318588
139. Li MT, Cao LH, Xiao N, Tang M, Deng B, Yang T, et al. Hub-organized parallel circuits of central circadian pacemaker neurons for visual photoentrainment in *Drosophila*. *Nat Commun*. (2018) 9:4247. doi: 10.1038/s41467-018-06506-5
140. Hanai S, Ishida N. Entrainment of *Drosophila* circadian clock to green and yellow light by Rh1, Rh5, Rh6 and CRY. *Neuroreport*. (2009) 20:755–8. doi: 10.1097/WNR.0b013e32832a7c4e
141. Helfrich-Forster C, Winter C, Hofbauer A, Hall JC, Stanewsky R. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron*. (2001) 30:249–61. doi: 10.1016/S0896-6273(01)00277-X
142. Mazzotta G, Rossi A, Leonardi E, Mason M, Bertolucci C, Caccin L, et al. Fly cryptochrome and the visual system. *Proc Natl Acad Sci USA*. (2013) 110:6163–8. doi: 10.1073/pnas.1212317110
143. Schlichting M, Rieger D, Cusumano P, Grebler R, Costa R, Mazzotta GM, et al. Cryptochrome interacts with actin and enhances eye-mediated light sensitivity of the circadian clock in *Drosophila* melanogaster. *Front Mol Neurosci*. (2018) 11:238. doi: 10.3389/fnmol.2018.00238
144. Ni JD, Baik LS, Holmes TC, Montell C. A rhodopsin in the brain functions in circadian photoentrainment in *Drosophila*. *Nature*. (2017) 545:340–4. doi: 10.1038/nature22325
145. Liang X, Ho MCW, Zhang Y, Li Y, Wu MN, Holy TE, et al. Morning and evening circadian pacemakers independently drive premotor centers via a specific dopamine relay. *Neuron*. (2019) 102:843–57.e844. doi: 10.1016/j.neuron.2019.03.028
146. Chen KF, Lowe S, Lamaze A, Kratschmer P, Jepson J. Neurocalcin regulates nighttime sleep and arousal in *Drosophila*. *Elife*. (2019) 8:e38114. doi: 10.7554/eLife.38114
147. Allen CN, Nitabach MN, Colwell CS. Membrane currents, gene expression, and circadian clocks. *Cold Spring Harb Perspect Biol*. (2017) 9:a027714. doi: 10.1101/cshperspect.a027714
148. Belle MD, Diekman CO, Forger DB, Piggins HD. Daily electrical silencing in the mammalian circadian clock. *Science*. (2009) 326:281–4. doi: 10.1126/science.1169657
149. Jackson AC, Yao GL, Bean BP. Mechanism of spontaneous firing in dorsomedial suprachiasmatic nucleus neurons. *J Neurosci*. (2004) 24:7985–98. doi: 10.1523/JNEUROSCI.2146-04.2004
150. Huang RC. Sodium and calcium currents in acutely dissociated neurons from rat suprachiasmatic nucleus. *J Neurophysiol*. (1993) 70:1692–703. doi: 10.1152/jn.1993.70.4.1692
151. Itri JN, Michel S, Vansteensel MJ, Meijer JH, Colwell CS. Fast delayed rectifier potassium current is required for circadian neural activity. *Nat Neurosci*. (2005) 8:650–6. doi: 10.1038/nn1448
152. Kudo T, Loh DH, Kuljis D, Constance C, Colwell CS. Fast delayed rectifier potassium current: critical for input and output of the circadian system. *J Neurosci*. (2011) 31:2746–55. doi: 10.1523/JNEUROSCI.5792-10.2011
153. Lawrence JJ, Saraga F, Churchill JF, Statland JM, Travis KE, Skinner FK, et al. Somatodendritic Kv7/KCNQ/M channels control interspike interval in hippocampal interneurons. *J Neurosci*. (2006) 26:12325–38. doi: 10.1523/JNEUROSCI.3521-06.2006
154. del Camino D, Yellen G. Tight steric closure at the intracellular activation gate of a voltage-gated K(+) channel. *Neuron*. (2001) 32:649–56. doi: 10.1016/S0896-6273(01)00487-1
155. Liu Y, Holmgren M, Jurman ME, Yellen G. Gated access to the pore of a voltage-dependent K+ channel. *Neuron*. (1997) 19:175–84. doi: 10.1016/S0896-6273(00)80357-8
156. Morais-Cabral JH, Zhou Y, MacKinnon R. Energetic optimization of ion conduction rate by the K+ selectivity filter. *Nature*. (2001) 414:37–42. doi: 10.1038/35102000
157. Grabe M, Bichet D, Qian X, Jan YN, Jan LY. K+ channel selectivity depends on kinetic as well as thermodynamic factors. *Proc Natl Acad Sci USA*. (2006) 103:14361–6. doi: 10.1073/pnas.0606662103
158. Ranjan R, Logette E, Marani M, Herzog M, Tache V, Scantamburlo E, et al. A kinetic map of the homomeric voltage-gated potassium channel (Kv) family. *Front Cell Neurosci*. (2019) 13:358. doi: 10.3389/fncel.2019.00358
159. Teka W, Stockton D, Santamaria F. Power-law dynamics of membrane conductances increase spiking diversity in a Hodgkin-Huxley model. *PLoS Comput Biol*. (2016) 12:e1004776. doi: 10.1371/journal.pcbi.1004776
160. Agrawal P, Houl JH, Gunawardhana KL, Liu T, Zhou J, Zoran MJ, et al. *Drosophila* CRY entrains clocks in body tissues to light and maintains passive membrane properties in a non-clock body tissue independent of light. *Curr Biol*. (2017) 27:2431–41.e2433. doi: 10.1016/j.cub.2017.06.064
161. Nitz DA, van Swinderen B, Tononi G, Greenspan RJ. Electrophysiological correlates of rest and activity in *Drosophila* melanogaster. *Curr Biol*. (2002) 12:1934–40. doi: 10.1016/S0960-9822(02)01300-3
162. Ruben M, Drapeau MD, Mizrak D, Blau J. A mechanism for circadian control of pacemaker neuron excitability. *J Biol Rhythms*. (2012) 27:353–64. doi: 10.1177/0748730412455918
163. Hodge JJ, Stanewsky R. Function of the Shaw potassium channel within the *Drosophila* circadian clock. *PLoS ONE*. (2008) 3:e2274. doi: 10.1371/journal.pone.0002274
164. Pimentel D, Donlea JM, Talbot CB, Song SM, Thurston AJF, Miesenbock G. Operation of a homeostatic sleep switch. *Nature*. (2016) 536:333–7. doi: 10.1038/nature19055
165. Donlea JM, Pimentel D, Miesenböck G. Neuronal machinery of sleep homeostasis in *Drosophila*. *Neuron*. (2014) 81:860–72. doi: 10.1016/j.neuron.2013.12.013
166. Buhl E, Bradlaugh A, Ogueta M, Chen KF, Stanewsky R, Hodge JJ. Quasimodo mediates daily and acute light effects on *Drosophila* clock neuron excitability. *Proc Natl Acad Sci USA*. (2016) 113:13486–91. doi: 10.1073/pnas.1606547113
167. Feng G, Zhang J, Li M, Shao L, Yang L, Song Q, et al. Control of sleep onset by Shal/Kv4 channels in *Drosophila* circadian neurons. *J Neurosci*. (2018) 38:9059–71. doi: 10.1523/JNEUROSCI.0777-18.2018
168. Fernandez-Chiappe F, Frenkel L, Colque CC, Ricciuti A, Hahm B, Cerredo K, et al. High-frequency neuronal bursting is essential for circadian and sleep behaviors in *Drosophila*. *J Neurosci*. (2021) 41:689–710. doi: 10.1523/JNEUROSCI.2322-20.2020
169. Cao G, Nitabach MN. Circadian control of membrane excitability in *Drosophila* melanogaster lateral ventral clock neurons. *J Neurosci*. (2008) 28:6493–501. doi: 10.1523/JNEUROSCI.1503-08.2008
170. Sheeba V, Gu H, Sharma VK, O'Dowd DK, Holmes TC. Circadian- and light-dependent regulation of resting membrane potential and spontaneous action potential firing of *Drosophila* circadian pacemaker neurons. *J Neurophysiol*. (2008) 99:976–88. doi: 10.1152/jn.00930.2007
171. Park D, Griffith LC. Electrophysiological and anatomical characterization of PDF-positive clock neurons in the intact adult *Drosophila* brain. *J Neurophysiol*. (2006) 95:3955–60. doi: 10.1152/jn.00117.2006

172. Gouwens NW, Wilson RI. Signal propagation in *Drosophila* central neurons. *J Neurosci*. (2009) 29:6239–49. doi: 10.1523/JNEUROSCI.0764-09.2009
173. Barber AF, Erion R, Holmes TC, Sehgal A. Circadian and feeding cues integrate to drive rhythms of physiology in *Drosophila* insulin-producing cells. *Genes Dev*. (2016) 30:2596–606. doi: 10.1101/gad.288258.116
174. Cavanaugh DJ, Geratowski JD, Wooltorton JR, Spaethling JM, Hector CE, Zheng X, et al. Identification of a circadian output circuit for rest:activity rhythms in *Drosophila*. *Cell*. (2014) 157:689–701. doi: 10.1016/j.cell.2014.02.024
175. Klarsfeld A, Malpel S, Michard-Vanhée C, Picot M, Chélot E, et al. Novel features of cryptochrome-mediated photoreception in the brain circadian clock of *Drosophila*. *J Neurosci*. (2004) 24:1468–77. doi: 10.1523/JNEUROSCI.3661-03.2004
176. Lin FJ, Song W, Meyer-Bernstein E, Naidoo N, Sehgal A. Photic signaling by cryptochrome in the *Drosophila* circadian system. *Mol Cell Biol*. (2001) 21:7287–94. doi: 10.1128/MCB.21.21.7287-7294.2001
177. Suri V, Qian Z, Hall JC, Rosbash M. Evidence that the TIM light response is relevant to light-induced phase shifts in *Drosophila* melanogaster. *Neuron*. (1998) 21:225–34. doi: 10.1016/S0896-6273(00)80529-2
178. Tang CH, Hinteregger E, Shang Y, Rosbash M. Light-mediated TIM degradation within *Drosophila* pacemaker neurons (s-LNvs) is neither necessary nor sufficient for delay zone phase shifts. *Neuron*. (2010) 66:378–85. doi: 10.1016/j.neuron.2010.04.015
179. Wang TA, Yu YV, Govindaiah G, Ye X, Artinian L, Coleman TP, et al. Circadian rhythm of redox state regulates excitability in suprachiasmatic nucleus neurons. *Science*. (2012) 337:839–42. doi: 10.1126/science.1222826
180. Chatterjee A, Lamaze A, De J, Mena W, Chelot E, Martin B, et al. Reconfiguration of a multi-oscillator network by light in the *Drosophila* circadian clock. *Curr Biol*. (2018) 28:2007–17.e2004. doi: 10.1016/j.cub.2018.04.064
181. Schlichting M, Weidner P, Diaz M, Menegazzi P, Dalla Benetta E, Helfrich-Forster C, et al. Light-mediated circuit switching in the *Drosophila* neuronal clock network. *Curr Biol*. (2019) 29:3266–76.e3263. doi: 10.1016/j.cub.2019.08.033
182. Fernandez MP, Pettibone HL, Bogart JT, Roell CJ, Davey CE, Pranevicius A, et al. Sites of circadian clock neuron plasticity mediate sensory integration and entrainment. *Curr Biol*. (2020) 30:2225–37.e2225. doi: 10.1016/j.cub.2020.04.025
183. Tranah GJ, Blackwell T, Stone KL, Ancoli-Israel S, Paudel ML, Ensrud KE, et al. Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. *Ann Neurol*. (2011) 70:722–32. doi: 10.1002/ana.22468
184. Wright KP, Lowry CA, Lebourgeois MK. Circadian and wakefulness-sleep modulation of cognition in humans. *Front Mol Neurosci*. (2012) 5:50. doi: 10.3389/fnmol.2012.00050
185. Van der Zee EA, Havekes R, Barf RP, Hut RA, Nijholt IM, Jacobs EH, et al. Circadian time-place learning in mice depends on Cry genes. *Curr Biol*. (2008) 18:844–8. doi: 10.1016/j.cub.2008.04.077
186. Fernandez F, Lu D, Ha P, Costacurta P, Chavez R, Heller HC, et al. Circadian rhythm. Dysrhythmia in the suprachiasmatic nucleus inhibits memory processing. *Science*. (2014) 346:854–7. doi: 10.1126/science.1259652
187. Ruby NF, Fernandez F, Garrett A, Klima J, Zhang P, Sapolsky R, et al. Spatial memory and long-term object recognition are impaired by circadian arrhythmia and restored by the GABAAAntagonist pentylenetetrazole. *PLoS ONE*. (2013) 8:e72433. doi: 10.1371/journal.pone.0072433
188. Girardeau G, Inema I, Buzsaki G. Reactivations of emotional memory in the hippocampus-amygdala system during sleep. *Nat Neurosci*. (2017) 20:1634–42. doi: 10.1038/nn.4637
189. Lee AK, Wilson MA. Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron*. (2002) 36:1183–94. doi: 10.1016/S0896-6273(02)01096-6
190. Ego-Stengel V, Wilson MA. Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. *Hippocampus*. (2010) 20:1–10. doi: 10.1002/hipo.20707
191. Girardeau G, Benchenane K, Wiener SI, Buzsaki G, Zugaro MB. Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci*. (2009) 12:1222–3. doi: 10.1038/nn.2384
192. Roux L, Hu B, Eichler R, Stark E, Buzsaki G. Sharp wave ripples during learning stabilize the hippocampal spatial map. *Nat Neurosci*. (2017) 20:845–53. doi: 10.1038/nn.4543
193. van de Ven GM, Trouche S, McNamara CG, Allen K, Dupret D. Hippocampal offline reactivation consolidates recently formed cell assembly patterns during sharp wave-ripples. *Neuron*. (2016) 92:968–74. doi: 10.1016/j.neuron.2016.10.020
194. Norimoto H, Makino K, Gao M, Shikano Y, Okamoto K, Ishikawa T, et al. Hippocampal ripples down-regulate synapses. *Science*. (2018) 359:1524–7. doi: 10.1126/science.aao0702
195. Chaudhury D, Wang LM, Colwell CS. Circadian regulation of hippocampal long-term potentiation. *J Biol Rhythms*. (2005) 20:225–36. doi: 10.1177/0748730405276352
196. Brancaccio M, Patton AP, Chesham JE, Maywood ES, Hastings MH. Astrocytes control circadian timekeeping in the suprachiasmatic nucleus via glutamatergic signaling. *Neuron*. (2017) 93:1420–35.e1425. doi: 10.1016/j.neuron.2017.02.030
197. McCauley JP, Petroccione MA, D'Brant LY, Todd GC, Affinnih N, Wisnoski JJ, et al. Circadian modulation of neurons and astrocytes controls synaptic plasticity in hippocampal area CA1. *Cell Rep*. (2020) 33:108255. doi: 10.1016/j.celrep.2020.108255
198. Blum ID, Keles MF, Baz ES, Han E, Park K, Luu S, et al. Astroglial calcium signaling encodes sleep need in *Drosophila*. *Curr Biol*. (2021) 31:150–62.e157. doi: 10.1016/j.cub.2020.10.012
199. Donlea JM, Thimgan MS, Suzuki Y, Gottschalk L, Shaw PJ. Inducing sleep by remote control facilitates memory consolidation in *Drosophila*. *Science*. (2011) 332:1571–6. doi: 10.1126/science.1202249
200. Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, et al. Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell*. (1994) 79:49–58. doi: 10.1016/0092-8674(94)90399-9
201. Sakai T, Inami S, Sato S, Kitamoto T. Fan-shaped body neurons are involved in period-dependent regulation of long-term courtship memory in *Drosophila*. *Learn Mem*. (2012) 19:571–4. doi: 10.1101/lm.028092.112
202. Vecsey CG, Baillie GS, Jaganath D, Havekes R, Daniels A, Wimmer M, et al. Sleep deprivation impairs cAMP signalling in the hippocampus. *Nature*. (2009) 461:1122–5. doi: 10.1038/nature08488
203. Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, et al. Optogenetic disruption of sleep continuity impairs memory consolidation. *Proc Natl Acad Sci USA*. (2011) 108:13305–10. doi: 10.1073/pnas.1015633108
204. Vandewalle G, Balteau E, Phillips C, Degueldre C, Moreau V, Sterpenich V, et al. Daytime light exposure dynamically enhances brain responses. *Curr Biol*. (2006) 16:1616–21. doi: 10.1016/j.cub.2006.06.031
205. Vandewalle G, Gais S, Schabus M, Balteau E, Carrier J, Darsaud A, et al. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure. *Cereb Cortex*. (2007) 17:2788–95. doi: 10.1093/cercor/bhm007
206. Perrin F, Peigneux P, Fuchs S, Verhaeghe S, Laureys S, Middleton B, et al. Nonvisual responses to light exposure in the human brain during the circadian night. *Curr Biol*. (2004) 14:1842–6. doi: 10.1016/j.cub.2004.09.082
207. Warthen DM, Wiltgen BJ, Provencio I. Light enhances learned fear. *Proc Natl Acad Sci USA*. (2011) 108:13788–93. doi: 10.1073/pnas.1103214108
208. Shan LL, Guo H, Song NN, Jia ZP, Hu XT, Huang JF, et al. Light exposure before learning improves memory consolidation at night. *Sci Rep*. (2015) 5:15578. doi: 10.1038/srep15578
209. LeGates TA, Altimus CM, Wang H, Lee HK, Yang S, Zhao H, et al. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature*. (2012) 491:594–8. doi: 10.1038/nature11673

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 April 2021
doi: 10.3389/fnins.2021.642376

Suppression of Circadian Timing and Its Impact on the Hippocampus

*Norman F. Ruby**

Biology Department, Stanford University, Stanford, CA, United States

In this article, I describe the development of the disruptive phase shift (DPS) protocol and its utility for studying how circadian dysfunction impacts memory processing in the hippocampus. The suprachiasmatic nucleus (SCN) of the Siberian hamster is a labile circadian pacemaker that is easily rendered arrhythmic (ARR) by a simple manipulation of ambient lighting. The DPS protocol uses room lighting to administer a phase-advancing signal followed by a phase-delaying signal within one circadian cycle to suppress clock gene rhythms in the SCN. The main advantage of this model for inducing arrhythmia is that the DPS protocol is non-invasive; circadian rhythms are eliminated while leaving the animals neurologically and genetically intact. In the area of learning and memory, DPS arrhythmia produces much different results than arrhythmia by surgical ablation of the SCN. As I show, SCN ablation has little to no effect on memory. By contrast, DPS hamsters have an intact, but arrhythmic, SCN which produces severe deficits in memory tasks that are accompanied by fragmentation of electroencephalographic theta oscillations, increased synaptic inhibition in hippocampal circuits, and diminished responsiveness to cholinergic signaling in the dentate gyrus of the hippocampus. The studies reviewed here show that DPS hamsters are a promising model for translational studies of adult onset circadian dysfunction in humans.

Keywords: Siberian, hamster, theta, acetylcholine, dentate gyrus, memory

OPEN ACCESS

Edited by:
Christopher S. Colwell, University of California, Los Angeles, United States

Reviewed by:
Ethan Buhr, University of Washington, United States
Dipesh Chaudhury, New York University Abu Dhabi, United Arab Emirates

***Correspondence:**
Norman F. Ruby
ruby@stanford.edu

Specialty section:
This article was submitted to Sleep and Circadian Rhythms, a section of the journal Frontiers in Neuroscience

Received: *16 December 2020*
Accepted: *17 March 2021*
Published: *08 April 2021*

Citation:
Ruby NF (2021) Suppression of Circadian Timing and Its Impact on the Hippocampus. Front. Neurosci. 15:642376. doi: 10.3389/fnins.2021.642376

INTRODUCTION

The formal properties of the circadian system of Siberian hamsters (a.k.a., Djungarian hamsters; *Phodopus sungorus*) have been thoroughly characterized over the last 50 years, mainly as a model species for studies of melatonin signaling of daylength (Bartness et al., 1993). Over those years, the circadian system of these hamsters has been evaluated for its responses to photic signals in a range of photoperiod studies, entrainment paradigms, and phase responses, all of which showed that circadian responses in this species are similar to those observed in other nocturnal rodents. And yet, when these animals are challenged by a photic phase-advancing stimulus, followed by a photic phase-delaying stimulus less than 24 h later, the opposing light signals completely eliminate circadian timing in the molecular clock in the suprachiasmatic nucleus (SCN) within just a few days.

The unique vulnerability to rhythm disruption in an otherwise typical circadian system suggests that there is a great deal more to learn about photic effects on circadian timing, but it also presents an opportunity to study the adverse impact of circadian disruption in a new way. Animal models of circadian arrhythmia have been limited in their relevance to human circadian disruption because they involve surgical ablation of the SCN or genomic modifications that are obviously

not present in humans, and because they have confounding effects on sleep and stress. By contrast, the circadian-arrhythmic (ARR) hamster lacks these confounds (cf., Larkin et al., 2004), thus allowing us to better model how a malfunctioning SCN affects the progression of human illnesses. The circadian-ARR hamster model has revealed how the loss of circadian timing impairs different types of memory (Fernandez et al., 2014; Müller et al., 2015), immune responses (Prendergast et al., 2013), and has furthered our understanding of sleep homeostasis (Larkin et al., 2004) and ultradian rhythm generation (Prendergast et al., 2012). This article presents the development of the disruptive phase shift (DPS) protocol, and shows how it is useful for modeling circadian influences on hippocampal memory processing, an issue that has become important recently in elderly populations (Tranah et al., 2011; Covell et al., 2012; Coogan et al., 2013).

DEVELOPMENT OF THE DPS MODEL: CHANCE FAVORS THE DISTRACTED MIND

I'd like to say that I developed the DPS model through a combination of deep thinking and meticulous deployment of circadian principles, but in truth, it was an accident. An undergraduate who worked in my laboratory prepared animals for a study, but had to abandon the project to attend to a family matter. After a few weeks, I delayed the light-dark (LD) cycle in the animal room by 5 h so that the lights would come on at the more user-friendly time of 0700 h instead 0200 h (**Figure 1A**). Being preoccupied with other more pressing studies, the project languished. When I plotted the actograms a few weeks later, I found that 10 animals were free-running *through the LD cycle* at periods ranging from 24.3–26.3 h (Ruby et al., 1996). One hamster became completely ARR immediately, while the remaining animal reentrained to the LD cycle (Ruby et al., 1996). If not for that last animal, I would have thrown the data in the trash and started over. But of course, if one animal reentrained, then the lights must be working properly, otherwise, to what zeitgeber was it entrained? Just in case, I went into the lab one morning to watch the lights come on at 7 AM, checked them every hour throughout the day, and waited 16 h to watch them shut off at 11 PM. The lights were fine. Moreover, there were no signs of relative coordination to suggest any photic modulation of the pacemaker.

To get some perspective on these observations, I sent the data to Ben Rusak and Jeff Elliott, to whom I am indebted for their thoughtful insights and encouragement. In addition, Jeff generously shared some of his unpublished data with me that shed light on the hamster entrainment problem. In a past study, Jeff entrained golden hamsters to *T*-cycles of 23 h (0.25 h of light: 22.75 h of dark). Most animals shortened their active phases (i.e., alpha, α) to <4 h (i.e., α compression), at which point entrainment was lost as the animals free-ran through the *T*-cycle. When Jeff subsequently transferred the animals into constant darkness (DD) and administered light pulses across their circadian cycles, he found that they had a flat phase response curve (PRC; Elliott, presented at the International Chronobiology Conference, 1999). Some animals, however, did not exhibit α compression or flat PRCs and thus remained entrained to the *T*-cycle.

In the Siberian hamsters, I had also observed that α compression preceded the free run through the LD cycle which, based on Jeff's work, suggested that the pacemaker was no longer responding to light (Ruby et al., 1996). To test for this, I decided against generating a PRC in the free-runners because their rhythms were a little too messy, so it would be easy for small phase shifts to go undetected. Instead, we put the animals in DD for 24 h, and then administered a light pulse (30 min) early in their active phase and probed the SCN for *c-fos* and *per1* mRNA induction because light pulses rapidly induce expression of these genes (Barakat et al., 2004). In contrast to the control animals, we found absolutely no mRNA induction in the SCN of the free-runners (**Figure 1B**). We placed groups of hamsters in several weeks of DD or constant light (LL), but neither condition had any effect on the period or phase of their rhythms (**Figures 1D2,D3**). We also checked for photic mRNA induction in a group of ARR animals, assuming we would find the same result as with the free-runners, but, as it turned out, the SCN of ARR hamsters was still responsive to light (**Figure 1B**; Barakat et al., 2005). Given that the SCN of ARR hamsters was sensitive to light, it is puzzling that light did not restore rhythms to these animals. As will be discussed later, it also suggested that the underlying mechanism of arrhythmia is different between LL and DPS-induced arrhythmia.

As most students of circadian rhythms know, light in the middle of the night can suppress the amplitude of the pacemaker (Jewett et al., 1991; Honma and Honma, 1999; Leloup and Goldbeter, 2001; Huang et al., 2006). If the light intensity and timing are just right, the light pulse sends the pacemaker into a state of arrhythmia (i.e., singularity). I house my hamsters in 16 h of light and 8 h of dark, thus, on the first day of a 5-h delay of the LD cycle, light extends through the middle of the night (**Figure 1A**), so I decided to test the idea that light exposure in the middle of the night could break entrainment (**Figure 1A**). My results matched Jeff's; animals that compressed α in response to the 5-h delay of the LD cycle lost entrainment, while animals that maintained an uncompressed α successfully reentrained (Ruby et al., 2004). By contrast to a 5-h delay, all hamsters easily reentrained to a 3-h delay of the LD cycle because, presumably, light did not impinge on the middle of the night (**Figure 1A**). To test that idea, hamsters were pre-treated with 2-h of light in the middle of the night, and then, on the next day, the LD cycle was delayed by 3 h to see if the light pulse would impair reentrainment (**Figure 1A**). As controls, I ran two additional groups that were given the 2-h light pulse early or late at night (**Figure 1A**). As it turned out, I was wrong. The light pulse late at night had the greatest impact on reentrainment—none of those animals reentrained. By contrast, all animals reentrained if they got the light pulse early at night, and 58% of animals reentrained if they got the light pulse in the middle of the night (**Figure 1C**, left panel). The percent of animals that reentrained also correlated with the extent of their α compression (**Figure 1C**, right panel). And just as Jeff found, when α was compressed to <4 h as in

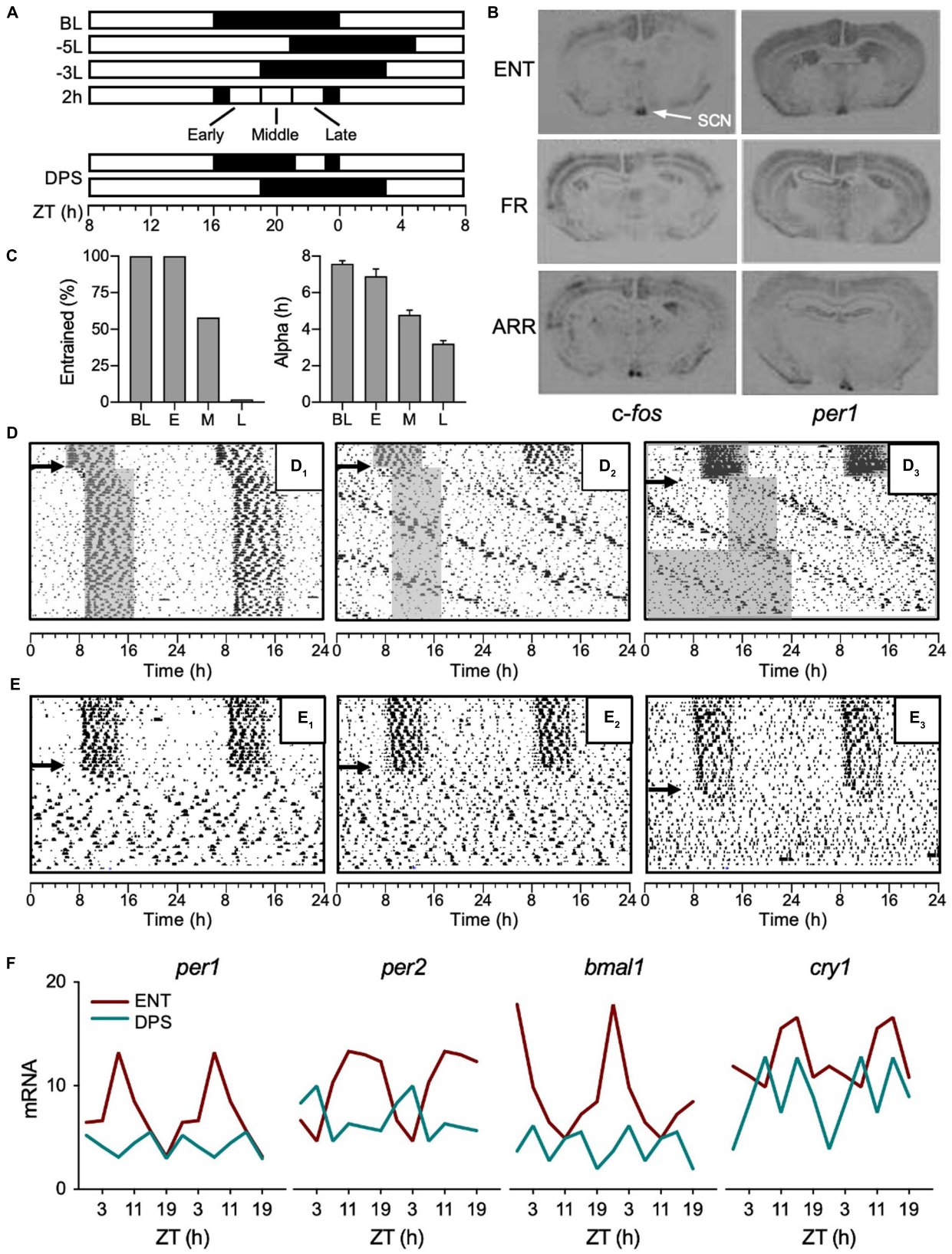

FIGURE 1 | Continued

FIGURE 1 | Development of the DPS protocol. **(A)** Light-dark (LD) cycles given by white and black rectangles, respectively. A phase advance or delay of the LD cycle is indicated by a plus (+) or min (–) sign, respectively, followed by phase shift duration in hours; L or D to indicate whether the shift was accomplished via a change in the light or dark phase, respectively (Aschoff et al., 1975). For example, a 5-h phase delay made by extending the light phase is indicated by –5L. ZT (zeitgeber time), ZT0 = baseline (BL) time of lights-on. **(B)** Coronal brain sections of mRNA hybridization for *c-fos* and *per1* from hamsters that were entrained (ENT), free ran in the LD cycle (FR), or were circadian-arrhythmic (ARR). **(C)** The percent of animals that reentrained (left panel) and the duration of their active phases (α, right panel) after given a 2-h light pulse given early (E), middle (M), or late (L) in the night, and followed by a -3L shift on the following day. **(D)** Representative actograms with consecutive days double plotted from top to bottom of hamsters that reentrained **(D1)** or free-ran after -5L **(D2,D3)**; arrows indicate day of -5L, gray shading indicates nighttime to visualize phase shift. **(E)** Representative actograms of animals that became arrhythmic after the DPS protocol (**E1–E3**, indicated by arrows). **(F)** Mean mRNA values of clock genes (*per1*, *per2*, *bmal1*, and *cry1*) quantified by RT-PCR from the SCN of ENT (red) and ARR (green) hamsters. Error bars removed for clarity. ZT0 = light onset in 16:8 LD cycle. Figures modified from Ruby et al., 1996, 1998, 2004, Barakat et al. (2004, 2005) and Grone et al. (2011).

our "late" group, none of the animals reentrained (**Figure 1C**). Of those animals, ∼30% free-ran and ∼70% became ARR (see **Figures 1D,E** for examples).

A similar finding was reported by Steinlechner et al. (2002) using a comparable method, but in their protocol, hamsters were given a 15-min light pulse late at night, and then another light pulse early on the following night. The combination of advancing, then delaying, signals caused a rapid compression of α followed by arrhythmia in body temperature, locomotor activity, and melatonin secretion. Since these studies were published, a similar relationship between α compression and entrainment was found in an experiment on selective breeding in Siberian hamsters. Animals with a significant delay in their time of their nightly activity onset were bred to increase this phenotype in a laboratory population (Weinert and Schöttner, 2007). The most common phenotype exhibited a spontaneous gradual compression of α over a period of several weeks (Weinert and Schöttner, 2007). A number of animals compressed α down to <3 h. At that point, some animals began to free run with periods close to 25 h, whereas other hamsters became ARR.

To summarize, severe α compression precedes both loss of entrainment and arrhythmia. In Siberian hamsters, the key to arrhythmia is about maximizing alpha compression, and the most severe alpha compression results from the combined effects of a phase-advancing and a phase-delaying signal occurring (in that order) within a single circadian cycle. Furthermore, housing free-running or ARR hamsters in DD does not restore entrainment (**Figure 1D3**; Steinlechner et al., 2002; Barakat et al., 2004). The combination of a 2-h light pulse late at night, followed by a 3-h delay of the LD cycle on the next day, termed the DPS protocol by Prendergast et al. (2012), reliably induces arrhythmia in 40–65% of hamsters in any given cohort.

WHY ARRHYTHMIA? WHY THIS SPECIES?

Steinlechner et al. (2002) explain the progression from alpha compression to arrhythmia in the central pacemaker in the context of the two-oscillator model originally described by Pittendrigh and Daan (1976) and also used by Elliott and Tamarkin (1994) to describe circadian control of wheel-running and melatonin secretion in Syrian hamsters. In nocturnal rodents, α is determined by the phase-angle difference (ψ_{EM}) between the evening (E) and morning (M) oscillators which control activity onset and offset, respectively, in a multioscillator pacemaker. The key to the model is the coupling strength between these oscillators. Tight coupling means that an advancing or delaying light signal phase-shifts both E and M in unison, without any changes in ψ_{EM} (Pittendrigh and Daan, 1976). Loose coupling allows for changes in ψ_{EM} and in α because E and M can phase shift independently of one another. Photic signals compress α by delaying E and/or by advancing M, which then decreases ψ_{EM}. Steinlechner et al. (2002) suggest that arrhythmia arises when ψ_{EM} is driven to zero. In that condition, the pacemaker can be considered to be in a state of singularity with zero amplitude.

We found evidence for the amplitude suppression hypothesis by quantifying clock gene expression in the SCN of DPS hamsters (Grone et al., 2011). DPS animals lacked daily rhythms in expression of *per1*, *per2*, *bmal1*, and *cry1* mRNA (**Figure 1F**). Moreover, the mRNA levels of those genes were suppressed to a basal level of expression. This is an important point because if SCN neurons were oscillating, but desynchronized from one another, mRNA levels in DPS hamsters would be expressed well above basal levels. In mice made ARR by LL, neuronal desynchrony in the SCN was reported using *Per1:GFP* fluorescence to monitor individual cells (Ohta et al., 2005). In those animals, individual SCN neurons continued to oscillate at circadian periodicities, but phase synchrony was widely dispersed in the neuronal population, resulting in ARR locomotor activity patterns. Taken together, the underlying mechanisms of arrhythmia caused by LL versus the DPS protocol appear to be quite different. If ARR hamsters truly are an instance of amplitude suppression, then it suggests that the clock in each SCN neuron stopped oscillating, which is an idea that was unheard of not that long ago.

I have been asked many times if the DPS protocol could be adapted for mice. I am doubtful that it can, mainly because most mice have such small phase advance zones, if any at all. Worse, one would need to compress α by putting mice into a photoperiod with nights much shorter than 12 h, which would make the phase advance zone even smaller (i.e., reduce PRC amplitude; cf., Elliott, 1976). And even if one could figure out how to make the advance zone larger, oscillator coupling in the SCN would have to be loosened up, although there might be practical ways to do so (Herzog et al., 2017). I also would not suggest using LL because it is stressful and not reliable. I once housed a group of C57BL/6 mice in LL under 1,000 lux of light and all of them free ran for 6 months without any signs of arrhythmia. Having said all this, producing arrhythmia in a rodent is not impossible (Honma and Honma, 1999), but keeping them ARR for months while housed under a standard LD cycle might be.

For the sake of posterity, I would like to point out that Stephan Steinlechner and myself both discovered the phenomenon of using advancing and delaying light signals to induce long-term arrhythmia independently and without knowledge of the other's work. At a meeting of the Society for Research on Biological Rhythms (1998) where I presented some of these research findings, Franziska Wollnik informed me that Stephan Steinlechner had already been making Djungarian hamsters ARR for years, which was news to me. I was never able to confirm this with him, but I assume that Franziska was right.

It is difficult to explain all of these data according to current theories of entrainment, and harder yet to provide a unifying physiological mechanism that ties all the data together, but there is a related question we should consider. If circadian rhythms and their entrainment to light are so adaptive, then why is entrainment, and the pacemaker itself, so vulnerable to light, particularly in a seasonal species that depends on proper entrainment to changing daylengths for its survival? I suspect that what we are seeing in the laboratory is a window into some adaptation, one that promotes survival in animals from harsh environments. During the breeding season, nights become very short at the high northern latitudes from where Siberian hamsters originate. Females of this species experience high energetic demands as they can be pregnant and nursing a litter of pups simultaneously. Short nights limit foraging time, so in periods of food scarcity, it might be beneficial to forgo strict nocturnality and risk predation to forage during the day. In this situation, a labile circadian pacemaker would be adaptive to permit daytime activity. In Siberian hamsters, reducing food intake weakens circadian timing in body temperature and activity as the animals redistribute their activity into the daylight hours (Ruby and Zucker, 1992; Challet et al., 2000). By contrast, the same reduction in food intake in Syrian (golden) hamsters does not (Challet et al., 2000). Rather, that species, which originates from a more temperate climate, maintains strict nocturnality, but compensates for food shortages by reducing daily amounts of locomotor activity. This speculation raises a worthwhile issue: if a weakened circadian system is needed to permit foraging into the daytime hours, then impairments in circadian timing should have no effect on spatial memory or on hippocampal function which are required to form a cognitive map of food sources. But, as we shall see, the relationship between circadian rhythms and the hippocampus is not so simple.

SCN LESIONS IMPROVE AND IMPAIR MEMORY, OR NEITHER

Research on circadian rhythms and memory goes back nearly 50 years, all of which has been well summarized in a pair of excellent reviews (Smarr et al., 2014; Krishnan and Lyons, 2015), so there is no need to summarize that work here. As those reviews show, the majority of work performed with rodents employed memory tasks that critically depend on the hippocampus. In general, those studies found that task performance varies across the day and night, and perturbations to circadian timing, like simulated chronic jet-lag, impair task performance. These findings led to the idea that the circadian system exerts some degree of control over information processing in the hippocampus. To directly test this hypothesis, several studies have evaluated memory performance in animals in which the SCN had been surgically ablated (SCNx). But as I will argue, the bulk of the data show that SCN ablation does not impair memory. In fact, some studies show that SCN ablation *improved* memory.

A summary of memory tasks performed with SCNx animals reveals some trends across studies (**Table 1**). First, in 11 of 14 memory tests from 11 publications, the authors reported no memory impairments in SCNx animals. Second, three of these studies can easily be interpreted to show that SCN ablation improved memory (**Figure 2**). In the study by Mistlberger et al. (1996), rats were trained to use time of day as the discrimination cue for obtaining food by pressing a lever from two food dispensers, one in the morning and one in the afternoon. The data over the first ten training sessions shows that SCNx animals acquired the time-of-day memory faster than SCN-intact animals (**Figure 2A**). In a passive-avoidance test by Stephan and Kovacevic (1978), memory recall was optimal 24 h after training, but poor at other non-circadian intervals. SCN lesions eliminated this time-of-day effect by improving performance at those non-circadian times (**Figure 2B**). In a time-memory task where rats were trained to respond to a sound after a fixed time interval, SCNx animals acquired the conditioned reflex significantly faster than SCN-intact animals (**Figure 2C**; Vodolazhskaya et al., 2001).

TABLE 1 | Memory tests performed in SCNx animals.

Memory task	Model	Recovery time (days)	Reported memory Impairment?	References
NOR	B6/C3H mouse	>21	No	Chuluun et al., 2020
NOR	Siberian hamster	>28	No	Fernandez et al., 2014
SA	Siberian hamster	>28	No	Fernandez et al., 2014
PA	Golden hamster	>21	No	Cain et al., 2012
CPA	Golden hamster	>21	No	Cain and Ralph, 2009
MWM	C57BL/6 mouse	14	No	Phan et al., 2011
cTPL	C57BL6/J mouse	>17	No	Mulder et al., 2014
TMh	Wistar rat	>21	No	Maruyama et al., 2007
PA	Sprague-Dawley rat	7–9	No	Stephan and Kovacevic, 1978
TDF	Wistar rat	?	No	Mistlberger et al., 1996
CR	Mongrel white rat	?	No	Vodolazhskaya et al., 2001
NOR	C57BL/6 mouse	14	Yes*	Phan et al., 2011
CFC	C57BL/6 mouse	14	Yes*	Phan et al., 2011
NOR	C57BL/6 mouse	>14	Yes*	Shimizu et al., 2016

TDF, time of day discrimination for food reward; PA, passive avoidance; NOR, novel object recognition with 24 h training-testing interval; SA, spontaneous alternation; CPA, conditioned place avoidance; CR, conditioned reflex; TMh, time memory to heat exposure; MWM, Morris water maze; cTPL, circadian time place memory; CFC, contextual fear conditioning; and RT, recovery time allowed after SCN lesion surgery (days).

**non-cognitive or methodological factors qualify the result (see text for details).*

?, information unavailable. SCNx studies of food entrainment and of individual human medical cases were excluded.

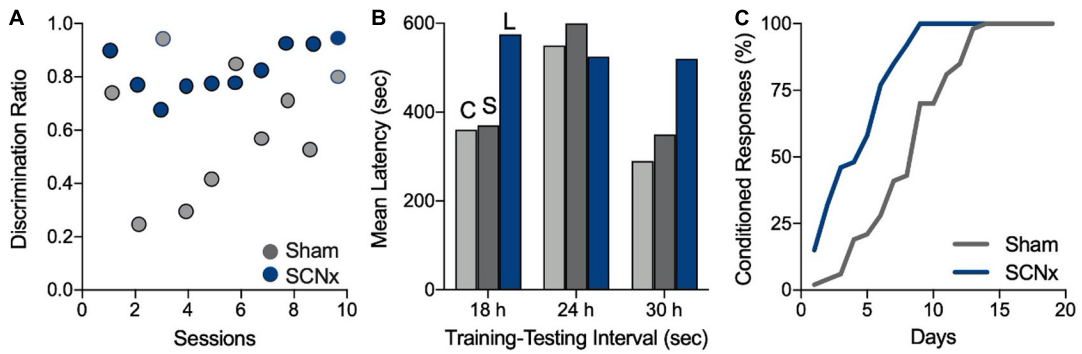

FIGURE 2 | SCN lesions appear to improve memory. **(A)** Rats were trained in a discrimination task that required them to obtain a food reward from one feeder in the morning and another feeder in the afternoon. Higher discrimination scores indicate better memory retention. Circles represent mean scores for each group by session. SCNx rats (blue) appeared to acquire the discrimination in fewer sessions than did sham-operated rats (gray). Figure modified from Mistlberger et al. (1996). **(B)** Rats in a passive-avoidance task learned to avoid entering a dark box where they previously received a foot shock. Longer latencies to reenter the dark box signify better memory of the shock. SCN lesions resulted in longer latencies and appeared to improve memory of the shock at 18 or 30 h after training (C, control; S, sham-lesioned; and L, SCN-lesioned). Figure modified from Stephan and Kovacevic (1978). **(C)** Rats were trained to replace a conditioned reflex to sound with one conditioned to a fixed time interval of 20 s. Training sessions consisted of 20 trials/day over 1 month. The authors statistical analysis showed that SCNx rats acquired the response significantly faster than sham-operated animals. Figure modified from Vodolazhskaya et al. (2001). All figures redrawn from published data and are for illustration purposes only.

Taken at face value, these three studies show that SCN ablation improved memory, thus contradicting the expectation that SCN lesions should impair memory processing.

If SCN lesions can improve memory, can they also impair memory? Memory impairments were reported for SCNx mice by Phan et al. (2011), but closer evaluation allows a different interpretation. In their test of novel object recognition (NOR), mice were allowed to explore two identical objects for 5 min on each of two consecutive days. On the third day, one object was replaced with a new unfamiliar object. In a typical NOR task, control animals will spend about twice as much time exploring the new object compared to the familiar one, indicating a memory for the familiar object. In this study, SCNx mice spent the same amount of time with the old familiar object as they did with the novel one, indicating a failure to either encode or recall the "familiar" objects. However, the control (sham-operated) animals also failed to recognize the familiar object 24 h after training. This might be due to the brief amount of time allowed for exploration (5 min). Most NOR studies with mice allow 10–15 min for object exploration because their latency to engage the objects can be several minutes, even with habituation to the arena. As a result, studies which allow at least 10 min of object exploration time routinely retain object memory for 24 h (c.f., Fernandez et al., 2007; Antunes and Biala, 2012; Lueptow, 2017; Chuluun et al., 2020). By contrast, Siberian hamsters perform successfully in the NOR task with only 5 min of exploration time because they tend to engage objects almost immediately upon entry into the arena (Ruby et al., 2008).

In a test of contextual fear conditioning (Phan et al., 2011), SCNx mice froze significantly less and were more active than controls in the test session, suggesting impairment of context association for the footshock. It is possible, however, that the decrease in freezing was due to the relatively short postsurgical recovery time of 14 days, which is shorter than similar studies (**Table 1**). Alternatively, few chronobiologists know that SCN lesions make some animals hyperactive, which would explain the decrease in freezing. We screen our SCNx and sham-operated animals for hyperactivity by checking for postsurgical increases in home cage locomotor activity. More puzzling though, is that SCNx mice performed just as well as controls in the Morris water maze (MWM), showing no differences in escape latencies, which is the gold standard for evaluating hippocampal-guided navigation in the MWM. Thus, the normal escape latency shows that navigational functions in the hippocampus and exploration behavior of SCNx animals were intact.

There is another intriguing facet to the Phan et al. (2011) study. The authors suggest that the loss of circadian rhythms in mitogen-activated protein kinase (MAPK) in the hippocampus is a cause of the putative memory impairments. But there is another way to interpret the data. The unimpaired performance of SCNx mice in the MWM indicates the persistence of robust spatial memory and hence, intact hippocampal processing. Taken together with the inconclusive evidence from the NOR and fear conditioning tasks, one could just as easily conclude that circadian rhythms in the hippocampus are unnecessary for information processing in that structure. Furthermore, if we assume that SCN lesions also eliminated MAPK rhythms in all the other memory studies of SCNx animals (**Table 1**), then the lack of memory impairments in the other 11 memory tests allow us to conclude that that the hippocampus does not require circadian rhythms in MAPK for successful performance in those memory tasks. MAPK is an important component of memory storage, but based on all of the SCN lesions studies (**Table 1**), daily cycles in its expression do not appear necessary for the hippocampus to function normally.

Results from the remaining SCNx studies in **Table 1** are at best contradictory. The study by Shimizu et al. (2016) is the outlier. The authors reported that SCNx mice recalled a familiar object

after 8 min, but not after 24 h. The actual exploration times are not given, so it is possible that the SCNx mice were less interested in the objects than were the control animals, or that they had longer latencies to explore the objects. This study set a minimum time of 10 s total for exploration of both objects (i.e., total duration of physical interaction with the objects), whereas a 20 s minimum is recommended (Lueptow, 2017). Moreover, trisomic (Down Syndrome) Ts65Dn mice exhibit memory deficits in object recognition (Fernandez et al., 2007), but when trained and tested weeks after SCN ablation and given 10 min of exploration time, they were able to recall the memory of an object 24 h after exploring it (Chuluun et al., 2020). More to the point here, SCN ablation did not impair 24-h recall in the control group of 2N diploid littermates (Chuluun et al., 2020).

Attributing memory deficits to SCN ablation requires ruling out all non-cognitive explanations, but this cuts both ways—the aforementioned memory improvements in SCNx animals require the same scrutiny. Did SCN lesions really improve memory in those three studies? Cain and Ralph (2009) performed a replication of the study by Stephan and Kovacevic (1978) in which it appeared that SCN lesions improved memory, but they were unable to replicate that finding. To explain this discrepancy, Cain and Ralph (2009) suggest that the high performance (i.e., long step through latencies) of SCNx animals in the study by Stephan and Kovacevic (1978) was not due to improved memory, but was an artifact of the size and placement of the lesions which may have damaged the optic tracts. Such damage could impair light perception, thereby reducing the aversiveness of the brightly illuminated start box and hence, increasing the latency to escape. In regards to the study by Mistlberger et al. (1996), the putative memory improvement in SCNx animals might be explained by the fact that SCNx animals underwent more training sessions than did the SCN-intact controls (Mistlberger, personal communication). This piece of information was not reported because the rate of discrimination acquisition was not relevant to the goals of that study. Vodolazhskaya et al. (2001) appears to be the only study to show a bona fide improvement in memory performance in SCNx animals. However, we do not know if time of day might have affected acquisition of the conditioned response in the control animals, and, therefore, whether the sham-operated animals would have performed better had they been trained and tested at other times of day.

Methodological differences among these studies that could explain the seemingly contradictory results such as lesion size and placement, postsurgical recovery time, amount of training, latency to engage the task, and time of day that tests were done, etc., still might not resolve these issues. Discrepant findings in behavioral studies have plagued many areas of neuroscience, despite extraordinary efforts to standardize conditions across laboratories (Crabbe et al., 1999). The standardization of behavioral tests has been suggested to minimize this problem—and that approach has its advantages, but it is doubtful that differences among laboratories can be minimized to the point where they are no longer problematic (Wahlsten, 2001). Although discrepancies exist, there is far more consistency across laboratories in primary findings (Crabbe et al., 1999). Thus, the interpretation of individual studies of SCN lesions and memory, no matter how comprehensive they appear, must still be evaluated within the larger context of all other relevant SCN lesion studies in order to minimize differences in testing procedures and conditions that vary across laboratories. In that context, it is clear that the overwhelming weight of evidence shows that SCN lesions do not impair memory processing in hippocampal-dependent tasks (**Table 1**).

A MALFUNCTIONING SCN IS WORSE THAN NO SCN

If SCN lesions do not impair memory then why have so many studies reported that chronic disruption of circadian timing impairs memory? Further, why is there mounting clinical evidence that circadian dysfunction contributes to cognitive decline in aging (Tranah et al., 2011; Covell et al., 2012; Coogan et al., 2013)? I propose that the answer to these questions is that an intact, but malfunctioning, SCN interferes with cholinergic transmission in the septohippocampal pathway. In this section, I will discuss how the DPS model has shed light on the functional relationship between the SCN and hippocampus, and describe a mechanism to explain how a dysfunctional SCN interferes with memory.

We evaluated memory in DPS hamsters with the NOR task and by spontaneous alternation (SA), which assays spatial working memory. In the NOR task, we allowed the hamsters 5 min to sample two identical objects (sample phase), and then returned them to the arena after a fixed time interval (i.e., 1, 20, or 60 min, or 24 h), and allowed them to explore two objects for 5 min (test phase); one object was from the sample phase (i.e., familiar) and the other was different (i.e., novel). Entrained hamsters spent an equal amount of time with both objects in the sample phase, but spent nearly twice as much time with the new object, thus indicating memory retention of the familiar object (Ruby et al., 2008).

The SA task is less well known than the NOR task, but is better suited as a behavioral assay of hippocampal function, as will be discussed later. We used a continuous trials version of the SA test in which animals are placed in a start box at the base of a T-maze for 1 min, and then allowed to explore the maze for 7 min. Alternation behavior is the tendency of animals to alternate left-right arm choices with each trip from the start box to the maze intersection at the top of the "T." An alternation event is when an animal chooses, for example, the left arm on its first trip through the maze, then the right arm on its next trip. Performance is scored as the proportion of trips from the stem to the intersection where an animal alternates arm choices, compared to when it chooses the same arm (i.e., non-alternation).

Both memory tasks showed that there is a daily rhythm in performance in ENT hamsters, which was poor early in the day, but successful at the remaining time points (**Figures 3A1,B1**). DPS hamsters, however, failed at all time points. By contrast, exploratory behavior, given as the time spent exploring objects (NOR) or as the number of arm entries in the maze (SA), was constant across all time points (**Figures 3A2,B2**). Moreover, the amount of exploration was the same among ENT and DPS

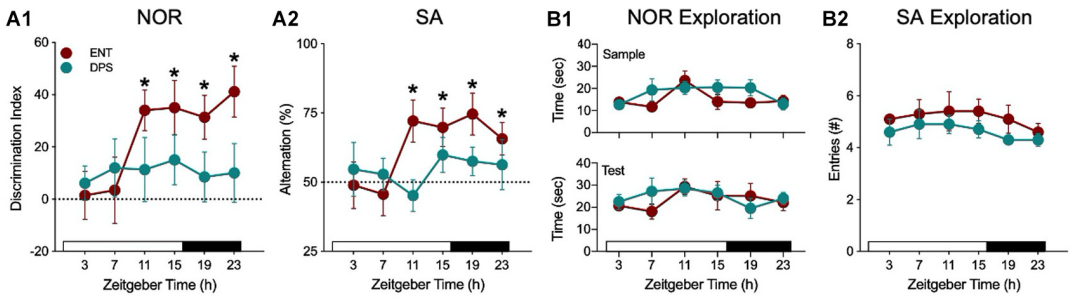

FIGURE 3 | Recognition and spatial working memory were impaired in DPS hamsters. **(A)** Performance of ENT hamsters (red) and DPS (green) hamsters in the NOR **(A1)** and SA **(A2)** tasks at different times of day. * indicates that task performance was significantly different from chance ($P < 0.05$; dotted line). In contrast to task performance, exploration behavior, defined as the amount of time spent exploring objects **(B1)** and number of arm entries **(B2)**, did not change across the day and did not differ among ENT and DPS hamsters. NOR task used a 60 min interval between sample and test phases. Figure modified from Ruby et al. (2008, 2013).

hamsters. Both tasks reveal two important features of circadian involvement in memory tests of exploration. First, while test performance varies across the day, exploration behavior does not; it remains constant across the day and night. Second, loss of circadian timing did not impair the motivation to explore the objects and environments. DPS hamsters fail, but not for lack of trying; they explore just as much as ENT hamsters.

Given the observed differences in SCNx animals and DPS hamsters in memory performance, we decided that a head-to-head test of both types of arrhythmia in the same species, in the same laboratory, and under identical conditions might clarify the role of the SCN in the NOR and SA tasks. As expected, we found that performance in the NOR and SA tasks was fully intact in SCNx animals, but impaired in DPS hamsters (Fernandez et al., 2014). There were also no differences between these groups in their behavior during the tasks; both groups spent the same amount of time exploring objects and the maze (Fernandez et al., 2014). Those results suggested that memory impairments in DPS animals were caused by arrhythmia in the SCN. We tested this hypothesis by evaluating whether ablation of the SCN could rescue memory in DPS animals (**Figure 4A**). A group of hamsters was first tested while they were entrained (ENT), after which, they were exposed to the DPS protocol. Hamsters that were completely ARR 4 weeks later were tested a second time. In the third phase, those DPS hamsters underwent SCN lesion surgery. After 4 weeks of recovery from surgery, this group of hamsters were tested a third time on both memory tasks (**Figure 4B**). Complete SCN ablation rescued memory in DPS hamsters. Hamsters that sustained complete bilateral ablation of the SCN performed as well as sham-operated animals in both tasks (**Figure 4C**). There were no significant changes in exploration behavior across the three phases of the experiment (**Figure 4C**).

Among the lesioned hamsters were a subgroup of animals that sustained only partial damage to the SCN (20–60% ablated).

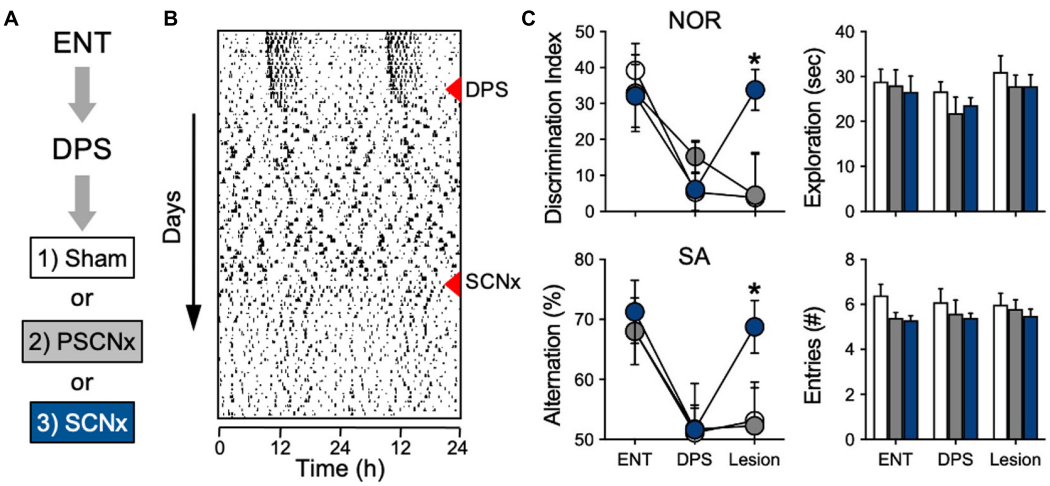

FIGURE 4 | SCN lesions rescue object recognition and spatial memory in DPS hamsters. **(A)** Sequence of experiment stages. ENT hamsters performed the NOR and SA tasks, followed by exposure to the DPS protocol (red triangle). 4 weeks later, arrhythmic animals underwent SCN ablation or Sham surgeries (red triangle). **(B)** Representative actogram of an SCNx animal; memory tasks were performed at the end of each experimental stage. NOR task used a 24-h interval between sample and test phases. **(C)** Memory was rescued only in animals with complete SCN ablation (blue circles). No differences were found in NOR exploration times or in SA arm entries; all tests were performed within 3 h before dark onset. Figure modified from Fernandez et al. (2014).

In that group with partial SCN lesions (PSCNx), damage to the SCN did not rescue memory (**Figure 4C**). This finding was not entirely unexpected. It is well documented that partial ablation of the SCN does not eliminate circadian timing, with some studies showing that only 10% of the SCN is sufficient for circadian rhythms to persist (cf., Ruby, 2003). The PSCNx animals showed that—just as with circadian rhythm generation—a small subset of SCN neurons are sufficient to maintain function. But in this case, that function is memory impairment. Taken together, these experiments show that SCN lesions do not impair long-term object recognition or spatial working memory. Moreover, they also show that an ARR SCN can interfere with memory processing even when most of the nucleus is destroyed.

SPONTANEOUS ALTERNATION: A GREAT MEMORY TASK FOR CHRONOBIOLOGISTS

Spontaneous alternation behavior is a memory task well-suited to circadian studies, so it is worth taking a moment to provide some background. SA behavior is mainly used as an assay of spatial working memory. As such, it is an excellent metric for the immediate and long-term effects of a treatment, such as jet-lag, on memory. SA tasks allow for frequent (daily) re-testing of individual animals without decrements in performance (Douglas, 1989). Unlike fear conditioning or food rewards, SA does not produce arousal or require training sessions, all of which can produce daily timing signals. It is also highly sensitive to disturbances in cholinergic pathways from the medial septum (MS) to the dentate gyrus (DG) of the hippocampus. The only known projection of the SCN to the entire limbic system is not to the hippocampus, but to the ventrolateral subregion of the lateral septum (LSv) which has reciprocal projections to the SCN (Watts et al., 1987; Watts, 1991; Morin et al., 1994; Kriegsfeld et al., 2004; **Figure 5**). GABAergic fibers from the LSv target the MS (Risold and Swanson, 1997), which is a major source of acetylcholine (Ach; Dutar et al., 1995; Teles-Grilo Ruivo and Mellor, 2013). Cholinergic projections from the MS innervate the DG and CA regions of the hippocampus (Haam and Yakel, 2017), but it is the projections to the dentate that underlie SA behavior (Gold, 2003; Solari and Hangya, 2018). Because this circuit is essential for SA behavior, the SA task is essentially a biometric readout of the integrity of this circuit.

I am indebted to my colleague, Fabian-Xosé Fernandez, for introducing me to the literature on SA behavior and for his collaborative efforts in developing our working model describing the functional connectivity between the SCN and hippocampus (Ruby et al., 2008; Fernandez et al., 2014). A more detailed primer on SA behavior compiled by Dr. Fernandez may be found in Ruby et al. (2017, Supplementary Material). Briefly, SA behavior was first observed in rats over 100 years ago (Hunter, 1914). Since that time, it has been reported for insects, crustaceans,

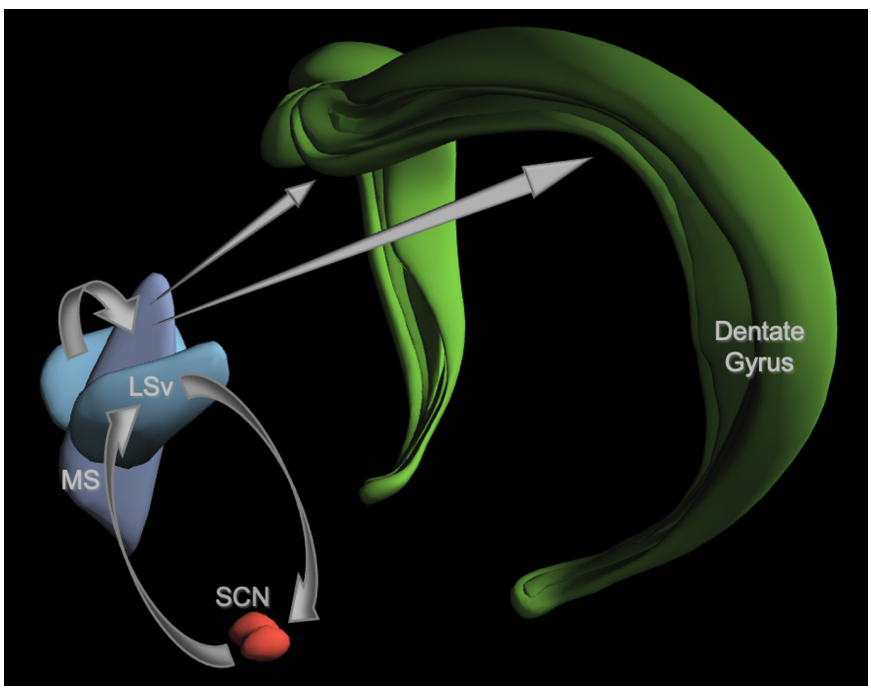

FIGURE 5 | A neuroanatomical model of a proposed SCN-septum-dentate circuit. For the sake of clarity, only the elements of the model discussed in the text are illustrated here. The projections from each structure are shown as gray arrows. The SCN has reciprocal connections with the ventral lateral septum (LSv). GABAergic fibers from the LSv innervate the medial septum (MS) shown here without the diagonal band of Broca. MS fibers containing Ach, GABA, and glutamate project to all subregions of the hippocampus, but only the dentate gyrus is shown. Fibers from the LSv to the SCN are primarily vasopressinergic (VP). See text for details and citations. Image credit: Allen Institute.

fish, reptiles, birds, rodents, and humans (Hughes, 1989; Lewis et al., 2017). Animals are intrinsically motivated to keep track of information relating to the location of food, predators, and potential mates (Barnett, 1958; Berlyne, 1966). These behaviors, collectively termed *exploration*, ultimately result in the creation of a cognitive map, which is a complex internal representation of the environment's physical space (Tolman, 1948; O'Keefe and Nadel, 1978, 1979). Animals continue to update their spatial representations of places over time, maintaining the accuracy of these representations via regular bouts of patrolling (Nadel, 1990). If the environment they are patrolling is given a specific spatial framework, such as a T-maze, they will travel systematically from one endpoint of the apparatus to the next, alternating more than two-thirds of the time (Tolman, 1925; Gerlai, 1998).

A synthesis of work done over the past five decades suggests that cholinergic input from the MS to the DG is critical for maintaining this function. The developmental emergence of SA behavior and of hippocampal maturation occur in parallel in several species (Douglas et al., 1973; Bronstein et al., 1974; Frederickson and Frederickson, 1979; Dumas, 2004; Blair et al., 2013), *including humans* (Vecera et al., 1991). The latest developing subfield of the hippocampus is the DG; the granule cells there (*fascia dentata*) still divide after birth in many species to sculpt a portal of information flow from the medial entorhinal cortex (MEC)—another component of the brain's navigation system—to the hippocampus. In the rat, SA rates increase as the dentate matures (Douglas et al., 1973). The achievement of adult-like SA performance at the end of the third postnatal week coincides with increases in the synaptic strength of the MEC-DG perforant path (Dumas, 2004).

Rodents with electrolytic or excitotoxic lesions of the MS do not alternate significantly above a chance rate of 50% (Douglas and Raphelson, 1966; Clody and Carlton, 1969; Dalland, 1970; Johnson et al., 1977; Thomas, 1979; Brito and Thomas, 1981; Hepler et al., 1985; Chang and Gold, 2004). Animals treated systemically with anticholinergic drugs, such as scopolamine, also perform poorly in a T or Y-maze task (Squire, 1969; Leaton and Utell, 1970; Douglas and Truncer, 1976; Kokkinidis and Anisman, 1976; McNaughton and Feldon, 1980; for a comprehensive review, see Klinkenberg and Blokland, 2010). These data—collated from adults—complement studies from developing rat pups, which show that SA behavior comes online along the same time-course with which the maturing hippocampus is innervated by the septum (Kirkby, 1967; Egger et al., 1973; Hess and Blozovski, 1987). Research by the Gold lab suggests that the septohippocampal pathway is highly active during SA and is taxed more when SA behavior is tested in mazes with more arms. Rats exhibit significant spikes in hippocampal Ach release during SA in a four-arm plus maze that is sustained throughout the testing period (a 50–70% increase relative to baseline as determined by *in vivo* microdialysis; Ragozzino et al., 1994, 1996, 1998; Ragozzino and Gold, 1995; Stefani and Gold, 2001).

In summary, the septohippocampal pathway—particularly its cholinergic projections—is considered to be the critical structure for expression of alternation behavior because: (1) there is a close association between maturation of septal inputs to the DG, and development of the dentate itself, that is necessary for SA behavior, (2) only brain lesions that destroy or alter the circuits associated with this pathway impair SA behavior, whereas damage to several other brain regions outside of the hippocampal system do not, and (3) pharmacological inhibition of septal inputs to the dentate impair SA behavior.

THE IMPORTANCE OF BEING NON-CIRCADIAN: THETA OSCILLATIONS

The deficits in the SA task observed in DPS hamsters implied that there should be functional deficits in cholinergic septohippocampal circuitry as manifested in the expression of theta rhythms. Theta oscillations in this circuit coordinate the firing patterns of neuronal ensembles across diverse brain regions. This synchronized activity allows information stored in anatomically distant regions to be shared and processed as coherent memories (Colgin, 2016). Theta rhythms dominate the electroencephalogram (EEG) during activities that support working memory and the encoding of episodic memories, such as when animals explore novel objects or navigate their environment. Ach signaling supports spatial working memory (Ragozzino et al., 1998; Gold, 2003; Teles-Grilo Ruivo and Mellor, 2013) via its release from medial septal neurons onto cells in the DG and CA1 (Dutar et al., 1995; Teles-Grilo Ruivo and Mellor, 2013). This phasic release of Ach from septal neurons is highly correlated with theta frequency (Lee et al., 2005; Zhang et al., 2010). Furthermore, optogenetic activation of septal cholinergic neurons enhances theta oscillations in hippocampal neurons (Vandercasteele et al., 2014). Thus, we examined whether the loss of circadian timing disrupted theta episodes during object exploration.

Activity in the EEG delta band (0.5–4.0 Hz) reflects a restful state whereas theta (5–8 Hz) reflects a state of attention and active information processing. Stimuli that increase theta also suppress adjacent frequency bands, thus, the theta/delta (T/D) ratio provides an index of active engagement with environmental stimuli. A single theta episode was defined as the number of consecutive EEG epochs (4 s) in which the T/D ratio was >1.0 such that, for example, two consecutive epochs would be a single theta episode of 8 s. We found that the relative power densities in the EEG spectra did not differ among ENT, DPS, SCNx, or Sham-lesioned animals (**Figure 6A**), indicating that theta-generating mechanisms were intact in all four groups. The mean duration of individual theta episodes in both DPS and SCNx groups were, however, shortened to half the duration observed in ENT animals (**Figure 6B3**; Loewke et al., 2020), but DPS and SCNx animals differed in two important respects. First, the total amount of time that theta dominated the EEG during exploration in DPS hamsters was less than it was for ENT animals, whereas SCNx hamsters spent twice as much time in theta (**Figure 6B1**). Second, the number of theta episodes was the same for ENT and DPS hamsters, but was 3-fold greater for SCNx animals (**Figure 6B2**).

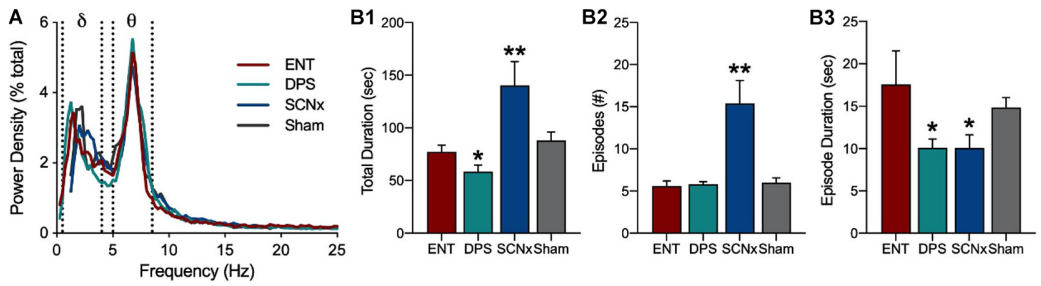

FIGURE 6 | Loss of circadian timing fragments theta oscillations. **(A)** Power density in the EEG averaged over 5 min of object exploration. Delta (δ) and theta (θ) power bands defined by dotted lines. Data for ENT, DPS, SCNx, and Sham groups are aligned by peaks in theta power. Theta dominance was defined by a theta/delta (T/D) ratio > 1.0 within each EEG epoch. **(B)** Theta dominance in the EEG during exploration of a novel object given for **(B1)** total amount of time T/D > 1.0, **(B2)** number of theta-dominated episodes, and **(B3)** the mean duration of individual episodes where T/D was > 1.0. Data are mean (± SEM). * indicates $P < 0.05$, $^{**}P < 0.01$ compared to ENT. Figure modified from Loewke et al. (2020).

This shortening, or fragmenting, of theta episodes was a consequence of circadian arrhythmia for both DPS and SCNx groups. Such fragmentation suggests that circadian arrhythmia disrupted signaling in the septohippocampal pathway, possibly by also fragmenting cholinergic signaling in that pathway (Loewke et al., 2020), or by disrupting synchronized firing among neuronal populations (Buzsáki and Moser, 2013; Harris and Gordon, 2015), or perhaps theta fragmentation represents fragmentation in the chunks of information carried within a theta sequence (Gupta et al., 2012; Teng et al., 2018). Deficits in any of these mechanisms of information processing would result in the misrepresentation of spatial information encoded in memory and, perhaps, in the erroneous recall of spatial representations.

The key difference between DPS and SCNx hamsters is that SCN lesions allow the animals to compensate for theta fragmentation by increasing the number of theta episodes through increased cognitive effort, an effect which can increase Ach release from the MS (Ragozzino et al., 1998; Gold, 2003; Roland et al., 2014). The increased frequency of these episodes might support memory by shortening the intervals between theta episodes thus allowing greater continuity in encoding object information. Alternatively, more frequent theta episodes might increase the repetition of information carried in fragmented theta sequences. Regardless of the underlying mechanism, the data show that disruptions to SCN functioning interfere with the normal expression of theta oscillations during active exploration of unfamiliar objects.

THE ARRHYTHMIC SCN: SHIFTING THE BALANCE IN THE HIPPOCAMPUS

The model we developed to explain the memory impairments in the DPS hamsters predicts that these animals should exhibit impairments in cholinergic signaling in the DG of the hippocampus. This assertion is based on data from the theta study discussed above, and on the well-established role of cholinergic projections from the MS/diagonal band to the granule cells in the dentate, which are critical for hippocampal theta, SA behavior, and for spatial working memory (Dember and Richman, 1989; Kesner, 2007; Khakpai et al., 2013; Haam and Yakel, 2017; Sasaki et al., 2018).

Contrary to my expectations, we found no differences between DPS and ENT controls in long-term potentiation (LTP) in the DG (or in CA1) despite recording from a relatively large number of animals (n = 23 DPS, n = 24 ENT; data not shown; McMartin et al., 2021). Likewise, there were no differences in excitatory postsynaptic field potentials (fEPSPs) in the dendritic field of the dentate (**Figure 7A1**), or in population spikes (POP) in the dentate granule (somatic) layer (**Figure 7A2**), or even in fEPSPs during bath application of the muscarinic cholinergic agonist carbachol (**Figure 7A1**). However, we began to see differences between the ENT and DPS groups when we examined the effects of carbachol on the POP spike. Carbachol enhanced facilitation of the spike, but that effect was attenuated in the DPS group (**Figure 7B**). That finding was of interest to us because the postsynaptic signal travels from the dendritic field to the granule cell bodies, and the magnitude of the POP spike generally covaries with the fEPSP that drives them, but that did not happen here. Instead, carbachol had no effect on the fEPSP, but facilitated the POP spike. This suggested that the effects of carbachol on the POP spike must be downstream from the postsynaptic dendritic fields. The most likely explanation for this was that carbachol reduced inhibitory inputs to the granule cell bodies where the POP spike occurs.

This hypothesis was confirmed by direct measures of inhibitory influences on dentate granule cells. Under control conditions, there were no differences among ENT and DPS groups in the amplitude of evoked inhibitory postsynaptic potentials (IPSPs), which is a measure of evoked inhibition (**Figure 7B1**). Carbachol suppressed IPSP amplitudes in cells from ENT animals, as expected from its known actions on muscarinic receptors (Bell et al., 2013; McQuiston, 2014), but had no effect on cells from DPS animals (**Figure 7B1**). Furthermore, spontaneous IPSP events, which are a measure of tonic inhibition, were twice as frequent in cells from DPS animals compared to the ENT group (**Figure 7B2**, for intervals <500 ms). Carbachol increased the frequency of these spontaneous IPSP events, but the magnitude of this increase did not differ among ENT and DPS groups (**Figure 7B2**). Thus, arrhythmia in the SCN resulted

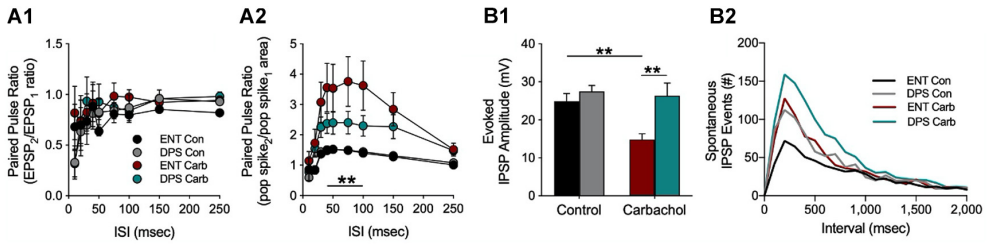

FIGURE 7 | Increased synaptic inhibition and reduced cholinergic responsiveness in the dentate gyrus of DPS hamsters. **(A)** Paired pulse ratios from granule cells in the dentate gyrus at different interstimulus intervals (ISI) during simultaneous recordings of **(A1)** field excitatory postsynaptic potentials (fEPSPs) and **(A2)** populations spikes (POP) under control (Con) or carbachol bath (Carb, 10 μM) conditions. Note that we use area rather than amplitude to quantify the POP spike. This is because amplitude can be confounded both by the number of cells excited by the stimulus and by the synchrony of those cells whereas POP spike area is not. The carbachol-induced population spike increase was attenuated in cells from DPS hamsters compared to cells from ENT animals at ISIs from 40–100 Hz (**$P < 0.01$). **(B1)** Carbachol normally suppresses evoked inhibition as it did here in the control group (ENT, red); however carbachol had no effect on cells from DPS hamsters (green) where evoked inhibition remained high (**$P < 0.001$). **(B2)** The number of spontaneous IPSP events in cells from DPS hamsters was 2x that observed in cells from ENT animals under control conditions at intervals < 500 ms (KS test, $P = 0.014$). Carbachol increased spontaneous IPSPs by the same magnitude in both groups. Figure modified from McMartin et al. (2021).

in more than a doubling of the amount of inhibition occurring in dentate circuits, and substantially attenuated the ability of cholinergic agonism to suppress inhibition. This shift toward inhibition would have downstream effects. Dentate granule cell mossy fibers project exclusively to hippocampal area CA3, a microcircuit that is important for spatial memory (Prince et al., 2016; Sasaki et al., 2018; Senzai, 2019). Thus, in DPS animals, there would likely be reduced excitation in that microcircuit, the net result of which would be to impair spatial memory (Flasbeck et al., 2018; Senzai, 2019), which is what we observed in DPS hamsters (**Figure 3**).

We further considered whether the increased synaptic inhibition in DPS hamsters might be associated with decreases in Ach signaling from the MS to the dentate so, as part of a pilot study, we performed an *in vivo* microdialysis study to measure Ach in the DG while a hamster was given cotton nesting material in the microdialysis chamber. The nest material serves as a novel object but also requires sustained attention to construct a nest. Siberian hamsters respond almost immediately by separating and fluffing the cotton to construct a nest. Lesions of the septum and hippocampus, among other brain areas, impair nest building (Deacon et al., 2002), thus, we used nesting to promote neural activity in the MS while we collected Ach samples from the DG (see **Figure 8A** for timeline). Microdialysis samples collected from a single hamster before and after the DPS protocol showed elevations in Ach as soon as the animal was placed in the chamber, then declined over the next 45 min (**Figure 8B**), at which point, nesting material was provided. Nesting elevated Ach concentrations in the ENT, but not DPS, condition (**Figure 8B**). Direct observation of the animal did

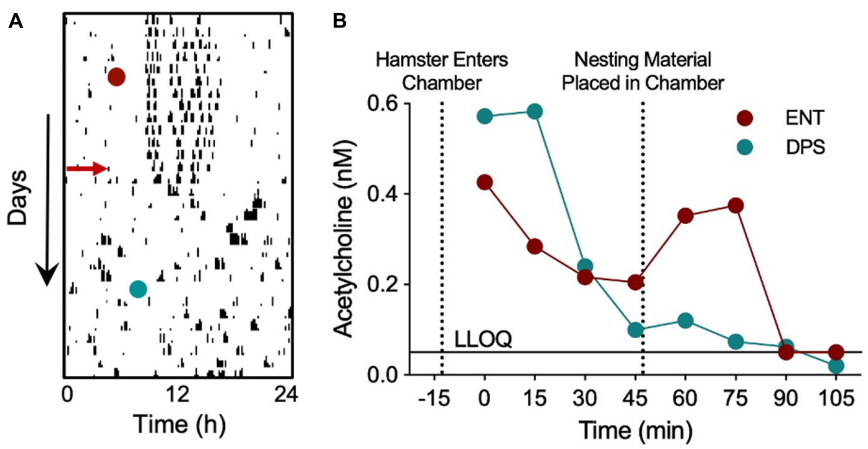

FIGURE 8 | Nest building failed to elicit increases in dentate Ach in a DPS hamster. *In vivo* microdialysis was used to quantify Ach concentrations during nest building in a single hamster before and after the DPS protocol. A microdialysis probe inserted into the dentate gyrus withdrew cerebrospinal fluid (CSF) samples at 15 min intervals, beginning 45 min before nesting material was provided, and continuing for 60 min afterward. Neostigmine (200 nM) was added to the samples to prevent enzymatic degradation of Ach. **(A)** Single-plotted actogram of a hamster before and after the DPS protocol (red arrow). Days and times of microdialysis given (red and green circles). **(B)** Ach concentrations (nM). Dotted lines indicate time when the hamster was placed in the microdialysis chamber and when nesting material was provided. LLOQ, lower level of quantification. Samples quantified courtesy of Brains On-Line (San Francisco, CA, United States).

not reveal any obvious differences in its locomotor activity or engagement of the nesting material in the different conditions. As this was only a pilot study, one must interpret the data with caution, but the lack of Ach release suggests that SCN arrhythmia was involved in Ach suppression, despite behavioral engagement with the nest building task.

PUTTING THE PIECES TOGETHER

The collective data from the EEG, hippocampus, and microdialysis experiments suggest a model to explain the memory deficits in DPS hamsters. The encoding of spatial information begins when exploration triggers theta rhythms in the septal nuclei which, in turn, cause the rhythmic release of Ach from MS neurons onto dentate cells (Lee et al., 2005; Zhang et al., 2010). Normally, those cholinergic signals would be filtered and processed in the dentate, and then conveyed through the CA fields, but when those signals arrive in the dentate of DPS hamsters, they hit a roadblock in the form of elevated synaptic inhibition. It seems likely that such a barrier would not only disrupt spatial memory encoding in the dentate-CA3 microcircuit, but would also disrupt the return signals from the hippocampus back to the septal complex, resulting in theta fragmentation. The microdialysis data further suggest that theta oscillations may be propagated along the septohippocampal pathway without concomitant release of Ach from septal neuronal terminals. This lack of Ach release is important because reductions in hippocampal Ach are a common cause of dementia (Teles-Grilo Ruivo and Mellor, 2013; Anand et al., 2017). Whether SCNx animals exhibit similar increases in synaptic inhibition or in cholinergic release is unknown, but their ability to compensate for theta fragmentation likely involves cholinergic signaling that is improved by increased cognitive effort.

I have purposely omitted any discussion of the medial prefrontal cortex (mPFC) in this model since it has not been addressed empirically in the hamsters. However, synchronous activity between the hippocampus and mPFC is critical to short-term spatial memory (Euston et al., 2012). The necessity of that interaction suggests that fragmented theta oscillations in septohippocampal circuits might convey misrepresentations of spatial information to the cortex, thus providing an additional locus of dysfunctional memory encoding. This model is just one way to think about the data. I have offered it—and done so with informal language—because my intention is to make the overall findings accessible to readers outside the fields of chronobiology and neuroscience. More detailed and formal presentations of these ideas may be found in the original research publications.

There is another facet of the DPS memory deficits that I think is important, but easily overlooked, and that is the severity of the memory deficits. It is clear that an active ARR SCN is capable of causing widespread dysfunction in the hippocampal formation. What is not immediately clear, is that memory deficits are so severe, that the circadian- ARR hamster is a distinct cognitive phenotype; an ARR SCN doesn't just interfere with hippocampal memory, it obliterates it. The performance of DPS hamsters on memory tasks is not simply decreased by a few percent compared to controls; rather, they fail completely, never performing better than chance in the NOR and SA tasks. Even though we made the NOR task as easy as possible, with two very different objects in the test phase, they still failed, even when the time between the sample and test phases was only 20 min (Ruby et al., 2008). In the T-maze, DPS hamsters are unable to recall which arm of the T-maze they previously entered, even when that entry occurred only seconds earlier. In fear conditioning work currently underway, we are seeing the same phenomenon. Testing memory in ENT and DPS hamsters produces binary effects—one group passes, the other fails, with no middle ground.

CONCLUSION

In humans, impaired cholinergic signaling is a hallmark of dementia and cognitive decline, which is accelerated by circadian dysfunction. The research summarized here suggests multiple loci where circadian rhythms and cognitive function intersect. The main benefit of the DPS model for translational studies is that it more closely resembles human circadian dysfunction than models employing SCN lesions or that manipulate genetics to alter SCN function. My colleagues and I were able to develop this model because we took an approach that was grounded in comparative physiology rather than in modern genetic techniques. The result is a simple method that uses light to break circadian timing in the SCN, but that also leaves the animals neurologically and genetically intact, all while the animals sit undisturbed in their home cages. In addition to the work we have done, other laboratories have used ARR Siberian hamsters to document impairments in social memory and object recognition (Müller et al., 2015; Müller and Weinert, 2016), as well as impairments in immune function (Prendergast et al., 2012). The DPS model can, in principle, be used to study the impact of circadian dysfunction on any physiological system or in the progression of disease and, unlike methods that employ constant reentrainment to phase-shifted LD cycles or LL to disrupt rhythms, we know that DPS animals do not incur any sleep loss (Larkin et al., 2004).

One of the goals of phototherapy is to shore up a weakened circadian system, a condition that is common among the aged. The DPS model might be valuable in achieving this goal. After all, if we can suppress circadian timing, why can't we use targeted phototherapy to bring it back? This goal seems possible, particularly given some of the new phototherapeutic approaches under development (Najjar and Zeitzer, 2016; Negelspach et al., 2018; Kaladchibachi et al., 2019). In a number of unpublished failed experiments, I had no luck in restoring rhythms by daily melatonin injections (14 days), daily scheduled feeding (21 days), 2-deoxyglucose injections (to lower brain temperature and possibly reset the SCN), constant dark, and white light pulses. If a light treatment to restore circadian rhythms were developed in DPS animals, it would not only be of interest from a phototherapeutic perspective,

but it would allow us to turn the SCN clock on and off with light. With such a system, one could essentially perform optogenetic experiments without surgery or any genomic modifications to the animals.

One of the reasons I turned to the field of learning and memory with the DPS model was that the neural circuitry that underlies so many memory tasks is well delineated. Unlike the SCN, output pathways from the hippocampus and related structures are well defined, so the task for the chronobiologist is to figure out how the SCN fits into those circuits. Having been in chronobiology for so many years, it still astounds me that we know so little about the functional outputs of the SCN. I consider it to be a gross oversight in our field because it continues to hold us back from better integrating into the medical community (Fernandez et al., 2021). We cannot claim a role for the circadian system in cognitive impairments such as Alzheimer's disease and then shrug when asked how the SCN influences the hippocampus, or feel that it is sufficient to appeal to oscillator misalignment and expect that to satisfy medical professionals. The circuitry within the hippocampal formation is complex, but its connection to the SCN is not. Understanding how the SCN exerts control over the hippocampus is a manageable problem, one that will satisfy the goals of chronobiologists and clinicians alike.

AUTHOR CONTRIBUTIONS

NFR was solely responsible for this manuscript.

FUNDING

This research herein was supported by grants from the National Institute of Mental Health (MH60385 and MH095837). Preparation of this manuscript was supported by the National Cancer Institute (CA231122).

ACKNOWLEDGMENTS

The author thanks Drs. Jeffrey A. Elliott and Benjamin Rusak for helpful discussions in the development of the DPS protocol, and Dr. Elliott for freely sharing his unpublished data. The author also thank Dr. Fabian-Xosé Fernandez for his helpful comments on an earlier version of this manuscript and for his collaborative contributions to this research. Dr. Stephanie Perreau-Lenz performed the surgeries and data collection for the microdialysis project and acetylcholine samples were quantified courtesy of Brains On-Line (San Francisco, CA, United States).

REFERENCES

Anand, A., Patience, A. A., Sharma, N., and Khurana, N. (2017). The present and future of pharmacotherapy of Alzheimer's disease: a comprehensive review. *Eur. J. Pharmacol.* 815, 364–375. doi: 10.1016/j.ejphar.2017.09.043

Antunes, M., and Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modificaitons. *Cogn. Process* 13, 93–110. doi: 10.1007/s10339-011-0430-z

Aschoff, J., Hoffman, K., Pohl, H., and Wever, R. (1975). Re-entrainment of circadian rhythms after phase-shifts of the zeitgeber. *Chronobiologia* 2, 23–78.

Barakat, M. T., O'Hara, B. F., Cao, V. H., Heller, H. C., and Ruby, H. C. (2005). Light induces c-fos and per1 in the suprachiasmatic nucleus of arrhythmic hamsters. *Am. J. Physiol.* 289, R1381–R1386.

Barakat, M. T., O'Hara, B. F., Cao, V. H., Larkin, J. E., Heller, H. C., and Ruby, N. F. (2004). Light pulses do not induce c-fos or per1 in the SCN of hamsters that fail to reentrain to the photocycle. *J. Biol. Rhythms* 19, 287–297. doi: 10.1177/0748730404266771

Barnett, S. A. (1958). Exploratory behaviour. *Br. J. Psychol.* 49, 289–310.

Bartness, T. J., Powers, J. B., Hastings, M. H., Bittman, E. L., and Goldman, B. D. (1993). The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J. Pineal. Res.* 15, 161–190. doi: 10.1111/j.1600-079x.1993.tb00903.x

Bell, L. A., Bell, K. A., and McQuiston, A. R. (2013). Synaptic muscarinic response types in hippocampal CA1 interneurons depend on different levels of presynaptic activity and different muscarinic receptor subtypes. *Neuropharmacol* 73, 160–173. doi: 10.1016/j.neuropharm.2013.05.026

Berlyne, D. E. (1966). Exploration and curiosity. *Science* 153, 25–33.

Blair, M. G., Nguyen, N. N., Albani, S. H., L'Etoile, M. M., Andrawis, M. M., Owen, L. M., et al. (2013). Developmental changes in structural and functional properties of hippocampal AMPARs parallels the emergence of deliberative spatial navigation in juvenile rats. *J. Neurosci.* 33, 12218–12228. doi: 10.1523/jneurosci.4827-12.2013

Brito, G. N., and Thomas, G. J. (1981). T-maze alternation, response patterning, and septo-hippocampal circuitry in rats. *Behav. Brain Res.* 3, 319–340. doi: 10.1016/0166-4328(81)90003-6

Bronstein, P. M., Dworkin, T., and Bilder, B. H. (1974). Age-related differences in rats' spontaneous alternation. *Anim. Learn. Behav.* 2, 285–288. doi: 10.3758/bf03199196

Buzsáki, G., and Moser, E. I. (2013). Memory, navigation and theta rhythms in the hippocampal-entorhinal system. *Nat. Neurosci.* 16, 130–138. doi: 10.1038/nn.3304

Cain, S. W., Chalmers, J. A., and Ralph, M. R. (2012). Circadian modulation of passive avoidance is not eliminated in arrhythmic hamsters with suprachiasmatic nucleus lesions. *Behav. Brain Res.* 230, 288–290. doi: 10.1016/j.bbr.2012.02.022

Cain, S. W., and Ralph, M. R. (2009). Circadian modulation of conditioned place preference in hamsters does not require the suprachiasmatic nucleus. *Neurobiol. Learn. Mem.* 91, 81–84. doi: 10.1016/j.nlm.2008.10.005

Challet, E., Kolker, D. E., and Turek, F. W. (2000). Metabolic influences on circadian rhythmicity in Siberian and Syrian hamsters exposed to long photoperiods. *J. Neuroendocrinol.* 12, 69–78. doi: 10.1046/j.1365-2826.2000.00424.x

Chang, Q., and Gold, P. E. (2004). Impaired and spared cholinergic functions in the hippocampus after lesions of the medial septum/vertical limb of the diagonal band with 192 IgG-saporin. *Hippocampus* 14, 170–179. doi: 10.1002/hipo.10160

Chuluun, B., Pittaras, E., Hong, H., Fisher, N., Colas, D., Ruby, N. F., et al. (2020). Suprachiasmatic lesions restore object recognition in Down syndrome model mice. *Neurobiol. Sleep Circadian Rhythms* 8:100049. doi: 10.1016/j.nbscr.2020.100049

Clody, D. E., and Carlton, P. L. (1969). Behavioral effects of lesions of the medial septum of rats. *J. Comp. Physiol. Psychol.* 67, 344–351. doi: 10.1037/h0026756

Colgin, L. L. (2016). Mechanisms and functions of theta rhythms. *Nat. Rev.* 176, 239–249.

Coogan, A. N., Schutová, B., Husung, S., Furczyk, K., Baune, B. T., Kropp, P., et al. (2013). The circadian system in Alzheimer's disease: disturbances, mechanisms, and opportunities. *Biol. Psychiatry* 74, 333–339. doi: 10.1016/j.biopsych.2012.11.021

Covell, G. E. S., Dhawan, P. S., Iannotti, J. K. L., Hoffman-Snyder, C. R., Wellik, K. E., Caselli, R. J., et al. (2012). Disrupted daytime activity and altered sleep-wake patterns may predict transition to mild cognitive impairment or dementia. *Neurologist* 18, 426–429. doi: 10.1097/nrl.0b013e318272f7ef

Crabbe, J. C., Wahlsten, D., and Dudek, B. C. (1999). Genetic of mouse behavior: interactions with laboratory environment. *Science* 284, 1670–1672. doi: 10.1126/science.284.5420.1670

Dalland, T. (1970). Response and stimulus perseveration in rats with septal and dorsal hippocampal lesions. *J. Comp. Physiol. Psychol.* 71:114. doi: 10.1037/h0028956

Deacon, R. M. J., Croucher, A., and Rawlins, J. N. P. (2002). Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. *Behav. Brain Res.* 132, 203–213. doi: 10.1016/s0166-4328(01)00401-6

Dember, W. N., and Richman, C. L. (1989). *Spontaneous Alternation Behavior*. New York, NY: Springer-Verlag.

Douglas, R. J. (1989). "Using SAB as a tool: advice from a veteran," in *Spontaneous Alternation Behavior*, eds W. N. Dember and C. L. Richman (New York, NY: Springer), 145–160. doi: 10.1007/978-1-4613-8879-1_8

Douglas, R. J., Peterson, J. J., and Douglas, D. P. (1973). The ontogeny of a hippocampus-dependent response in two rodent species. *Behav. Biol.* 8, 27–37. doi: 10.1016/s0091-6773(73)80003-3

Douglas, R. J., and Raphelson, A. C. (1966). Spontaneous alternation and septal lesions. *J. Comp. Physiol. Psychol.* 62, 320–322. doi: 10.1037/h0023657

Douglas, R. J., and Truncer, P. C. (1976). Parallel but independent effects of pentobarbital and scopolamine on hippocampus-related behavior. *Behav. Biol.* 18, 359–367. doi: 10.1016/s0091-6773(76)92327-0

Dumas, T. C. (2004). Early eyelid opening enhances spontaneous alternation and accelerates the development of perforant path synaptic strength in the hippocampus of juvenile rats. *Dev. Psychobiol.* 45, 1–9. doi: 10.1002/dev.20011

Dutar, P., Bassant, M. H., Senut, M. C., and Lamour, Y. (1995). The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol. Rev.* 75, 393–427. doi: 10.1152/physrev.1995.75.2.393

Egger, G. J., Livesey, P. J., and Dawson, R. G. (1973). Ontogenetic aspects of central cholinergic involvement in spontaneous alternation behavior. *Dev. Psychobiol.* 6, 289–299. doi: 10.1002/dev.420060402

Elliott, J. A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. *Fed. Proc.* 35, 2339–2346.

Elliott, J. A., and Tamarkin, L. (1994). Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. *J. Comp. Physiol.* 174, 469–484.

Euston, D. R., Gruber, A. J., and McNaughton, B. L. (2012). The role of medial prefrontal cortex in memory and decision making. *Neuron* 76, 1057–1070. doi: 10.1016/j.neuron.2012.12.002

Fernandez, F., Lu, D., Ha, P., Costacurta, P., Chavez, R., Heller, H. C., et al. (2014). Dysrhythmia in the suprachiasmatic nucleus inhibits memory processing. *Science* 346, 854–857. doi: 10.1126/science.1259652

Fernandez, F., Morishita, W., Zuniga, E., Nguyen, J., Blank, M., Malenka, R. C., et al. (2007). Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat. Neurosci.* 10, 411–413. doi: 10.1038/nn1860

Fernandez, F. X., Kaladchibachi, S., and Negelspach, D. C. (2021). Resilience in the suprachiasmatic nucleus: implications for aging and Alzheimer's disease. *Exp. Gerontol.* 147:111258. doi: 10.1016/j.exger.2021.111258

Flasbeck, V., Atucha, E., Nakamura, N. H., Yoshida, M., and Sauvage, M. M. (2018). Spatial information is preferentially processed by the distal part of CA3: implications for memory retrieval. *Behav. Brain Res.* 354, 31–38. doi: 10.1016/j.bbr.2018.07.023

Frederickson, C. J., and Frederickson, M. H. (1979). Emergence of spontaneous alternation in the kitten. *Dev. Psychobiol.* 12, 615–621. doi: 10.1002/dev.420120610

Gerlai, R. (1998). A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. a strain comparison and lesion study. *Behav. Brain Res.* 95, 91–101. doi: 10.1016/s0166-4328(97)00214-3

Gold, P. E. (2003). Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiol. Learn. Mem.* 80, 194–210. doi: 10.1016/j.nlm.2003.07.003

Grone, B. P., Chang, D., Bourgin, P., Cao, V., Fernald, R. D., Heller, H. C., et al. (2011). Acute light exposure suppresses circadian rhythms in clock gene expression. *J. Biol. Rhythms* 26, 78–81. doi: 10.1177/0748730410388404

Gupta, A. S., van der Meer, M. A. A., Touretzky, D. S., and Redish, A. D. (2012). Segmentation of spatial experience by hippocampal theta sequences. *Nat. Neurosci.* 15, 1032–1039. doi: 10.1038/nn.3138

Haam, J., and Yakel, J. L. (2017). Cholinergic modulation of the hippocampal region and memory function. *J. Neurochem.* 142, 111–121. doi: 10.1111/jnc.14052

Harris, A. Z., and Gordon, J. A. (2015). Long-range neural synchrony in behavior. *Ann. Rev. Physiol.* 38, 171–194. doi: 10.1146/annurev-neuro-071714-034111

Hepler, D. J., Olton, D. S., Wenk, G. L., and Coyle, J. T. (1985). Lesions in nucleus basalis magnocellularis and medial septal area of rats produce qualitatively similar memory impairments. *J. Neurosci.* 5, 866–873. doi: 10.1523/jneurosci.05-04-00866.1985

Herzog, E. D., Hermanstyne, T., Smyllie, N. J., and Hastings, M. H. (2017). Regulating the suprachiasmatic nucleus (SCN) circadian clockwork: interplay between cell-autonomous and circuit-level mechanisms. *Cold Spring Harb. Perspect. Biol.* 9:a027706. doi: 10.1101/cshperspect.a027706

Hess, C., and Blozovski, D. (1987). Hippocampal muscarinic cholinergic mediation of spontaneous alternation and fear in the developing rat. *Behav. Brain Res.* 24, 203–214. doi: 10.1016/0166-4328(87)90058-1

Honma, S., and Honma, K. I. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 276, R1390–R1396.

Huang, G., Wang, L., and Liu, Y. (2006). Molecular mechanism of suppression of circadian rhythms by a critical stimulus. *EMBO J.* 25, 5349–5357. doi: 10.1038/sj.emboj.7601397

Hughes, R. N. (1989). "Phylogenetic comparisons," in *Spontaneous Alternation Behavior*, eds W. N. Dember and C. L. Richman (New York, NY: Springer), 39–58. doi: 10.1007/978-1-4613-8879-1_3

Hunter, W. S. (1914). The auditory sensitivity of the white rat. *J. Animal Behav.* 4, 215–222. doi: 10.1037/h0072405

Jewett, M. E., Kronauer, R. E., and Czeisler, C. A. (1991). Light induced suppression of endogenous circadian amplitude in humans. *Nature* 350, 59–62. doi: 10.1038/350059a0

Johnson, C. T., Olton, D. S., Gage, F. H. III, and Jenko, P. G. (1977). Damage to hippocampus and hippocampal connections: effects on DRL and spontaneous alternation. *J. Comp. Physiol. Psychol.* 91, 508–522. doi: 10.1037/h0077346

Kaladchibachi, S., Negelspach, D. C., Zeitzer, J. M., and Fernandez, F. (2019). Optimiazation of circadian responses with shorter and shorter millisecond flashes. *Biol. Lett.* 15:20190371. doi: 10.1098/rsbl.2019.0371

Kesner, R. P. (2007). A behavioral analysis of dentate gyrus function. *Prog. Brain Res.* 163, 567–576. doi: 10.1016/S0079-6123(07)63030-1

Khakpai, F., Nasehi, M., Haeri-Rohani, A., Eidi, A., and Zarrindast, M. R. (2013). Septo-hippocampo-septal loop and memory formation. *Basic Clin. Neurosci.* 4, 5–23.

Kirkby, R. J. (1967). A maturation factor in spontaneous alternation. *Nature* 215:784. doi: 10.1038/215784a0

Klinkenberg, I., and Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neurosci. Biobehav. Rev.* 34, 1307–1350. doi: 10.1016/j.neubiorev.2010.04.001

Kokkinidis, L., and Anisman, H. (1976). Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. *Psychopharmacology* 48, 261–270. doi: 10.1007/bf00496859

Kriegsfeld, L. J., Leak, R. K., Yackulic, C. B., LeSauter, J., and Silver, R. (2004). Organization of suprachiasmatic nucleus projections in Syrian hamsters (Mesocricetus auratus): an anterograde and retrograde analysis. *J. Comp. Neurol.* 468, 361–379. doi: 10.1002/cne.10995

Krishnan, H. C., and Lyons, L. C. (2015). Synchrony and desynchrony in circadian clocks: impacts on learning and memory. *Learn. Mem.* 22, 426–437. doi: 10.1101/lm.038877.115

Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P., and Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *Am. J. Physiol.* 287, R104–R111.

Leaton, R. N., and Utell, M. J. (1970). Effects of scopolamine on spontaneous alternation following free and forced trials. *Physiol. Behav.* 5, 331–334. doi: 10.1016/0031-9384(70)90106-x

Lee, M. N., Hassani, O. K., Alonso, A., and Jones, B. E. (2005). Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *J. Neurosci.* 25, 4365–4369. doi: 10.1523/jneurosci.0178-05.2005

Leloup, J. C., and Goldbeter, A. (2001). A molecular explanation for the long-term suppression of circadian rhythms by a single light pulse. *Am. J. Physiol.* 280, R1206–R1212.

Lewis, S. A., Negelspach, D. C., Kaladchibachi, S., Cowen, S. L., and Fernandez, F. (2017). Spontaneous alternation: a potential gateway to spatial working memory in Drosophila. *Neurobiol. Learn. Mem.* 142, 230–235. doi: 10.1016/j.nlm.2017.05.013

Loewke, A. C., Garrett, A., Steiger, A., Fisher, N., Heller, H. C., Colas, D., et al. (2020). Loss of circadian timing disrupts theta episodes during object recognition. *Clocks Sleep* 2, 523–525. doi: 10.3390/clockssleep2040038

Lueptow, L. M. (2017). Novel object recognition test for the investigation of learning and memory in mice. *J. Vis. Exp.* 126:e55718.

Maruyama, M., Hara, T., Katara, M., Hashimoto, M., Haque, A., Li, G., et al. (2007). Contribution of the suprachiasmatic nucleus to the formation of a time memory for heat exposure in rats. *Physiol. Sci.* 57, 107–114. doi: 10.2170/physiolsci.rp014506

McMartin, L., Kiraly, M., Heller, H. C., Madison, D. V., and Ruby, N. F. (2021). Disruption of circadian timing increases synaptic inhibition and reduces cholinergic responsiveness in the dentate gyrus. *Hippocampus* 31, 422–434.

McNaughton, N., and Feldon, J. (1980). Spontaneous alternation of body turns and place: differential effects of amylobarbitone, scopolamine and septal lesions. *Psychopharmacology* 68, 201–206. doi: 10.1007/bf00432142

McQuiston, A. R. (2014). Acetylcholine release and inhibitory interneuron activity in hippocampal CA1. *Front. Synaptic Neurosci.* 6:20. doi: 10.3389/fnsyn.2014.00020

Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M., and Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Res.* 739, 12–18. doi: 10.1016/s0006-8993(96)00466-0

Morin, L. P., Goodless-Sanchez, N., Smale, L., and Moore, R. Y. (1994). Projections of the suprachiasmatic nuclei, subparaventricular zone, and retrochiasmatic area in the golden hamster. *Neuroscience* 61, 391–410. doi: 10.1016/0306-4522(94)90240-2

Mulder, C. K., Papantoniou, C., Gerkema, M. P., and Van Der Zee, E. A. (2014). Neither the SCN nor the adrenals are required for circadian time-place learning in mice. *Chronobio Intl.* 31, 1075–1092. doi: 10.3109/07420528.2014.944975

Müller, L., Fritzsche, P., and Weinert, D. (2015). Novel object recognition of Djungarian hamsters depends on circadian time and rhythmic phenotype. *Chronobiol. Intl.* 32, 458–467. doi: 10.3109/07420528.2014.992526

Müller, L., and Weinert, D. (2016). Individual recognition of social rank and social memory performance depends on a functional circadian system. *Behav. Processes* 132, 85–93. doi: 10.1016/j.beproc.2016.10.007

Nadel, L. (1990). Varieties of spatial cognition. *Ann. N.Y. Acad. Sci.* 608, 613–636.

Najjar, R. P., and Zeitzer, J. M. (2016). Temporal integration of light flashes by the human circadian system. *J. Clin. Invest.* 126, 938–947. doi: 10.1172/jci82306

Negelspach, D. C., Kaladchibachi, S., and Fernandez, F. (2018). The circadian activity rhythm is reset by nanowatt pulses of ultraviolet light. *Proc. Biol. Sci.* 285:20181288. doi: 10.1098/rspb.2018.1288

Ohta, H., Yamazaki, S., and McMahon, D. G. (2005). Constant light desynchronizes mammalian clock neurons. *Nat. Neurosci.* 8, 267–269. doi: 10.1038/nn1395

O'Keefe, J., and Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford: Oxford University Press.

O'Keefe, J., and Nadel, L. (1979). Precis of O'Keefe & Nadel's The hippocampus as a cognitive map. *Behav. Brain Sci.* 2, 487–494.

Phan, T. H., Chan, G. C.-K., Sindreu, C. B., Eckel-Mahan, K. L., and Storm, D. R. (2011). The diurnal oscillation of MAP (mitogen-activated protein) kinase and adenylyl cyclase activities in the hippocampus depends on the suprachiasmatic nucleus. *J. Neurosci.* 31, 10640–10647. doi: 10.1523/jneurosci.6535-10.2011

Pittendrigh, C. S., and Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: a clock for all seasons. *J. Comp. Physiol.* 106, 333–355. doi: 10.1007/bf01417860

Prendergast, B. J., Cable, E. J., Patel, P. N., Pyter, L. M., Onishi, K. G., Stevenson, T. J., et al. (2013). Impaired leukocyte trafficking and skin inflammatory responses in hamsters lacking a functional circadian system. *Brain Behav. Immun.* 32, 94–104. doi: 10.1016/j.bbi.2013.02.007

Prendergast, B. J., Cisse, Y. M., Cable, E. J., and Zucker, I. (2012). Dissociation of ultradian and circadian phenotypes in female and male Siberian hamsters. *J. Biol. Rhythms* 27, 287–298. doi: 10.1177/0748730412448618

Prince, L. Y., Bacon, T. J., Tigaret, C. M., and Mellor, J. R. (2016). Neuromodulation of the feedforward dentate gyrus-CA3 microcircuit. *Front. Synaptic Neurosci.* 8:32. doi: 10.3389/fnsyn.2016.00032

Ragozzino, M. E., and Gold, P. E. (1995). Glucose injections into the medial septum reverse the effects of intraseptal morphine infusions on hippocampal acetylcholine output and memory. *Neuroscience* 68, 981–988. doi: 10.1016/0306-4522(95)00204-v

Ragozzino, M. E., Pal, S. N., Unick, K., Stefani, M. R., and Gold, P. E. (1998). Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections. *J. Neurosci.* 18, 1595–1601. doi: 10.1523/jneurosci.18-04-01595.1998

Ragozzino, M. E., Unick, K. E., and Gold, P. E. (1996). Hippocampal acetylcholine release during memory testing in rats: augmentation by glucose. *Proc. Natl. Acad. Sci. U.S.A.* 93, 4693–4698. doi: 10.1073/pnas.93.10.4693

Ragozzino, M. E., Wenk, G. L., and Gold, P. E. (1994). Glucose attenuates a morphine-induced decrease in hippocampal acetylcholine output: an in vivo microdialysis study in rats. *Brain Res.* 655, 77–82. doi: 10.1016/0006-8993(94)91599-7

Risold, P. Y., and Swanson, L. W. (1997). Connections of the rat lateral septal complex. *Brain Res. Rev.* 24, 115–195. doi: 10.1016/s0165-0173(97)00009-x

Roland, J. J., Stewart, A. L., Janke, K. L., Gielow, M. R., Kostek, J. A., Savage, L. M., et al. (2014). Medial septum-diagonal band of Broca (MSDB) GABAergic regulation of hippocampal acetylcholine efflux is dependent on cognitive demands. *J. Neurosci.* 34, 506–514. doi: 10.1523/jneurosci.2352-13.2014

Ruby, N. F. (2003). Hibernation: when good clocks go cold. *J. Biol. Rhythms* 18, 275–286. doi: 10.1177/0748730403254971

Ruby, N. F., Barakat, M. T., and Heller, H. C. (2004). Phenotypic differences in re-entrainment behavior and sensitivity to nighttime light pulses in Siberian hamsters. *J. Biol. Rhythms* 19, 530–541. doi: 10.1177/0748730404268055

Ruby, N. F., Fernandez, F., Garrett, A., Klima, J., Zhang, P., Sapolsky, S., et al. (2013). Spatial memory and object recognition are impaired by circadian arrhythmia and restored by the GABAA antagonist pentylenetetrazole. *PLoS One* 8:e72433. doi: 10.1371/journal.pone.0072433

Ruby, N. F., Fisher, N., Patton, D. F., Paul, M. J., Fernandez, F., and Heller, H. C. (2017). Scheduled feeding restores memory and modulates c-Fos expression in the suprachiasmatic nucleus and septohippocampal complex. *Sci. Rep.* 7:6755.

Ruby, N. F., Hwang, C. E., Wessells, C., Fernandez, F., Zhang, P., Sapolsky, R., et al. (2008). Hippocampal-dependent learning requires a functional circadian system. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15593–15598. doi: 10.1073/pnas.0808259105

Ruby, N. F., Joshi, N., Heller, H. C. (1998). Phase shift magnitude and direction determine whether Siberian hamsters reentrain to the photocycle. *J. Biol. Rhythms* 13, 506–517. doi: 10.1177/074873049801300606

Ruby, N. F., Saran, A., Kang, T., Franken, P., and Heller, H. C. (1996). Siberian hamsters free run or become arrhythmic after a phase delay of the photocycle. *Am. J. Physiol.* 271, R881–R890.

Ruby, N. F., and Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *Am. J. Physiol.* 263, R353–R362.

Sasaki, T., Piatti, V. C., Ahmadi, S., Lisman, J. E., Leutgeb, S., and Leutgeb, J. K. (2018). Dentate network activity is necessary for spatial working memory by supporting CA3 sharp-wave ripple generation and prospective firing of CA3 neurons. *Nat. Neurosci.* 21, 258–269. doi: 10.1038/s41593-017-0061-5

Senzai, Y. (2019). Function of local circuits in the hippocampal dentate gyrus-CA3 system. *Neurosci. Res.* 140, 43–52. doi: 10.1016/j.neures.2018.11.003

Shimizu, K., Kobayashi, Y., Nakatsuji, E., Yamazaki, M., Shimba, S., Sakimura, K., et al. (2016). SCOP/PHLPP1β mediates circadian regulation of long-term recognition memory. *Nat. Comm.* 7:12926.

Smarr, B. L., Jennings, K. J., Driscoll, J. R., and Kriegsfeld, L. J. (2014). A time to remember: the role of circadian clocks in learning and memory. *Behav. Neurosci.* 128, 283–303. doi: 10.1037/a0035963

Solari, N., and Hangya, B. (2018). Cholinergic modulation of spatial learning, memory and navigation. *Eur. J. Neurosci.* 48, 2199–2230. doi: 10.1111/ejn.14089

Steinlechner, S., Stieglitz, A., and Ruf, T. (2002). Djungarian hamsters: a species with a labile circadian pacemaker? Arrhythmicity under a light-dark cycle induced by short light pulses. *J. Biol. Rhythms* 17, 248–258. doi: 10.1177/074873040201700308

Stephan, F. K., and Kovacevic, N. S. (1978). Multiple retention deficit in passive avoidance in rats is eliminated by suprachiasmatic lesions. *Behav. Biol.* 22, 456–462. doi: 10.1016/S0091-6773(78)92565-8

Squire, L. R. (1969). Effects of pretrial and posttrial administration of cholinergic and anticholinergic drugs on spontaneous alternation. *J. Comp. Physiol. Psychol.* 69, 69–75. doi: 10.1037/h0027937

Stefani, M. R., and Gold, P. E. (2001). Intrahippocampal infusions of k-atp channel modulators influence spontaneous alternation performance: relationships to acetylcholine release in the hippocampus. *J. Neurosci.* 21, 609–614. doi: 10.1523/jneurosci.21-02-00609.2001

Teles-Grilo Ruivo, L. M., and Mellor, J. R. (2013). Cholinergic modulation of hippocampal network function. *Front. Synaptic Neurosci.* 5:2.

Teng, X., Tian, X., Doelling, K., and Poeppel, D. (2018). Theta band oscillations reflect more than entrainment: behavioral and neural evidence demonstrates an active chunking process. *Eur. J. Neurosci.* 48, 2770–2782. doi: 10.1111/ejn.13742

Thomas, G. J. (1979). Comparison of effects of small lesions in posterodorsal septum on spontaneous and rerun correction (contingently reinforced) alternation in rats. *J. Comp. Physiol. Psychol.* 93, 685–694. doi: 10.1037/h0077608

Tolman, E. C. (1948). Cognitive maps in rats and men. *Psych. Rev.* 55, 189–208. doi: 10.1037/h0061626

Tolman, E. C. (1925). Purpose and cognition: the determiners of animal learning. *Psychol. Rev.* 32, 285–297. doi: 10.1037/h0072784

Tranah, G. J., Blackwell, T., Stone, K. L., Ancoli-Israel, S., Paudel, M. L., Ensrud, K. E., et al. (2011). Circadian activity rhythms and risk incident dementia and mild cognitive impairment in older women. *Ann. Neurol.* 70, 722–732. doi: 10.1002/ana.22468

Vandercasteele, M., Varga, V., Berényi, A., Papp, E., Barthó, P., Venance, L., et al. (2014). Optogenetic activation of septal cholinergic neurons suppresses sharp wave ripples and enhances theta oscillations in the hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13535–13540. doi: 10.1073/pnas.1411233111

Vecera, S. P., Rothbart, M. K., and Posner, M. I. (1991). Development of spontaneous alternation in infancy. *J. Cogn. Neurosci.* 3, 351–354. doi: 10.1162/jocn.1991.3.4.351

Vodolazhskaya, M. G., Beier, E. V., and Popov, A. V. (2001). Functional interactions of the suprachiasmatic nuclei and the hippocampus during the acquisition and recovery of a conditioned reflex to time in rats. *Neurosci. Behav. Physiol.* 31, 481–488.

Wahlsten, D. (2001). Standardizing tests of mouse behavior: reasons, recommendations, and reality. *Physiol. Behav.* 73, 695–704. doi: 10.1016/s0031-9384(01)00527-3

Watts, A. G. (1991). "The efferent connections of the suprachiasmatic nucleus: anatomical insights into the control of circadian rhythms," in *Suprachiasmatic Nucleus: The Mind's Clock*, eds D. C. Klein, R. Y. Moore, and S. M. Reppert (New York: Oxford University Press), 77–106.

Watts, A. G., Swanson, L. W., and Sanchez-Watts, G. (1987). Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport Phaseolus vulgaris leucoagglutinin in the rat. *J. Comp. Neurol.* 258, 204–229. doi: 10.1002/cne.902580204

Weinert, D., and Schöttner, K. (2007). An inbred lineage of Djungarian hamsters with a strongly attenuated ability to synchronize. *Chronobiol. Intl.* 24, 1065–1079. doi: 10.1080/07420520701791588

Zhang, H., Lin, S.-C., and Nicolelis, M. A. L. (2010). Spatiotemporal coupling between hippocampal acetylcholine release and theta oscillations in vivo. *J. Neurosci.* 30, 13431–13440. doi: 10.1523/jneurosci.1144-10.2010

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 Psychiatry

PERSPECTIVE
published: 13 May 2021
doi: 10.3389/fpsyt.2021.652161

Should We Re-think Regulations and Standards for Lighting at Workplaces? A Practice Review on Existing Lighting Recommendations

Oliver Stefani [1,2]* *and Christian Cajochen* [1,2]

[1] *Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Basel, Switzerland,* [2] *Transfaculty Research Platform Molecular and Cognitive Neurosciences (MCN), University of Basel, Basel, Switzerland*

Nowadays lighting projects often include temporal variations of the light, both spectrally and in terms of intensity to consider non-visual effects of light on people. However, as of today there are no specific regulations. Compliance with common lighting standards that address visual aspects of light, often means that only little non-visually effective light reaches the eye. In this practice review we confront existing regulations and standards on visual lighting aspects with new recommendations on non-visual aspects and highlight conflicts among them. We conclude with lighting recommendations that address both aspects.

Keywords: lighting, workplace, standards, circadian rhythms, non-image forming effects of light

OPEN ACCESS

Edited by:
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Building Research Establishment, United Kingdom
Kevin William Houser,
Oregon State University,
United States

***Correspondence:**
Oliver Stefani
oliver.stefani@unibas.ch

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Psychiatry

Received: *11 January 2021*
Accepted: *14 April 2021*
Published: *13 May 2021*

Citation:
Stefani O and Cajochen C (2021) Should We Re-think Regulations and Standards for Lighting at Workplaces? A Practice Review on Existing Lighting Recommendations. Front. Psychiatry 12:652161. doi: 10.3389/fpsyt.2021.652161

INTRODUCTION

The advent of electric lighting made it possible to decouple working hours by means of shift work from times when daylight was available. Concomitant to alterations in working hours, sleep-wake times are very often irregular in shift workers thereby impacting on the endogenous circadian timing system with negative health consequences (1). Light as the principal synchronizer (i.e., Zeitgeber) of human circadian rhythms, is "seen" during the biological night while at work, which might lead to circadian rhythms disturbance such as shift work sleep disorder (SWD). SWD is a circadian rhythm sleep disorder characterized by insomnia and excessive sleepiness affecting people whose work hours typically occur during the habitual sleep period (1). In particular light with high proportions of short wavelengths in the blue spectral range in the evening and at night suppress the secretion of the night hormone melatonin, a marker of circadian rhythmicity in humans. Additionally to negative light effects during the night, low illuminances during the day can destabilize circadian rhythms (2).

Continuously disturbing the entrainment of endogenous circadian rhythms with external diurnal Zeitgeber rhythms with can weaken the regenerative ability of the organism (3, 4). Night work is associated with negative consequences for somatic and mental health (5) and persistent desynchronization of endogenous rhythms limits cognitive performance (6). Thus, with the increase in flexible working times, innovative lighting concepts that take particular account of non-visual lighting effects become particularly important.

With the increased time spent in buildings, the length of time during which people are exposed to high amounts of daylight decreases. Although, lighting standards for workplaces ensure that we can see well, specifications for artificial lighting correspond to twilight conditions outdoors (7). 500 lx of artificial light indoors corresponds to ∼0.5% of the light on a cloudless day. Measurements

at workplaces have shown that workers are usually exposed to illuminance of only 100 lx for more than 50% of the day (8), which fall far below the recent recommendation of daylight illuminance exposure (9).

VISUAL EFFECTS OF LIGHT – REGULATIONS, STANDARDS, AND ENERGY ASPECTS

Where life safety is threatened (e.g., in emergency situations like building fires) interior lighting projects must comply with requirements imposed by regulations regarding minimum levels of illuminance. Different regions of the world approach regulations, recommended practices, and standards differently. For the design and operation of workplaces, the German "Technical Rules for Workplaces" (ASR) for example, reflect the state of the art for occupational medicine, occupational hygiene, as well as other reliable ergonomic findings for setting up and operating workplaces. According to the German Workplace Ordinance, workplaces must receive as much daylight as possible and be equipped with artificial lighting that is appropriate for the safety and health protection of employees. The Technical Rules for Workplaces ASR A3.4 (10) "Lighting" specify the requirements of the Workplaces Ordinance for setting up and operating the lighting in workplaces as well as the requirements for glare protection when exposed to sunlight. In Germany for example, an artificial lighting system for workplaces must meet requirements concerning illuminance, limitation of glare, color rendering, flickering, or pulsation as well as shadows. DGUV Informative Publication 215-210 "Natural and Artificial Lighting of Workplaces" of the German Social Accident Insurance (DGUV) offers also assistance to employers in implementing the ASR A3.4 "Lighting."

Standards differ from regulations. In general, lighting professionals are expected to appraise each design situation and develop criteria for illuminance, color rendering quality, uniformity, correlated color temperature (CCT) etc., that are appropriate for a project, and although, there is no obligation to comply to standards, they provide valuable guidance. Workplace lighting in particular should consider these standards. One example is the German DIN EN 12464-1:2011-08 (11). This standard specifies the planning principles for lighting systems, but does not specify the requirements for the safety and health protection of employees at work. This standard gives illuminance recommendations for the task area, the immediate surroundings, background area, and for walls and ceilings. In a new draft (prEN 12464-1:2019), higher illuminances are recommended in order to allow for adjustments. In the new draft, the horizontal illuminance of 500 lx for workplaces e.g., is now specified as a minimum requirement. A higher value (1000 lx) is specified, which is to be used e.g., in rooms with elderly persons with lower eyesight. The demands on lighting quality are determined by the visual tasks that the human eye has to master. The classic quality features of lighting can be divided into three basic quality features, which are weighted differently depending on the use of the room and the desired appearance: Visualization, visual comfort, and visual ambience. The following applies:

- Visual performance is influenced by the illuminance and the limitation of direct and reflected glare.
- Good color rendering and a harmonious brightness distribution ensure visual comfort.
- Visual ambience is determined by CCT, light direction, and modeling (i.e., the distribution of light and shadows).

Good lighting systems are also characterized by energy efficiency. However, according to DIN EN 12464-1 (11), the quality of light should not be reduced for lower energy consumption.

The purpose of the Swiss SIA 2024 (12) is to standardize assumptions about room usage, in particular about personal occupancy, and equipment usage. These assumptions should be applied in calculations and verifications according to the standards of energy and building services engineering if there is no more accurate information available. The requirements are regarded as standard values for the design of plants or factories in an early planning phase. Finally, typical values are given for power and energy requirements in the areas of appliances, lighting, ventilation, etc.,

Further, important features are flicker-free lighting and the possibility of changing brightness and CCT. Luminaires that are too bright in the field of view can cause glare. Therefore, light sources must be shielded in a suitable way. Glare is a complex topic that cannot be discussed in rigorous detail in this work. Other work, such as the DIN EN 12464-1 should be referred to.

When working with PC screens, care should be taken that the luminance ratio between the working field and its immediate surroundings is no greater than 3:1. The luminance ratio between the work surface and the more distant surfaces should not exceed 10:1. Due to higher luminances and improved anti-reflective coatings, modern PC screens can tolerate much higher environmental luminance levels than their predecessors. DIN EN 12464-1 describes the permissible limit values for avoiding reflected glare. For screens with a luminance of $L \leq 200$ cd/m^2 luminances of up to 1500 cd/m^2 are permissible for luminaires. For monitors with a monitor luminance $L > 200$ cd/m^2 (typical for offices with good to very good daylight supply and correspondingly adapted flat screens) luminance values of up to 3000 cd/m^2 are permissible. The ambient contrast ratio (A-CR) is a key metric to achieve a high image quality of displays when considering bright ambient lighting (13). While OLED (organic light-emitting device) based displays exhibit several attractive features, such as self-emission, high brightness, and a high contrast ratio, when operated under bright ambient light, most of the incident light is reflected and decreases the A-CR which makes the application under high illuminances difficult. Today there are several methods to eliminate reflected ambient light in OLEDs (e.g., by a circular polarizer or a destructive interference layer). A-CR is generally defined as

$$ACR = \frac{L_{\text{on}} + L_{\text{ambient}} \cdot R_{\text{L}}}{L_{\text{off}} + L_{\text{ambient}} \cdot R_{\text{L}}}$$

where L_{on} (L_{off}) represents the on-state (off-state) luminance value of an LCD or OLED, and $L_{ambient}$ is ambient luminance (14). R_L is the luminous reflectance of the display panel.

For a balanced luminance in the room all surfaces must be taken into account. Surface luminances can be determined by the reflectance of the surfaces and the illuminance on the surfaces. According to DIN EN 12464-1 recommended reflectances are:

- ceiling: 0.7 to 0.9
- walls: 0.5 to 0.8
- floor: 0.2 to 0.4

Regarding good color rendering it is commonly recommended to have a CRI of Ra > 80. We have recently found evidence for positive effects of a high CRI (Ra 97 vs. 80) on visual comfort, daytime wakefulness, well-being, and nighttime sleep (15). A newer and better system for evaluating a light source's color rendering property is IES TM-30-15. The fidelity index and the gamut index of IES TM-30-15 of the LED causing the before mentioned positive effects were Rf.97 and Rg.101, respectively, and for the poorer performing LED it was Rf.81 and Rg.94.

Without shadows objects are only two-dimensional images. Only the correct distribution of light and shadow guarantees that faces and gestures, surfaces, and structures can be easily recognized (11). A pleasant lighting climate is created when people, architecture, and room furniture are illuminated in such a way that shapes and surface structures are clearly visible. Distances can be easily estimated and orientation in the room is made easier. Good visual communication requires that faces are easily and quickly recognized. In areas where good visual communication is important, for example in offices and meeting areas, DIN EN 12464-1 recommends a higher average cylindrical illuminance of 150 lx. The cylindrical illuminance is the average of all vertical light on an imaginary cylinder. DIN EN 12464-1 cites "modeling" as an important quality feature for the perception of people and objects. Modeling is the relationship between cylindrical and horizontal illuminance and should be between 0.30 and 0.60.

The international WELL Building Institute (16) aims for advancing health and well-being in buildings. It also provides recommendations for lighting to support visual acuity and is mainly based on the American National Standards Institute (ANSI) and Illuminating Engineering Society (IES) RP-1-20 (17) standard and on the standard of the Ontario Ministry of Labour, Computer Ergonomics: Workstation Layout and Lighting (18).

At workstations or desks requirements are met when

1. The ambient lighting system is able to maintain an average light intensity of 215 lx or more, measured on the horizontal work plane. The lights may be dimmed in the presence of daylight, but they should be able to independently achieve these levels.
2. The ambient lighting system is zoned in independently controlled banks no larger than 46.5 m² or 20% of open floor area of the room (whichever is larger).
3. If average ambient light is below 300 lx, task lights providing 300 to 500 lx at the work surface are available upon request.

The American National Standards Institute and Illuminating Engineering Society of North America. RP-1-20 provides recommended luminance ratios for offices:

1. Luminance ratios should not exceed 3:1 between a paper task and an adjacent visual display terminal.
2. For ceiling luminance ratios, 10:1 is the maximum acceptable ratio.
3. Luminance ratios should not exceed 10:1 between a task and a remote surface.

In the latest version of the WELL v2 pilot (Q1 2021) it is recommended that indoor spaces should comply with one of the lighting reference guidelines (IES Lighting Handbook 10th Edition, EN 12464-1: 2011, ISO 8995-1:2002(E) (CIE S 008/E:2001), or GB50034-2013).

NON-VISUAL EFFECTS OF LIGHT

It is only known since 2002 that the human eye has a third photoreceptor for processing ambient light in addition to the two classical photoreceptor types, the rods, and cones (19, 20). Effects of visible radiation, which are mainly controlled by this newly discovered photoreceptor, but make a minor contribution to classical visual information processing, are also called non-visual light effects. Berson and colleagues reported that those ganglion cells containing the photopigment melanopsin project almost exclusively to the nucleus suprachiasmaticus (SCN), the central pacemaker driving circadian rhythms. They are also able to transmit light signals into the cortex without the help of the classical photoreceptors. Thus, these ganglion cells have been termed "intrinsically photosensitive retinal ganglion cells (ipRGC)" with melanopsin maximally sensitive to visible short-wave radiation (21).

Provencio et al. (22) reported that a coarsely resolved network of photosensitive ganglion cells extends over the retina of mice, which has the task of detecting brightness. Later, it was found that these melanopsin-containing ganglion cells are distributed not only in the fovea but also over the entire retina with a density of 3–5 cells/mm² and have their maximum concentration of 20–25 cells/mm² in the area surrounding the fovea (20, 23). IpRGCs are not evenly distributed across the retina but have a higher density in the lower half so that light that falls into the eye from above and impinges the lower half of the retina suppresses the nocturnal release of melatonin more than light from below (24, 25).

While blue-enriched light can contribute to increased alertness, especially in the evening and at night, nocturnal exposure to light reduces melatonin secretion. Melatonin secretion is especially reduced by light at night if people are exposed to only a low dose of light during the day (26). Today, people often live their lives in isolation from their natural environment with a high risk to develop circadian disorders (27). Severe circadian disturbances are found in people who are forced to chronically change their lifestyle by adapting their sleep-wake schedules to the imposed work schedules (e.g., rotating shift workers) (1). A milder form of circadian disorder occurs in many people due to too short night's sleep on working days

and a delayed onset and prolonged duration of sleep on non-working days. Over 80% of people in western industrialized countries show this altered sleep behavior and that nightly sleep on non-working days is delayed by an average of 90 min (28).

Since circadian rhythms only have an approximate period length of 24 h, they require daily synchronization with the environment (29, 30). The rotation of the earth and thus the regular light-dark change is the most important environmental signal for the synchronization of circadian rhythms (31). For the interpretation of ambient brightness, photoreceptors continuously calculate the intensity, and spectral composition of the light entering our eyes (32). Thus, when light with increased short-wave radiation enters the eye, ipRGCs signal a bright phase of day to the SCN. In addition, the times of change from dark to light phase and vice versa (i.e., dawn and dusk) provide a crucial input for the SCN and the synchronization of circadian rhythms with the environment (31, 33, 34). Bright white light at night can shift the circadian phase backward by up to 3 h in the next 24-h cycle. In contrast, early morning exposure to light can shift the circadian phase forward by up to 2 h in the next 24-h cycle (35, 36).

Some studies document acute effects of bright light on the subjective feeling of alertness at night and during usual sleep periods (37–39), before falling asleep (40) and immediately after waking up in the morning (41, 42) but also during daytime (43). Although, most of these studies compare very low illuminances (5–50 lx) with high illuminances (1,000–5,000 lx) of fluorescent white light, alerting effects were estimated to occur already at around 100 lx during the night and at 500 lx during the evening. Thus, there is evidence, that subjective wakefulness due to bright light can basically occur at any time of the day. Other research with objective measures has so far only been able to prove a wakefulness inducing effect of bright light at night (44) and results of studies conducted during the day provided inconsistent results (43, 45, 46), possibly due to smaller differences in the light levels compared. Nevertheless, bright light during the day makes the circadian system less sensitive to nocturnal light (26, 47–49). Although the protocols of these studies differ from each other, illuminances that were compared during the day were significantly different from each other (i.e., at least 10-fold up to 400-fold different). A dark phase of several hours can increase sensitivity to light. Thus, early morning exposure to light immediately after awakening (i.e., after several hours of nightly sleep in darkness) can phase advance the circadian phase by 1–3 h (50, 51).

In many cases, the alerting effect of light, especially in the evening and at night, is also associated with an increase in attention and working memory performance (41, 52). Non-visual effects also include the mood enhancing effect of bright light (53–55), with required light doses being around 2500 lx-h. There is also evidence that the current mood reflects a person's level of alertness and the immediate effect of bright light on mood is mediated by the wakefulness inducing effect (56).

LIGHTING CONCEPTS THAT ADDRESS NON-VISUAL EFFECTS OF LIGHT

Considering our experience with around 100 years of electric lighting, people have been exposed to this artificial creation 5,000 times shorter than to the light at night from fire. The first evidence for the use of handcrafted light sources comes from archaeological findings from around 500,000 years ago. Fire was used as a source of light at night, but life and work still depended on daylight. Daylight is perhaps the purest form of human centric lighting since our eyes have had several million times longer to optimize to daylight than to LEDs. It is therefore reasonable to assert, from the evolutionary standpoint, that human eyes, and behavior are not yet optimized to electric light.

New lighting technologies that try to mimic continuously changing CCT and illuminance of sunlight according to the time of day are often termed HCL (Human Centric Lighting). According to manufacturers of these lighting technologies, it is possible to provide people indoors with artificial light similar to daylight in such a way that they can benefit from the beneficial effects natural daylight would provide. These include increased alertness, concentration, and performance. A publication summarizing the benefits of HCL on humans shows that it has sound motivations (57). The authors conclude that "bright days and dark nights are a good starting point," and suggest that apart from electric lighting, architecture should be driven by daylight design principles. A conclusion that we support. Since HCL is increasingly being promoted and used for work places or private homes due to their postulated effects, the Swiss State Secretariat for Economic Affairs (SECO), and the Federal Office of Public Health (FOPH) have commissioned the Centre for Chronobiology at the University of Basel to evaluate scientific literature on HCL (58). The central question was whether this light can influence physiological, cognitive, or subjective effects in humans, i.e., the effects perceived by humans themselves.

The Basel study (58) has shown that only a few studies have investigated whether HCL can influence the above mentioned effects. Therefore, the University of Basel has extended the assessment and additionally evaluated studies on physiological, cognitive, or subjective effects of artificial light that affects people during the day during office hours (from 7:00 a.m. to 5:00 p.m.) but does not continuously adapt to the properties of daylight. A total of 45 studies met the inclusion criteria. On the basis of these studies, it was possible to check for 33 different effect variables whether they depend on the light intensity and CCT of artificial light that affects people during the day.

The Basel study (58) shows that neither the light intensity nor CCT significantly influence physiological parameters such as pulse rate and brain waves during normal office hours. In the case of cognitive effects, however, it was shown that light intensity and CCT had an influence on the reaction time of people. In addition, the spectrum of light influences the accuracy with which people solve tasks. In the subjective effects, light intensity and light spectrum had an influence on the concentration, tiredness and drowsiness perceived by the persons themselves. Overall, however, the observed effect strengths of the light effect

during office hours were rather small. Nevertheless, the authors of the study come to the conclusion that high light intensity and higher CCT during daytime hours are advantageous in artificially illuminated interiors, even if these advantages are only evident in cognitive and subjective effects but not in physiological parameters. During the night, the effects of higher CCT are more prominent even in field studies. While blue-enriched white light sources can adjust circadian rhythm to night-shiftwork, reduce sleepiness, and enhance cognitive performance of night-shift workers (59) this concept should be applied cautiously and only if workers must work very concentrated (e.g., in control rooms).

While there are numerous studies providing evidence of non-visual effects of light during the evening and at night, results might not be translatable to the day. In line with the Basel study (58), a literature review on daytime non-visual effects of light on alertness (60) concludes that the present literature provides inconclusive results on alerting effects of light during daytime, particularly for objective measures and correlates of alertness. The authors suggest that the alerting potential of exposure to more intense white light should still be investigated. Another systematic review assessed effects of light on alertness and mood in daytime workers (61). Although, they conclude that light with a high CCT may improve alertness during the day, they suggest that additional studies are still needed because all findings are based on low-quality evidence.

Impacts of two dynamic LED lighting concepts with illuminance and CCT gradually decreasing between 1:30 p.m. and 5 p.m. were investigated on its effects on sleep and well-being (62). In one setting illuminance changed from 700 to 500 lx and CCT from 6000 to 3500 K, in the other from 500 to 300 lx, and CCT from 5000 to 3000 K. The settings were compared to static light (500 lx, 5000 K and 300 lx, 4000 K). A significant increase in subjective alertness was observed at 1 p.m., indicating a potential solution to reduce the subjective sleepiness in the afternoon. On the other hand, a significant decrease in perceived sleep quality and sleep duration was reported after subjects were exposed to dynamic lighting. No significant differences were observed for mental stress, productivity, visual comfort, or perceived naturalness.

A different approach with custom-built desktop luminaires intended to support office occupants' entrainment while supporting their alertness during the day. The luminaires were designed to deliver three lighting interventions. First saturated blue light (455 nm, 50 lx) in the morning (6–12 a.m.), Then polychromatic white (6500 K, 200 lx) light at midday (12 a.m.−1:30 p.m.) provided a smooth transition from the first to the third intervention. The third intervention was saturated red light (634 nm, 50 lx) in the afternoon (1:30–5 p.m.). In their results the authors observed advances in sleep start and sleep end times and hence they suggest that the participants were better entrained to the local 24-h light dark cycle while at the same time reporting increased subjective alertness in the afternoon with red light (63).

The effect of dynamic light during shift work on the quality of sleep and melatonin secretion was examined with staff of an Intensive Care Unit (ICU) and compared with staff from a similar ICU with standard light (64). CCT controlled ceiling luminaires with light tubes (2700 and 6500 K) and indirect lighting with RGBW lights "to imitate the reflection of the sun" were used but no information about the spectral characteristics is reported. The light changed color and intensity. The nightlight between 10 p.m. and 5 a.m. was dim (68 lx), and short-wavelength depleted causing the light to appear "unnaturally red." Between 5 and 6 a.m., the light gradually changed to a daylight scenario (525 lx). In the afternoon from 3 p.m. light levels decreased. Between 8 and 10 p.m. a change occurred "toward a mix of primarily red, green, and white." Since no precise spectral measurements are available but only RGB percentages it is difficult to replicate the lighting conditions. Nevertheless, the intervention group reported to be more rested and assessed their condition on awakening as better than the control group. The study, however, found no significant differences in sleep efficiency and melatonin levels. Subjectively, nurses from the intervention group assessed their sleep as more effective than participants from the control group. In a different field study, bright fluorescent lighting (1500–2000 lx) when compared to standard lighting (300 lx) in hospitals decreased sleepiness of ICU nurses working a 10-h night shift (65).

In a field experiment, effects of dynamic lighting on office workers were tested (66). In the dynamic lighting condition, employees experienced a gradually changing lighting scenario (changing twice a day between 8 and 12 a.m. and 1:30 and 4 p.m. from 700 to 500 lx and 4700 to 3000 K). The static condition provided an illuminance of 500 lx and CCT of 3000 K While employees were more satisfied with the dynamic lighting there were no significant differences for need for recovery, vitality, alertness, headache and eyestrain, mental health, sleep quality, or subjective performance.

Under strictly controlled laboratory conditions, we examined whether dynamic light across the day influences cognitive performance, visual comfort, melatonin secretion, sleepiness, and sleep (67). Volunteers either woke up with static daylight LED (100 lx at the pillow and 4000 K, melEDI 69 lx) or with a dynamic daylight LED that changed CCT (2700–5000 K) and intensity (0–100 lx at the pillow, melEDI 0.4–76 lx) across the day (daylight here refers to the spectral characteristics of the Toshiba TRI-R LED). Participants underwent a 49-h laboratory protocol. They spent the first 5-h in the evening under standard lighting, followed by an 8-h nocturnal "baseline" sleep episode at habitual bedtimes. Thereafter, they spent a scheduled 16-h waking day under one of the lighting conditions. Following a 8-h nocturnal "treatment" sleep episode, the volunteers spent another 12 h either under static or dynamic light. Horizontal illuminance at desk height ranged, depending on the position, between 150 and 650 lux, which corresponds to standard office lighting. Under dynamic light, evening melatonin levels were less suppressed 1.5 h prior to usual bedtime, and participants felt less vigilant in the evening compared to static light. Sleep latency was significantly shorter compared to the static light condition while sleep structure, sleep quality, cognitive performance, and visual comfort did not significantly change. These results support the recommendation of using blue-depleted light and low illuminances in the late evening, which can be achieved by a dynamically changing LED solution. Since illuminance

decreased to around 1 lx, this lighting concept can only be applied in domestic areas but if concentration at work in the late evening and at night is required, this lighting concept would be counterproductive.

To date, only a few field studies investigated the influence of dynamic lighting solutions during shift work. A field study surveying the state of subjective alertness and fatigue in 542 employees during three-shift work (8 h working shifts) in ongoing production operations compared dynamic light with a static lighting condition (68). In a first round, 256 respondents evaluated the static lighting concept by completing a structured questionnaire. In a second round, 287 respondents commented on the alternating lighting concept. Fourty one percent of the participants who experienced the alternating lighting concept took part in the survey on the static lighting concept. They worked in three shifts (morning, late, and night) for 8 h each. The alternating lighting concept featured a high horizontal illuminance on the workplace (850 lx) and a high CCT (5300 K) during daytime. The resulting vertical illuminance at eye height was 237 lx (melEDI 164 lx) and CRI was Ra 77. During nighttime, a reduced illuminance (580 lx) with low CCT (3400 K) and CRI of Ra 85 was deployed. This resulted in a vertical illuminance of 158 lx (melEDI 71 lx) at eye height. Illuminance and CCT changed gradually between 5 and 8 p.m. and between 5 and 9 a.m. This setting was compared to the static lighting condition (horizontal at the workplace 760 lx, vertical at the eye 210 lx (melEDI 128 lx), 4600 K, CRI Ra 82). All participants assessed specific lighting characteristics (such as CCT, brightness, color rendering, appeal) using a seven-point Likert scale. No significant differences were found between the participants' rating of the characteristics surveyed in terms of alternating and static lighting conditions. The transitions from day to night conditions and vice versa had no disturbing effect on the participants' rating of the lighting criteria surveyed ($p > 0.05$). Thus, it was concluded that shift workers accepted the alternating lighting system. In addition, there were no significant differences in alertness and fatigue between the early and late shifts for both lighting conditions. This survey indicated the potential usefulness of a dynamic lighting solution for shift-work without major impact on the worker's alertness and fatigue level. However, objective measures such as salivary melatonin levels, and reaction time measures to assess vigilant attention are clearly mandatory for future studies to test the usefulness of dynamic lighting solutions in shift work environments.

With the goal to provide adequate light for visual tasks while lessening disruption of the human circadian system, Moore-Ede et al. (69) derived a spectral sensitivity curve with a peak at 477 nm and a full-width half-maximum of 438 to 493 nm. While there are other products commercially available that notch the spectrum in the melanopsin region, they specifically call it "steadystate circadian potency spectral sensitivity" and suggest that it "permits the development of spectrally engineered LED light sources to minimize circadian disruption and address the health risks of light exposure at night in our 24/7 society, by alternating between daytime circadian stimulatory white light spectra and nocturnal circadian protective white light spectra." They further suggest, that it could provide attractive and energy-efficient white electric light that minimizes circadian disruption if violet LED dies with peak wavelengths of 410 to 420 nm replace the typical 450 nm blue peak emissions of conventional LEDs. Since short-wavelength light is known to have alerting-, performance-, and mood enhancing properties (70, 71), they suggest that this light at night could be used to reduce human error, without the risk of circadian disruption and health disorders. The alerting effect of short-wavelength light could be retained because there is evidence by a single study that the alerting effects of 420 nm violet light are even greater than 440 or 470 nm blue light (72).

In an approach to mimic certain aspects of daylight (i.e., direct warm sunlight and diffuse cool skylight), Aalborg University proposed a combination of directional task lighting and diffuse ambient lighting, with respective intensities and CCTs to create naturally perceived luminous variations. Such lighting concepts mimicking the combination of light from the sun and sky date back to 1952 (73). Aalborg University conducted a pilot study with four participants that worked for 4 months in such static and dynamic lighting. Visual comfort, perceived atmosphere, and work engagement were evaluated with interviews and questionnaires. The tentative results indicate that dynamic lighting has a positive effect on visual comfort, perceived atmosphere, and work engagement compared to static lighting (74). Another study (75) from the same author investigated the quality of light in an office after adding ceiling-mounted spotlights to traditional diffuse ceiling panels with the intention to complement the directionality of the natural daylight inflow from windows. The visual light quality and perceived atmosphere of the office environment was tested with 30 volunteers through questionnaires, reaction cards and semi-structured interviews. The authors report: "The direct flow of light is recommended to be more than 15% of the total illuminance at the work-plane to provide the distinct visual appearance of modeling and a cozier atmosphere, which is preferable for socializing, and <45% to avoid glare and high contrast for visual tasks. Direct warm and diffuse cool lighting were perceived as the most natural but were not always preferred. There is a slight preference for cooler ambient lighting in clear sky situations and warmer ambient lighting in overcast situations. Strong individual preferences for combinations of color temperatures was identified. . . ."

Effects on well-being and motivation by changing light distribution were investigated by Fleischer (76) in 2001. Lighting consisted of luminaires that slowly changed between direct and indirect light. The ratio between direct and indirect lighting was changed according to the time of the day or to weather conditions. It was shown that pleasure rises with higher illuminance and a large indirect component. This might be due to a "sky-like" impression of the bright ceiling. The preference for a large indirect component was also found by Houser et al. (77) who report a subtle overall preference, when the indirect contribution to horizontal illuminance was 60% or greater. With an increase of the direct component and an increase of illuminance arousal rises. The direct component results in a darker ceiling but brighter desk. Apparently this contradicts the findings of (23) and (25) that find higher sensitivity of ipRGCs

for light coming from above. The results of Fleischer, however, may not be explained by NIF effects (by ipRGCs) but solely by visual effects.

EXISTING RECOMMENDATIONS FOR NON-VISUAL AND CIRCADIAN ASPECTS

From a non-visual and circadian perspective, compliance with the above-mentioned standards cannot guarantee that enough biological active light reaches the eye (78). Some studies (45, 79, 80) report that corneal illuminance levels of at least 1000 lx for several hours are necessary to achieve non-visual effects during the day. Many of these studies investigated the effects of light therapy in the morning while others also found effects with illuminances ranging from 1000 to 1700 lx during various normal office hours (when compared to 165–200 lx). Thus, lighting standards addressing visual aspects are currently not designed to account for non-visual light effects during the day.

Today, an evaluation of the non-visual effectiveness of radiation is based on a radiometric characterization of the radiation entering the eye, and the corneally measured spectral irradiance is weighted with the spectral sensitivity of all five photoreceptors and referenced to the spectrum D65 (standard illuminant) (81). The International Standard of the International Commission on Illumination (CIE) CIE S 026:2018 (82) "CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light" defines spectral sensitivity functions, quantities, and metrics to describe the ability of optical radiation to stimulate each of the five photoreceptor types (S-cone, M-cone, L-cone, rhodopsin, and melanopsin). This standard also denotes a quantity named the "melanopic equivalent daylight illuminance" (melanopic EDI or melEDI), that is expressed in Lux. The melanopic EDI of a light condition expresses how much daylight results in the same melanopic irradiance as the test light condition. Nowadays lighting projects often include temporal variations of the light, both spectrally and in terms of intensity. Lighting projects that consider the possible effects of changing light on people, try to optimize well-being. However, as of today there are no specific regulations. Recommended practices sprout everywhere but experts in the field criticize them.

Seven examples for recommendations for circadian lighting are (in alphabetical order):

1. Chartered Institution of Building Services Engineers (CIBSE) and Building Research establishment (BRE) Research Insight Circadian lighting (83).
2. CIE S 026:2018 (82) "CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light."
3. DGUV 215-210 "Non-visual Effects of Light on Humans" (84).
4. DIN SPEC 67600:2013-04 (85) (technical report) "Biologically Effective Lighting - Planning Recommendations."
5. Recommendations for Healthy Daytime, Evening, and Night-Time Indoor Light Exposure (9).
6. UL DG 24480 (86) "Design Guideline for Promoting Circadian Entrainment with Light for Day-Active People."
7. The WELL standard "CIRCADIAN LIGHTING DESIGN" and the update WELL v2 pilot (87).

CIBSE and BRE Research Insight Circadian lighting

Based on a literature review (83) and their results from a field-study they suggest these tentative recommendations:

1. "From mid-morning until early afternoon, use higher than normal levels of light with increased blue light. Current high color temperature light sources such as LEDs and some types of fluorescent light give high outputs of blue light. There is still scope to tailor their spectra further in the future to fit the peak response of the ipRGC sensors in the eye and maximize their circadian impact.
2. Toward the end of the day, dim the lighting (while retaining enough light to meet visual task recommendations) and lower its color temperature ("warmer," redder light, similar to that in a domestic setting). There is also future scope to alter the spectrum of existing LEDs to give very low circadian stimulus in the evening or at night. Even warm white LEDs often have a small peak of blue light which can stimulate the ipRGCs.
3. Maximize reflected light from room surfaces by using light fittings with an upward light component, and "wall washing" to illuminate the walls directly. This will give more light to people facing the walls.
4. As light levels will be higher than normal for part of the day, use high quality fittings to minimize glare and avoid all flicker. Have a balanced visual environment, for example by avoiding very light-colored desks.
5. Vary the lighting gradually, to avoid disturbing the occupants. Controls need to be reliable.
6. People vary in their preferences for lighting; conventional good practice is to offer individual control but this can negate the circadian effects. There is no obvious way round this.
7. Explain to the occupants what the lighting system is doing and the purpose of varying the lighting."

CIE S 026:201861 "CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light"

CIE recommends to spend adequate time outdoors during the day since it is associated with better health and well-being and also recommends to not restrict daylight within indoor settings. Although, no specific quantities are given, CIE recommends a high melEDI during the day to support alertness, the circadian rhythm, and good sleep during the night in a position statement on Non-Visual Effect of Light. During the evening and at night a low melEDI facilitates sleep initiation and consolidation (88).

DGUV 215-210 "Non-visual Effects of Light on Humans"

This DGUV (German statutory accident insurance) information brochure (84) provides advice on hazards to safety and health at work, how they can be avoided and how opportunities for maintaining health can be exploited with modern lighting concepts. Since scientific knowledge about the non-visual effects

of light on humans is not yet complete, as the brochure says, it is not yet possible to derive any generally valid quantitative statements regarding non-visual effects, for example numerical values for illuminance or CCT. This brochure gives the advice that daylight should be used first and foremost. For this reason, workplaces should preferably be located close to windows. The better the inner clock is synchronized by daylight, the less sensitive it is to disturbing factors, such as artificial light in the evening. Only if little daylight is available at workplaces, bright artificial lighting or lighting with high blue components should be used as a supplement during the day. Light sources with high CCTs are usually favorable for this purpose. This light can achieve similar non-visual lighting effects as daylight, but cannot replace it. In the evening, bright light and light with high blue components should be avoided. This should be done at least 2 h before the usual start of sleep. During this time, the light should primarily illuminate the work surface relevant to the visual task and not fall directly into the eye. Looking directly into the light source and at very brightly illuminated surfaces should be avoided. When working on a computer, tablet or smartphone, special blue light filter programs (e.g., flux, Night Shift or other manufacturer-specific blue light filter apps) should be used at least 2 h before the usual start of sleep. Furthermore, advice is given, that during the day, bright walls, and ceilings should enhance the non-visual effects through indirect light components. In the evening, the necessary brightness at the workplace should be limited. The lower indirect share of light on the ceiling and walls should reduce non-visual effects.

DIN SPEC 67600:2013-04 "Biologically Effective Illumination - Design Guidelines"

The German DIN SPEC 67600:2013-04 "Biologically effective illumination - Design guidelines" recommends: Illuminance at the eye $\geq$ 250 lx at CCT = 8000 K or Illuminance at the eye $\geq$ 290 lx at CCT = 6500 K. The Commission for Occupational Health and Safety and Standardization (KAN) (represents occupational health and safety interests in the standardization process) criticizes (89):

> "Contents of the already published DIN SPEC 67600:2013-04 (technical report) "Biologically Effective Lighting - Planning Recommendations" are partly based on insufficiently secured findings, therefore a misinterpretation during its application cannot be excluded … the planning recommendations of DIN SPEC 67600 (technical report) do not form a secure basis for the implementation of the Technical Regulation for Lighting ASR A3.4 in operation."

CIBSE and BRE concluded in a literature review: "*The existing recommendations in DIN SPEC 67600 should be treated with caution.*"

Recommendations for Healthy Daytime, Evening, and Night-Time Indoor Light Exposure

A recent publication (9) from experts in lighting, neurophysiological photometry and sleep and circadian research provides an expert consensus for healthy daytime and evening/night-time light environments. They come to the conclusion that "Throughout the daytime, the recommended minimum melEDI is 250 lx at the eye measured in the vertical plane at ∼1.2 m height (i.e., vertical illuminance at eye level when seated). If available, daylight should be used in the first instance to meet these levels. If additional electrical lighting is required, the polychromatic white light should ideally have a spectrum that, like natural daylight, is enriched in shorter wavelengths close to the peak of the melanopic action spectrum. During the evening and at home, Brown et al. (9) recommend to reduce melEDI to around 10 lx at least 3 h before bedtime. During sleep the recommended maximum melEDI is 1 lx.

UL DG 2448022 "Design Guideline for Promoting Circadian Entrainment With Light for Day-Active People"

UL DG 2448022 "Design Guideline for Promoting Circadian Entrainment with Light for Day-Active People" recommends: "The amount of light equivalent to that after 1 h of exposure, capable of suppressing the production of melatonin at night by 30 per cent … should be continuously available at the occupant's eyes for a minimum of 2 h during the daytime." This would translate into a vertical illuminance at the eye of about 350 lx for warm light (CCT < 3000 K) and ∼200 lx for cool light (CCT > 5000 K) sources. Here the question arises, how a suppression of melatonin at night relates to a measure of light during the day. UL DG 2448022 is commented by the IES (90) as follows:

> "It is important to note that UL Design Guideline 24480 is not a consensus (ANSI) document. The IES maintains the position that any Recommended Practice related to light and health should be a consensus document developed through an accredited American National Standards Institute process. Without the full rigor of an ANSI approved Standard, non-consensus based information cannot be deemed to have been fully vetted and lacks the authority to provide public guidance regarding means or methods that affect public health. The IES urges the lighting industry to exercise caution when considering a non-consensus document for design, application, product qualification or regulatory purposes."

The Well Standard and Well v2 Pilot

The WELL standard recommends for melanopic light intensity at work areas: "Light models or light calculations demonstrate that at least one of the following requirements is met":

1. At 75% or more of workstations, at least 200 equivalent melanopic lx (EML) is present, measured on the vertical plane facing forward, 1.2 m above finished floor (to simulate the view of the occupant). This light level may incorporate daylight, and is present for at least the hours between 9:00 a.m. and 1:00 p.m. for every day of the year.
2. For all workstations, electric lights provide maintained illuminance on the vertical plane facing forward (to simulate the view of the occupant) of 150 EML or greater.

The newer WELL v2 pilot recommends these levels for all spaces (at least 150 EML) and adds the corresponding EDI value (136

melEDI). In case that 218 melEDI or more are achieved, the space would achieve a better score in a system based on points. These light levels vertical plane at the eye should be achieved at least between the hours of 9 a.m. and 1 p.m. and may be lowered after 8 p.m. at night.

CIBSE and BRE concluded in a literature review (91): "*The existing recommendations in the WELL Building Standard should be treated with caution.*"

CONCLUSION

The circadian timing system in humans is genetically timed in such way that we are active and awake during daytime and inactive and asleep during the night (i.e., diurnal species). Thus, during the biological night the hormone melatonin is actively secreted in a circadian fashion usually peaking 2–3 h after habitual bedtime. As melatonin is important for many physiological processes in the human body (e.g., antioxidant and regulating sleep-wake timing), its secretion should not be suppressed or altered in the evening and at night by light. Avoiding light at night during shift work, however, is rather difficult, especially if workers need to be fully concentrated. Thus, ideal lighting conditions during night shifts is always a trade-off between optimal light for visual tasks, safety, alertness, and well-being and optimal light for non-visual effects avoiding circadian phase shifts and melatonin suppression.

Exposure to brighter light and light with high proportions of short wavelengths in the blue spectral range during the day can improve subjective alertness, concentration, the reaction time, and accuracy with which individuals solve tasks. It reduces tiredness and drowsiness and helps to maintain circadian rhythms and improve sleep quality as compared to darker and blue depleted light. Physiological measures (e.g., EEG), however, are less likely to be affected by light during the day, most probably related to the fact that we are a diurnal species. The pathway of light for affecting circadian rhythms, sleep-wake behavior, alertness and well-being in individuals is predominantly expected to be through the eye and the stimulation of ipRGCs, which then send signals to the suprachiasmatic nucleus (SCN) and other areas in the brain implicated in the regulation of different neurobehavioral domains. Therefore, it can be assumed that melEDI is a suitable measure for predicting melatonin suppression and other non-visual effects in humans. A precise quantity of melEDI to trigger these effects however is difficult to determine and may depend on the output domain (e.g., alertness, melatonin, sleep, circadian phase shifts etc.,) one is interested in. Based on the expert consensus (9) mentioned above, we recommend to aim for 250 lx melEDI during usual daytime office hours (in the vertical plane at 1.2 m height) for everyone working inside buildings, even if this requires more energy.

Notably, luminous efficacy is calculated in Lumens per Watt because Lumens are based on visual brightness perception (V lambda curve). Non-visual aspects, however, should be considered too. Non-visual effects have a different spectral sensitivity curve compared to the visual perception of brightness and are therefore not considered in the common calculation of energy efficiency; therefore, we would recommend not only considering Lumens per Watt as a measure for luminous efficacy but also a measure that considers "non-visual luminous efficacy" during the day (e.g., melanopic EDI per Watt). When light is capable to reinforce normal circadian patterns of alertness during the day and sleep at night, studies have either used very high light levels (approximately 1000 lx) or strongly blue enriched light (CCT 17000 K). Notably, most field studies have not controlled for the position of individuals within a room and hence for their precise light exposure levels at the eye.

A lighting concept that reduces melatonin suppression during the night while still allowing for high concentration would be ideal. Since melatonin suppression is mainly linked to the melEDI (92, 93) and not necessarily to CCT, metameric light sources that reduce melatonin suppression could be an innovative solution (94). By optimizing the light spectrum metamerically, daytime alertness could also be improved (95). Additionally, the light distribution could be changed between night and day. Since direct light increases arousal (76) and iPRGCs are probably more sensitive to indirect light from the ceiling (25), these factors could be considered in the lighting design for day and night. Following existing guidelines to avoid glare and yet achieving a high amount of melEDIs at workplaces during the day could be achieved by three workarounds:

1. Optimizing the spectrum by using light sources with a relatively high melEDI (and a high CRI).
2. Optimizing vertical illuminances at the eye by optimizing the light distribution (also considering the reflection of surrounding surfaces. Optimized lighting design can provide higher light levels at the eye (vertical illuminances) for the same horizontal illuminance. Often, downwards oriented lighting from ceiling mounted luminaires intended for rooms with PC screen result in relatively low vertical illuminances. To achieve higher vertical illuminance and hence more light at the eyes, suspended or floor standing luminaires with indirect light distribution (that direct light onto the ceiling) and "wall washing" luminaires can be used. Additional white vertical elements can increase vertical illuminances.
3. Since the melanopsin-containing ganglion cells in the eye are distributed over a large area of the retina, it can be assumed that the non-visual effect of light is greatest when light comes from a large-area source. In nature, this light comes from the sky. If only a small area of the retina is illuminated, as is the case with the directional light of a spot, a weaker non-visual effect is assumed.

During the evening we share the opinion with Brown and colleagues (9) of reducing melEDI to around 10 lx, of course, only if no safety-relevant activities have to be carried out (i.e., at home before bedtime).

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.

AUTHOR CONTRIBUTIONS

OS wrote the main manuscript text. CC provided critical review of and revisions to the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

OS disclosed receipt of the following financial support for the research, authorship, and/or publication of this article. The study was partly supported by Schweizerische Bundesbahnen AG, (SBB), Switzerland.

REFERENCES

1. Cheng P, Drake C. Shift work disorder. *Neurol Clin.* (2019) 37:563–77. doi: 10.1016/j.ncl.2019.03.003
2. Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, et al. Seasonal affective disorder: a description of the syndrome and preliminary findings with light therapy. *Arch Gen Psychiatry.* (1984) 41:72–80. doi: 10.1001/archpsyc.1984.01790120076010
3. Tarocco A, Caroccia N, Morciano G, Wieckowski MR, Ancora G, Garani G, et al. Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care. *Cell Death Dis.* (2019) 10:317. doi: 10.1038/s41419-019-1556-7
4. Hildebrandt G, Moser M, Lehofer M. *Chronobiologie und Chronomedizin*. Stuttgart: Hippokrates (1998).
5. Amlinger-Chatterjee. *Psychische Gesundheit in der Arbeitswelt - Atypische Arbeitszeiten*. Dortmund: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (2016).
6. Ahmad M, Md Din NSB, Tharumalay RD, Che Din N, Ibrahim N, Amit N, et al. The effects of circadian rhythm disruption on mental health and physiological responses among shift workers and general population. *Int J Environ Res Public Health.* (2020) 17:7156. doi: 10.3390/ijerph17197156
7. Lam H, Gani S, Mawson R, Young J, Potma E. A practical tool for ambient illumination comparisons at dusk/dawn. *Proc Hum Factors Ergon Soc Annu Meet.* (2016) 60:470–4. doi: 10.1177/1541931213601107
8. Hébert M, Dumont M, Paquet J. Seasonal and diurnal patterns of human illumination under natural conditions. *Chronobiol Int.* (1998) 15:59–70. doi: 10.3109/07420529808998670
9. Brown T BG, Cajochen C, Czeisler C, Hanifin J, Lockley S, Lucas R, et al. recommendations for healthy daytime, evening, and night-time indoor light exposure. (2020). doi: 10.20944/preprints202012.0037.v1. [Epub ahead of print].
10. *ASR A3.4 Beleuchtung. Technische Regel für Arbeitsstätten*. (2011). p. 287. Available online at: https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/ASR/ASR-A3-4.html (accessed April 29, 2020).
11. IN Deutsches Institut für Normung. *DIN EN 12464-1 Light and lighting - Lighting of work places - Part 1: Indoor work places*. (2019). Available online at: https://www.din.de/en/getting-involved/standards-committees/fnl/drafts/wdc-beuth:din21:302583817?destinationLanguage=&sourceLanguage= (accessed April 29, 2020).
12. SIA (2024). *Space Utilization Data for Energy and Building Technology*. (2015). Available online at: http://shop.sia.ch/normenwerk/architekt/sia%202024/d/2015/D/Product (accessed April 29, 2021).
13. Edward F. Kelley ML, Penczek J. Display daylight ambient contrast measurement methods and daylight readability. *J Soc Inf Display.* (2006) 14:1019–30. doi: 10.1889/1.2393026
14. Haiwei Chen GT, Wu S-T. Ambient contrast ratio of LCDs and OLED displays. *Opt Express*. (2017) 25:33643–56. doi: 10.1364/OE.25.033643
15. Cajochen C, Freyburger M, Basishvili T, Garbazza C, Rudzik F, Renz C, et al. Effect of daylight LED on visual comfort, melatonin, mood, waking performance and sleep. *Light Res Technol.* (2019) 51:1044–62. doi: 10.1177/1477153519828419
16. WELL. *The WELL Building Standard, WELL v2*, (2021). Available online at: https://v2.wellcertified.com/v/en/light (accessed April 29, 2020).
17. Recommended Practice. *Lighting Office Spaces*. New York, NY: Illuminating Engineering Society (2020).
18. Ontario Ministry of Labour. Computer ergonomics: workstation layout and lighting. In: *Health and Safety Guidelines*. (2004).
19. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* (2002) 295:1070–3. doi: 10.1126/science.1067262
20. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science.* (2002) 295:1065–70. doi: 10.1126/science.1069609
21. Paul KN, Saafir TB, Tosini G. The role of retinal photoreceptors in the regulation of circadian rhythms. *Rev Endocr Metab Disord.* (2009) 10:271–8. doi: 10.1007/s11154-009-9120-x
22. Provencio I, Rollag MD, Castrucci AM. Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. *Nature.* (2002) 415:493. doi: 10.1038/415493a
23. Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, Pokorny J, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature.* (2005) 433:749–54. doi: 10.1038/nature03387
24. Lasko TA, Kripke DF, Elliot JA. Melatonin suppression by illumination of upper and lower visual fields. *J Biol Rhythms.* (1999) 14:122–5. doi: 10.1177/074873099129000506
25. Glickman G, Hanifin JP, Rollag MD, Wang J, Cooper H, Brainard GC. Inferior retinal light exposure is more effective than superior retinal exposure in suppressing melatonin in humans. *J Biol Rhythms.* (2003) 18:71–9. doi: 10.1177/0748730402239678
26. Hébert M, Martin SK, Lee C, Eastman CI. The effects of prior light history on the suppression of melatonin by light in humans. *J Pineal Res.* (2002) 33:198–203. doi: 10.1034/j.1600-079X.2002.01885.x
27. Smolensky MH, Sackett-Lundeen LL, Portaluppi F. Nocturnal light pollution and underexposure to daytime sunlight: Complementary mechanisms of circadian disruption and related diseases. *Chronobiol Int.* (2015) 32:1029–48. doi: 10.3109/07420528.2015.1072002
28. Foster RG, Peirson SN, Wulff K, Winnebeck E, Vetter C, Roenneberg T. Chapter eleven - sleep and circadian rhythm disruption in social jetlag and mental illness. In: Gillette MU, editor. *Progress in Molecular Biology and Translational Science*. Oxford; Amsterdam; Waltham, MA; San Diego, CA: Academic Press (2013). vol. 119. p. 325–46. doi: 10.1016/B978-0-12-396971-2.00011-7
29. Aschoff J. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb Symp Quant Biol.* (1960) 25:11–28. doi: 10.1101/SQB.1960.025.01.004
30. de Mairan JJ. Observation botanique. In: *Historie de l'Academie Royale des Sciences*. Paris (1729).
31. Peirson SN, Halford S, Foster RG. The evolution of irradiance detection: melanopsin and the non-visual opsins. *Philos Trans R Soc Lond B Biol Sci.* (2009) 364:2849–65. doi: 10.1098/rstb.2009.0050
32. Markwell EL, Feigl B, Zele AJ. Intrinsically photosensitive melanopsin retinal ganglion cell contributions to the pupillary light reflex and circadian rhythm. *Clin Exp Optom.* (2010) 93:137–49. doi: 10.1111/j.1444-0938.2010.00479.x
33. Foster RG, Wulff K. The rhythm of rest and excess. *Nat Rev Neurosci.* (2005) 6:407–14. doi: 10.1038/nrn1670
34. Hatori M, Panda S. The emerging roles of melanopsin in behavioral adaptation to light. *Trends Mol Med.* (2010) 16:435–46. doi: 10.1016/j.molmed.2010.07.005
35. Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. A phase response curve to single bright light pulses in human subjects. *J Physiol.* (2003) 549(Pt. 3):945–52. doi: 10.1113/jphysiol.2003.040477

36. Minors DS, Waterhouse JM, Wirz-Justice A. A human phase-response curve to light. *Neurosci Lett.* (1991) 133:36–40. doi: 10.1016/0304-3940(91)90051-T
37. Badia P, Myers B, Boecker M, Culpepper J, Harsh JR. Bright light effects on body temperature, alertness, EEG, and behavior. *Physiol Behav.* (1991) 50:583–8. doi: 10.1016/0031-9384(91)90549-4
38. Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. *Behav Brain Res.* (2000) 115:75–83. doi: 10.1016/S0166-4328(00)00236-9
39. Campbell SS, Dawson D. Enhancement of nighttime alertness and performance with bright ambient light. *Physiol Behav.* (1990) 48:317–20. doi: 10.1016/0031-9384(90)90320-4
40. Myers BL, Badia P. Immediate effects of different light intensities on body temperature and alertness. *Physiol Behav.* (1993) 54:199–202. doi: 10.1016/0031-9384(93)90067-P
41. Gaggioni G, Maquet P, Schmidt C, Dijk DJ, Vandewalle G. Neuroimaging, cognition, light and circadian rhythms. *Front Syst Neurosci.* (2014) 8:126. doi: 10.3389/fnsys.2014.00126
42. LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. *Nat Rev Neurosci.* (2014) 15:443–54. doi: 10.1038/nrn3743
43. Phipps-Nelson J, Redman JR, Dijk DJ, Rajaratnam SM. Daytime exposure to bright light, as compared to dim light, decreases sleepiness, and improves psychomotor vigilance performance. *Sleep.* (2003) 26:695–700. doi: 10.1093/sleep/26.6.695
44. Souman JL, Tinga AM, Te Pas SF, van Ee R, Vlaskamp BNS. Acute alerting effects of light: a systematic literature review. *Behav Brain Res.* (2018) 337:228–39. doi: 10.1016/j.bbr.2017.09.016
45. Gornicka GB. *Lighting at work : environmental study of direct effects of lighting level and spectrum on psychophysiological variables* [Phd Thesis 1 (Research TU/e / Graduation TU/e)]. Eindhoven: Technische Universiteit Eindhoven, Netherlands (2008).
46. Segal AY, Sletten TL, Flynn-Evans EE, Lockley SW, Rajaratnam SMW. Daytime exposure to short- and medium-wavelength light did not improve alertness and neurobehavioral performance. *J Biol Rhythms.* (2016) 31:470–82. doi: 10.1177/0748730416659953
47. Chang A-M, Scheer FAJL, Czeisler CA. The human circadian system adapts to prior photic history. *J Physiol.* (2011) 589(Pt. 5):1095–102. doi: 10.1113/jphysiol.2010.201194
48. Kozaki T, Kubokawa A, Taketomi R, Hatae K. Effects of day-time exposure to different light intensities on light-induced melatonin suppression at night. *J Physiol Anthropol.* (2015) 34:27. doi: 10.1186/s40101-015-0067-1
49. Smith KA, Schoen MW, Czeisler CA. Adaptation of human pineal melatonin suppression by recent photic history. *J Clin Endocrinol Metab.* (2004) 89:3610–4. doi: 10.1210/jc.2003-032100
50. Avery DH, Eder DN, Bolte MA, Hellekson CJ, Dunner DL, Vitiello MV, et al. Dawn simulation and bright light in the treatment of SAD: a controlled study. *Biol Psychiatry.* (2001) 50:205–16. doi: 10.1016/S0006-3223(01)01200-8
51. Gabel V, Maire M, Reichert CF, Chellappa SL, Schmidt C, Hommes V, et al. Effects of artificial dawn and morning blue light on daytime cognitive performance, well-being, cortisol., and melatonin levels. *Chronobiol Int.* (2013) 30:988–97. doi: 10.3109/07420528.2013.793196
52. Vandewalle G, Dijk D-J. Neuroimaging the effects of light on non-visual brain functions. In: Nofzinger E, Thorpy MJ, Maquet P, editors. *Neuroimaging of Sleep and Sleep Disorders*. Cambridge: Cambridge University Press (2013). p. 171–8. doi: 10.1017/CBO9781139088268.023
53. Boivin DB, Czeisler CA, Dijk DJ, Duffy JF, Folkard S, Minors DS, et al. Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Arch Gen Psychiatry.* (1997) 54:145–52. doi: 10.1001/archpsyc.1997.01830140055010
54. Golden RN, Gaynes BN, Ekstrom RD, Hamer RM, Jacobsen FM, Suppes T, et al. The efficacy of light therapy in the treatment of mood disorders: a review and meta-analysis of the evidence. *Am J Psychiatry.* (2005) 162:656–62. doi: 10.1176/appi.ajp.162.4.656
55. Mårtensson B, Pettersson A, Berglund L, Ekselius L. Bright white light therapy in depression: a critical review of the evidence. *J Affect Disord.* (2015) 182:1–7. doi: 10.1016/j.jad.2015.04.013
56. Stephenson KM, Schroder CM, Bertschy G, Bourgin P. Complex interaction of circadian and non-circadian effects of light on mood: shedding new light on an old story. *Sleep Med Rev.* (2012) 16:445–54. doi: 10.1016/j.smrv.2011.09.002
57. Houser K, Boyce P, Zeitzer J, Herf M. Human-centric lighting: myth, magic or metaphor? *Light Res Tech.* (2021) 53:97–118. doi: 10.1177/1477153520958448
58. Federal Office of Public Health FOPH, Artificially generated daylight for interiors. Künstlich erzeugtes Tageslicht für Innenräume. Bern: FOPH (2019). Available online at: https://www.bag.admin.ch/bag/de/home/gesund-leben/umwelt-und-gesundheit/strahlung-radioaktivitaet-schall/elektromagnetische-felder-emf-uv-laser-licht/licht_beleuchtung.html (accessed April 29, 2020).
59. Motamedzadeh M, Golmohammadi R, Kazemi R, Heidarimoghadam R. The effect of blue-enriched white light on cognitive performances and sleepiness of night-shift workers: a field study. *Physiol Behav.* (2017) 177:208–14. doi: 10.1016/j.physbeh.2017.05.008
60. Lok R, Smolders KCHJ, Beersma DGM, de Kort YAW. Light, alertness, and alerting effects of white light: a literature overview. *J Biol Rhythms.* (2018) 33:589–601. doi: 10.1177/0748730418796443
61. Pachito DV, Eckeli AL, Desouky AS, Corbett MA, Partonen T, Rajaratnam SMW, et al. Workplace lighting for improving alertness and mood in daytime workers. *Cochrane Database Syst Rev.* (2018) 3:CD012243. doi: 10.1002/14651858.CD012243.pub2
62. Zhang R, Campanella C, Aristizabal S, Jamrozik A, Zhao J, Porter P, et al. Impacts of dynamic LED lighting on the well-being and experience of office occupants. *Int J Environ Res Public Health.* (2020) 17:7217. doi: 10.3390/ijerph17197217
63. Figueiro M, Steverson B, Heerwagen J, Yucel R, Roohan C, Sahin L, et al. Light, entrainment, and alertness: a case study in offices. *Light Res Technol.* (2020) 52:736–50. doi: 10.1177/1477153519885157
64. Jensen HI, Markvart J, Holst R, Thomsen TD, Larsen JW, Eg DM, et al. Shift work and quality of sleep: effect of working in designed dynamic light. *Int Arch Occup Environ Health.* (2016) 89:49–61. doi: 10.1007/s00420-015-1051-0
65. Griepentrog JE, Labiner HE, Gunn SR, Rosengart MR. Bright environmental light improves the sleepiness of nightshift ICU nurses. *Crit Care.* (2018) 22:295. doi: 10.1186/s13054-018-2233-4
66. de Kort Y, Smolders K. Effects of dynamic lighting on office workers: first results of a field study with monthly alternating settings. *Light Res Technol.* (2010) 42:345–60. doi: 10.1177/1477153510378150
67. Stefani O, Freyburger M, Veitz S, Basishvili T, Meyer M, Weibel J, et al. Changing color and intensity of LED lighting across the day impacts on circadian melatonin rhythms and sleep in healthy men. *J Pineal Res.* (2021) 70:e12714. doi: 10.1111/jpi.12714
68. Schöllhorn I PA, Braun M, Seiler S, Stefani, O. *Evaluation eiNes Alternierenden Beleuchtungskonzepts in Einem Produktionsbetrieb. Arbeit interdisziplinär Analysieren, bewerten, gestalten : 65 Kongress der Gesellschaft für Arbeitswissenschaft; 27. Februar - 1. März.* (2019). Dresden: Gesellschaft für Arbeitswissenschaft (GfA) (2019).
69. Moore-Ede M, Heitmann A, Guttkuhn R. Circadian potency spectrum with extended exposure to polychromatic white LED light under workplace conditions. *J Biol Rhythms.* (2020) 35:405–15. doi: 10.1177/0748730420923164
70. Rahman SA, Flynn-Evans EE, Aeschbach D, Brainard GC, Czeisler CA, Lockley SW. Diurnal spectral sensitivity of the acute alerting effects of light. *Sleep.* (2014) 37:271–81. doi: 10.5665/sleep.3396
71. Viola AU, James LM, Schlangen LJ, Dijk DJ. Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. *Scand J Work Environ Health.* (2008) 34:297–306. doi: 10.5271/sjweh.1268
72. Revell VL, Arendt J, Fogg LF, Skene DJ. Alerting effects of light are sensitive to very short wavelengths. *Neurosci Lett.* (2006) 399:96–100. doi: 10.1016/j.neulet.2006.01.032
73. Kelly R. Lighting as an integral part of architecture. *Coll Art J.* (1952) 12:24–30. doi: 10.2307/773361
74. Hansen EK, Bjørner T, Xylakis E, Pajuste M. An experiment of double dynamic lighting in an office responding to sky and daylight: perceived effects on comfort, atmosphere and work engagement. *Indoor Built Environ.* (2021). doi: 10.1177/1420326X21991198
75. Hansen E, Pajuste M, Xylakis E. Flow of light: balancing directionality and cct in the office environment. *Leukos.* (2020). doi: 10.1080/15502724.2020.1808014. [Epub ahead of print].

76. Fleischer S. *The Psychological Effect of Changeable Artificial Lighting Situations on Humans. ETH Zurich Research Collection Zurich (Switzerland)*. Zurich: ETH (2001).
77. Houser K, Tiller D, Bernecker C, Mistrick R. The subjective response to linear fluorescent direct/indirect lighting systems. *Light Res Technol.* (2002) 34:243–60. doi: 10.1191/1365782802li039oa
78. Hubalek S, Brink M, Schierz C. Office workers' daily exposure to light and its influence on sleep quality and mood. *Light Res Technol.* (2010) 42:33–50. doi: 10.1177/1477153509355632
79. Huiberts LM, Smolders KCHJ, de Kort YAW. Non-image forming effects of illuminance level: exploring parallel effects on physiological arousal and task performance. *Physiol Behav.* (2016) 164:129–39. doi: 10.1016/j.physbeh.2016.05.035
80. Smolders KCHJ, de Kort YAW, Cluitmans PJM. A higher illuminance induces alertness even during office hours: findings on subjective measures, task performance and heart rate measures. *Physiol Behav.* (2012) 107:7–16. doi: 10.1016/j.physbeh.2012.04.028
81. ISO/CIE DIS 11664-2 [PREN ISO/CIE 11664-2]. *ICS 17.180.20 Colorimetry - Part 2: CIE Standard Illuminants*. (2020). Available online at: https://www.iso.org/standard/77215.html (accessed April 29, 2020).
82. /E:2018 CS. CIE system for metrology of optical radiation for ipRGC-influenced responses to light. *Color Res Appl.* (2018) 44:316. doi: 10.1002/col.22350
83. Ticleanu. *Research Insight Circadian lighting*. London: CIBSE and BRE (2020).
84. DGUV. *Deutsche Gesetzliche Unfallversicherung e.V.* (2018). Available online at: https://publikationen.dguv.de/widgets/pdf/download/article/3247 (accessed April 29, 2021).
85. DIN SPEC 67600:2013-04. *Biologically Effective Illumination - Design guidelines [Biologisch wirksame Beleuchtung - Planungsempfehlungen]*. (2013). Available online at: https://www.beuth.de/en/technical-rule/din-spec-67600/170956045 (accessed April 29, 2020).
86. Underwriters Laboratories (UL). *Design Guideline for Promoting Circadian Entrainment with Light for Day-Active People*. (2020). Available online at: https://www.shopulstandards.com/ProductDetail.aspx?UniqueKey=36592 (accessed April 29, 2021).
87. The WELL Building Standard. *Circadian Lighting Design*. (2020). Available online at: https://standard.wellcertified.com/light/circadian-lighting-design (accessed April 29, 2020).
88. CIE. *Position Statement on Non-Visual Effects of Light - Recommending Proper Light at the Proper Time, 2nd ed.* (2019). Available online at: https://cie.co.at/publications/position-statement-non-visual-effects-light-recommending-proper-light-proper-time-2nd (accessed April 29, 2021).
89. KAN. *KAN-Positionspapier zum Thema künstliche,biologisch wirksame Beleuchtung und Normung*. (2017). Available online at: https://www.kan.de/fileadmin/Redaktion/Dokumente/Basisdokumente/de/Deu/KAN-Position_Beleuchtung_2017.pdf (accessed April 29, 2021).
90. IES. *PS-12-19: IES Position On UL RP 24480 Regarding Light and Circadian Entrainment*. (2020). Available online at: https://www.ies.org/about-outreach/position-statements/ps-12-19-ies-position-on-ul-rp-24480-regarding-light-and-circadian-entrainment/ (accessed April 29, 2021).
91. CIBSE. *Literature Review on Circadian Lighting*. (2017). Available online at: https://www.cibse.org/knowledge/knowledge-items/detail?id=a0q0O00000CF7o9QAD (accessed April 29, 2021).
92. Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res.* (2020) 69:e12655. doi: 10.1111/jpi.12655
93. Nowozin C, Wahnschaffe A, Rodenbeck A, de Zeeuw J, Hädel S, Kozakov R, et al. Applying melanopic lux to measure biological light effects on melatonin suppression and subjective sleepiness. *Curr Alzheimer Res.* (2017) 14:1042–52. doi: 10.2174/1567205014666170523094526
94. Allen AE, Hazelhoff EM, Martial FP, Cajochen C, Lucas RJ. Exploiting metamerism to regulate the impact of a visual display on alertness and melatonin suppression independent of visual appearance. *Sleep.* (2018) 41:zsy100. doi: 10.1093/sleep/zsy100
95. de Zeeuw J, Papakonstantinou A, Nowozin C, Stotz S, Zaleska M, Hädel S, et al. Living in biological darkness: objective sleepiness and the pupillary light responses are affected by different metameric lighting conditions during daytime. *J Biol Rhythms.* (2019) 34:410–31. doi: 10.1177/0748730419847845

Conflict of Interest: The authors declare the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: OS is listed as an inventor on the following patents: US8646939B2—Display system having circadian effect on humans; DE102010047207B4—Projection system and method for projecting image content; US8994292B2—Adaptive lighting system; WO2006013041A1—Projection device, and filter thereof; WO2016092112A1—Method for the selective adjustment of a desired brightness and/or color of a specific spatial area, and data processing device thereof. OS is a member of the Daylight Academy. OS has had the following commercial interests in the last four years (2017–20) related to lighting: Investigator-initiated research grants from Derungs, Audi, VW, Porsche, Festo, ZDF, Toshiba, and SBB; Speaker fees for invited seminars from Merck, Fraunhofer, Firalux, and Selux. CC has had the following commercial interests in the last four years (2017–2020) related to lighting: honoraria, travel, accommodation and/or meals for invited keynote lectures, conference presentations or teaching from Toshiba Materials, Velux, Firalux, Lighting Europe, Electrosuisse, Novartis, Roche, Elite, Servier, and WIR Bank. CC is a member of the Daylight Academy.

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REVIEW
published: 14 May 2021
doi: 10.3389/fnins.2021.640113

Comparative Neurology of Circadian Photoreception: The Retinohypothalamic Tract (RHT) in Sighted and Naturally Blind Mammals

*Jens Hannibal**

Department of Clinical Biochemistry, Bispebjerg Frederiksberg Hospital, University of Copenhagen, Copenhagen, Denmark

The mammalian eye contains two systems for light perception: an image detecting system constituted primarily of the classical photoreceptors, rods and cones, and a non-image forming system (NIF) constituted of a small group of intrinsically photosensitive retinal ganglion cells driven by melanopsin (mRGCs). The mRGCs receive input from the outer retina and NIF mediates light entrainment of circadian rhythms, masking behavior, light induced inhibition of nocturnal melatonin secretion, pupillary reflex (PLR), and affect the sleep/wake cycle. This review focuses on the mammalian NIF and its anatomy in the eye as well as its neuronal projection to the brain. This pathway is known as the retinohypothalamic tract (RHT). The development and functions of the NIF as well as the knowledge gained from studying gene modified mice is highlighted. Furthermore, the similarities of the NIF between sighted (nocturnal and diurnal rodent species, monkeys, humans) and naturally blind mammals (blind mole rats *Spalax ehrenbergi* and the Iberian mole, *Talpa occidentalis*) are discussed in relation to a changing world where increasing exposure to artificial light at night (ALAN) is becoming a challenge for humans and animals in the modern society.

Keywords: photoreceptors, circadian rhythms, neurotransmitters, entrainment, pupil reflex

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***Correspondence:**
Jens Hannibal
j.hannibal@dadlnet.dk

Specialty section:
This article was submitted to Sleep and Circadian Rhythms, a section of the journal Frontiers in Neuroscience

Received: *14 December 2020*
Accepted: *29 March 2021*
Published: *14 May 2021*

Citation:
Hannibal J (2021) Comparative Neurology of Circadian Photoreception: The Retinohypothalamic Tract (RHT) in Sighted and Naturally Blind Mammals. Front. Neurosci. 15:640113. doi: 10.3389/fnins.2021.640113

INTRODUCTION

The daily shift between day and night due to the rotation of the earth toward the sun defines the astronomical day of 24 h, which has shaped almost all life forms on the planet. In mammals, light is perceived through the retina and used for image formation primarily based on the classical photoreceptors, rods and cones, and a non-image forming (NIF) system using the photoreceptor, melanopsin (Do and Yau, 2010). The circadian timing system, which is fundamental for survival by driving physiology (hormone secretion, core body temperature, heart rate) and behavior (sleep/wake, eating) into distinct time and periods of the solar cycle, is a major target for the NIF system. The circadian system is orchestrated by a biological "master" clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus and is constituted by approximately 20,000 neurons (Mohawk and Takahashi, 2011). Each individual SCN neuron exhibits a biological clock with an intrinsic period and phase different from other neurons, but their rhythm is coupled to each other

to produce a coherent SCN output rhythm. Output signals (neuronal, hormonal) synchronize the peripheral circadian clocks located in the tissues and organs (Yamaguchi et al., 2003; Welsh et al., 2010). The intracellular molecular machinery driving the circadian oscillation in the majority of the SCN neurons consists of interlocked transcriptional and translational feedback loops involving several clock genes and their products (Mohawk and Takahashi, 2011; Takahashi, 2016). However, the endogenous period length of the SCN clock deviates from the astronomical day of exactly 24 h and therefore, the clock needs a daily resetting by light to align the endogenous phase of the clock with the solar cycle, a process known as photoentrainment (Golombek and Rosenstein, 2010). Photoentrainment in mammals is solely dependent on the retina (Nelson and Zucker, 1981). The neuronal pathway transmitting light to the SCN is known as the retinohypothalamic tract (RHT) (Hannibal, 2002). Other NIF functions mediated by the RHT are light suppression of nocturnal melatonin secretion, as well as masking behavior, and regulation of the pupillary reflex (Do and Yau, 2010).

Within the last 20 years, studies of anatomy and physiology using different mammalian species and, in particular, gene modified mice, have shed light on NIF. Through the discovery of specific neurotransmitters (glutamate and pituitary adenylate cyclase activating polypeptide (PACAP) (Hannibal, 2002) and the photoreceptor melanopsin expressed in subpopulations of retinal ganglion cells (mRGCs) constituting the RHT, a fundamental system in the mammalian eye mediating NIF to the brain was characterized (Do and Yau, 2010).

This review will focus on NIF and studies in the circadian photoentrainment, masking behavior and pupillary light reflex in animal models, neuroanatomy, and physiology in gene modified mice lacking elements of the NIF pathway to the brain such as photoreceptors, neurotransmitters, and their receptors. Furthermore, the different aspects of the effects of light on the physiology between sighted (nocturnal and diurnal rodent species, monkeys, humans) and blind mammals (the naturally blind mole rat *Spalax ehrenbergi and* the Iberian mole, *Talpa occidentalis*) will be discussed.

NON-IMAGE FORMING PHOTOPERCEPTION (NIF): LIGHT ENTRAINMENT; PHOTIC PHASE RESPONSE CURVE (PRC), MASKING, AND PUPILLARY LIGHT REFLEX (PLR)

The NIF system of the mammalian eye can be considered as an irradiance detector or "light meter" system used for light entrainment of the circadian system, regulation of masking behavior, and the pupillary light reflex (PLR) (La Morgia et al., 2018; Foster et al., 2020). Furthermore, NIF is involved in sleep and core body temperature regulation. When studying the NIF physiology, the focus has mainly been on light entrainment, masking behavior, and PLR.

Light Entrainment

Light has a profound effect on the phase of the circadian clock which is fundamental for the ability of the species to stay entrained with the solar cycle. This phase shifting capacity is time dependent which means that light stimulation during daytime in normal feed animals have a little effect on the clock phase while light stimulation during the early part of the subjective night slows down the clock speed by inducing phase delays (Hannibal, 2002). Light stimulation at the end of the night, on the other hand, speeds up the clock resulting in a phase advance of the clock phase (**Figure 1**). This time dependent effect of light on the clock phase is known as the phase-response curve to light stimulation and is the fundamental ability of the clock to stay entrained with the circadian light/dark cycle as well as the annual cycle (Hughes et al., 2015; Foster et al., 2020). Importantly, light intensity as well as the wavelength and duration of the stimulus determine the size of the phase shift (Hannibal and Fahrenkrug, 2006; Duffy and Czeisler, 2009; Golombek and Rosenstein, 2010).

Masking

Light can also directly influence the rhythmic physiology and behavior, and this effect is called masking (Mrosovsky, 1999). While nocturnal animals generally become more active in response to darkness (positive masking), light at night has the opposite effect making the animals less active (negative masking). Negative masking causes the inhibition of locomotor activity observed in nocturnal animals when exposed to light at night (Redlin, 2001). While masking is independent of the SCN clock (Redlin and Mrosovsky, 1999), it is an important factor controlling the rhythmic behavior and physiology in arrhythmic animals due to clock gene mutation (Bunger et al., 2000; Husse et al., 2014) or disrupted synchronization of individual clock neurons (Bechtold et al., 2008; Hannibal et al., 2011). A direct inhibition of the nocturnal secretion of the night hormone melatonin can be considered as a "negative" masking by light (Mrosovsky, 1999; Pevet and Challet, 2011).

Pupillary Light Reflex (PLR)

As early as the late 1920s, Clyde Keeler described a strain of blind house mice which, despite lacking the outer retina, still had light perception and an intact PLR (Keeler, 1924; Keeler et al., 1928). This NIF function was "re-discovered" in the early 1990s by Russell Foster and colleagues (Foster et al., 1991), and the PLR is considered an important parameter when examining the mammalian NIF function. The use of light with different wavelengths can discriminate functional defects located in the outer (i.e., rod and cone defects) or the inner retina (melanopsin defects) (La Morgia et al., 2018).

THE RETINO-HYPOTHALAMIC TRACT (RHT) IN MAMMALS

In 1972, a distinct monosynaptic neuronal pathway to the SCN was described in mammals for the first time (Hendrickson et al., 1972; Moore and Lenn, 1972). These pioneering observations

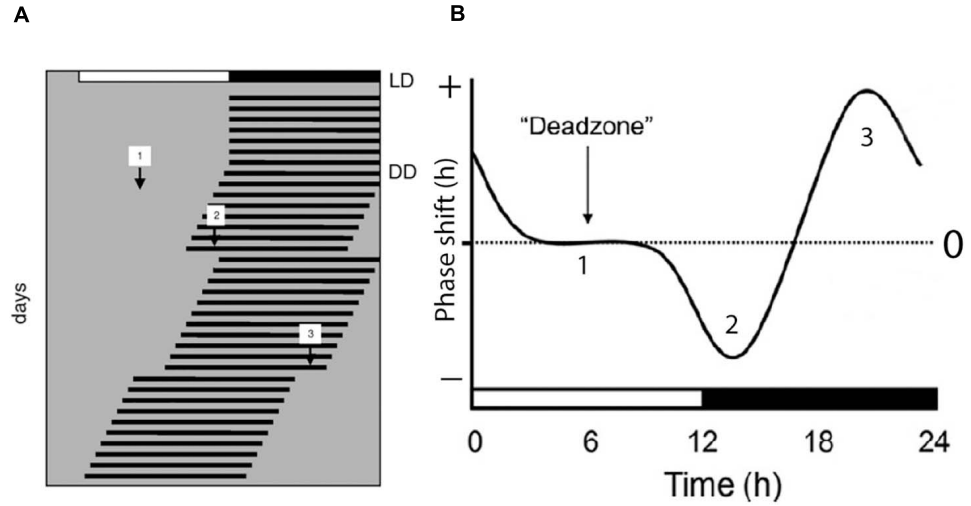

FIGURE 1 | Light regulation of circadian rhythms; the photic phase response curve (PRC). **(A)** Schematic presentation of the activity rhythms of a nocturnal animal where each horizontal line represents the activity of the animal in 1 day. The animal is entrained to a light/dark photoperiod (LD) as represented on the top of the record. The animal is then released into constant darkness (DD) and the activity rhythm is now "free-running." During the free-running period, the animal experiences light pulses during subjective day [1], early subjective night [2], and late subjective night [3]. The light pulse given during the day has little or no effect on the phase of the endogenous rhythm. Light pulse given in the early subjective night results in a phase delay of the overt rhythm as indicated by [2] in panels **(A,B)**, and a light pulse given in the late subjective night results in a phase advance of the overt rhythm as indicated by [3] in panels **(A,B)**. A complete phase response curve to light stimulation during a 24 h period is drawn in **(B)**. Phase delays are plotted in the negative direction (downward) and phase advances are plotted in the upwards direction. The horizontal axis in **(B)** represents one circadian cycle (from Hannibal, 2020).

were subsequently confirmed by studies using the subunit B of cholera toxin (CtB) as an anterograde tracer. CtB tracing resulted in a detailed visualization of the RHT projections in several mammalian species (reviewed in Hannibal, 2002). More recently, direct conjugation of different fluorophores to CtB have made it possible to characterize the contralateral and ipsilateral natures of the RHT projections in the mammalian brain into details (Guler et al., 2008; Langel et al., 2015). The RHT originates from a distinct subpopulation of retinal ganglion cells (RGCs) widely distributed in the entire retina (Moore et al., 1995; Hannibal et al., 1997). RHT nerve terminals reach the ventral bilateral SCN (Morin and Allen, 2005), and some of these axons bifurcated and reach the intergeniculate leaflet (IGL) of the lateral geniculate complex which is considered part of the circadian timing system (Pickard, 1985). Nerve fibers from the RHT target several areas in the forebrain which are not directly involved in the circadian timing but are considered as the neuronal pathway of NIF (Canteras et al., 2011). One area, the ventral preoptic area (VLPO), is involved in the homeostatic regulation of sleep (Saper et al., 2005a) and light indirectly affects the hormone secretion dependent of sleep homeostasis (Saper et al., 2005b). Furthermore, RHT directly targets the nerve fibers in the hypothalamic subparaventricular zone (SubPVN), which has been suggested to play a role in light regulated masking behavior (see above) (Shuboni et al., 2012) (see also below). A minor terminal field of the RHT nerve terminals is found in the lateral hypothalamic area dorsal to the supraoptic nucleus (Hannibal and Fahrenkrug, 2004b; Hattar et al., 2006; Canteras et al., 2011). This part of the RHT is supposed to influence the masking effects on specific behaviors such as defensive, drinking, and reproductive behaviors (Swanson, 2000;Canteras et al., 2011).

The Neuropeptide PACAP: A Marker for the RHT in Mammals

In 1997, a distinct marker labeling all neurons and projections of the RHT to the brain was demonstrated by showing the localization of the neuropeptide PACAP in all RGCs of the rat RHT (Hannibal et al., 1997). The neuropeptide PACAP was discovered in 1989–1990 due to its ability to stimulate cyclic AMP in pituitary cells (Miyata et al., 1989; Miyata et al., 1990). PACAP is, due to its sequence similarities, placed in the family of vasoactive intestinal polypeptide (VIP), secretin and glucagon (Vaudry et al., 2000). PACAP has its own specific receptor, the PACAP type 1 (PAC1) receptor, and shares the VIP types 1 and 2 receptors (VPAC1 and VPAC2) with VIP (Harmar et al., 2012). Retinal PACAP projections densely innervate the bilateral SCN and all other non-visual (NIF) areas of the brain, most intensely the lateral geniculate nucleus and especially the intergeniculate nucleus (IGL) and the ventral geniculate nucleus (VGL), the pretectum including the olivary pretectal nucleus (OPN), and with little innervation of the superior colliculus (SC) (Hannibal and Fahrenkrug, 2004b). The IGL is known as a part of the "circadian visual system" (Morin and Allen, 2005; Morin, 2013), and the OPN is a part of the PLR (Morin, 2013). PACAP immunostaining in combination with CtB injection in the eye has proven useful in the characterization of RHT projections in nocturnal animals such as the rat (Hannibal et al., 1997; Hannibal and Fahrenkrug, 2004b;

Engelund et al., 2010), hamster (Bergström et al., 2003), and mouse (Engelund et al., 2012), diurnal rodent (*Arvicantis niloticus*) (Langel et al., 2015), and as well as the monkey (Hannibal et al., 2014). Interestingly, in almost every target area reached by the PACAP nerve terminals, a minor number of retinal projections (CtB positive) not co-storing PACAP were identified. Furthermore, no distinct difference of the melanopsin/PACAP projections were found when comparing the nocturnal and diurnal species (Langel et al., 2015), although, the time of the development of the retinal projections may differ slightly (Todd et al., 2012).

Identification of Melanopsin in RGCs of the RHT

In the 1990s, Russel Foster and colleagues described two mouse models, the rd/rd (rodless/rodless) and the rd/cl (rodless/coneless) mice, characterized by the loss of all rods or both rods and cones during early development (Foster et al., 1991, 1993). Both strains of mice had an intact RHT and were able to photoentrain their circadian rhythm to the light/dark cycle as their littermates (Foster et al., 1991; Freedman et al., 1999). Furthermore, the rd/cl mice photoentrain and suppress nocturnal secretion of melatonin in response to monochromatic light of wavelength of approximately 500 nanometers, indicating that the mammalian retina had additional ocular photoreceptors in the inner retina (Lucas and Foster, 1999; Lucas et al., 1999). In the rd/rd mice with a normal photoentrainment, light induces the transcription factor FOS in neurons of the SCN and in a small population of RGCs (Masana et al., 1996). In rats, light was shown to induce and sustain FOS expression selectively in PACAP expressing RGCs given that the light was ON (both during the subjective day and night). This response was unexpected and unique compared to other RGCs and amacrine cells (in which FOC decreases within 1–2 h) (Hannibal et al., 2001b). This finding was the first direct indication that RGCs of the RHT could be intrinsically photosensitive (Hannibal et al., 2001b). This idea was furthermore supported by the identification of melanopsin, which was initially cloned from frog skin (Provencio et al., 1998). Melanopsin, named from the cells of which it was first isolated (melanophore cells causing the ability of frog skin to shift color), were demonstrated in a small population of RGCs in the mammalian retina shortly after its cloning (Provencio et al., 2000). This observation led to several studies confirming that melanopsin was located exclusively in the surface membrane of RGCs of the RHT (Hannibal et al., 2002a; Hattar et al., 2002; **Figure 2**). At the same time, the melanopsin-expressing RGCs (mRGCs) were shown to be intrinsically photosensitive (Berson et al., 2002) and shortly hereafter, melanopsin was established as a new mammalian photoreceptor (Berson, 2003; Rollag et al., 2003; Do and Yau, 2010) being sensitive to blue light in a wavelength of approximately 480 nm (Lucas et al., 2014).

Using the melanopsin promoter and knockin of the *tau-lacZ* gene, which codes for the β-galactosidase enzyme fused to a sequence of tau protein and promotes axonal transport of the marker enzyme, Hattar et al. (2002, 2006) were able to visualize the axon projections of the mRGCs (RHT) in the mouse brain, which were very similar to that of the rat (Hattar et al., 2002, 2006; Hannibal and Fahrenkrug, 2004b). Interestingly, using another melanopsin gene (Opn4) knockin ($Opn4^{Cre/+;Z/AP}$) mouse in which CRE-recombinase were combined with an enhanced placental alkaline phosphatase that increases the sensitivity of visualization of melanopsin expressing cells and their projections in the brain, the number of mouse mRGCs were found to be more than twice the number of mRGCs which were initially visualized by immunostaining in mice (Ecker et al., 2010). Brain areas targeted by retinal projections from mRGCs involved in vision processing that were not previously identified [the lateral geniculate complex (LGN) and superior colliculus (SC)], were now identified (Brown et al., 2010; Ecker et al., 2010). These observations were aligned with studies in primates, in which retrograde and anterograde tracing revealed melanopsin projections to both the LGN and SC (Dacey et al., 2005; Hannibal et al., 2014).

The Diversity of mRGCs

The melanopsin expressing RGCs (mRGCs) were a more heterogeneous group of mRGCs than what was initially suggested and represented by 5–7 subtypes of mRGCs in both rodents and primates, including humans (Baver et al., 2008; Berson et al., 2010; Ecker et al., 2010; Sand et al., 2012; Hannibal et al., 2017). The total number of mRGCs were 0.6–1% of the total number of RGCs in the mammalian retina (Hannibal et al., 2002a, 2017; Hattar et al., 2002). The different subtypes of mRGCs are based on the pattern of dendritic aberration in the inner layers of the inner plexiform layer (IPL) and inner nuclear layer (INL), which form two distinct networks of dendritic projections: one inner stratifying layer located in the sublamina I of the IPL and one outer stratifying layer located in sublaminar V in the IPL close to the INL (**Figure 3**; Schmidt et al., 2011a; Reifler et al., 2014; Hannibal et al., 2017). These mRGCs differ in the morphology (size, number of dendrites), electrophysical response (Ecker et al., 2010; Schmidt et al., 2011b; Lucas et al., 2014), expression of melanopsin (Pires et al., 2009), and expression of the transcription factor Brn3b (Badea et al., 2009; Chen et al., 2011). The different levels of melanopsin expression in mice seem to be a result of the expression of two isoforms of melanopsin from Opn4 locus, a long isoform (Opn4L) and a short isoform (Opn4S). Both Opn4L and Opn4S are expressed in the mRGCs and Opn4S seems to be 40 times more abundant than Opn4L (Pires et al., 2009). Both isoforms contribute to different functions of the mRGCs (Hughes et al., 2012; Jagannath et al., 2015) (see also below).

Anatomical Connectivity Between mRGCs and the Outer Retina

In addition to being directly responsive to light, the mRGCs receive light information from the rods and cones as well (Guler et al., 2008). The information is integrated in the mRGCs via bipolar and amacrine cells (Belenky et al., 2003; Jusuf et al., 2007; Ostergaard et al., 2007; Grunert et al., 2011; Lucas et al., 2012; Hannibal et al., 2017). Differences in the dendritic morphology of the mRGCs support the fact that afferent connections differ

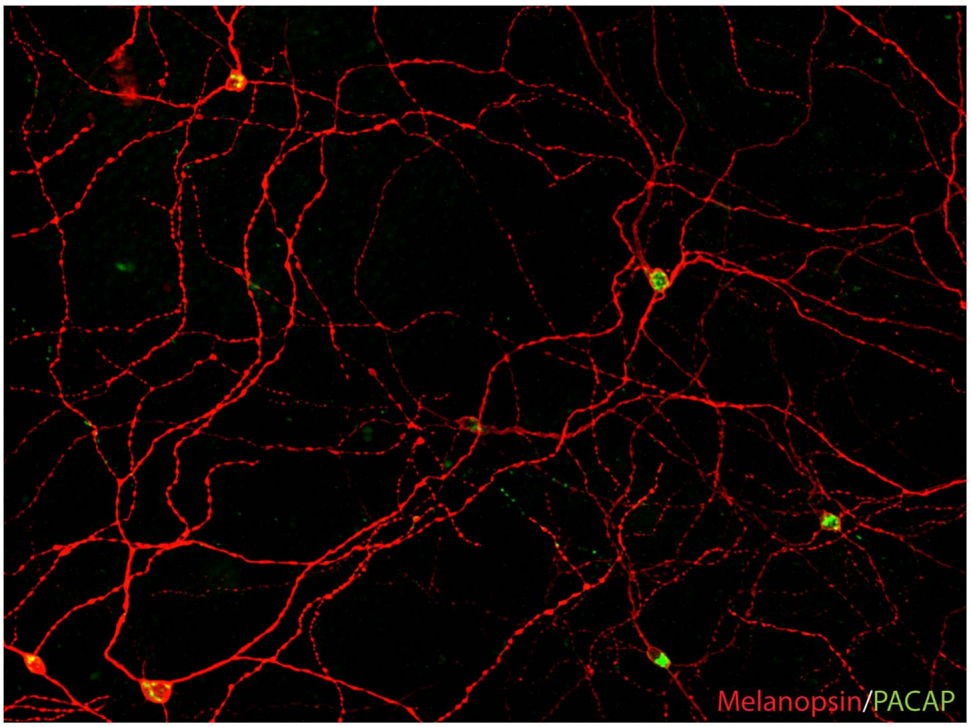

FIGURE 2 | PACAP is found in melanopsin-immunoreactive RGCs in mammals (image from the macaque modified from Hannibal et al., 2014). The image represents a montage of a Z-stack covering the depth required to ensure that both inner and outer stratifying melanopsin processes are visible.

qualitatively, making the NIF system sensitive to the broad spectrum of light found from sunrise to sunset (Dacey et al., 2005; Lucas et al., 2014).

Melanopsin in Naturally Blind Mole Rat: The *Spalax ehrenbergi* and the Iberian Mole, the Talpa Occidentalis

A large number of mammalian species have adopted a subterranean lifestyle, which in some species have led to natural blindness due to the regression of the eyes and in others, are covered by the skin (Nevo, 1999). Although visually blind, it seems that the eyes of these animals are able to provide information about the circadian and annual cycles (David-Gray et al., 1998; Nevo et al., 2001). The *Spalax ehrenbergi,* which is a blind subterranean mole rat with rudimentary eyes (diameter less than 1 mm) located under the skin, responds to light stimulation and adapts behavior and physiology to both circadian and annual lightnings (David-Gray et al., 1998; Nevo et al., 2001). While the lens of the *Spalax* is pigmented and severely degenerated, the retina is well-organized in the inner and outer layers (Cernuda-Cernuda et al., 2002). The eyes are located in the enlarged harderian gland with an optic nerve that contains less than 900 axons with no image-forming vision (Cooper et al., 1993a). The *Spalax* eye can therefore be considered as a light meter corresponding to the NIF system found in the sighted eye (Cooper et al., 1993b; Hannibal et al., 2002b; Esquiva et al., 2016). Tract tracing from the *Spalax* eye to the brain demonstrates the areas involved in visual perception which receives a significantly reduced retinal projections whereas the brain areas involved in the NIF functions such as the SCN and the ventral geniculate nucleus (VGL) are innervated as found in sighted animals (Bronchti et al., 1991; Cooper et al., 1993b). A detailed study revealed the complex wiring of the classical photoreceptors (rods and L/M cones), amacrine and bipolar cells, and mRGCs, which seem to be similar to that of the sighted mammals (Esquiva et al., 2016; **Figure 4**). However, while mRGCs represent approximately 1% of all RGCs in sighted animals and humans, nearly 90% of a total of 900 RGCs are mRGCs. As in other mammalian species, mRGCs co-store PACAP, which can be found in retinal target areas in the *Spalax* brain (Hannibal et al., 2002b). Based on the anatomy, the *Spalax* eye seems to represent a functional "light meter" that could resemble what seems to be one of two systems of light perception found in the sighted eye, a system in which classical photoreceptors and mRGCs together constitute the NIF. Interestingly, as found in sighted animals, a minor fraction of *Spalax* RGCs (10%) expressing Brn3a and calretinin, but not melanopsin (**Figure 4**), projects to the NIF areas in the brain suggesting that non-melanopsin light signaling mediates NIF as well (Esquiva et al., 2016).

The eyes of the Iberian mole, like the *Spalax*, is covered by skin and severely regressed (Carmona et al., 2010). The Iberian mole eye differentiates to form a well-structured retina with all layers as found in a seeing eye [ganglion cell layer (GCL), inner nuclear cell layer (INL), outer nuclear cell layer (ONL), and photoreceptor layer] (Carmona et al., 2010). Although not quantified, a high

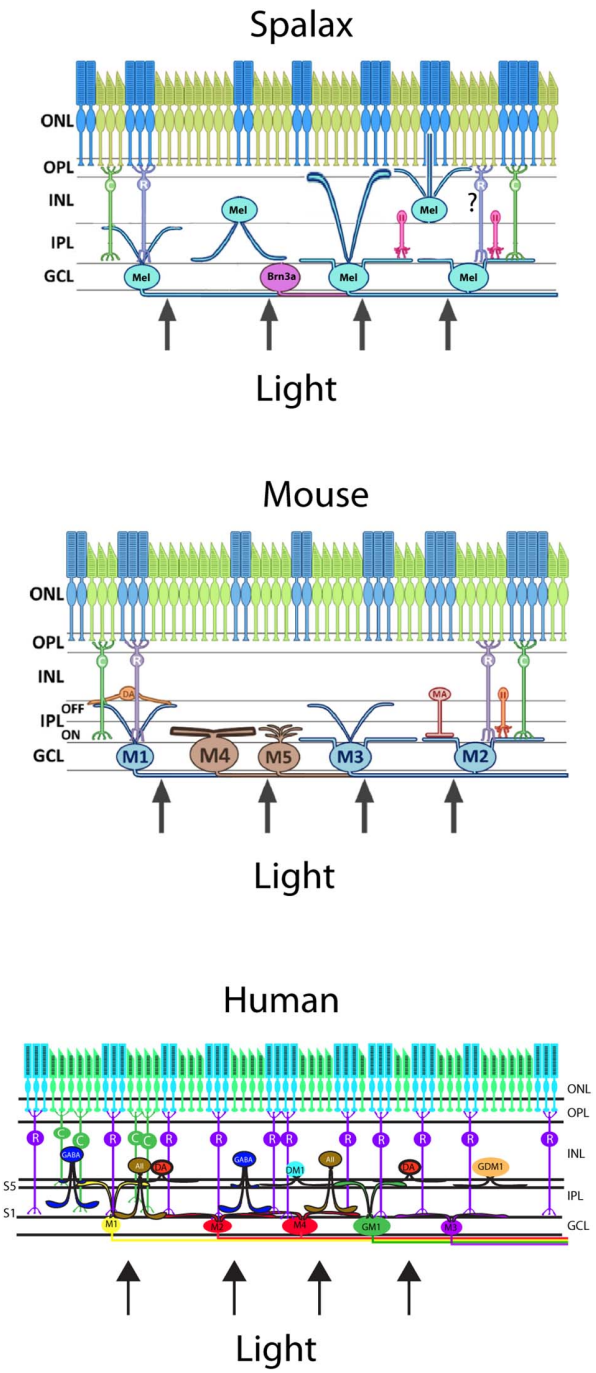

FIGURE 3 | Schematic vertical section of a Spalax (top), mouse (middle), and human (bottom) retina showing the rods and cones (green and blue) and the diverse melanopsin RGC subtypes. Human melanopsin immunoreactive retinal ganglion cells were sub-classified revealing the M1,–displaced M1 (DM1), –M2, –M3, and –M4 cell subtypes and in mouse, the not previously reported gigantic M1 (GM1) and gigantic displaced M1 (GDM1) cells. Melanopsin RGCs receive input from dopamine amacrine cells (DA) and GABAergic amacrine cells found in S5 of the inner plexiform layer (IPL). All amacrine cell and rod bipolar cell axons innervate the subtypes of melanopsin RGCs located in the ganglion cell layer (GCL). ONL; outer nuclear layer, OPL; outer plexiform layer, INL; inner nuclear layer (modified from Hannibal et al., 2017).

proportion of RGCs express melanopsin and only a minor part of RGCs express Brn3a (Carmona et al., 2010), as also reported in the *Spalax* (Esquiva et al., 2016).

mRGCs Are Responsive to Light From Birth

Melanopsin is expressed in the rat retina from prenatal day 18 (Fahrenkrug et al., 2004), whereas the classical photoreceptors occur and are functional from approximately postnatal day 10, 2–3 days before the eyes open (Ratto et al., 1991). In both rats and mice, NIF is established from P0, a timepoint where light induces FOS in mRGCs and where RHT nerve fibers can be identified in the ventral SCN (Hannibal and Fahrenkrug, 2004a). From this time point, light induces the expression of FOS in the SCN (Hannibal and Fahrenkrug, 2004a; Sekaran et al., 2005; Lupi et al., 2006; **Figure 5**). Although it has been established that neonatal photoentrainment overrides maternal entrainment from P8, light seems to influence the SCN function from P0 (Takahashi and Deguchi, 1983; Duncan et al., 1986) and may be involved in the development of SCN neuronal networks and retinal pathways (both non-image forming and image forming) in the neonatal brain.

Melanopsin Expression Is Regulated by Light and the Circadian Clock

Already from birth, the melanopsin expression is regulated both in the pigmented and albino retina, in a process that prenatally are independent of rods and cones (Hannibal et al., 2007). In adults, light has a strong impact on both the melanopsin protein and mRNA expression in the albino retina (Castrucci et al., 2004; Hannibal et al., 2005; Hannibal, 2006a) which seems to override a circadian regulation (Hannibal et al., 2005). In the pigmented retina circadian expression of melanopsin, mRNA seems to be more pronounced than in the albino retina (Sakamoto et al., 2004; Hannibal et al., 2013) and to some extent, dependent of the outer retina and classical photoreceptors (Sakamoto et al., 2004; Wan et al., 2006). However, prolonged exposure to light down regulates the melanopsin protein, whereas prolonged periods of darkness increase the melanopsin protein expression (Hannibal et al., 2013). The functional significance of the melanopsin gene expression regulation remains to be fully clarified but most likely, the changing levels of melanopsin involved in the retinal adaption to environmental light and darkness creating a maximal light sensitivity during the solar cycle.

FUNCTIONAL SIGNIFICANCE OF MRGCS—LEARNING FROM THE KNOCKOUT MODELS

mRGC Are the Primary RGCs Mediating NIF

By the discovery of melanopsin in a subpopulation of RGCs making them intrinsically photosensitive (mRGCs), several questions were asked. Is melanopsin the only photoreceptor for the NIF functions? Rod- and cone-less mice had an intact

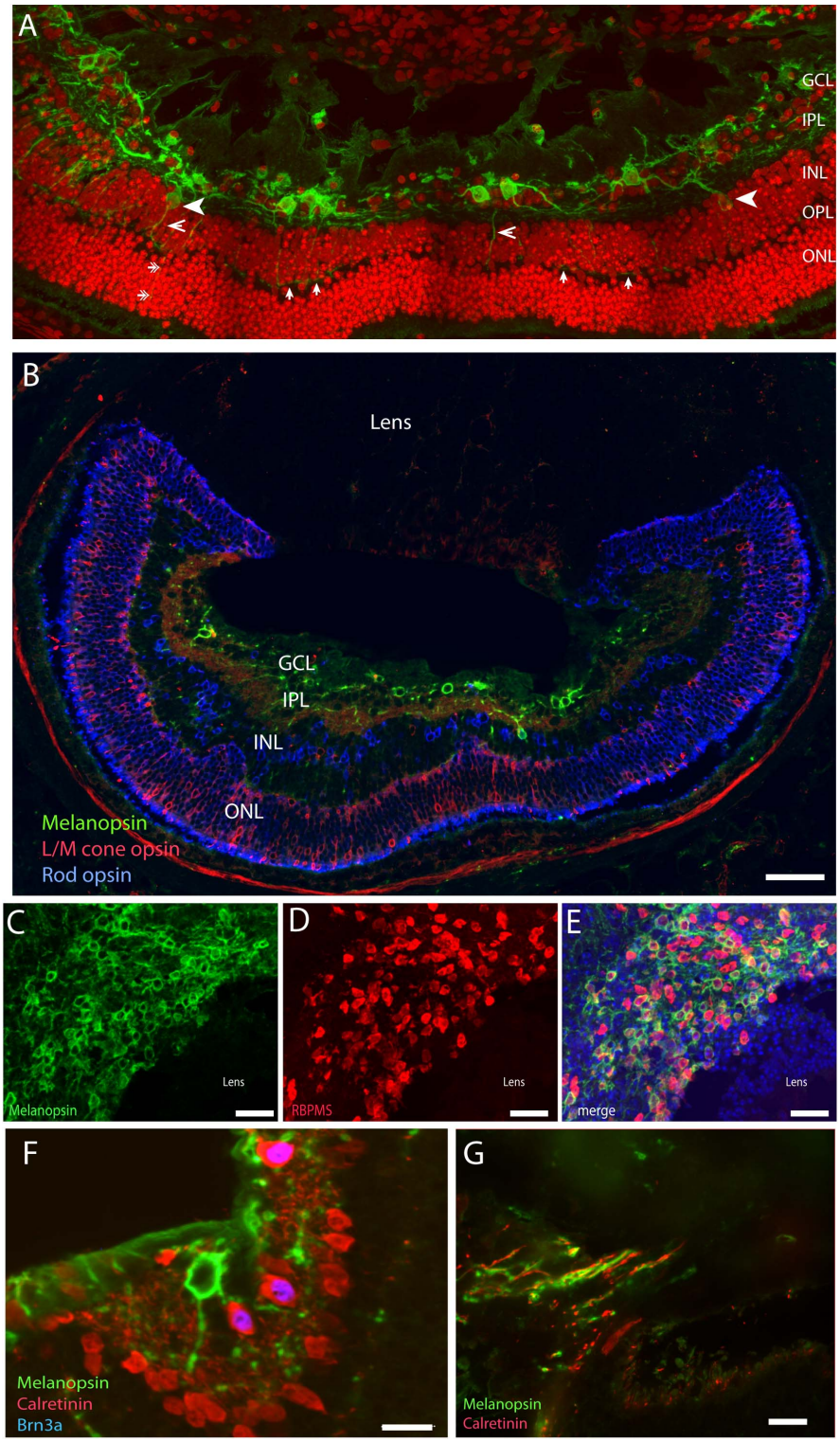

FIGURE 4 | Melanopsin in the naturally blind mole rat, the *Spalax ehrenbergi*. (**A**) Confocal photomicrographs of melanopsin (green) RGC (mRGCs) and DAPI nuclear counterstaining (red) in the *Spalax* retina. Melanopsin RGCs are located in the ganglion cell layer (GCL) and few displaced RGCs are found in the inner nuclear cell layer (INL) (indicated by arrowhead). mRGCs project mainly into the IPL, but also to the outer plexiform layer (OPL) (exemplified by open arrowhead) where they form an outer plexus (indicated by single arrows in **(A) (B)** Melanopsin (green), L/M coneopsin (red), and rhodopsin (blue) in *Spalax*. **(C–E)** Ganglion cell marker RBPMS (red) in combination with melanopsin (green) and DAPI nuclear counterstaining (blue) in horizontal sections through the GCL. Brn3a (blue) is found in all non-melanopsin RGCs co-storing calretinin (red) **(F)**. Panel **(G)** shows the optic nerve containing melanopsin and calretinin positive axons. Scalebars: **(A)**: 40 μm, **(B)**: 15 μm, **(C–E)**: 50 μm, **(E,F)**: 15 μm. GCL; ganglion cell layer, IPL; inner plexiform layer, INL; inner nuclear layer (modified from Esquiva et al., 2016).

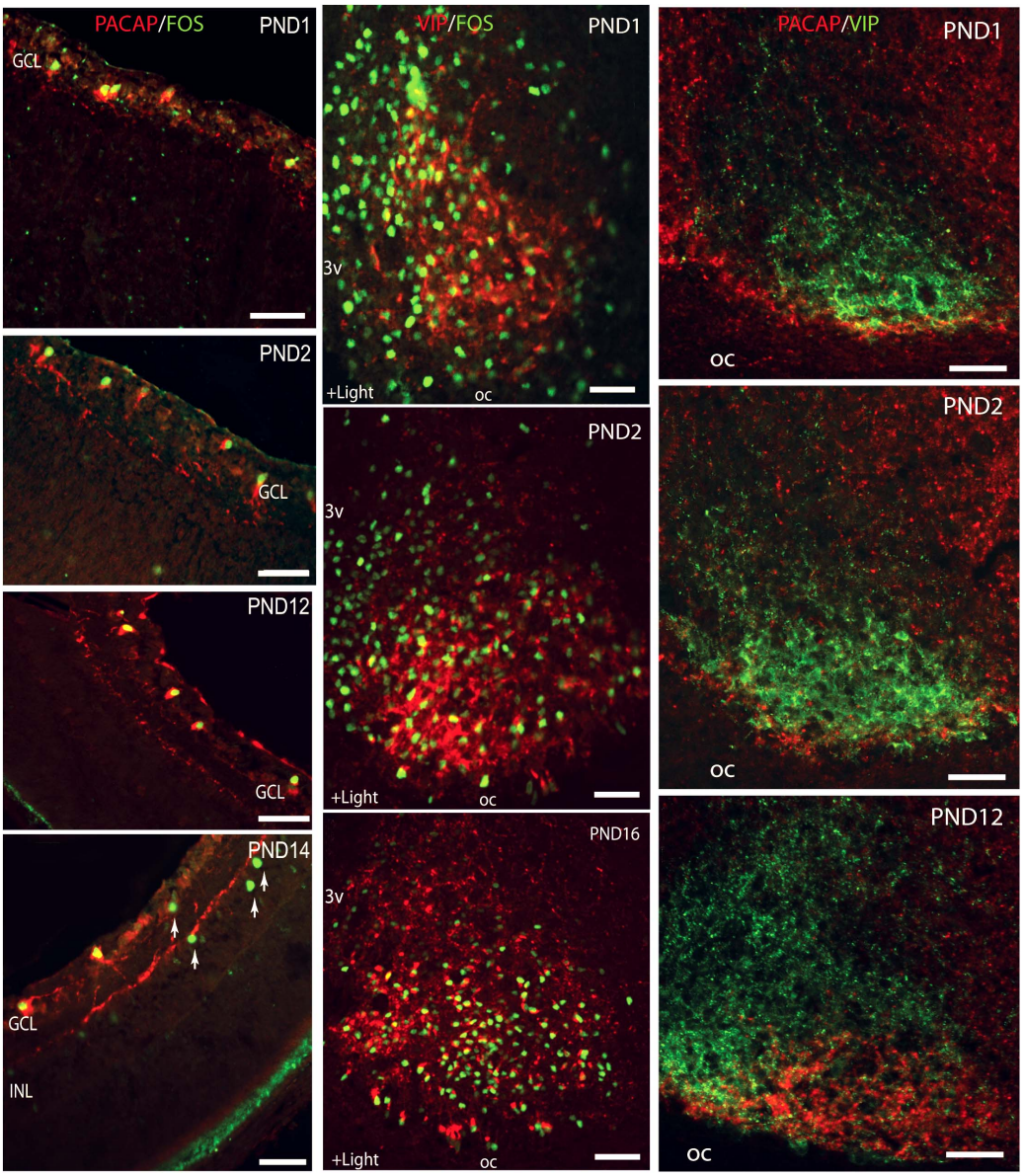

FIGURE 5 | Left lane: Light induction of FOS in melanopsin expressing retinal ganglion cells (RGCs) during postnatal development. Confocal photomicrographs showing double immunostaining for melanopsin (red) and FOS (green) in cross sections of rat retinae obtained from postnatal day (PND1) to PND14. Middle lane: Light stimulation at subjective dawn induces FOS in the SCN from the first postnatal day. Confocal photomicrographs showing double immunostaining for FOS (green) and VIP (red) in sections through the unilateral mid SCN from animals kept in darkness from subjective dawn and receiving light for 1.5 h beginning at subjective dawn. Right lane: Double immunostaining showing postnatal development of the innervation of the ventral SCN (VIP staining in green) by the retinohypothalamic tract (PACAP staining in red). PACAP positive nerve fibers are present in the SCN already on the first postnatal day and an adult pattern is found from PND12. Ages are indicated in each photomicrograph. Oc, optic chiasma. Bars = 50 mm (modified from Hannibal and Fahrenkrug, 2004a).

NIF such as photoentrainment, negative masking behavior, and suppression of nocturnal melatonin (Freedman et al., 1999; Lucas and Foster, 1999; Lucas et al., 1999). Generation of melanopsin deficient mice clarified that these mice had a normal circadian rhythm and negative masking compared to their littermates but with a significant blunted phase shift response to nocturnal light pulses indicating an important role of melanopsin in photoentrainment (Panda et al., 2002). Electrophysical examination revealed that that RGCs of melanopsin knockout mice were no longer intrinsically photosensitive (Lucas et al., 2003). These mice have a normal PLR at low light intensities, but the PLR at high light intensity was severely compromised indicating that both the melanopsin and classical photoreceptors were complementary for a normal NIF (Lucas et al., 2003). Mating of melanopsin knockout mice and rodless (rd/rd) mice showed that the double mutant mice lost all the NIF

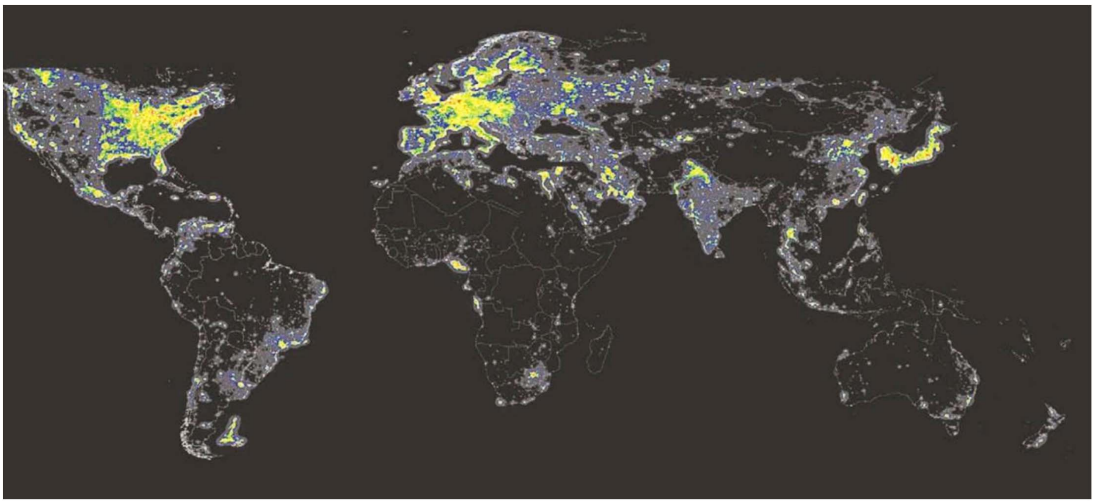

FIGURE 6 | Artificial light at night (ALAN). ALAN levels detected by the US-DMPS satellite sensors in 2010. Note that the areas emitting the highest ALAN levels are marked in red, less lit areas are marked in orange and yellow. Areas with no stable light appear in black (Cinzano et al., 2001). Source: mapped using DMSP (2014) data.

functions [photoentrainment, negative masking behavior, light inhibition of AA-NAT (the enzyme responsible for nocturnal melatonin secretion and prolongation of the endogenous period length during constant light)] (Panda et al., 2003). However, if melanopsin and the classical photoreceptors (rods and cones) mediate NIF to the brain, is it then by a separate neuronal pathway? Anatomically, the mRGCs were identified as the RGCs of the RHT. Tract tracing studies demonstrated that only a few non-melanopsin retinal projections were targeting the NIF areas in the brain innervated by melanopsin neuronal projection (see above). To clarify this question, it was necessary to eliminate the melanopsin expressing RGCs. The Hattar group used the approach to knock-in a gene (aDTA) encoding the attenuated diphtheria toxin A subunit (aDTA) into the mouse gene locus encoding melanopsin (Guler et al., 2008). In the homozygote knock-in mice, an almost complete loss of mRGCs was observed and neurologically, these mice were circadian blind with the loss of all NIF functions while image formation was left intact (Guler et al., 2008). A slightly different approach was used by the Panda group which used a Cre-inducible diphtheria toxin receptor expressed exclusively in the mRGC resulting in a selective loss of mRGCs after the injection of the Diphtheria toxin (DT, which crossed the blood-brain barrier) (Hatori et al., 2008). This approach removed almost all mRGCs and led to a loss of photoentrainment, negative masking behavior, and prolongation of the endogenous period length during constant light as well as the PLR (Hatori et al., 2008). These observations demonstrated that mRGCS are responsible for the NIF function in mammals.

Different Subtypes of mRGCs Mediate Different Functions

The initial discovery of melanopsin in one subtype of RGCs was modified based on the morphology, expression of melanopsin, dendritic projections/connections, and electrophysical properties. Today, 6–7 subtypes of melanopsin have been identified (Schmidt et al., 2011a; Sand et al., 2012; Reifler et al., 2015; see above and **Figure 3**). These different subtypes respond to light with slightly different patterns making it likely that they are involved in different functionalities. By dividing mRGCs in M1 and non-M1 subtypes, Chen et al. (2011) found that M1 subtypes can be differentiated based on the expression of Brn3b, a POU domain transcription factor (Badea et al., 2009). Brn3b positive M1 RGCs are functionally different from the Brn3b negative mRGCs. Brn3b-positive mRGCs innervate all other brain targets known to be involved in NIF, including the olivary pretectal nucleus, whereas Brn3b-negative M1 RGCs innervate the suprachiasmatic nucleus (SCN). Selective ablation of Brn3b-positive mRGCs severely disrupts the PLR, but does not impair the circadian photoentrainment consistent with these innervation patterns (Chen et al., 2011). These observations indicate that distinct subpopulations of the M1 subtype of mRGCs innervate different brain regions to execute specific light-induced functions despite being morphologically and electrophysiologically similar. Other melanopsin RGC subtypes (M2, M4, M5, and M6) seem to increase the dynamic range and spectral bandpass of the NIF as well as to shape vision perception (contrast, color, etc.) (Estevez et al., 2012; Weng et al., 2013; Schmidt et al., 2014; Stabio et al., 2018; Quattrochi et al., 2019).

Different Isoforms of Melanopsin Regulate Different NIF Functions

In mice, melanopsin can be found in a long form (OPN4L) and a short form (OPN4S), both encoding an active photopigment (Jagannath et al., 2015) (see above). The study demonstrated that OPN4S mediates light-induced pupillary constriction whereas the OPN4L regulates negative masking. However, both isoforms contribute to light entrainment and light induced sleep induction (Jagannath et al., 2015). The observations show that

different splice variants of a single receptor gene can regulate different behaviors.

NEUROTRANSMITTERS OF THE RHT: FUNCTIONAL SIGNIFICANCE AND LEARNING FROM THE KNOCKOUT MODELS

Nerve fiber terminals of the RHT are found in the retino-recipient areas of the brain including the SCN, IGL, ventrolateral preoptic nucleus (VLPO) regulating sleep, subparaventicular zone of the PVN, lateral hypothalamic area dorsal to the SON (Hannibal and Fahrenkrug, 2004b; Canteras et al., 2011), and several nuclei in the pretectal area, of which, the most densely innervated is the olivary pretectal nucleus (OPN) controlling the pupillary reflex (Hannibal and Fahrenkrug, 2004b; Hattar et al., 2006; Hannibal et al., 2014). In rat, these projections co-store two neurotransmitters, the classic neurotransmitter glutamate and PACAP (Hannibal et al., 2000; Engelund et al., 2010), targeting several subtypes of glutamate receptors and the PACAP specific receptor, the PAC1 receptor located in the SCN neurons (Hannibal, 2002, 2006b). Glutamate is considered as the primary neurotransmitter having a "light like" phase shifting capacity on the SCN neurons (Hannibal, 2002), while PACAP is considered as a neuromodulator gating the effects of glutamate induced resetting of the circadian phase (Hannibal, 2006b, 2016).

NIF in Mice Lacking the PACAP or PAC1 Receptor

In vivo and *in vitro* studies of the behavior and gene expression using PACAP and PAC1 deficient mice have increased our understanding of the role of PACAP and the PAC1 receptor in NIF (light entrainment, negative masking behavior, and PLR). *In vitro* electrophysiological studies indicate that PACAP can potentiate glutamate induced phase delay during early night and decrease glutamate induced phase advance at late night (Chen et al., 1999; Bergström et al., 2003; Hannibal, 2006b). *In vitro* studies in rat and hamster have provided evidence that PACAP in nanomolar concentrations induces phase shifts similar to light, whereas micromolar concentrations seem to modulate glutamate induced phase shifts (Chen et al., 1999; Harrington et al., 1999). Three independent groups have generated mice lacking the PACAP gene, one group mice lacking the PAC1 receptor gene.

PACAP deficient mice show normal light entrainment although a significant reduced phase shift of the circadian rhythm after a high light intensity stimulation (<100 lux) (Kawaguchi et al., 2003; Colwell et al., 2004). This finding was aligned with the *in vitro* results using PACAP in a micromolar concentration (Chen et al., 1999; Harrington et al., 1999). PACAP KO mice also demonstrated a compromised masking response whereas the PLR seemed unaffected (Kawaguchi et al., 2010). A third strain of PACAP KO mice showed similar free-running periods and normal photoentrainment (Beaule et al., 2009). However, although this strain of PACAP KO mice did not display a phase-advance after single light pulses at late night, the PACAP KO mice did entrain to a 23-h T-cycle (Beaule et al., 2009). In addition, PACAP KO mice needs several LD cycles to re-entrain after a 6-h phase advance of the LD cycle (Beaule et al., 2009). These results indicate that PACAP is required for the normal integration of the phase advancing light signal by the SCN.

PACAP receptor 1 (PAC1) deficient mice showed a normal photoentrainment as the PACAP KO mice, indicating that both genotypes have a stable biological clock (Hannibal et al., 2001a, 2008). PAC1 KO mice entrain to LD cycles but have a significantly reduced response to light stimulation at early subjective night (Hannibal et al., 2008). When placed in T-cycles (circadian day length of 21–26 h), PAC1 KO mice reach the limit of entrainment that is most pronounced at low light intensities (Hannibal et al., 2008). In accordance, PAC1 KO mice significantly needs more time (LD cycles) to re-entrain after an 8-h phase shift of the external LD cycle at low light intensities (jetlag experiment) (Hannibal et al., 2008). Furthermore, the PAC1 KO mice show an impaired negative masking that is most significant at a low light intensity (Hannibal et al., 2008). Together, *in vivo* and *in vitro* studies in mice lacking either PACAP or the PAC1 receptor show that PACAP plays an important role in light regulation of the SCN activity especially at low light intensities.

Light stimulation at early night induces, in mice, large phase delays of the circadian rhythm (see **Figure 1**) and small phase advances at late night, while in hamsters, light induces large phase advances at late night and small phase delays at early night (Hannibal, 2002). These differences are determined by the length of the endogenous period (tau). Mice, which have a fast running clock (tau shorter than 24 h), need to slow down the clock (phase delay) in order to stay entrained whereas hamsters, having a slow running clock (tau longer than 24 h), need to speed up their clock (phase advance) to stay entrained with the LD cycle (see also section "Light Entrainment") (Golombek and Rosenstein, 2010). It is possible that the low sensitivity for light in mice at late night explains these phase shift differences at this time point in both the PACAP KO and PAC1 KO mice compared to wild type mice (Golombek and Rosenstein, 2010). The OPN, another NIF target areas which controls the PLR, receives retinal PACAP input and expresses the PAC1 receptor (Engelund et al., 2012). Compared to the wild type mice, the PLR of PAC1 KO mice is significantly attenuated and the difference is significantly increased with higher light intensities (Engelund et al., 2012), indicating a role of the PAC1 receptor signaling in the PLR. However, some discrepancy is found in the PLR comparing the PACAP KO and PAC1 KO mice. It is important to note that although melanopsin in mRGCs are necessary for an intact PLR, mRGCs receive input from the classical photoreceptors (Guler et al., 2008). When studying PLR in PACAP KO mice, they were exposed to blue light [(λ) 460–490 nm] (Kawaguchi et al., 2010). This wave length selectively stimulates the melanopsin photoreceptor (Do and Yau, 2010). PAC1 KO mice were stimulated using white light (Engelund et al., 2012), which also activates rods and cones (Do and Yau, 2010). Recent studies indicate that the PLR is controlled by Brn3b-positive mRGCs (Chen et al., 2011). These mRGCs may be dependent on the input signals from the outer retina (rods and cones) as well as being more sensitive to white light (Chen et al., 2011). Most likely, the melanopsin/PACAP mRGCs that

are involved in the regulation of the PLR are different from the mRGCs regulating light entrainment and masking (Chen et al., 2011). The PLR of PAC1 KO is significantly more altered when exposed to light at a higher intensity (Engelund et al., 2012) while entrainment and negative masking in these animals are more affected at a low light intensity (Hannibal et al., 2008), supporting the involvement of the different subtypes of mRGCs.

NIF in Mice Lacking the Vesicular Glutamate Transporter 2 (VGLUT2) (Glutamate)

The VGLUT2 transporter is important for the process that packs glutamate into synaptic vesicles (Takamori, 2006). The VGLUT2 transporter shows a distinct expression within the CNS and retina (Fujiyama et al., 2003) and both the VGLUT2 (Johnson et al., 2007) and PACAP are co-stored in the melanopsin containing mRGCs (Engelund et al., 2010). Mice with a specific loss of VGLUT2 in mRGCs were investigated at different light intensities (Gompf et al., 2014; Purrier et al., 2014). VGLUT2 KO mice entrained to the LD cycle at 900 lux and all VGLUT2 KO mice showed a normal free-running activity when exposed to constant darkness, indicating an intact circadian clock (Purrier et al., 2014). VGLUT2 KO mice showed a decreased ability to re-entrain after an 8 h shift in the external LD cycle (jetlag experiment) (Purrier et al., 2014), and furthermore, VGLUT2 KO mice failed to entrain to a skeleton photoperiod (1 h of light in the morning and 1 h in the evening, simulating dawn and dusk). These finding suggests that VGLUT2 KO mice had a disturbed setting of signals for dusk and dawn (Purrier et al., 2014). A more severe affected phenotype of the VGLUT2 KO mice was described by Gompf et al. (2014). These mice failed to entrain to the LD cycle (Gompf et al., 2014) and when exposed to light at early night, the VGLUT2 KO mice showed no shift in the circadian phase and no induction of FOS in the SCN, which indicates a strongly compromised light sensitivity in these mice (Gompf et al., 2014). The decreased light sensitivity in these animals is substantiated during constant light conditions. Normal wild type changes their free running tau, which becomes longer than 24 h while the tau of VGLUT2 KO mice remained unchanged during constant darkness (Gompf et al., 2014). When exposing the population of VGLUT2 KO mice to strong constant light (2,000 lux), the tau was increased, indicating that light sensitivity remains, although with a higher threshold in this subpopulation of VGLUT2 KO mice. In both stains of the VGLUT2 KO mice, negative masking was impaired despite the light intensities used (Gompf et al., 2014; Purrier et al., 2014). The PLR was found to be significantly attenuated in VGLUT2 KO mice (Purrier et al., 2014).

The investigations of mice lacking PACAP (PACAP and PAC1 KO mice) and glutamate signaling (VGLUT2 KO mice) confirm the behavior, physiologically and gene expression studies *in vitro* and *in vivo* (reviewed in Hannibal, 2002, 2006b), showing that glutamate and PACAP are the neurotransmitters in the RHT mediating NIF information to the brain. Furthermore, the studies referred to emphasize the role of PACAP as a co-transmitter in the RHT acting together with glutamate, the primary neurotransmitter of the RHT (Hannibal, 2006b). Future studies are needed to clarify the stimulus conditions which release glutamate and PACAP from the RHT nerve terminals due to the stimulation of the photoreceptors (rods, cones, melanopsin). Not only light intensity but also irradiance and wave length (color) are of major importance in the regulation of NIF (Walmsley et al., 2015).

Photoreceptors and Neurotransmitters Involved in the PLR

Different subtypes of mRGCs are involved in the different NIF functions (Chen et al., 2011; Schmidt et al., 2011a). In a recent study, the PLR was used to investigate the contribution of photoreceptors that initiates light signaling and release of the two neurotransmitters, glutamate and PACAP (Keenan et al., 2016). The study used a combination of mice lacking rods, cones, melanopsin-, PACAP-, and VGLUT2 deficient mice. The results demonstrated a complex interaction between photoreceptors and neurotransmitters in the RHT (Keenan et al., 2016). The transient pupillary response was found to be driven by rod photoreceptors during dim and moderate light intensities. The process was found to be mediated by glutamate and was able to adapt within minutes. The sustained pupillary responses are in contrast, dominated by melanopsin phototransduction in mRGCs and mediated by PACAP, providing a stable pupil maintenance across the day (Keenan et al., 2016). These findings demonstrate how one NIF function (PLR) in the visual system is able to accomplish a high sensitivity, transient, as well as integrative and long-term responses. Other NIF functions such as photoentrainment may be differently regulated and potentially dependent on different subtypes of mRGCs.

ARTIFICIAL LIGHT AT NIGHT (ALAN) AND NIF

All NIF functions including the circadian timing system have evolved in mammals long before the occurrence of modern society. While many people on earth still lives in rural areas where light is coming primarily from daylight, people in most parts of the industrial world are exposed to artificial white light at night (ALAN) (**Figure 6**). Electrical white light has changed the lives of humans by extending the time of light during the 24 h solar cycle. This has been an important factor for the industrial growth in our society. However, recent studies have shown that light at night could have a great impact on health, potentially playing an essential role in the development of lifestyle-associated diseases such as metabolic syndrome, sleep problems, and cancer (Navara and Nelson, 2007; Panda, 2016). Since hormone levels vary during the light/dark cycle corresponding to metabolic, reproductive, and immunological functions, ALAN is a potential disruptor of the endocrine functions due to its impact on the circadian regulation and by direct effects of light independent of the circadian system (Hannibal, 2020) (see above section "Masking"). Nocturnal melatonin secretion is the most sensitive

neuroendocrine hormone axis to be affected by ALAN (Ouyang et al., 2018). Such disruption in nocturnal melatonin has been associated with an increased risk of developing different forms of cancer (Hansen, 2001; Kloog et al., 2010; Van Dycke et al., 2015). Similarly, a higher occurrence of health problems has been found in people at shift work, such as metabolic syndrome, sleep disturbances, and depression (Shivers et al., 1991; Navara and Nelson, 2007; Fonken et al., 2010).

PERSPECTIVES

Photoentrainment of the circadian clock is fundamental for the stable regulation of neuroendocrine systems leading to the secretion of hormones into the temporal niche controlling physiological functions such as metabolism, sleep, immune systems, and reproduction. Light directly suppresses melatonin secretion independent of the circadian system with an impact on several neuroendocrine axes. In modern societies, ALAN seems to affect NIF including the circadian and neuroendocrine systems. Therefore, it should be taken into serious consideration when trying to gain a better understanding of health problems in the industrialized human population.

AUTHOR CONTRIBUTIONS

JH wrote the manuscript.

FUNDING

This work was supported by the Danish Biotechnology Center for Cellular Communication.

ACKNOWLEDGMENTS

The author thank to Ida Stangerup, Elisabeth Obara, and Birgitte Georg for their critical and constructive comments.

REFERENCES

Badea, T. C., Cahill, H., Ecker, J., Hattar, S., and Nathans, J. (2009). Distinct roles of transcription factors brn3a and brn3b in controlling the development, morphology, and function of retinal ganglion cells. *Neuron* 61, 852–864. doi: 10.1016/j.neuron.2009.01.020

Baver, S. B., Pickard, G. E., Sollars, P. J., and Pickard, G. E. (2008). Two types of melanopsin retinal ganglion cell differentially innervate the hypothalamic suprachiasmatic nucleus and the olivary pretectal nucleus. *Eur. J. Neurosci.* 27, 1763–1770. doi: 10.1111/j.1460-9568.2008.06149.x

Beaule, C., Mitchell, J. W., Lindberg, P. T., Damadzic, R., Eiden, L. E., and Gillette, M. U. (2009). Temporally restricted role of retinal PACAP: integration of the phase-advancing light signal to the SCN. *J. Biol. Rhythms* 24, 126–134. doi: 10.1177/0748730409332037

Bechtold, D. A., Brown, T. M., Luckman, S. M., and Piggins, H. D. (2008). Metabolic rhythm abnormalities in mice lacking VIP-VPAC2 signaling. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R344–R351.

Belenky, M. A., Smeraski, C. A., Provencio, I., Sollars, P. J., and Pickard, G. E. (2003). Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *J. Comp. Neurol.* 460, 380–393. doi: 10.1002/cne.10652

Bergström, A. L., Hannibal, J., Hindersson, P., and Fahrenkrug, J. (2003). Light-induced phase shift in the Syrian hamster (*Mesocricetus auratus*)is attenuated by the PACAP receptor antagonist PACAP6-38 or PACAP immunoneutralization. *Eur. J. Neurosci.* 18, 2552–2562. doi: 10.1046/j.1460-9568.2003.03000.x

Berson, D. M. (2003). Strange vision: ganglion cells as circadian photoreceptors. *Trends Neurosci.* 26, 314–320. doi: 10.1016/s0166-2236(03)00130-9

Berson, D. M., Castrucci, A. M., and Provencio, I. (2010). Morphology and mosaics of melanopsin-expressing retinal ganglion cell types in mice. *J. Comp. Neurol.* 518, 2405–2422.

Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295, 1070–1073. doi: 10.1126/science.1067262

Bronchti, G., Rado, R., Terkel, J., and Wollberg, Z. (1991). Retinal projections in the blind mole rat: a WGA-HRP tracing study of a natural degeneration. *Brain Res. Dev. Brain Res.* 58, 159–170. doi: 10.1016/0165-3806(91)90002-z

Brown, T. M., Gias, C., Hatori, M., Keding, S. R., Semo, M., Coffey, P. J., et al. (2010). Melanopsin contributions to irradiance coding in the thalamo-cortical visual system. *PLoS Biol.* 8:e1000558. doi: 10.1371/journal.pbio.1000558

Bunger, M. K., Wilsbacher, L. D., Moran, S. M., Clendenin, C., Radcliffe, L. A., Hogenesch, J. B., et al. (2000). Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell* 103, 1009–1017. doi: 10.1016/s0092-8674(00)00205-1

Canteras, N. S., Ribeiro-Barbosa, E. R., Goto, M., Cipolla-Neto, J., and Swanson, L. W. (2011). The retinohypothalamic tract: comparison of axonal projection patterns from four major targets. *Brain Res. Rev.* 65, 150–183. doi: 10.1016/j.brainresrev.2010.09.006

Carmona, F. D., Glosmann, M., Ou, J., Jimenez, R., and Collinson, J. M. (2010). Retinal development and function in a 'blind' mole. *Proc. Biol. Sci.* 277, 1513–1522. doi: 10.1098/rspb.2009.1744

Castrucci, A. D. L., Ihara, N., Doyle, S. E., Rollag, M. D., Provencio, I., and Menaker, M. (2004). Light regulation of melanopsin-positive retinal ganglion cells in the albino hamster. *ARVO Meeting Abstracts* 45:4645.

Cernuda-Cernuda, R., DeGrip, W. J., Cooper, H. M., Nevo, E., and Garcia-Fernandez, J. M. (2002). The retina of *Spalax ehrenbergi*: novel histologic features supportive of a modified photosensory role. *Invest. Ophthalmol. Vis. Sci.* 43, 2374–2383.

Chen, D., Buchanan, G. F., Ding, J. M., Hannibal, J., and Gillette, M. U. (1999). PACAP: a pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13409–13414.

Chen, S. K., Badea, T. C., and Hattar, S. (2011). Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. *Nature* 476, 92–95. doi: 10.1038/nature10206

Cinzano, P., Falchi, F., and Elvidge, C. D. (2001). The first world atlas of the artificial night sky brightness. *Monthly Notice R. Astronom. Soc.* 328, 689–707. doi: 10.1046/j.1365-8711.2001.04882.x

Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Tam, J., Lelievre, V., et al. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R1194–R1201.

Cooper, H. M., Herbin, M., and Nevo, E. (1993a). Ocular regression conceals adaptive progression of the visual system in a blind subterranean mammal. *Nature* 361, 156–159. doi: 10.1038/361156a0

Cooper, H. M., Herbin, M., and Nevo, E. (1993b). Visual system of a naturally microphthalmic mammal: the blind mole rat, *Spalax ehrenbergi*. *J. Comp. Neurol.* 328, 313–350. doi: 10.1002/cne.903280302

Dacey, D. M., Liao, H. W., Peterson, B. B., Robinson, F. R., Smith, V. C., Pokorny, J., et al. (2005). Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature* 433, 749–754. doi: 10.1038/nature03387

David-Gray, Z. K., Janssen, J. W., DeGrip, W. J., Nevo, E., and Foster, R. G. (1998). Light detection in a 'blind' mammal. *Nat. Neurosci.* 1, 655–656. doi: 10.1038/3656

Do, M. T., and Yau, K. W. (2010). Intrinsically photosensitive retinal ganglion cells. *Physiol. Rev.* 90, 1547–1581.

Duffy, J. F., and Czeisler, C. A. (2009). Effect of light on human circadian physiology. *Sleep Med. Clin.* 4, 165–177. doi: 10.1016/j.jsmc.2009.01.004

Duncan, M. J., Banister, M. J., and Reppert, S. M. (1986). Developmental appearance of light-dark entrainment in the rat. *Brain Res.* 369, 326–330. doi: 10.1016/0006-8993(86)90544-5

Ecker, J. L., Dumitrescu, O. N., Wong, K. Y., Alam, N. M., Chen, S. K., LeGates, T., et al. (2010). Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. *Neuron* 67, 49–60. doi: 10.1016/j.neuron.2010.05.023

Engelund, A., Fahrenkrug, J., Harrison, A., and Hannibal, J. (2010). Vesicular glutamate transporter 2 (VGLUT2) is co-stored with PACAP in projections from the rat melanopsin-containing retinal ganglion cells. *Cell Tissue Res.* 340, 243–255. doi: 10.1007/s00441-010-0950-3

Engelund, A., Fahrenkrug, J., Harrison, A., Luuk, H., and Hannibal, J. (2012). Altered pupillary light reflex in PACAP receptor 1-deficient mice. *Brain Res.* 1453, 17–25. doi: 10.1016/j.brainres.2012.03.005

Esquiva, G., Avivi, A., and Hannibal, J. (2016). Non-image forming light detection by melanopsin, rhodopsin, and long-middlewave (L/W) cone opsin in the subterranean blind mole rat, spalax ehrenbergi: immunohistochemical characterization, distribution, and connectivity. *Front. Neuroanat.* 10:61. doi: 10.3389/fnana.2016.00061

Estevez, M. E., Fogerson, P. M., Ilardi, M. C., Borghuis, B. G., Chan, E., Weng, S., et al. (2012). Form and function of the M4 cell, an intrinsically photosensitive retinal ganglion cell type contributing to geniculocortical vision. *J. Neurosci.* 32, 13608–13620. doi: 10.1523/jneurosci.1422-12.2012

Fahrenkrug, J., Nielsen, H. S., and Hannibal, J. (2004). Expression of melanopsin during development of the rat retina. *Neuroreport* 15, 781–784. doi: 10.1097/00001756-200404090-00008

Fonken, L. K., Workman, J. L., Walton, J. C., Weil, Z. M., Morris, J. S., Haim, A., et al. (2010). Light at night increases body mass by shifting the time of food intake. *Proc. Natl. Acad. Sci. U S A.* 107, 18664–18669. doi: 10.1073/pnas.1008734107

Foster, R. G., Argamaso, S., Coleman, S., Colwell, C. S., Lederman, A., and Provencio, I. (1993). Photoreceptors regulating circadian behavior: a mouse model. *J. Biol. Rhythms* 8(Suppl.), S17–S23.

Foster, R. G., Hughes, S., and Peirson, S. N. (2020). Circadian photoentrainment in mice and humans. *Biology (Basel)* 9:180. doi: 10.3390/biology9070180

Foster, R. G., Provencio, I., Hudson, D., Fiske, S., De Grip, W., and Menaker, M. (1991). Circadian photoreception in the retinally degenerate mouse (rd/rd). *J. Comp. Physiol.* 169, 39–50. doi: 10.1007/bf00198171

Freedman, M. S., Lucas, R. J., Soni, B., von Schantz, M., Muñoz, M., David-Gray, Z., et al. (1999). Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 284, 502–504.

Fujiyama, F., Hioki, H., Tomioka, R., Taki, K., Tamamaki, N., Nomura, S., et al. (2003). Changes of immunocytochemical localization of vesicular glutamate transporters in the rat visual system after the retinofugal denervation. *J. Comp. Neurol.* 465, 234–249. doi: 10.1002/cne.10848

Golombek, D. A., and Rosenstein, R. E. (2010). Physiology of circadian entrainment. *Physiol. Rev.* 90, 1063–1102. doi: 10.1152/physrev.00009.2009

Gompf, H. S., Fuller, P. M., Hattar, S., Saper, C. B., and Lu, J. (2014). Impaired circadian photosensitivity in mice lacking glutamate transmission from retinal melanopsin cells. *J. Biol. Rhythms* 30, 35–41. doi: 10.1177/0748730414561545

Grunert, U., Jusuf, P. R., Lee, S. C., and Nguyen, D. T. (2011). Bipolar input to melanopsin containing ganglion cells in primate retina. *Vis. Neurosci.* 28, 39–50. doi: 10.1017/s095252381000026x

Guler, A. D., Ecker, J. L., Lall, G. S., Haq, S., Altimus, C. M., Liao, H. W., et al. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature* 453, 102–105. doi: 10.1038/nature06829

Hannibal, J. (2002). Neurotransmitters of the retino-hypothalamic tract. *Cell Tissue Res.* 309, 73–88. doi: 10.1007/s00441-002-0574-3

Hannibal, J. (2006a). Regulation of melanopsin expression. *Chronobiol. Int.* 23, 159–166. doi: 10.1080/07420520500464544

Hannibal, J. (2006b). Roles of PACAP-containing retinal ganglion cells in circadian timing. *Int. Rev. Cytol.* 251, 1–39. doi: 10.1016/s0074-7696(06)51001-0

Hannibal, J. (2016). "PACAP in the circadian timing system: learning from knockout models," in *Pituitary Adenylate Activating Polypeptide - PACAP*, eds D. Reglodi, and A. Tamas (Switzerland: Springer International Publisher), 227–237. doi: 10.1007/978-3-319-35135-3_14

Hannibal, J., Brabet, P., and Fahrenkrug, J. (2008). Mice lacking the PACAP type I receptor have impaired photic entrainment and negative masking. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R2050–R2058.

Hannibal, J., Brabet, P., Jamen, F., Nielsen, H. S., Journot, L., and Fahrenkrug, J. (2001a). Dissociation between light induced phase shift of the circadian rhythm and clock gene expression in mice lacking the PACAP type 1 receptor (PAC1). *J. Neurosci.* 21, 4883–4890. doi: 10.1523/jneurosci.21-13-04883.2001

Hannibal, J., Vrang, N., Card, J. P., and Fahrenkrug, J. (2001b). Light dependent induction of c-Fos during subjective day and night in PACAP containing retinal ganglion cells of the retino-hypothalmic tract. *J. Biol. Rhythms* 16, 457–470. doi: 10.1177/074873001129002132

Hannibal, J., Christiansen, A. T., Heegaard, S., Fahrenkrug, J., and Kiilgaard, J. F. (2017). Melanopsin expressing human retinal ganglion cells: subtypes, distribution, and intraretinal connectivity. *J. Comp. Neurol.* 525, 1934–1961. doi: 10.1002/cne.24181

Hannibal, J., Ding, J. M., Chen, D., Fahrenkrug, J., Larsen, P. J., Gillette, M. U., et al. (1997). Pituitary adenylate cyclase activating peptide (PACAP) in the retinohypothalamic tract. a daytime regulator of the biological clock. *J. Neurosci.* 17, 2637–2644. doi: 10.1523/jneurosci.17-07-02637.1997

Hannibal, J., and Fahrenkrug, J. (2004a). Melanopsin containing retinal ganglion cells are light responsive from birth. *Neuroreport* 15, 2317–2320. doi: 10.1097/00001756-200410250-00003

Hannibal, J., and Fahrenkrug, J. (2004b). Target areas innervated by PACAP immunoreactive retinal ganglion cells. *Cell Tissue Res.* 316, 99–113. doi: 10.1007/s00441-004-0858-x

Hannibal, J., and Fahrenkrug, J. (2006). Neuronal input pathways to the brain's biological clock and their functional significance. *Adv. Anat. Embryol. Cell Biol.* 182, 1–71. doi: 10.1007/3-540-27789-7_1

Hannibal, J., Georg, B., and Fahrenkrug, J. (2007). Melanopsin changes in neonatal albino rat independent of rods and cones. *Neuroreport* 18, 81–85. doi: 10.1097/wnr.0b013e328010ff56

Hannibal, J., Georg, B., and Fahrenkrug, J. (2013). Differential expression of melanopsin mRNA and protein in brown Norwegian rats. *Exp. Eye Res.* 106, 55–63. doi: 10.1016/j.exer.2012.11.006

Hannibal, J., Georg, B., Hindersson, P., and Fahrenkrug, J. (2005). Light and darkness regulate melanopsin in the retinal ganglion cells of the albino wistar rat. *J. Mol. Neurosci.* 27, 147–156. doi: 10.1385/jmn:27:2:147

Hannibal, J., Hindersson, P., Knudsen, S. M., Georg, B., and Fahrenkrug, J. (2002a). The photopigment melanopsin is exclusively present in PACAP containing retinal ganglion cells of the retinohypothalamic tract. *J. Neurosci.* 22:RC191.

Hannibal, J., Hindersson, P., Nevo, E., and Fahrenkrug, J. (2002b). The circadian photopigment melanopsin is expressed in the blind subterranean mole rat. *Spalax. Neuroreport.* 13, 1411–1414. doi: 10.1097/00001756-200208070-00013

Hannibal, J., Hsiung, H. M., and Fahrenkrug, J. (2011). Temporal phasing of locomotor activity, heart rate rhythmicity, and core body temperature is disrupted in VIP receptor 2-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R519–R530. doi: 10.1152/ajpregu.00599.2010

Hannibal, J., Kankipati, L., Strang, C. E., Peterson, B. B., Dacey, D., and Gamlin, P. D. (2014). Central projections of intrinsically photosensitive retinal ganglion cells in the macaque monkey. *J. Comp. Neurol.* 522, 2231–2248. doi: 10.1002/cne.23555

Hannibal, J., Moller, M., Ottersen, O. P., and Fahrenkrug, J. (2000). PACAP and glutamate are co-stored in the retinohypothalamic tract. *J. Comp. Neurol.* 418, 147–155. doi: 10.1002/(sici)1096-9861(20000306)418:2<147::aid-cne2>3.0.co;2-#

Hannibal, J. (2020). "Action of light on the neuroendocrine axis," in *Neuroendocrine Clocks and Calenders*, eds F. J. P. Ebling, and H. D. Piggins (Berlin: Springer Verlag), 163–176. doi: 10.1007/978-3-030-55643-3_8

Hansen, J. (2001). Light at night, shiftwork, and breast cancer risk. *J. Natl. Cancer Inst.* 93, 1513–1515. doi: 10.1093/jnci/93.20.1513

Harmar, A. J., Fahrenkrug, J., Gozes, I., Laburthe, M., May, V., Pisegna, J. R., et al. (2012). Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR review 1. *Br. J. Pharmacol.* 166, 4–17. doi: 10.1111/j.1476-5381.2012.01871.x

Harrington, M. E., Hoque, S., Hall, A., Golombek, D., and Biello, S. (1999). Pituitary adenylate cyclase activating peptide phase shifts circadian rhythms in a manner similar to light. *J. Neurosci.* 19, 6637–6642. doi: 10.1523/jneurosci.19-15-06637.1999

Hatori, M., Le, H., Vollmers, C., Keding, S. R., Tanaka, N., Schmedt, C., et al. (2008). Inducible ablation of melanopsin-expressing retinal ganglion cells reveals their central role in non-image forming visual responses. *PLoS One.* 3:e2451. doi: 10.1371/journal.pone.0002451

Hattar, S., Kumar, M., Park, A., Tong, P., Tung, J., Yau, K. W., et al. (2006). Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J. Comp. Neurol.* 497, 326–349. doi: 10.1002/cne.20970

Hattar, S., Liao, H. W., Takao, M., Berson, D. M., and Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* 295, 1065–1070. doi: 10.1126/science.1069609

Hendrickson, A. E., Wagoner, N., and Cowan, W. M. (1972). An autoradiographic and electron microscopic study of retino-hypothalamic connections. *Z. Zellforsch. Mikrosk. Anat.* 135, 1–26. doi: 10.1007/bf00307084

Hughes, S., Jagannath, A., Hankins, M. W., Foster, R. G., and Peirson, S. N. (2015). Photic regulation of clock systems. *Methods Enzymol.* 552, 125–143. doi: 10.1016/bs.mie.2014.10.018

Hughes, S., Welsh, L., Katti, C., Gonzalez-Menendez, I., Turton, M., Halford, S., et al. (2012). Differential expression of melanopsin isoforms Opn4L and Opn4S during postnatal development of the mouse retina. *PLoS One* 7:e34531. doi: 10.1371/journal.pone.0034531

Husse, J., Leliavski, A., Tsang, A. H., Oster, H., and Eichele, G. (2014). The light-dark cycle controls peripheral rhythmicity in mice with a genetically ablated suprachiasmatic nucleus clock. *FASEB J.* 28, 4950–4960. doi: 10.1096/fj.14-256594

Jagannath, A., Hughes, S., Abdelgany, A., Pothecary, C. A., Di Pretoro, S., Pires, S. S., et al. (2015). Isoforms of melanopsin mediate different behavioral responses to light. *Curr. Biol.* 25, 2430–2434. doi: 10.1016/j.cub.2015.07.071

Johnson, J., Fremeau, R. T. Jr., Duncan, J. L., Renteria, R. C., Yang, H., et al. (2007). Vesicular glutamate transporter 1 is required for photoreceptor synaptic signaling but not for intrinsic visual functions. *J. Neurosci.* 27, 7245–7255. doi: 10.1523/jneurosci.0815-07.2007

Jusuf, P. R., Lee, S. C., Hannibal, J., and Grunert, U. (2007). Characterization and synaptic connectivity of melanopsin-containing ganglion cells in the primate retina. *Eur. J. Neurosci.* 26, 2906–2921. doi: 10.1111/j.1460-9568.2007.05924.x

Kawaguchi, C., Isojima, Y., Shintani, N., Hatanaka, M., Guo, X., Okumura, N., et al. (2010). PACAP-deficient mice exhibit light parameter-dependent abnormalities on nonvisual photoreception and early activity onset. *PLoS One* 5:e9286. doi: 10.1371/journal.pone.0009286

Kawaguchi, C., Tanaka, K., Isojima, Y., Shintani, N., Hashimoto, H., Baba, A., et al. (2003). Changes in light-induced phase shift of circadian rhythm in mice lacking PACAP. *Biochem. Biophys. Res. Commun.* 310, 169–175. doi: 10.1016/j.bbrc.2003.09.004

Keeler, C. E. (1924). The inheritance of a retinal abnormality in white mice. *Proc. Natl. Acad. Sci. U S A.* 10, 329–333. doi: 10.1073/pnas.10.7.329

Keeler, C. E., Sutcliffe, E., and Chaffee, E. L. (1928). Normal and "Rodless" retinae of the house mouse with respect to the electromotive force generated through stimulation by light. *Proc. Natl. Acad. Sci. U S A.* 14, 477–484.

Keenan, W. T., Rupp, A. C., Ross, R. A., Somasundaram, P., Hiriyanna, S., Wu, Z., et al. (2016). A visual circuit uses complementary mechanisms to support transient and sustained pupil constriction. *eLife* 5:e15392.

Kloog, I., Stevens, R. G., Haim, A., and Portnov, B. A. (2010). Nighttime light level co-distributes with breast cancer incidence worldwide. *Cancer Causes Control.* 21, 2059–2068. doi: 10.1007/s10552-010-9624-9624

La Morgia, C., Carelli, V., and Carbonelli, M. (2018). Melanopsin retinal ganglion cells and pupil: clinical implications for neuro-ophthalmology. *Front. Neurol.* 9:1047. doi: 10.3389/fneur.2018.01047

Langel, J. L., Smale, L., Esquiva, G., and Hannibal, J. (2015). Central melanopsin projections in the diurnal rodent, *Arvicanthis niloticus*. *Front. Neuroanat.* 9:93. doi: 10.3389/fnana.2015.00093

Lucas, R. J., and Foster, R. G. (1999). Neither functional rod photoreceptors nor rod or cone outer segments are required for the photic inhibition of pineal melatonin. *Endocrinology* 140, 1520–1524. doi: 10.1210/endo.140.4.6672

Lucas, R. J., Freedman, M. S., Munoz, M., Garcia-Fernandez, J. M., and Foster, R. G. (1999). Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* 284, 505–507. doi: 10.1126/science.284.5413.505

Lucas, R. J., Hattar, S., Takao, M., Berson, D. M., Foster, R. G., and Yau, K. W. (2003). Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* 299, 245–247. doi: 10.1126/science.1077293

Lucas, R. J., Lall, G. S., Allen, A. E., and Brown, T. M. (2012). How rod, cone, and melanopsin photoreceptors come together to enlighten the mammalian circadian clock. *Prog. Brain Res.* 199, 1–18. doi: 10.1016/b978-0-444-59427-3.00001-0

Lucas, R. J., Peirson, S. N., Berson, D. M., Brown, T. M., Cooper, H. M., Czeisler, C. A., et al. (2014). Measuring and using light in the melanopsin age. *Trends Neurosci.* 37, 1–9. doi: 10.1016/j.tins.2013.10.004

Lupi, D., Sekaran, S., Jones, S. L., Hankins, M. W., and Foster, R. G. (2006). Light-evoked FOS induction within the suprachiasmatic nuclei (SCN) of melanopsin knockout (Opn4(-/-)) mice: a developmental study. *Chronobiol. Int.* 23, 167–179. doi: 10.1080/07420520500545870

Masana, M. I., Benloucif, S., and Dubocovich, M. L. (1996). Light-induced c-fos mRNA expression in the suprachiasmatic nucleus and the retina of C3H/HeN mice. *Brain Res. Mol. Brain Res.* 42, 193–201. doi: 10.1016/s0169-328x(96)00031-9

Miyata, A., Arimura, A., Dahl, R. R., Minamino, N., Uehara, A., Jiang, L., et al. (1989). Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem. Biophys. Res. Commun.* 164, 567–574. doi: 10.1016/0006-291x(89)91757-9

Miyata, A., Jiang, L. J., Dahl, R. D., Kitada, C., Kubo, K., Fujino, M., et al. (1990). Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochem. Biophys. Res. Commun.* 170, 643–648. doi: 10.1016/0006-291x(90)92140-u

Mohawk, J. A., and Takahashi, J. S. (2011). Cell autonomy and synchrony of suprachiasmatic nucleus circadian oscillators. *Trends Neurosci.* 34, 349–358. doi: 10.1016/j.tins.2011.05.003

Moore, R. Y., and Lenn, N. J. (1972). A retinohypothalamic projection in the rat. *J. Comp. Neurol.* 146, 1–14. doi: 10.1002/cne.901460102

Moore, R. Y., Speh, J. C., and Card, J. P. (1995). The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. *J. Comp. Neurol.* 352, 351–366. doi: 10.1002/cne.903520304

Morin, L. P. (2013). Neuroanatomy of the extended circadian rhythm system. *Exp Neurol.* 243, 4–20. doi: 10.1016/j.expneurol.2012.06.026

Morin, L. P., and Allen, C. N. (2005). The circadian visual system, 2005. *Brain Res. Rev.* 51, 1–60. doi: 10.1016/j.brainresrev.2005.08.003

Mrosovsky, N. (1999). Masking: history, definitions, and measurement. *Chronobiol. Int.* 16, 415–429.

Navara, K. J., and Nelson, R. J. (2007). The dark side of light at night: physiological, epidemiological, and ecological consequences. *J. Pineal. Res.* 43, 215–224. doi: 10.1111/j.1600-079X.2007.00473.x

Nelson, R. J., and Zucker, I. (1981). Absence of extraocular photoreception in diurnal and nocturnal rodents exposed to direct sunlight. *Comp. Biochem. Physiol.* 69A, 145–148.

Nevo, E. (1999). *Mosaic Evolution of Subterranean Mammals: Regression, Progression and Global convergence*. Oxford: Oxford University Press.

Nevo, E., Ivaniskaya, I., and Beiles, A. (2001). *Adaptive Radiation of Blind Subterrancan Mole Rats: Naming and Revisiting the Four Siblings Species of the Spalax Ehrenbergi Superspecies in Israel: Spalax gallili (2n=52), S. Golani (2n=54), S. carmeli (2n=58) and S. judaei (2n=60)*. Leiden: Bachkhuys Publisher.

Ostergaard, J., Hannibal, J., and Fahrenkrug, J. (2007). Synaptic contact between melanopsin-containing retinal ganglion cells and rod bipolar cells. *Invest. Ophthalmol. Vis. Sci.* 48, 3812–3820.

Ouyang, J. Q., Davies, S., and Dominoni, D. (2018). Hormonally mediated effects of artificial light at night on behavior and fitness: linking endocrine mechanisms with function. *J. Exp. Biol.* 221:jeb156893.

Panda, S. (2016). Circadian physiology of metabolism. *Science* 354, 1008–1015. doi: 10.1126/science.aah4967

Panda, S., Provencio, I., Tu, D. C., Pires, S. S., Rollag, M. D., Castrucci, A. M., et al. (2003). Melanopsin is required for non-image-forming photic responses in blind mice. *Science* 301, 525–527.

Panda, S., Sato, T. K., Castrucci, A. M., Rollag, M. D., DeGrip, W. J., Hogenesch, J. B., et al. (2002). Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* 298, 2213–2216.

Pevet, P., and Challet, E. (2011). Melatonin: both master clock output and internal time-giver in the circadian clocks network. *J. Physiol. Paris.* 105, 170–182. doi: 10.1016/j.jphysparis.2011.07.001

Pickard, G. E. (1985). Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suprachiasmatic nucleus and the intergeniculate leaflet of the thalamus. *Neurosci. Lett.* 55, 211–217.

Pires, S. S., Hughes, S., Turton, M., Melyan, Z., Peirson, S. N., Zheng, L., et al. (2009). Differential expression of two distinct functional isoforms of melanopsin (Opn4) in the mammalian retina. *J. Neurosci.* 29, 12332–12342.

Provencio, I., Jiang, G., De Grip, W. J., Hayes, W. P., and Rollag, M. D. (1998). Melanopsin: an opsin in melanophores, brain, and eye. *Proc. Natl. Acad. Sci. U S A.* 95, 340–345.

Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., and Rollag, M. D. (2000). A novel human opsin in the inner retina. *J. Neurosci.* 20, 600–605.

Purrier, N., Engeland, W. C., and Kofuji, P. (2014). Mice deficient of glutamatergic signaling from intrinsically photosensitive retinal ganglion cells exhibit abnormal circadian photoentrainment. *PLoS One* 9:e111449. doi: 10.1371/journal.pone.0111449

Quattrochi, L. E., Stabio, M. E., Kim, I., Ilardi, M. C., Michelle Fogerson, P., Leyrer, M. L., et al. (2019). The M6 cell: a small-field bistratified photosensitive retinal ganglion cell. *J. Comp. Neurol.* 527, 297–311. doi: 10.1016/j.neuron.2017.11.030

Ratto, G. M., Robinson, D. W., Yan, B., and McNaughton, P. A. (1991). Development of the light response in neonatal mammalian rods. *Nature* 351, 654–657.

Redlin, U. (2001). Neural basis and biological function of masking by light in mammals: suppression of melatonin and locomotor activity. *Chronobiol. Int.* 18, 737–758.

Redlin, U., and Mrosovsky, N. (1999). Masking by light in hamsters with SCN lesions. *J. Comp. Physiol.* 184, 439–448.

Reifler, A. N., Chervenak, A. P., Dolikian, M. E., Benenati, B. A., Meyers, B. S., Demertzis, Z. D., et al. (2014). The rat retina has five types of ganglion-cell photoreceptors. *Exp. Eye Res.* 130C, 17–28.

Reifler, A. N., Chervenak, A. P., Dolikian, M. E., Benenati, B. A., Meyers, B. S., Demertzis, Z. D., et al. (2015). The rat retina has five types of ganglion-cell photoreceptors. *Exp. Eye Res.* 130, 17–28.

Rollag, M. D., Berson, D. M., and Provencio, I. (2003). Melanopsin, ganglion-cell photoreceptors, and mammalian photoentrainment. *J. Biol. Rhythms* 18, 227–234.

Sakamoto, K., Liu, C., and Tosini, G. (2004). Classical photoreceptors regulate melanopsin mRNA levels in the rat retina. *J. Neurosci.* 24, 9693–9697.

Sand, A., Schmidt, T. M., and Kofuji, P. (2012). Diverse types of ganglion cell photoreceptors in the mammalian retina. *Prog. Retin. Eye Res.* 31, 287–302.

Saper, C. B., Cano, G., and Scammell, T. E. (2005a). Homeostatic, circadian, and emotional regulation of sleep. *J. Comp. Neurol.* 493, 92–98.

Saper, C. B., Scammell, T. E., and Lu, J. (2005b). Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437, 1257–1263.

Schmidt, T. M., Alam, N. M., Chen, S., Kofuji, P., Li, W., Prusky, G. T., et al. (2014). A role for melanopsin in alpha retinal ganglion cells and contrast detection. *Neuron* 82, 781–788.

Schmidt, T. M., Chen, S. K., and Hattar, S. (2011a). Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends Neurosci.* 34, 572–580.

Schmidt, T. M., Do, M. T., Dacey, D., Lucas, R., Hattar, S., and Matynia, A. (2011b). Melanopsin-positive intrinsically photosensitive retinal ganglion cells: from form to function. *J. Neurosci.* 31, 16094–16101.

Sekaran, S., Lupi, D., Jones, S. L., Sheely, C. J., Hattar, S., Yau, K. W., et al. (2005). Melanopsin-dependent photoreception provides earliest light detection in the mammalian retina. *Curr. Biol.* 15, 1099–1107.

Shivers, B. D., Gorcs, T. J., Gottschall, P. E., and Arimura, A. (1991). Two high affinity binding sites for pituitary adenylate cyclase- activating polypeptide have different tissue distributions. *Endocrinology* 128, 3055–3065.

Shuboni, D. D., Cramm, S., Yan, L., Nunez, A. A., and Smale, L. (2012). Acute behavioral responses to light and darkness in nocturnal *Mus musculus* and diurnal *Arvicanthis niloticus*. *J. Biol. Rhythms* 27, 299–307. doi: 10.1177/0748730412449723

Stabio, M. E., Sabbah, S., Quattrochi, L. E., Ilardi, M. C., Fogerson, P. M., Leyrer, M. L., et al. (2018). The M5 cell: a color-opponent intrinsically photosensitive retinal ganglion cell. *Neuron* 97:e154.

Swanson, L. W. (2000). Cerebral hemisphere regulation of motivated behavior. *Brain Res.* 886, 113–164. doi: 10.1016/s0006-8993(00)02905-x

Takahashi, J. S. (2016). "Molecular architecture of the circadian clock in mammals," in *A Time for Metabolism and Hormones*, eds P. Sassone-Corsi and Y. Christen (Berlin: Springer), 13–24.

Takahashi, K., and Deguchi, T. (1983). Entrainment of the circadian rhythms of blinded infant rats by nursing mothers. *Physiol. Behav.* 31, 373–378.

Takamori, S. (2006). VGLUTs: 'exciting' times for glutamatergic research? *Neurosci. Res.* 55, 343–351. doi: 10.1016/j.neures.2006.04.016

Todd, W. D., Gall, A. J., Weiner, J. A., and Blumberg, M. S. (2012). Distinct retinohypothalamic innervation patterns predict the developmental emergence of species-typical circadian phase preference in nocturnal Norway rats and diurnal nile grass rats. *J. Comp. Neurol.* 520, 3277–3292.

Van Dycke, K. C., Rodenburg, W., van Oostrom, C. T., van Kerkhof, L. W., Pennings, J. L., Roenneberg, T., et al. (2015). Chronically alternating light cycles increase breast cancer risk in mice. *Curr. Biol.* 25, 1932–1937. doi: 10.1016/j.cub.2015.06.012

Vaudry, D., Gonzalez, B. J., Basille, M., Yon, L., Fournier, A., and Vaudry, H. (2000). Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev.* 52, 269–324.

Walmsley, L., Hanna, L., Mouland, J., Martial, F., West, A., Smedley, A. R., et al. (2015). Colour as a signal for entraining the mammalian circadian clock. *PLoS. Biol.* 13:e1002127. doi: 10.1371/journal.pbio.1002127

Wan, J., Zheng, H., Hu, B. Y., Xiao, H. L., She, Z. J., Chen, Z. L., et al. (2006). Acute photoreceptor degeneration down-regulates melanopsin expression in adult rat retina. *Neurosci. Lett.* 400, 48–52.

Welsh, D. K., Takahashi, J. S., and Kay, S. A. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. *Annu. Rev. Physiol.* 72, 551–577.

Weng, S., Estevez, M. E., and Berson, D. M. (2013). Mouse ganglion-cell photoreceptors are driven by the most sensitive rod pathway and by both types of cones. *PLoS One* 8:e66480. doi: 10.1371/journal.pone.0066480

Yamaguchi, S., Isejima, H., Matsuo, T., Okura, R., Yagita, K., Kobayashi, M., et al. (2003). Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302, 1408–1412.

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 Neuroscience

PERSPECTIVE
published: 07 June 2021
doi: 10.3389/fnins.2021.637221

Biologically Relevant Lighting: An Industry Perspective

Robert Soler and Erica Voss*

BIOS Lighting, Carlsbad, CA, United States

Innovations in LED lighting technology have led to tremendous adoption rates and vastly improved the metrics by which they are traditionally evaluated–including color quality, longevity, and energy efficiency to name a few. Additionally, scientific insight has broadened with respect to the biological impact of light, specifically our circadian rhythm. Indoor electric lighting, despite its many attributes, fails to specifically address the biological responses to light. Traditional electric lighting environments are biologically too dim during the day, too bright at night, and with many people spending much of their lives in these environments, it can lead to circadian dysfunction. The lighting industry's biological solution has been to create bluer days and yellower nights, but the technology created to do so caters primarily to the cones. A better call to action is to provide biologically brighter days and biologically darker nights within the built environment. However, current lighting design practices have specified the comfort and utility of electric light. Brighter intensity during the day can often be uncomfortable or glary, and reduced light intensity at night may compromise visual comfort and safety, both of which will affect user compliance. No single lighting solution will effectively create biologically brighter days and biologically darker nights, but rather a variety of parameters need to be considered. This paper discusses the contributions of spectral power distribution, hue or color temperature, spatial distribution, as well as architectural geometry and surface reflectivity, to achieve biologically relevant lighting.

Keywords: social jet lag, circadian, lighting, melanopsin, Opn4

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Edited by:
Jamie Zeitzer,
Stanford University, United States

Reviewed by:
Michael Herf,
f.lux Software LLC, United States
Daniel S. Joyce,
University of Nevada, Reno, United States

****Correspondence:***
Robert Soler
rsoler@bioslighting.com

Specialty section:
This article was submitted to Sleep and Circadian Rhythms, a section of the journal Frontiers in Neuroscience

Received: *04 December 2020*
Accepted: *05 May 2021*
Published: *07 June 2021*

Citation:
Soler R and Voss E (2021) Biologically Relevant Lighting: An Industry Perspective. Front. Neurosci. 15:637221. doi: 10.3389/fnins.2021.637221

INTRODUCTION

The discovery of a novel Intrinsically Photosenstive Retinal Ganglion Cell (ipRGC) in the eye has changed our understanding of the role of light in our everyday lives (Provencio and Foster, 1995; Provencio et al., 1998; Brainard et al., 2001; Thapan et al., 2001; Berson et al., 2002; Panda et al., 2002; Dacey et al., 2005; Güler et al., 2008; Hughes et al., 2016). These ipRGCs contain a photopigment melanopsin with an *in-vitro* spectral sensitivity around 480 nm. However, after lens transmission this spectral sensitivity is shifted to longer wavelengths between 487 and 496 nm (Spitschan, 2019), and it is now understood that these ipRGCs are responsible for several physiological effects such as circadian synchronization, tracking seasonal changes, acute alertness, working memory improvements and mood improvements (Gaggioni et al., 2014; LeGates et al., 2014; Rodgers et al., 2016; Fernandez et al., 2018; Brown, 2020). The role of rods and cones on ipRGC response is still being debated. Some studies have shown no contribution of S-cone

(Spitschan et al., 2019) while others show an exposure time dependent contribution of S-cone (Brown et al., 2021) when evaluating melatonin suppression. Further studies show a reduced circadian impact of blue colored environments (Mouland et al., 2019). Less information is available on rod and cone contributions for other ipRGC driven responses.

In order to apply a biologically relevant lighting solution within the built environment, we must first understand the challenge at hand. Today, we spend most of our time indoors, removed from daylight which contains an important natural and robust daytime and nighttime signal (Knoop et al., 2020). Daylight has been replaced with electric lighting which has been engineered and optimized for visual efficiency and can be switched on or off at any time of the day. Moreover, today's light level recommendations (while sufficient for visual tasks) are insufficient for proper circadian signaling. We find ourselves immersed in environments where the lighting is too biologically dim for our brains to receive a proper daytime signal and too biologically bright to provide a proper nighttime signal. This lack of delineation between day and night can lead to circadian drift and exacerbate social jet lag (Roenneberg and Merrow, 2016), which have been associated with a whole host of negative health outcomes: decreases in learning and attention, increased risk of obesity, addiction, and cardiovascular disease (Roenneberg et al., 2012; Zarrinpar et al., 2015; Roenneberg and Merrow, 2016; Sulli et al., 2018). Moreover, this appears to be a widespread phenomenon that affects the majority of people. In fact, it has been shown that 87% of non-shift workers have some form of circadian dysfunction and the associated health risks previously mentioned (Roenneberg and Merrow, 2016), illustrating that the current lighting environment may be insufficient for proper circadian entrainment and amplitude. Therefore, biologically relevant lighting should provide biologically brighter days and biologically darker nights within the built environment. However, in practice there are additional constraints. Energy consumption, visual comfort (i.e., glare) associated with brighter days, and visual acuity and safety related to darker nights. It should be noted that while everyone should benefit from brighter days and darker nights, it may not be additive, there may be more benefit from darker nights compared to brighter days (Skeldon et al., 2017), and vice versa, depending on existing conditions and population type. We do know that large differences exist between individuals for nighttime melatonin suppression (Phillips et al., 2019).

In order to create truly biologically relevant lighting, the following factors must be considered in conjunction with one another: spectral composition, color, intensity, and distribution of the light, as well as the geometry and reflectivity of the built environment. As long as we remain in the built environment, there is no single strategy that can provide the optimum circadian lighting environment. Instead, we must use a multi-faceted approach to achieve biologically relevant lighting that is focused on biologically brighter days and darker nights. Ultimately, we need to understand how both circadian lighting factors and visual lighting factors can be addressed to create spaces that are both biologically relevant for day and night while still providing comfortable and well-designed spaces (Berman et al., 1991; Rea and Ouellette, 1991).

CURRENT INDUSTRY STANDARDS, METRICS AND RECOMMENDATIONS

The WELL Building Standard™ uses a series of design categories–Air, Water, Nourishment, Light, Movement, Thermal Comfort, Sound, Materials, Mind, Community, and Innovation– to create a point-based framework that determines how much wellness can be delivered to building occupants. A minimum of 40 points is required to achieve any type of WELL certification, with nine total points available from the Light concept. Circadian lighting and daylight exposure are key Features within the WELL™ "Light" concept. The Circadian Lighting Design Feature uses vertical melanopic equivalent daylight illuminance (EDI) as a criterion for minimum daytime circadian stimulus. Vertical melanopic EDI is measured based on the occupants' primary location within a space. Circadian light level measurements are meant to quantify the light reaching the occupants' eye and as such are taken 4′ above finished floor (or 18″ above the task plane) in the primary viewing direction of the occupant. The amount of vertical melanopic EDI required is dependent on how much daylight availability there is. It ranges from 109 vertical melanopic EDI (for one point) when adequate daylight is present to 218 vertical melanopic EDI (for three points) when sufficient daylight is not present.

Melanopic EDI originated from The International Commission on Illumination (CIE) issuance of the CIE S 026/E:2018, and uses the same melanopic sensitivity function as the Lucas toolbox, with peak sensitivity at 490 nm. Additionally, the CIE has proposed melanopic Daylight Efficiency Ratio (melanopic DER) as a metric for determining the biological potential for a light spectrum to activate melanopsin relative to visual illuminance (lumens/m^2) (Lucas et al., 2014; International Commission on Illumination, 2018).

THE ROLE OF LED TECHNOLOGY IN BIOLOGICALLY RELEVANT LIGHTING

Light emitting diodes (LEDs) lighting has seen tremendous adoption rates due to its energy efficiency and longevity. As of 2018 residential products saw LED penetration in 33% of A-lamps and 45% of recessed downlights, while office lighting saw LED penetration in 20% of linear installations (Navigant, 2019, 2020). How does LED lighting impact these newly discovered physiological responses to light? Some claim that LEDs contain a large blue peak, sending too many daytime signals and thus deeming them unsuitable for nighttime use (Tosini et al., 2016). Here I will demonstrate that the contrary is true and in fact, there exists a fundamental dip in the melanopic region (from 470 to 500 nm) due to the way LED white light spectrum is generated. This means that white LEDs actually perform poorly when it comes to producing the necessary melanopic stimulation relative to its perceived color temperature and visual brightness.

Color Matching Functions

Color matching functions shown in **Supplementary Figure 1** are used to convert any spectral power distribution to a (*x,y*) point on the CIE 1931 color space diagram. These (*x,y*) color points are how the lighting industry communicates color. When referring to white light, these color coordinates are binned based on the temperature of a black body radiator in Kelvin (referred to as K). The following equations are used to convert spectrum into Tristimulus *X*, *Y*, and *Z*, then to color coordinates (*x,y*). Tristimulus *Y* is also the luminous efficiency function, used to calculate lumens.

$$X = \sum_{\lambda=380\text{nm}}^{780\text{nm}} S(\lambda)\,\bar{x}(\lambda)\,\partial\lambda$$

$$Y = \sum_{\lambda=380\text{nm}}^{780\text{nm}} S(\lambda)\,\bar{y}(\lambda)\,\partial\lambda$$

$$Z = \sum_{\lambda=380\text{nm}}^{780\text{nm}} S(\lambda)\,\bar{z}(\lambda)\,\partial\lambda$$

Where

$S(\lambda)$ = Spectral power distribution of the light source
$\bar{x}(\lambda)$ = spectral tristimulus value from **Figure 1**.
$\bar{y}(\lambda)$ = spectral tristimulus value from **Figure 1**.
$\bar{z}(\lambda)$ = spectral tristimulus value from **Figure 1**.

$$x = \frac{X}{X+Y+Z}$$

$$y = \frac{\mathrm{Y}}{X+Y+Z}$$

Where

x = x-coordinate on CIE 1931 color space diagram.
y = y-coordinate on CIE 1931 color space diagram.

One way to think about this is as follows: *Z* stimulation results in bluer color perception, *Y* stimulation results in greener color perception, and *X* stimulation results in redder color perception. This blue perception from *Z* stimulation versus melanopic stimulation is key to disentangling visual perception from physiological effect. *Z* has highest sensitivity from 430 to 450 nm, whereas melanopic blue-green has highest sensitivity from 480 to 500 nm. Thus, a spectral power distribution can be derived with heightened melanopic stimulation without appearing cold in color temperature. Likewise, a spectral power distribution can be created that has less melanopic stimulation while still appearing cold in color temperature.

Traditional LED Technology

Light emitting diodes are semiconductors that utilize a material composition to emit a specific wavelength of light. There are two classes of LEDs; InGaN (blue) and AlInGaP (red), which are doped to produce a desired wavelength (Shaolin et al., 2015). However, a green gap exists in this doping process that makes green light very inefficient to produce from a discrete LED source (Auf Der Maur et al., 2016). Thus, a common approach to LED light is to utilize a monochromatic blue LED with peak emission between 445 and 455 nm combined with a combination of green and red phosphors that are excited by the blue wavelengths of the LED (Mueller-Mach et al., 2005; Nishiura et al., 2011; Liu et al., 2015). This combination is what provides the LED its significant energy efficiency and allows for a much wider range of CCTs and spectral control than previous technologies. An example of this approach is shown in **Supplementary Figure 2** for a 6,500°K LED spectra. What can be seen from this LED spectra is a peak emission that coincides with the peak sensitivity for *Z* from the color matching functions. By pinpointing the *Z* sensitivity, LEDs provide the most visually blue-looking light source with the least amount of energy appropriated in the blue region of the spectrum, relative to other light sources. This is an important tactic for energy efficiency, as the Luminous Efficiency Function (tristimulus *y*) is very low in the blue region. A common misconception about LED lighting is that they produce more blue light than other sources, due to the narrow, high-amplitude peak of the blue wavelengths. However, this is not the case, LED light is in fact engineered to produce the least amount of blue light per lumen for any given color temperature of light, in order to gain the highest luminous efficacy.

Spectral Simulation Method

Spectral simulation was created using an excel based spectrum database and calculator containing selectable Blue LED with scaler factor to increase or decrease LED simulated intensity. One Blue LED was selected and combined with various amounts of three unique phosphors. A combination of three (3) color points can produce any of the colors in the space, however, a fourth color point adds an additional degree of freedom that allows lumens to be maintained throughout simulations. Each spectral simulation shown in this study are within 1% of lumen and color point targets. Lumen target was chosen arbitrarily at 717 lumens. Color point targets were obtained as central points for each ANSI color bin, obtained from LED datasheets from Lumileds (San Jose, CA, United States) and are as follows:

1,800 K: (*x,y*) = (0.549, 0.408)
2,200 K: (*x,y*) = (0.502, 0.415)
2,700 K: (*x,y*) = (0.458, 0.410)
3,000 K: (*x,y*) = (0.434, 0.403)
3,500 K: (*x,y*) = (0.407, 0.392)
4,000 K: (*x,y*) = (0.380, 0.380)
5,000 K: (*x,y*) = (0.345, 0.355)
6,500 K: (*x,y*) = (0.312, 0.328)

Spectral Data

450 nm royal blue LED data was obtained from Lumileds was translated to 410, 420, 430, 440, 460, 470, 480, 490, and 500 nm data for this simulation. Phosphor data was obtained from Merck EMD (Darmstadt, Germany). Phosphors were selected to have longer wavelength while having capability to meet the desired color points within the CIE 1931 color space, which is used to determine white color points. This was done to minimize effect of phosphor on melanopic content. Phosphors have peak wavelengths at 547 nm (YYG-547-210), 585 nm (OGA-585), and

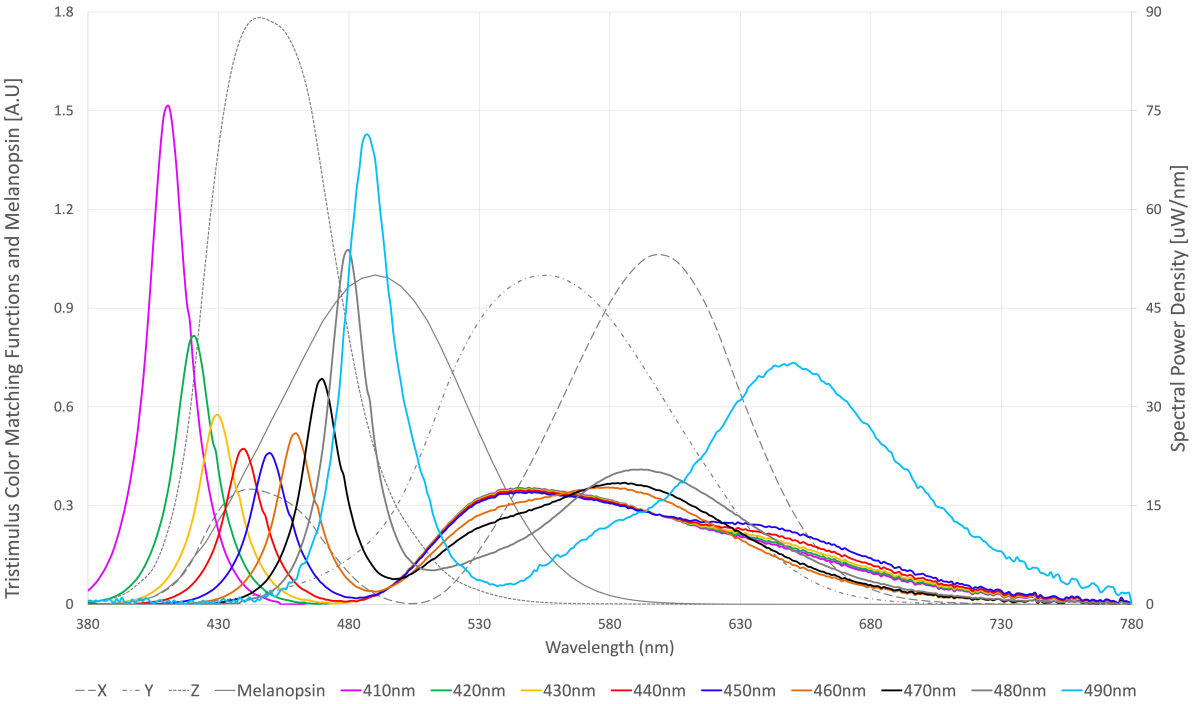

FIGURE 1 | Nine spectral metamers of light, each with different blue light peaks. 410 nm peaked metamer (purple), 420 nm peaked metamer (green), 430 nm peaked metamer (yellow), 440 nm peaked metamer (red), 450 nm peaked metamer (royal blue), 460 nm peaked metamer (orange), 470 nm peaked metamer (black), 480 nm peaked metamer (gray thick), and 490 nm peaked metamer (sky blue) are overlayed on top of tristimulus color matching functions X (gray–long dash), Y (gray–dash dot), and Z (gray–small dash) according to CIE1931, as well as melanopsin sensitivity function (gray–solid) according to CIE S 026. Each metamer produces visually the same 4,000 K color of light at the same lumens according to the tristimulus functions.

660 nm (YYG-660). Spectra and resulting color points for each component described in this section are plotted for reference in **Supplementary Figures 3, 4**. Color points are as follows:

410 nm LED: $(x,y) = (0.1704, 0.0191)$
420 nm LED: $(x,y) = (0.1668, 0.0206)$
430 nm LED: $(x,y) = (0.1627, 0.0236)$
440 nm LED: $(x,y) = (0.1570, 0.0300)$
450 nm LED: $(x,y) = (0.1491, 0.0423)$
460 nm LED: $(x,y) = (0.1370, 0.0666)$
470 nm LED: $(x,y) = (0.1169, 0.1166)$
480 nm LED: $(x,y) = (0.0857, 0.2183)$
490 nm LED: $(x,y) = (0.0509, 0.3943)$
500 nm LED: $(x,y) = (0.0415, 0.6034)$
YYG-547-210 Phosphor: $(x,y) = (0.4512, 0.5326)$
OGA-585 Phosphor: $(x,y) = (0.5581, 0.4410)$
YYG-660 Phosphor: $(x,y) = (0.686, 0.314)$

Working Color Space Analysis

Combinations of color points for 450 nm LED and three phosphors are bounded in **Supplementary Figure 5** to show possible combinations of colors possible with this color mix, creating a "working color space". ANSI color bins are also plotted for reference. These ANSI bins are the accepted areas by which a manufacturer can specify a white for a given color temperature. This "working color space" exercise is repeated for 410, 490, and 500 nm monochromatic peaks, shown in **Supplementary Figures 6–8**, respectively. From these charts, we see that not all color temperatures fall within the working spaces. Most notably, 500 nm cannot achieve any of the ANSI color bins from 6,500 to 2,700 K and was removed from analysis.

Spectral Optimization for Day and Night

Figure 1 illustrates nine (9) different metamers of light that produce the same 4,000°K white light with the same lumens. Each metamer has its peak blue wavelength shifted by 10 nm, from 410 to 490 nm. These spectra were evaluated for melanopic DER, shown in **Figure 2**. We see a DER of a 450 nm peaked 4,000 K light of 0.53, where a 410 nm peaked 4,000 K light has a DER of 0.43. This is a 19% reduction in melanopic DER by moving from a standard 450 nm blue to a 410 nm blue LED. A 490 nm peaked 4,000 K light has a DER of 1.42, a 2.7-fold increase in melanopic DER. Thus spectral optimization can be had for both day and night, however, more advantage might be gained from daytime spectral optimization.

It is important to consider how each of these spectra might render colors. Most of these metamers will not achieve color rendition characteristics needed for commercial viability and thus represent what is possible, but not practical. These metamers simply drive direction for spectrum strategies for day and night.

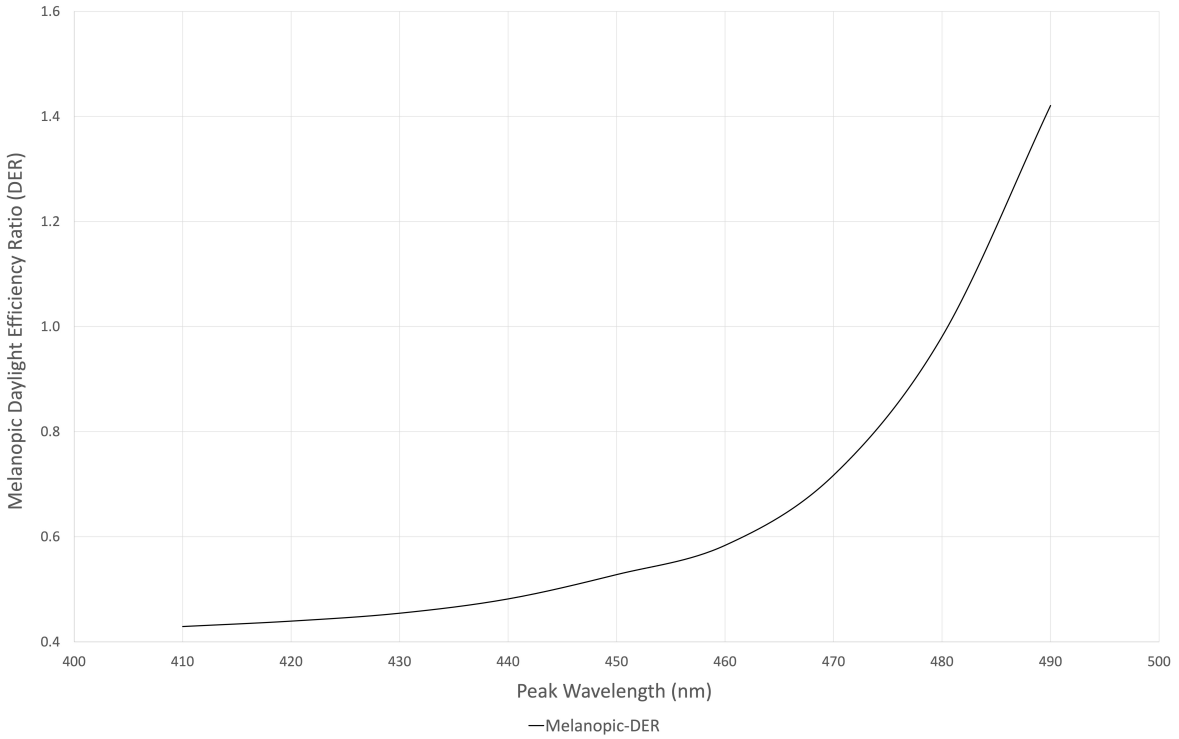

FIGURE 2 | Melanopic daylight efficiency ratio (DER) of the nine 4,000 K spectral metamers from **Figure 1** organized by their peak blue emission on the X-axis (410–490 nm).

When we control for a minimum color rendering index (CRI) of 80, the melanopic DER of a color-corrected 490 nm peaked 4,000 K spectrum falls to 0.83, compared to a 450 nm peaked spectrum that has a melanopic DER of 0.59. An example of this spectrum is shown in **Supplementary Figure 9**. This is a much more modest improvement of 41% in melanopic DER over the standard LED. Moreover, a 410 nm peaked spectrum only has a melanopic DER of 0.58, a 2% reduction compared to the standard LED. The trend can be seen in **Supplementary Figure 10**. Maximum benefit of 410 nm peak LED is gained at 2,700 K, where 410 nm 2,700 K spectrum has a melanopic DER of 0.33 compared to a standard LED 0.41, a 20% reduction. A larger reducation can be had with warmer color temperatures, such as 1,800 K (melanopic DER = 0.18) or 2,200 K (melanopic DER = 0.31), a 56 and 25% reduction, respectively.

On the colder color temperature end, standard 450 nm LED showed a melanopic DER of 0.73 and 0.86 for 5,000 and 6,500 K, respectively, an increase of 23 and 45%, respectively, over 4,000 K standard 450 nm LED. 490 nm peaked LED showed a melanopic DER of 0.93 and 1.12 for 5,000 and 6,500 K, an increase of 12 and 34% over the color-corrected 490 nm peaked 4,000 K LED spectrum. However, color-corrected 490 nm peaked 5,000 K had a 27% increase in melanopic DER over standard 450 nm 5,000 K LED and color-corrected 490 nm peaked 6,500 K had a 30% increase in melanopic DER over standard 450 nm 6,500 K LED.

Thus, spectrum has a larger impact than color temperature when striving for high melanopic DER and color temperature has a larger impact than spectrum when striving for low melanopic DER. Of course, both color temperature and spectrum should be employed whenever possible.

Other Biological Models

Other biological models have been proposed. The Circadian Stimulus (CS) Model has a peak sensitivity at 460 nm rather than 490 nm and includes interactions from rods and cones into the ipRGC response for color temperatures greater than 3,500 K that results in a subtraction in the green wavelengths. For color temperatures of 3,500 K and warmer, CS uses melanopsin as its sensitivity function (Figueiro et al., 2008). Circadian Potency Spectral Sensitivity (CPSS) has been proposed, suggesting a peak sensitivity at 477 nm with a narrower sensitivity band than melanopsin (Moore-Ede et al., 2020).

Each of the nine (9) 4,000 K metamers from **Figure 1** were applied to CS for color temperatures greater than 3,500 K (CS Cool) and CPSS (**Supplementary Figure 11**) and evaluated for relative biological potency relative to itself (**Supplementary Figure 12**) at 410 nm peaked 4,000 K. CS has the least amount of biological potency with the 420 and 430 nm spectra, but still we see a fivefold increase with the non-color-corrected 490 nm peaked LED spectrum compared to the 410 nm LED spectrum. This is a surprising result for a model with a peak sensitivity

at 460 nm. The reason is longer wavelength blues stimulate the *Y* tristimulus color matching function. This has to be counter-balanced by less green phosphor, which is primarily in the subtraction portion of the CS sensitivity curve.

The CPSS also has a minimum potency with the 420 nm LED spectrum, but still sees a 4.2-fold increase in biological potency from a non-color-corrected 490 nm peaked LED spectrum compared to a non-color-corrected 410 nm LED spectrum. Again this is a surprising effect from a curve with spectral sensitivity peak at 477 nm. This is due to the shape of the 490 nm LED, which has tremendous overlap of the 480 nm LED and 470 nm LED spectra.

In other words, the 490 nm peak is so large that it overshadows shorter wavelength LEDs. This large peak is required because the tristimulus color matching functions are much less sensitive at 490 nm compared to shorter wavelengths. In other words, the biological potential of a 490 nm peaked spectrum found in this analysis may not be due to the peak sensitivity of melanopsin at 490 nm, but rather due to the insensitivity of the tristimulus functions at longer wavelengths, allowing for more total radiant energy for the same visual lumens and perceived color.

OTHER KEY VARIABLES

While spectrum and color temperature of the light sources are of primary importance, there are secondary considerations that pertain to the biological relevance of the delivery of light. How the light exits the luminaire and how light bounces off the walls can contribute to biological relevance.

Geometry of the Built Environment on Vertical Illuminance

Commercial buildings often have a grid ceiling, often referred to as a drop ceiling, that include an array of lighting and heating, ventilation, and air conditioning (HVAC) systems integrated inside. This approach in architecture is the most cost-effective for wiring lighting and routing air ducts. This approach points lights downward toward desks and floors to optimize luminance and contrast of objects to be seen. This provides efficiency for visual applications, but lacks direct illumination into the occupants eyes, making this approach inefficient for providing the vertical illumination necessary for circadian daytime exposure.

Current lighting standards and recommendations for lighting are solely placed on the visual criteria as it relates to completing tasks that occur on a horizontal plane (i.e., on a desk). For example, an office may require 300 lumens per square meter (lux) to fall on the desk but make no criteria for what falls on the eyes of the occupants. Key factors related to how much vertical light you can achieve from these common luminaires are room geometry and wall reflectance. The ratio of wall area to floor area is referred to as the room cavity ratio (RCR), as this quantifies the opportunity for light to bounce off a vertical surface and redirect its weight from the horizontal plane to the vertical plane.

$$\mathrm{RCR} = 2.5 \times \frac{\text{Total wall area above work plane}}{\text{Floor area}}$$

Larger RCR values correspond to more wall area per floor area. For example, a private office may have an RCR of 5, while an open plan office has a RCR closer to 1.

Simulation of various RCRs at 50% wall reflectivity was achieved using AGi32 lighting design software from Lighting Analysts (Littleton, CO, United States) with typical lighting fixtures. Space types for the simulations included the following:

Open Office (46′ × 86′ × 9′) with recessed light fixtures, RCR = 0.8

Open Office (46′ × 86′ × 9′) with suspended light fixtures 18″ below the ceiling, RCR = 1.1

Classroom (20′ × 30′ × 9′) using recessed fixtures, RCR = 2.1

Classroom (20′ × 30′ × 9′) with suspended light fixtures 18″ below the ceiling, RCR = 2.7

Break Room (12′ × 20′ × 9′) with recessed light fixtures, RCR = 3.3

Break Room (12′ × 20′ × 9′) with suspended light fixtures 18″ below the ceiling, RCR = 4.3

Office/Conf. Room (12′ × 12′ × 9′) with recessed light fixtures, RCR = 4.2

Office/Conf. Room (12′ × 12′ × 9′) with suspended light fixtures 18″ below the ceiling, RCR = 5.4

Each space type outlined above also used the following luminaire types/manufacturers:

Recessed Downlights / Ledra Alphabet NU3RD (Tustin, CA, United States)

Recessed Wall Washer / Ledra Alphabet NU3RW (Tustin, CA, United States)

Recessed 2 × 2 Troffer / Pinnacle Lucen (Denver, CO, United States)

Recessed 2 × 4 Troffer / Pinnacle Lucen (Denver, CO, United States)

Direct Linear Pendant / Pinnacle Edge3 (Denver, CO, United States)

Indirect Linear Pendant /Axis Beam4 (Lasalle, QC, Canada)

Direct / Indirect Linear Pendant / Axis Surround Lite (Lasalle, QC, Canada)

Direct / Indirect Circular Area Pendant / Prudential P4000 (Los Angeles, CA, United States)

An example of these AGi32 calculations is shown in **Supplementary Figures 13–15**. **Supplementary Figure 16** illustrates the benefit in vertical to horizontal ratio using different fixture types versus RCR. In summary, an open plan office will provide 50 vertical lux at the occupant's eyes for every 100 lux on the desktop. Whereas a private office will provide 80 vertical lux at the occupant's eyes for every 100 lux on the desk surface, a 60% boost in vertical illumination.

These calculations were repeated for 70% wall reflectivity and 90% wall reflectivity. A boost in vertical to horizontal ratio was not observed due to wall reflectivity, however, a boost in total illuminance was observed. **Supplementary Figures 17, 18** illustrate these vertical illumination benefits of higher wall reflectivity as a function of RCR. These Figures illustrate that vertical illumination benefits were minor for small RCRs, but much stronger for larger RCRs. In other words, increasing wall reflectivity in a large open plan office will yield a meager benefit,

but increasing wall reflectivity in a private office will have a significant benefit to vertical illumination.

Luminaire Light Distribution

Light distribution is another characteristic that plays a critical role in achieving vertical illuminance. Pendants are a type of luminaire that are mounted to the ceiling and suspended in the space. Some pendants are fully luminous, such that it distributes light in all directions. We evaluated the Purelight Round Luminous pendant from Selux (Highland, NY, United States) that focus the majority of their energy downward toward the task plane. Our AGi32 analysis showed that these luminous surfaced pendants provided 22% more vertical illumination on average for the same amount of light on the task plane.

Different lighting strategies can also be applied at night. Typical LED "Edison-type" light bulbs do not have the same isotropic distribution as a standard incandescent light source. These light bulbs direct the majority of the light in the direction opposite of screw-in base. When applied into a standard table lamp with the screw base downward, light is directed onto the 80% reflective ceiling. Mirror light bulbs are fairly common and designed to reduce direct glare and can be used in place of these standard Edison LED bulbs to redirect the light from the 80% reflective ceiling toward a much lower reflectance floor. A study was conducted in a $14' \times 10' \times 8'$ (L × W × H) room, where a single table lamp was illuminated in a corner of the room using both a regular Edison-type light bulb and a mirror type light bulb. Vertical illumination was measured facing the lamp from $2'$ to $10'$ away using both a standard LED lamp and a mirror lamp of the same lumen output. Measurements are provided in supplemental **Supplementary Figure 19** which shows that this technique yielded a 35% reduction in vertical illumination, while still maintaining light availability.

Controls

Controls play an integral role in biologically relevant lighting. Controls should be configured to create brighter days and darker nights on a consistent and predictable cadence. This can be set to be in phase with the solar cycle or can be set to a specific time, such as 6 a.m. to 8 p.m. The later maybe preferred at higher latitudes. At a bare minimum, this control system would comprise of automatic dimmers that increase and decrease intensity according to time. However, a biologically relevant lighting system should consider intensity, spectrum, and distribution. Intensity and spectrum should be tied together to maximize day versus night delineation. Blending between a brighter intensity of blue enriched spectrum with peak emission at 490 nm of the highest accepted color temperature and dimmer intensity of blue depleted spectrum with peak emission between 410 and 450 nm of lowest acceptable color temperature. Further day/night delineation value can be obtained by including spatial distribution. Nighttime scenes should include no indirect light (lights pointed at the ceiling) and should have direct light (lights pointed at the task) with blue depleted spectrum of minimal intensity to achieve necessary tasks. Daytime scenes should include direct and indirect light, both with blue enriched spectrum with peak emission at 490 nm of highest accepted color temperature and of highest accepted intensity both in terms of comfort and energy constraints.

PERSONAL CIRCADIAN LIGHTING DEVICES

One final strategy to help provide biologically relevant lighting within a space is the use of personalized devices that can supply supplemental vertical lighting. These personalized devices could be located relatively close to the occupant and primarily provide vertical illumination, for example something akin to a light box or a table lamp. These types of devices could conceivably add 200 melanopic EDI or more at the eye of the occupant and could be tailored to the needs of an individual. Ideally, this type of intervention would be automated to dynamically transition between day and night illumination throughout the day.

DAYLIGHTING

The data presented here provides quantified strategies for implementing biologically brighter days and darker nights into the built environment. While not quantified in this analysis, daylight exposure is best for providing circadian benefits (Knoop et al., 2020), but it should be noted that vertical light exposure from windows drops off dramatically with distance from said window. Skylights and daylight harvesting strategies are encouraged to bring that daylight deeper into the space to maximize its benefits.

COMPOUNDING THE STRATEGIES FOR BRIGHTER DAYS AND DARKER NIGHTS

Standalone strategies for brighter days and darker nights are outlined in this article and while some individual interventions may not seem like they apply a space or application, the combination of these strategies compound their benefits to create a biologically relevant lighting environment.

For example, a person spends their day in the office under standard 3,500 K LED lighting with 300 lux at the task plane and 150 lux at the eye. A standard 3,500 K LED spectrum has a melanopic DER of about 0.51, thus the melanopic EDI at the eye is 76.5. The same person spends their evening in a home with luminaires populated with 2,700 K LED light bulbs. These luminaires provide approximately 50 lux at the eye. 2,700 K LED spectrum has a melanopic DER of about 0.4, thus a nighttime melanopic EDI of 20. This is a typical example of how light is biologically too dim for daytime use (76.5 melanopic EDI) and too bright for evening (20 melanopic EDI), with a day-to-night ratio of 3.8:1. Its not certain if more benefit would be gained from brighter days or darker nights, it is good practice to improve both and increase that day-to-night ratio.

At the office, the simple incorporation of a spectrally optimized daytime light source with a slightly cooler color temperature of 4,000°K will lead to a 58% boost in daytime

biological potency. This would increase 76.5 melanopic EDI from the example to 121 melanopic EDI. While at home, incorporating spatially optimized light bulbs with a slightly warmer color temperature of 2,200°K lighting provides a 60% reduction in nighttime biological potency. This would decrease 20 melanopic EDI from the example to eight melanopic EDI. Combining these two strategies would increase the day-to-night ratio in the example from 3.8:1 to 15.1:1. This is a 392% increase in the delineation of daytime versus nighttime.

The proposed goal is to combine as many of these individual strategies and put them into practice (when possible) while maintaining excellent light quality, appropriate design aesthetic, and achieving user/occupant compliance. These strategies are itemized here:

Daytime strategy	**Standalone benefit**
(1) Color-corrected spectrally optimized daytime spectrum	∼ (+41%)
(2) Colder color temperatures	∼ (+10%) per 500 K
(3) Spatially optimized luminaire	∼ (+22%)
(4) Private office	∼ (+60%)
(5) Private office with highly reflective walls	∼ (+150%)
(6) Personal circadian luminaire	+200 melanopic EDI
Nighttime strategy	**Standalone benefit**
(1) Warmer color temperatures	∼(−15%) per 500 K
(2) Spatially optimized luminaire	∼(−35%)
(3) Spectrally optimization	Up to −20%

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All spectral analysis, industry perspective, data collection, and analysis were done by RS. Lighting design simulation was done by EV, an employee of BIOS lighting.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.637221/full#supplementary-material

Supplementary Figure 1 | Tristimulus sensitivity according to CIE 1931 color matching functions and melanopsin sensitivity according to CIE S 026.

Supplementary Figure 2 | Standard off-the-shelf 6,500 K LED with peak blue emission at 450 nm and broad phosphor excitation emission from 480 to 780 nm compared to tristimulus sensitivity according to CIE 1931 and melanopsin sensitivity according to CIE S 026.

Supplementary Figure 3 | Spectral Power Distribution (SPD) for each component used in simulation. **(A)** SPD of 410 nm LED; **(B)** SPD of 420 nm LED; **(C)** SPD of 430 nm LED; **(D)** SPD of 440 nm LED; **(E)** SPD of 450 nm LED; **(F)** SPD of 460 nm LED; **(G)** SPD of 470 nm LED; **(H)** SPD of 480 nm LED; **(I)** SPD of 490 nm LED; **(J)** SPD of 500 nm LED; **(K)** SPD of 547 nm peaked phosphor YYG-547-210; **(L)** SPD of 585 nm peaked phosphor OGA-585; **(M)** SPD of 660 nm peaked phosphor YYG-660.

Supplementary Figure 4 | Color plots of each component used in simulation. Color points are labeled A through M, in reference to spectral power distributions of said color plot from **Supplementary Figure 3**.

Supplementary Figure 5 | Working space of 450 nm LED (E), 547 nm peaked phosphor YYG-547-210 (K), 585 nm peaked phosphor OGA-585 (L), and 660 nm peaked phosphor YYG-660 (M). Any color points within the triangle plotted here can be created. ANSI color temperature bins for 6,500, 5,000, 4,000, 3,500, 3,000, and 2,700 K white light are plotted for reference.

Supplementary Figure 6 | Working space of 410 nm LED (A), 547 nm peaked phosphor YYG-547-210 (K), 585 nm peaked phosphor OGA-585 (L), and 660 nm peaked phosphor YYG-660 (M). Any color points within the triangle plotted here can be created. ANSI color temperature bins for 6,500, 5,000, 4,000, 3,500, 3,000, and 2,700 K white light are plotted for reference.

Supplementary Figure 7 | Working space of 490 nm LED (I), 547 nm peaked phosphor YYG-547-210 (K), 585 nm peaked phosphor OGA-585 (L), and 660 nm peaked phosphor YYG-660 (M). Any color points within the triangle plotted here can be created. ANSI color temperature bins for 6,500, 5,000, 4,000, 3,500, 3,000, and 2,700 K white light are plotted for reference.

Supplementary Figure 8 | Working space of 500 nm LED (J), 547 nm peaked phosphor YYG-547-210 (K), 585 nm peaked phosphor OGA-585 (L), and 660 nm peaked phosphor YYG-660 (M). Any color points within the triangle plotted here can be created. ANSI color temperature bins for 6,500, 5,000, 4,000, 3,500, 3,000, and 2,700 K white light are plotted for reference.

Supplementary Figure 9 | Blended spectrum that achieves a minimum of 80 CRI and maximizes energy efficiency and melanopic daylight efficiency ratio (DER).

Supplementary Figure 10 | Melanopic DER versus color temperature of color-corrected optimized daytime spectrum (dashed line), color-corrected optimized nighttime spectrum (dash-dot line), and standard LED (solid line).

Supplementary Figure 11 | Nine spectral metamers of light, each with different blue light peaks. 410 nm peaked metamer (purple), 420 nm peaked metamer (green), 430 nm peaked metamer (yellow), 440 nm peaked metamer (red), 450 nm peaked metamer (royal blue), 460 nm peaked metamer (orange), 470 nm peaked metamer (black), 480 nm peaked metamer (dark gray), and 490 nm peaked metamer (sky blue), from **Figure 1**, are overlayed on top of melanopsin sensitivity function (gray–solid) according to CIE S 026, CS cool sensitivity function (gray–small dash) and CPSS (gray–dash dot). Each metamer produces visually the same 4,000 K color of light at the same lumens according to the tristimulus functions.

Supplementary Figure 12 | Relative weights of m-DER, CS, and CPSS of the nine spectral metamers from figure S10 (Y-axis) versus peak blue wavelength of metamer (X-axis). Weights are relative to themselves at the 410 nm peaked metamer.

Supplementary Figure 13 | Elevation view of AGi32 lighting simulation software and lighting calculation points of an open office layout. Calculation points are horizontal (with lines pointed up from points) and vertical (with lines pointed left from points). Blue lines reference building structure. Black lines reference furniture.

Supplementary Figure 14 | Plan view of AGi32 lighting simulation software and vertical lighting calculation points of an open office layout. Calculation points are vertical with lines pointed in direction of view. Blue lines reference building structure. Black lines reference furniture. Red lines reference light fixtures.

Supplementary Figure 15 | Rendering of isometric view using AGi32 lighting simulation software.

Supplementary Figure 16 | Vertical to Horizontal light ratio versus room cavity ratio with 90% reflective painted walls for of downlight luminaires (gray–solid), direct pendent luminaires (black–solid), wall washer luminaires (gray–dashed), indirect/direct pendent luminaires (black–dashed), indirect only pendent luminaires (gray–dash dot), and recessed general illumination luminaires (black–dash dot).

Supplementary Figure 17 | Boost in vertical melanopic EDI versus RCR of spaces with 90% wall reflectivity compared to spaces with 50% wall reflectivity of downlight luminaires (gray–solid), direct pendent luminaires (black–solid), wall washer luminaires (gray–dashed), indirect/direct pendent luminaires (black–dashed), indirect only pendent luminaires (gray–dash dot), and recessed general illumination luminaires (black–dash dot).

Supplementary Figure 18 | Boost in vertical melanopic EDI versus RCR of spaces with 70% wall reflectivity compared to spaces with 50% wall reflectivity of downlight luminaires (gray–solid), direct pendent luminaires (black–solid), wall washer luminaires (gray–dashed), indirect/direct pendent luminaires (black–dashed), indirect only pendent luminaires (gray–dash dot), and recessed general illumination luminaires (black–dash dot).

Supplementary Figure 19 | Measurements of vertical illumination (lux) at 40′′ height for a space with a single table lamp containing a standard LED A19 light bulb "upward lamp" (black–solid) or a A19 light bulb with a mirror coating "downward lamp" (black–dashed) versus distance away from lamp. Measurements taken facing the lamp.

REFERENCES

Auf Der Maur, M., Pecchia, A., Penazzi, G., Rodrigues, W., and Di Carlo, A. (2016). Efficiency drop in green InGaN/GaN light emitting diodes: the role of random alloy fluctuations. *Phys. Rev. Lett.* 116:027401. doi: 10.1103/PhysRevLett.116.027401

Berman, S. M., Jacobs, R. J., Bullimore, M. A., Bailey, L. L., Ghandi, N., and Greenhouse, D. S. (1991). *An Objective Measure of Discomfort Glare. First International Symposium On Glare*. New York, NY: Lighting Research Institute.

Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295, 1070–1073. doi: 10.1126/science.1067262

Brainard, G. C., Hanifin, J. P., Greeson, J. M., Byrne, B., Glickman, G., Gerner, E., et al. (2001). Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J. Neurosci. Off. J. Soc. Neurosci.* 21, 6405–6412.

Brown, T. M. (2020). *Melanopic illuminance* defines the magnitude of human circadian light responses under a wide range of conditions. *J. Pineal. Res.* 5:e12655. doi: 10.1111/jpi.12655

Brown, T. M., Thapan, K., Arendt, J., Revell, V. L., and Skene, D. J. (2021). S-cone contribution to the acute melatonin suppression response in humans. *J. Pineal Res.* doi: 10.1111/jpi.12719 *vol page,

Dacey, D. M., Liao, H., Peterson, B. B., Robinson, F. R., Smith, V. C., Pokorny, J., et al. (2005). Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature* 433, 749–754. doi: 10.1038/nature03344

Fernandez, D., Fogerson, P., Lazzerini, O., Thomsen, M., Layne, R., Severin, D., et al. (2018). Light affects mood and learning through distinct retina-brain pathways. *Cell* 175, 71–84.e18. doi: 10.1016/j.cell.2018.08.004

Figueiro, M. G., Bierman, A., and Rea, M. S. (2008). Retinal mechanisms determine the subadditive response to polychromatic light by the human circadian system. *Neurosci. Lett.* 438, 242–245. doi: 10.1016/j.neulet.2008.04.055

Gaggioni, G., Maquet, P., Schmidt, C., Dijk, D., and Vandewalle, G. (2014). Neuroimaging, cognitioon, light and circadian rhythms. *Front. Syst. Neurosci.* 8:126. doi: 10.3389/fnsys.2014.00126

Güler, A. D., Ecker, J. L., Lall, G. S., Haq, S., Altimus, C. M., Liao, H. W., et al. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature* 453, 102–105. doi: 10.1038/nature06829

Hughes, S., Jagannath, A., Rodgers, J., and Hankins, M. W. (2016). Signalling by expressing photosensitive retinal ganglion cells. *Nat. Publ. Group* 44, 1–8.

International Commission on Illumination (CIE) (2018). *CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light*. Vienna: Central Bureau CIE.

Knoop, M., Stefani, O., Bueno, B., Matusiak, B., Hobday, R., Wirz-Justice, A., et al. (2020). Daylight: What makes the difference? *Ligh. Res. Technol.* 52, 423–442. doi: 10.1177/1477153519869758

LeGates, T. A., Fernandez, D. C., and Hattar, S. (2014). Light as a central modulator of circadian rhythms, sleep and affect. *Nat. Rev. Neurosci.* 15:443.

Liu, G. H., Zhou, Z. Z., Shi, Y., Liu, Q., Wan, J. Q., and Pan, Y. B. (2015). Ce:YAG transparent ceramics for applications of high power LEDs: Thickness effects and high temperature performance. *Mater. Lett.* 139, 480–482. doi: 10.1016/j.matlet.2014.10.114

Lucas, R. J., Peirson, S. N., Berson, D. M., Brown, T. M., Cooper, H. M., Czeisler, C. A., et al. (2014). Measuring and using light in the melanopsin age. *Trends Neurosci.* 37, 1–9. doi: 10.1016/j.tins.2013.10.004

Moore-Ede, M., Heitmann, A., and Guttkuhn, R. (2020). Circadian potency spectrum with extended exposure to polychromatic white LED light under workplace conditions. *J. Biol. Rhyt.* 35, 405–415. doi: 10.1177/0748730420923164

Mouland, J. W., Martial, F., Watson, A., Lucas, R. J., and Brown, T. M. (2019). Cones support alignment to an inconsistent world by suppressing mouse circadian responses to the blue colors associated with twilight. *Curr. Biol. CB* 29, 4260–4267.e4. doi: 10.1016/j.cub.2019.10.02

Mueller-Mach, R., Mueller, G., Krames, M. R., Höppe, H. A., Stadler, F., Schnick, W., et al. (2005). Highly efficient all-nitride phosphor-converted white light emitting diode. *Phys. Status Soli.* 202, 1727–1732. doi: 10.1002/pssa.200590014

Navigant. (2019). *Energy Savings Forecast of Solid-State Lighting in General Illumination Applications. U.S. Department of Energy Report, DOE/EERE 2001*.

Navigant. (2020). *Adoption of Light-Emitting Diodes in Common Lighting Applications. U.S. Department of Energy Report*. 1–56. Available online at: https://www.energy.gov/sites/prod/files/2020/09/f78/ssl-led-adoption-aug2020.pdf (accessed January 20, 2021).

Nishiura, S., Tanabe, S., Fujioka, K., and Fujimoto, Y. (2011). Properties of transparent Ce:YAG ceramic phosphors for white LED. *Optical Mater.* 33, 688–691. doi: 10.1016/j.optmat.2010.06.005

Panda, S., Sato, T. K., Castrucci, A. M., Rollag, M. D., DeGrip, W. J., Hogenesch, J. B., et al. (2002). Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* 298, 2213–2216. doi: 10.1126/science.1076848

Phillips, A. J. K., Vidafar, P., Burns, A. C., McGlashan, E. M., Anderson, C., Rajaratnam, S. M. W., et al. (2019). High sensitivity and interindividual variability in the response of the human circadian system to evening light. *Proc. Natl. Acad. Sci.* 116, 12019–12024. doi: 10.1073/pnas.1901824116

Provencio, I., Cooper, H. M., and Foster, R. G. (1998). Retinal projections in mice with inherited retinal degeneration: implications for circadian photoentrainment. *J. Comp. Neurol.* 395, 417–439. doi: 10.1002/(sici)1096-9861(19980615)395:4<417::aid-cne1<3.0.co;2-4

Provencio, I., and Foster, R. G. (1995). Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. *Brain Res.* 694, 183–190. doi: 10.1016/0006-8993(95)00694-l

Rea, M. S., and Ouellette, M. J. (1991). Visual performance using reaction times: a basis for application. *Light. Res. Tech.* 23, 135–144.

Rodgers, J., Hankins, M. W., Peirson, S. N., and Foster, R. G. (2016). Signalling by melanopsin (OPN4) expressing photosensitive retinal ganglion cells. *Eye* 44, 1–8. doi: 10.1038/eye.2015.264

Roenneberg, T., Allebrandt, K. V., Merrow, M., and Vetter, C. (2012). Social jetlag and obesity. *Curr. Biol.* 22, 939–943. doi: 10.1016/j.cub.2012.03.038

Roenneberg, T., and Merrow, M. (2016). The circadian clock and human health. *Curr. Biol.* 26, R432–R443. doi: 10.1016/j.cub.2016.04.011

Shaolin, H., Sheng, L., Zhi, Z., Han, Y., Zhiyin, G., and Haisheng, F. (2015). A novel MOCVD reactor for growth of high-quality GaN-related LED layers. *J. Crystal Growth* 415, 72–77. doi: 10.1016/j.jcrysgro.2014.12.038

Skeldon, A. C., Phillips, A. J. K., and Dijk, D. (2017). The effects of self-selected light- dark cycles and social constraints on human sleep and circadian timing: a modeling approach. *Sci. Rep*. 7:45158. doi: 10.1038/srep45158

Spitschan, M. (2019). Melanopsin contributions to non-visual and visual function. *Curr. Opin. Behav. Sci.* 30, 67–72. doi: 10.1016/j.cobeha.2019.06.004

Spitschan, M., Lazar, R., Yetik, E., and Cajochen, C. (2019). No evidence for an S cone contribution to acute neuroendocrine and alerting responses to light. *Curr. Biol. CB* 29, R1297–R1298. doi: 10.1016/j.cub.2019.11.031

Sulli, G., Manoogian, E., Taub, P., and Panda, S. (2018). Training the circadian clock, clocking the drugs,and drugging the clock to prevent, manage, and treat chronic diseases. *Trends Pharmacol. Sci.* 39, 812–827. doi: 10.1016/j.tips.2018.07.003

Thapan, K., Arendt, J., and Skene, D. J. (2001). An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J. Physiol.* 535(Pt. 1), 261–267. doi: 10.1111/j.1469-7793.2001.t01-1-00261.x

Tosini, G., Ferguson, I., and Tsubota, K. (2016). Effects of bluelight ontheon the circadian system and eye physiology. *Mol. Vis.* 22, 61–72.

Zarrinpar, A., Chaix, A., and Panda, S. (2015). Daily eating patterns and their impact on health and disease. *Trends Endocrinol. Metabol.* 27, 69–83. doi: 10.1016/j.tem.2015.11.007

Conflict of Interest: RS is a founder of BIOS Lighting, a for-profit lighting manufacturer. EV is currently employed by the company BIOS Lighting, a for-profit company.

frontiers in Neurology

MINI REVIEW
published: 15 July 2021
doi: 10.3389/fneur.2021.654158

Time-Varying Light Exposure in Chronobiology and Sleep Research Experiments

Manuel Spitschan [1,2,3]*

[1] Department of Experimental Psychology, University of Oxford, Oxford, United Kingdom, [2] Centre for Chronobiology, Psychiatric Hospital of the University of Basel (UPK), Basel, Switzerland, [3] Transfaculty Research Platform Molecular and Cognitive Neurosciences, University of Basel, Basel, Switzerland

Light exposure profoundly affects human physiology and behavior through circadian and neuroendocrine photoreception primarily through the melanopsin-containing intrinsically photosensitive retinal ganglion cells. Recent research has explored the possibility of using temporally patterned stimuli to manipulate circadian and neuroendocrine responses to light. This mini-review, geared to chronobiologists, sleep researchers, and scientists in adjacent disciplines, has two objectives: (1) introduce basic concepts in time-varying stimuli and (2) provide a checklist-based set of recommendations for documenting time-varying light exposures based on current best practices and standards.

Keywords: time-varying light exposure, circadian photoreception, non-visual effects of light, temporal stimuli, ipRGCs, melanopsin, reporting

OPEN ACCESS

Edited by:
Narong Simakajornboon, Cincinnati Children's Hospital Medical Center, United States

Reviewed by:
Thomas Dye, Cincinnati Children's Hospital Medical Center, United States
James Timothy McKenna, Harvard Medical School, United States

***Correspondence:**
Manuel Spitschan
manuel.spitschan@psy.ox.ac.uk
orcid.org/0000-0002-8572-9268

Specialty section:
This article was submitted to Sleep Disorders, a section of the journal Frontiers in Neurology

Received: *15 January 2021*
Accepted: *06 May 2021*
Published: *15 July 2021*

Citation:
Spitschan M (2021) Time-Varying Light Exposure in Chronobiology and Sleep Research Experiments. Front. Neurol. 12:654158. doi: 10.3389/fneur.2021.654158

INTRODUCTION

Light profoundly affects human circadian and neuroendocrine physiology. Signals processed by the photoreceptors in the retina encode different aspects of environmental light. There are three classes of photoreceptors: the cones (of which there are three different spectral classes, the L, M, and S cones), the rods, and the melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs). The retinal photoreceptors are sensitive to different but overlapping wavelength ranges, with melanopsin playing the primary role in mediating the circadian and neuroendocrine effects of light (1). Furthermore, the different photoreceptor classes also differ in the way they respond to light stimuli that are patterned in time, such as a train of brief flashes or sinusoidal flicker. With recent studies showing that flashes of light lead to different effects on circadian and neuroendocrine physiology than continuous light exposure (2, 3), it is worth reviewing how light stimuli changing over time can be described parametrically.

This tutorial paper is targeted to chronobiologists, sleep researchers and scientists from adjacent disciplines, such as environmental psychology, who wish to develop an understanding of specifying light exposure in time. The focus will be on how time-varying light stimuli can be described quantitatively in the time and frequency domain, learn about major classes of time-varying stimuli and their properties, and some caveats in using time-varying stimuli. This paper is providing an introduction to readers with no specific background in signal processing and analyses of time-varying signals.

PARAMETRIC DESCRIPTIONS OF TIME-VARYING STIMULI

Basic Concepts

Time-Domain Representation

An intuitive way of thinking about time-varying stimuli is by representing their variation as a function of time, i.e., as a time course (**Figure 1A**). This representation is called the time domain. In this representation, stimulus values vary across different time points. In most cases, this representation is discrete, i.e., there is a set of time points for which stimulus values are specified. These stimulus values could be the luminance or illuminance, or some variant of radiance or illuminance, depending on the space in which the stimuli are specified. They could also be specified in terms of the contrast relative to an explicit or implicit reference light, such as an adaptation light.

Frequency-Domain Representation

An equivalent, but to some perhaps less intuitive way of representing time-varying stimuli is the frequency-domain representation (**Figure 1B**). While the time-domain representation shows the change of a signal over time, the frequency-domain representation describes the extent to which a given signal happens at different frequencies. For example, a sinusoidal change in intensity with a period length of 1 s has a frequency of 1 Hz but has no power at any other frequencies. The power a given signal has at all frequencies (within a given range) is called the power spectrum. Time-domain and frequency-domain representations can be converted between each other using the Fourier Transform, which decomposes the periodic signal into its constituent, single-frequency components (4).

Stimulus Spaces

The previous discussion mentioned two general classes of specifying waveforms: intensity and contrast. It is worth considering what actual quantities might be used for the specification of temporal waveforms and which units these might be expressed in. Here, we will review different ways of specifying the temporal waveform, focusing on absolute (intensity) and relative (contrast) units.

Intensity

The most basic representation of time-varying stimuli is a time course of spectral radiance or irradiance distributions, giving the radiance or irradiance of the stimulus at each wavelength for each point in time. At one point in time, this corresponds to a physical measurement (5) with no direct reference to the human photoreceptors. Spectra are weighted by the spectral sensitivities of the different photoreceptor classes (6), and then summed to arrive at alpha-opic radiance (for spectral radiance measurements) or alpha-opic irradiance (for spectral irradiance measurements). **Box 1** provides a glossary of relevant terms. This can be accomplished using an Excel-based toolbox provided by the CIE (7, 8) and the recently published platform-independent open-access and open-source web software *luox* (https://luox.app/) (9).

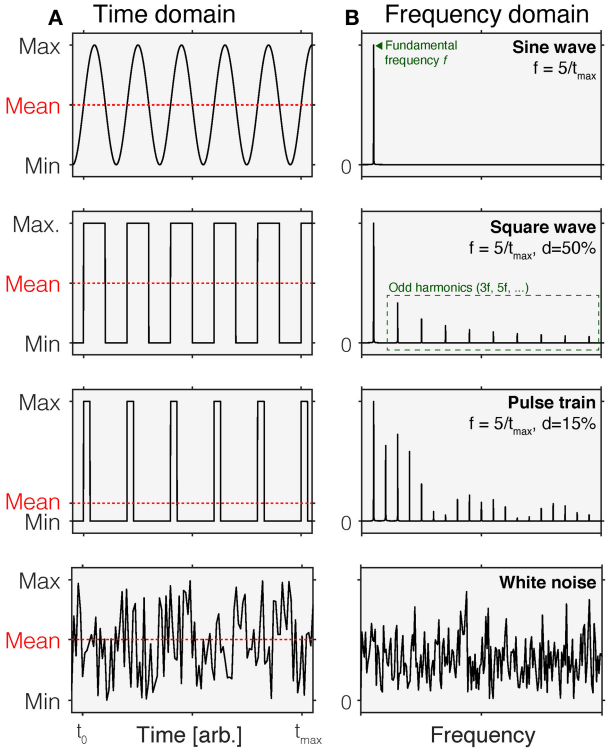

FIGURE 1 | Time-domain **(A)** and normalized frequency **(B)** representations of common time-varying stimuli. In each panel in **(A)**, the mean of each waveform is indicated by the dashed red line. Different waveforms in the time domain have different properties in the frequency domain. *Row 1*: A sine wave is a periodic waveform going between minimum and maximum values. It only contains frequency components at the fundamental frequency *f*. *Row 2*: A square wave varies in a discrete fashion between minimum and maximum values. It is composed of sine waves of different frequencies, including the fundamental frequency *f* and odd harmonics (3f, 5f,…). In a square wave, the duty cycle is 50%, which means that over time, the fraction of time at the maximum value is 50% within a period. *Row 3*: A pulse train can be thought of as a square wave with duty cycles that are <50%. This shorting of the time of that the wave is at maximum introduces additional harmonics. *Row 4*: White noise corresponds to a signal that is sampled from a uniform distribution. In the frequency domain, this corresponds to a flat curve, indicating that all frequencies are equally represented.

Contrast

In cases where there is a well-defined background light around which stimuli are presented, e.g., sinusoidally flickering lights against a mean background, it is useful to specify the contrast of the flickering stimuli as the relative change in (il)luminance or alpha-opic (ir)radiance relative to the background (which is the mean of the signal for sinusoidal flicker). In certain cases, time-varying contrast could be specified in a cone-opponent space (10). This is essential in cases where the photoreceptor excitation is actively controlled, such as using metameric lights changing over time, or stimuli generated using the method of silent substitution (11). In the method of silent substitution, lights are designed in such a way that they only produce differences in the activation of a selected class of photoreceptors, with no difference in the activation of the other photoreceptor classes, which are "silent" to the exchange of lights.

BOX 1 | Glossary.

Radiance-derived quantities

Spectral radiance: The spectral radiance is the absolute spectrum of light of a surface (self-emitting, e.g., displays, or reflecting, light reflected from a wall) per united solid angle per unit projected area. Spectral radiance is measured in W/m^2/sr/nm. From the radiance, we can calculate various quantities:

- **Luminance** (cd/m^2): Spectrum weighted by the photopic luminosity function.
- **α-opic radiances** (W/m^2/sr): Spectrum weighted by the five α-opic effect functions corresponding to the spectral sensitivities of the human photoreceptors and summed. As a consequence, there are α-opic radiances (the letter "α" is just a placeholder). The **melanopic radiance** (W/m^2/sr) is the spectrum weighted by the melanopsin spectral sensitivity function and summed.
- **α-opic equivalent daylight luminance (EDL)** (cd/m^2): Luminance of a D65 daylight spectrum that produces the same α-opic radiance is the spectrum in question. The **melanopic equivalent daylight luminance (mEDL)** is in reference to the melanopic radiance.

Irradiance-derived quantities

Spectral irradiance: The spectral radiance is the absolute spectrum of light of a received by a given area. Spectral irradiance is measured in W/m^2/nm. From the irradiance, we can calculate various quantities:

- **Illuminance** (lux): Spectrum weighted by the photopic luminosity function.
- **α-opic irradiances** (W/m^2): Spectrum weighted by the five α-opic effect functions corresponding to the spectral sensitivities of the human photoreceptors and summed. As a consequence, there are α-opic radiances (the letter "α" is just a placeholder). The **melanopic irradiance** (W/m^2) is the spectrum weighted by the melanopsin spectral sensitivity function and summed.
- **α-opic equivalent daylight luminance (EDL)** (lux): Illuminance of a D65 daylight spectrum that produces the same α-opic radiance is the spectrum in question. The **melanopic equivalent daylight illuminance (mEDI)** is in reference to the melanopic irradiance.

Chromaticity is an (il)luminance-independent way of specifying the color of an object, surface, or spectrum.

Correlated color temperature (CCT) is the temperature of a black-body radiator that matches the chromaticity of a spectrum in question.

Contrast is the relative different of activation (e.g., melanopic irradiance) between two different spectra.

Other Stimulus Spaces

It is important to note that time-varying stimuli defined in one stimulus space may not be linear when represented in another stimulus space. One such example is the mapping between correlated color temperature (CCT), a common way to specify the color appearance of lights relative to reference spectra (blackbody, <5,000 K or daylight spectrum, >5,000 K). A specification of CCT varying over time does not linearly map onto cone contrast changing over time, as CCT and cone activations are not linearly correlated. Consequently, such a non-linearity may lead to undesired frequency components in another space.

BASIC WAVEFORMS

Non-periodic Waveforms

Pulses

A pulse is a change in stimulus intensity or contrast that has a limited duration. For example, a brief light flash in an otherwise dim environment is a pulse. When the intensity is pulsed around a background light, the more intuitive way to specify the stimulus properties is in terms of contrast, i.e., the relative difference in stimulus intensity with respect to the background light.

Ramps

Ramps are increases or decreases in intensity or contrast up or down to a specified level. An example of such a stimulus is a gradual increase of light intensity in "dawn simulation" lights (12–19). The term ramp itself is ill-defined, as ramps can be linear, exponential, logarithmic, or modulated in some other way, e.g., using a cosine window [e.g., (20)]. Different ramps have different parameters in the frequency space. Ramps are often used to remove transient signals at the onset of a light, smoothing out the abrupt transition between different spectra.

Periodic Waveforms

Square-Wave Flicker

Square-wave flicker changes between two different intensity or contrast settings at a given frequency. A simple intuitive example is turning on the light switch in a room for 1 s, and turning it off again for 1 s.

Sinusoidal Flicker

Sinusoidal flicker is a light that is gradually changing intensity or contrast in accordance to the sine function. Sinusoidal flicker is parameterized by the frequency, phase, and the amplitude. In the frequency domain, sinusoidal flicker has the property that it only contains power at one frequency, the fundamental frequency. Square-wave flicker has power at odd harmonics as well.

Trains of Pulses

A sequence or train of pulses can be parameterized by the ratio between the duration that the light is on and the duration that the light is off. This is called the duty cycle and expresses the percentage that a light is turned on as part of the entire period. A duty cycle of 0% is of course simply no light, and a duty cycle of 100% is a continuous light. A repeating sequence of pulses with 50% duty cycle corresponds to the special case of square wave flicker (see above).

Other Waveforms

Of course, the space of possible waveforms is infinite. However, in studies employing stimuli parametrically, the waveforms above describe most use cases. Time courses sampled from different underlying distributions are called colored noise. For example, white noise (**Figure 1**, bottom row) has a flat frequency spectrum.

Descriptors for Complex Time-Varying Stimuli in Their Experimental Context

Documenting and reporting of lighting conditions is key to ensuring that studies can be reproduced, aggregated, and placed into context. While guidelines detailing how static aspects of light should be documented have been developed recently (5, 21) and are worth consulting for details on spectral characterization of light, technical details on time-varying light exposure are currently not typically documented and reported in standard form. Recently, the International Commission on Illumination (CIE) published technical note CIE TN 011:2020 (6), describing

which information should be captured in studies investigating non-visual responses to light. The document lists the following primary aspects that should be captured and documented: (1) the timeline of the experiment explained in detail, in clock time; (2) the duration of exposure in minutes or hours; (3) the sequence of exposures, including pre-experimental light and environmental exposures to the greatest degree of detail possible. The document expands this description to include (1) the overall duration of the experiment, (2) timing of light exposure (in clock time), (3) dim light exposure, (4) duration of the light exposure (in min or h), (5) duration of dim up and dim down (in min), and (6) pattern.

In an earlier document, CIE 213:2014 (22), the CIE suggested the following descriptors for dynamic light: (1) initial conditions (luminance, light source color, direction, etc.), specified as above; (2) intermediate and ending conditions, specified as above; (3) rate of change; (4) rate of cycle, if any; (5) change profile, particularly if the change is not linear; (6) movement of the observer's head and eyes relative to the light source or illuminated area. It is suggested to consult the two CIE documents already in the design stage of a research project.

In **Table 1**, we propose a reporting workflow based on the recommendations of CIE TN 011:2020 (6) and CIE 213:2014 (22) specifically for experiments in chronobiology and sleep research. In addition to naming specific quantities of interest and their derivatives, the proposed workflow also includes guidance on whether a specific item should be essential, optional, or recommended. The workflow is expandable and versatile. While it may be possible to derive this information from the full text of a given published study, future work should consider the development of a formalized schema which facilitates automated analyses based on stimulus descriptions as well as appropriate software tools that make it easy for investigators to provide this information. This may help make research future-proof (23) and more sustainable.

CONSIDERATIONS FOR USING TIME-VARYING STIMULI

Non-linearities in Light Output at Different Driving Inputs

Light sources are generally controlled using an input parameter, which we will call the input settings. In a conventional 8-bit RGB monitor, for example, three primary lights can be controlled at 255 levels. However, the output radiance is not linear with the input RGB settings but instead follows a non-linear gamma function. As a consequence, for example, an RGB triplet of [127 127 127] does not represent the expected 50% of the maximum output radiance, but less. To be able to control a monitor linearly then requires a measurement of the gamma function. The same of course applies to other light sources.

Unless they are corrected, non-linearities in output radiance lead to distortions in the output signal. Consider, for example, the case of a sinusoidal modulation that is displayed using a non-linear light source. In the time domain, the resulting waveform is no longer symmetric around the mean. In the frequency domain, it becomes obvious that the non-linearities have introduced additional frequencies that were not present in the target modulation.

In addition to non-linearities in output radiance at different input settings, light sources may also shift in their spectral output. This is most notable in LEDs, which can shift up to a few nanometers, depending on how they are driven. Of course, such a spectral shift will manifest in uncertainties in the effective stimulus and may also introduce undesirable artifacts (such as the unwanted stimulation of photoreceptors). As the direction and size of these spectral shifts cannot be predicted easily, a practical solution is to measure the spectral output at different input settings. These measurements can then be used to design stimuli that account for the spectral shifts or to characterize the uncertainty in stimulus presentation *post-hoc*, or both.

Temporal Resolution Limits and Time Constants

A historical precedent cautioning to calibrate the temporal output of light generators carefully was offered by Mollon and Polden (24). Measuring the time constants of tachistoscopes, which were devices enabling very brief light exposures pre-dating the common usage of computer displays in research, they found that the time constants were too slow to present stimuli at the millisecond scale accurately. More than 40 years later, accuracy of timing is still a topic in visual psychophysics, with each generation of novel display technology bringing its own potential idiosyncratic temporal artifacts (25–29).

PHYSIOLOGICAL RELEVANCE OF TIME-VARYING STIMULI

Temporal Properties of Distal vs. Proximal Stimuli

In the previous discussion, the near-exclusive focus has been on accurately capturing the temporal properties of the stimulus in the physical domain. In psychophysics, this is sometimes called the distal stimulus, while the pattern of light impinging on the retina is called the proximal stimulus. Notably, the distal stimulus and the proximal stimulus are related to one another through the optics of image formation and projection onto the retina, but they are not the same. Before light excites the photoreceptors, it is modified (relative to the cornea) by passing through the pupil and the ocular media.

Pupil Size

Pupil size, being the aperture of the eye, changes the overall retinal irradiance over a factor of ∼16×, or 1.2 log units, given by the ratio of the largest possible pupil area under full dark adaptation (8 mm) to the smallest possible pupil area under bright light conditions (2 mm) (30). The pupil is not static and responds to light in a wavelength- and time-specific fashion. Under time-varying stimuli, then, the pupil size is also dynamically changing, modifying the temporal properties of the distal stimulus in a way that is not very easy to predict. In addition, the pupil responds to other factors unrelated to light, such as cognitive processing (31), and displays spontaneous

TABLE 1 | Recommended reporting of time-varying stimuli.

Aspect	Form	Status
Timeline of experimental protocol, including exposure durations and sequence of exposures		
↳ Description of protocol timeline	Description in manuscript text	Essential
↳ Visualization of protocol timeline	Schematic visualization included as figure in main manuscript or supplementary figure	Optional, but recommended in particular for multiple-hour experiments
↳ Machine-readable tabular representation of protocol timeline	Data table included as supplementary material	Optional
Static spectral measurements of all discrete light scenarios		
↳ Spectral (ir)radiance distribution	Data table included as supplementary material	Essential
↳ CIE S026 quantities calculated from spectral measurements	Description in manuscript text or table in main text	Essential
OR		
Static CIE S026 referenced measurements of all discrete light scenarios		
↳ CIE S026 quantities	Description in manuscript text or table in main text	Essential
Time series of time-varying stimuli and exposures specified as spectral or melanopic (ir)radiance [including pre-experimental light and environmental exposures (6)]		
↳ Tabulated time series	Data table included as supplementary material	Essential
↳ Visualization of time series	Graph included as figure in main manuscript or supplementary figure	Optional, but recommended
↳ Representation of power spectra	Graph included as figure in main manuscript or supplementary figure	Optional
Behavioral demands		
↳ Instructions to participants regarding fixation and gaze direction	Description in manuscript text	Essential
↳ Verbatim text used to instruct participants	Description in manuscript text	Optional
Pupil size		
↳ Recording status (whether or not it was recorded)	Description in manuscript text	Essential
↳ Recording meta-data, including device details, sampling frequency, binocular or monocular recording	Description in manuscript text	Optional
↳ Mean pupil size per condition and statistical test showing whether they are different	Description in manuscript text	Optional
↳ Time series of pupil size data	Data table included as supplementary material	Optional

Expanded recommendations and checklist based on CIE documents (6, 22).
↳ refers to derivatives. Essential reporting items are shaded in yellow.

fluctuations (32, 33) which have been found to be related sleepiness (34). Furthermore, pupil size varies with age, with older people having smaller pupils on average (35), and is subject to diurnal variations (36–41).

As expected, when pupil size is controlled through pharmacological dilation and the stimulus is viewed through this maximally dilated pupil, the same corneal irradiance leads to more melatonin suppression compared with the undilated pupil (42). As a consequence, dose–response curves collected under undilated conditions [e.g., (43)] represent a mixture of two effects: a pupil size effect that modifies retinal light exposure, and a melatonin-suppressive effect. Special care must be taken when dose–response curves collected under different pupil conditions are compared (1, 44).

One solution to estimating retinal illuminance may be the use of mobile eye trackers during a given experiment [e.g., (45)], which enable the determination of pupil size at all time points. In conjunction with head-referenced irradiance or radiance measurements of the corneal irradiance, capturing an individual's "spectral diet" (46), the time course of light exposure at the cornea, it is then possible to determine the actual retinal irradiance, as this is the biologically relevant quantity. Development efforts for such a system are currently underway [e.g., (47)].

Eye Movements

Of course, observers are moving their trunk, head, and eyes during the waking day, thereby displacing the retinal image at a high frequency (48). Saccadic eye movements displace the retinal image (49), thereby repositioning different parts of the visual world into the fovea, thereby supporting various visually guided tasks and support various tasks (50, 51). Saccadic eye movements are guided by a variety of factors, including salience and higher-level factors (52). In free-viewing of pictures, saccades can make up to 20% of time (53). In addition, during periods of fixation, three types of fixational eye movements occur: tremor, also called physiological nystagmus, occurring at ∼90 Hz, drift, and microsaccades, occurring at 1–2 Hz (54). The extent to which the trunk, head, and eye movements decorrelate the distal from the proximal (retinal) stimulus depends on the spatial characteristics of the scene. One can imagine two extremes. In a completely unarticulated homogenous environment such as those produced by a ganzfeld stimulus, the retinal stimulus to an observer may be nearly constant. On the

BOX 2 | Recommended further reading.

- The CIE has published two documents on documenting lighting and light exposures: CIE 213:2014 (22) and CIE TN 011:2020 (6). The articles published Spitschan et al. (5) and Knoop et al. (21) represent independent proposal for standardized ways of reporting lighting conditions in experimental situations.
- Watson (73) provides a solid introduction into linear-systems modeling of temporal sensitivity in human psychophysics.
- Kronauer et al. (56) synthesize temporal non-linearities in the circadian system's response to light and subject it to rigorous modeling.
- Schlangen & Price (74) provide an extensive introduction in measuring the lighting environment.
- Münch et al. (75) and Knoop et al. (76) provide recent syntheses on the status of daylight for humans, which may serve as a reference when thinking about naturalistic light exposure and its temporal properties.

other extreme, a point light source in an otherwise dark room under free-viewing conditions will stimulate different retinal locations between and—due to fixational eye movements—during fixation, thereby producing very large spatial and temporal contrast.

Temporal Integration in Circadian and Neuroendocrine Photoreception

Ultimately, the use of temporally patterned stimuli biases processing in the retina to a specific set of photoreceptors. While previous work has examined how different photoreceptors drive the pupillary light reflex in different temporal regimes (55), there are at present no parametric measurements for the effects of photoreceptor-selective stimuli varying in their frequency properties on neuroendocrine and circadian physiology. A few studies (2, 3, 56) address the question of temporal integration, though a lot remains unknown in humans. The growing body of literature in animal models [rodents: (57–60), *Drosophila*: (61, 62)] can serve as a useful starting point.

It may be desirable to derive a summary metric for time-varying stimuli. For pulse stimuli, one intuitive way to summarize illuminance or irradiance and duration is the product of the two. This yields a summary quantity with units in e.g., "lux minutes." This approach has been used in literature to describe total light doses [e.g., (12–14, 63–65)], in line with the notion that the circadian system acts as a photon counter (66). The extent to which this is a useful metric, however, is not necessarily clear. It relies on reciprocity, which refers to the notion that irradiance and duration can be traded off without any difference in effect. Further data mapping out how intensity and duration can be traded off (67) are required before the adoption of such a summary metric.

In field experiments where both light exposure and an outcome metric (such as sleep timing and duration, or other circadian, neuroendocrine, behavioral, and cognitive outputs) are measured conjointly, summarizing the pattern of light exposure is often a necessary step to relate input to output. A data-driven approach to summarizing light exposures in these contexts is to use *time above threshold* (TAT), as a well as *distributional characteristics* of the light exposure above the threshold, such as the mean and standard deviation (68).

An Outlook

As the temporal properties of the proximal (retinal) stimulus cannot easily be determined, understanding the properties of the distal stimulus is a key first step. One strategy is to quantitatively characterize the propagation of light in specific study context using simulation software (69). This can be achieved using specialized architectural lighting design tools. An integrated workflow to go from physically realistic simulations of illuminated spaces to physiologically plausible simulations of retinal illumination [such as those available in the iSETBIO toolbox (70–72)] is a promising and exciting path forward. For readers wishing to learn more about the fundamentals touched upon in this review, **Box 2** provides advanced reading for readers with a background in signal processing and analyses of time-varying signals but no specific background in chronobiology and sleep research.

AUTHOR CONTRIBUTIONS

MS: conceptualization, methodology, software, resources, writing—original draft preparation, writing—review and editing, and visualization.

FUNDING

This work was supported by Wellcome Trust 204686/Z/16/Z and 204686/Z/16/C.

REFERENCES

1. Brown TM. Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res.* (2020) 69:e12655. doi: 10.1111/jpi.12655
2. Najjar RP, Zeitzer JM. Temporal integration of light flashes by the human circadian system. *J Clin Invest.* (2016) 126:938–47. doi: 10.1172/JCI82306
3. Rahman SA, St Hilaire MA, Chang AM, Santhi N, Duffy JF, Kronauer RE, et al. Circadian phase resetting by a single short-duration light exposure. *JCI Insight.* (2017) 2:e89494. doi: 10.1172/jci.insight.89494
4. Smith SW. *The Scientist and Engineer's Guide to Digital Signal Processing.* 1st ed. San Diego, CA: California Technical Pub. (1997).
5. Spitschan M, Stefani O, Blattner P, Gronfier C, Lockley SW, Lucas RJ. How to report light exposure in human chronobiology and sleep research experiments. *Clocks Sleep.* (2019) 1:280–9. doi: 10.3390/clockssleep1030024
6. CIE. *CIE TN 011:2020: What to Document and Report in Studies of ipRGC-Influenced Responses to Light.* Vienna: CIE Central Bureau (2020). doi: 10.25039/TN.011.2020
7. CIE. *CIE S 026 Toolbox (v1.49a - November 2020) [Computer Program].* Vienna: CIE (2020). doi: 10.25039/S026.2018.TB
8. CIE. *User Guide to the α-opic Toolbox for Implementing CIE S 026 [Computer Program].* Vienna: CIE (2020). doi: 10.25039/S026.2018.UG
9. Spitschan M, Mead J, Roos C, Lowis C, Griffiths B, Mucur P, et al. luox: novel open-access and open-source web platform for calculating and sharing

physiologically relevant quantities for light and lighting. *Wellcome Open Res.* (2021) 6:1. doi: 10.12688/wellcomeopenres.16595.1
10. Brainard DH. Cone contrast and opponent modulation color spaces. In: Kaiser B, Boynton R, editors. *Human Color Vision.* 2nd ed. Washington, DC: Optical Society of America (1996).
11. Spitschan M, Woelders T. The method of silent substitution for examining melanopsin contributions to pupil control. *Front Neurol.* (2018) 9:941. doi: 10.3389/fneur.2018.00941
12. Terman M, Terman JS. Controlled trial of naturalistic dawn simulation and negative air ionization for seasonal affective disorder. *Am J Psychiatry.* (2006) 163:2126. doi: 10.1176/ajp.2006.163.12.2126
13. Palmer CR, Kripke DF, Savage HC, Cindrich LA, Loving RT, Elliott JA. Efficacy of enhanced evening light for advanced sleep phase syndrome. *Behav Sleep Med.* (2003) 1:213–26. doi: 10.1207/S15402010BSM0104_4
14. Danilenko KV, Wirz-Justice A, Krauchi K, Cajochen C, Weber JM, Fairhurst S, et al. Phase advance after one or three simulated dawns in humans. *Chronobiol Int.* (2000) 17:659–68. doi: 10.1081/CBI-100101072
15. Gabel V, Maire M, Reichert CF, Chellappa SL, Schmidt C, Hommes V, et al. Dawn simulation light impacts on different cognitive domains under sleep restriction. *Behav Brain Res.* (2015) 281:258–66. doi: 10.1016/j.bbr.2014.12.043
16. Thompson A, Jones H, Gregson W, Atkinson G. Effects of dawn simulation on markers of sleep inertia and post-waking performance in humans. *Eur J Appl Physiol.* (2014) 114:1049–56. doi: 10.1007/s00421-014-2831-z
17. Bromundt V, Wirz-Justice A, Boutellier M, Winter S, Haberstroh M, Terman M, et al. Effects of a dawn-dusk simulation on circadian rest-activity cycles, sleep, mood and well-being in dementia patients. *Exp Gerontol.* (2019) 124:110641. doi: 10.1016/j.exger.2019.110641
18. Van De Werken M, Giménez MC, De Vries B, Beersma DG, Van Someren EJ, Gordijn MC. Effects of artificial dawn on sleep inertia, skin temperature, and the awakening cortisol response. *J Sleep Res.* (2010) 19:425–35. doi: 10.1111/j.1365-2869.2010.00828.x
19. Kavanau JL. Twilight transitions and biological rhythmicity. *Nature.* (1962) 194:1293–5. doi: 10.1038/1941293a0
20. Spitschan M, Bock AS, Ryan J, Frazzetta G, Brainard DH, Aguirre GK. The human visual cortex response to melanopsin-directed stimulation is accompanied by a distinct perceptual experience. *Proc Natl Acad Sci USA.* (2017) 114:12291–6. doi: 10.1073/pnas.1711522114
21. Knoop M, Broszio K, Diakite A, Liedtke C, Niedling M, Rothert I, et al. Methods to describe and measure lighting conditions in experiments on non-image-forming aspects. *Leukos.* (2019) 15:163–79. doi: 10.1080/15502724.2018.1518716
22. CIE. *CIE 213:2014: Guide to Protocols for Describing Lighting.* Vienna: CIE Central Bureau (2014).
23. Spitschan M. Opinion: future-proofing circadian research. *Light Res Technol.* (2019) 51:818–9. doi: 10.1177/1477153519873223
24. Mollon JD, Polden PG. On the time constants of tachistoscopes. *Quart J Exp Psychol.* (2018) 30:555–68. doi: 10.1080/00335557843000133
25. MacInnes WJ, Taylor TL. Millisecond timing on PCs and Macs. *Behav Res Methods Instrum Comput.* (2001) 33:174–8. doi: 10.3758/BF03195363
26. Plant RR, Quinlan PT. Could millisecond timing errors in commonly used equipment be a cause of replication failure in some neuroscience studies? *Cogn Affect Behav Neurosci.* (2013) 13:598–614. doi: 10.3758/s13415-013-0166-6
27. Rohr M, Wagner A. How monitor characteristics affect human perception in visual computer experiments: CRT vs. LCD monitors in millisecond precise timing research. *Sci Rep.* (2020) 10:6962. doi: 10.1038/s41598-020-63853-4
28. Elze T. Misspecifications of stimulus presentation durations in experimental psychology: a systematic review of the psychophysics literature. *PLoS ONE.* (2010) 5:12792. doi: 10.1371/journal.pone.0012792
29. Elze T. Achieving precise display timing in visual neuroscience experiments. *J Neurosci Methods.* (2010) 191:171–9. doi: 10.1016/j.jneumeth.2010.06.018
30. Watson AB, Yellott JI. A unified formula for light-adapted pupil size. *J Vis.* (2012) 12:12. doi: 10.1167/12.10.12
31. Mathôt S. Pupillometry: psychology, physiology, and function. *J Cogn.* (2018) 1:16. doi: 10.5334/joc.18
32. Yoss RE, Moyer NJ, Hollenhorst RW. Hippus and other spontaneous rhythmic pupillary waves. *Am J Ophthalmol.* (1970) 70:935–41. doi: 10.1016/0002-9394(70)92470-0
33. Bouma H, Baghuis LC. Hippus of the pupil: periods of slow oscillations of unknown origin. *Vision Res.* (1971) 11:1345–51. doi: 10.1016/0042-6989(71)90016-2
34. Wilhelm B, Wilhelm H, Lüdtke H, Streicher P, Adler M. Pupillographic assessment of sleepiness in sleep-deprived healthy subjects. *Sleep.* (1998) 21:258–65.
35. Winn B, Whitaker D, Elliott DB, Phillips NJ. Factors affecting light-adapted pupil size in normal human subjects. *Invest Ophthalmol Vis Sci.* (1994) 35:1132–7.
36. Fosnaugh JS, Bunker EB, Pickworth WB. Daily variation and effects of ambient light and circadian factors on the human light reflex. *Methods Find Exp Clin Pharmacol.* (1992) 14:545–53.
37. Loving RT, Kripke DF, Glazner LK. Circadian rhythms in the human pupil and eyelid. *Am J Physiol.* (1996) 271:R320–4. doi: 10.1152/ajpregu.1996.271.2.R320
38. Ranzijn R, Lack L. The pupillary light reflex cannot be used to measure sleepiness. *Psychophysiology.* (1997) 34:17–22. doi: 10.1111/j.1469-8986.1997.tb02411.x
39. Wilhelm B, Giedke H, Lüdtke H, Bittner E, Hofmann A, Wilhelm H. Daytime variations in central nervous system activation measured by a pupillographic sleepiness test. *J Sleep Res.* (2001) 10:1–7. doi: 10.1046/j.1365-2869.2001.00239.x
40. Yu M, Kautz MA, Thomas ML, Johnson D, Hotchkiss ER, Russo MB. Operational implications of varying ambient light levels and time-of-day effects on saccadic velocity and pupillary light reflex. *Ophthalmic Physiol Opt.* (2007) 27:130–41. doi: 10.1111/j.1475-1313.2006.00450.x
41. Van Egroo M, Gaggioni G, Cespedes-Ortiz C, Ly JQM, Vandewalle G. Steady-state pupil size varies with circadian phase and sleep homeostasis in healthy young men. *Clocks Sleep.* (2019) 1:240–58. doi: 10.3390/clockssleep1020021
42. Gaddy JR, Rollag MD, Brainard GC. Pupil size regulation of threshold of light-induced melatonin suppression. *J Clin Endocrinol Metab.* (1993) 77:1398–401. doi: 10.1210/jc.77.5.1398
43. Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J Physiol.* (2000) 526:695–702. doi: 10.1111/j.1469-7793.2000.00695.x
44. Bracke P, Van de Putte E, Ryckaert WR. Comment concerning the effects of light intensity on melatonin suppression in the review "light modulation of human clocks, wake, and sleep" by A. Prayag et al. *Clocks Sleep.* (2021) 3:181–8. doi: 10.3390/clockssleep3010011
45. Kassner M, Patera W, Bulling A. Pupil: an open source platform for pervasive eye tracking and mobile gaze-based interaction. *UbiComp '14 Adjunct.* (2014) 2014:1151–60. doi: 10.1145/2638728.2641695
46. Webler FS, Spitschan M, Foster RG, Andersen M, Peirson SN. What is the 'spectral diet' of humans? *Curr Opin Behav Sci.* (2019) 30:80–6. doi: 10.1016/j.cobeha.2019.06.006
47. Lazar R, Spitschan M. Measurement of light-mediated changes in pupil size under real-world conditions. *OSF Home [Preprint].* (2020). doi: 10.17605/OSF.IO/RNP2Z
48. Brainard GC, Rollag MD, Hanifin JP. Photic regulation of melatonin in humans: ocular and neural signal transduction. *J Biol Rhythms.* (1997) 12:537–46. doi: 10.1177/074873049701200608
49. Rayner K. Eye movements in reading and information processing. *Psychol Bullet.* (1978) 85:618–60. doi: 10.1037/0033-2909.85.3.618
50. Land M, Mennie N, Rusted J. The roles of vision and eye movements in the control of activities of daily living. *Perception.* (1999) 28:1311–28. doi: 10.1068/p2935
51. Land MF, Hayhoe M. In what ways do eye movements contribute to everyday activities? *Vis Res.* (2001) 41:3559–65. doi: 10.1016/S0042-6989(01)00102-X
52. Schutz AC, Braun DI, Gegenfurtner KR. Eye movements and perception: a selective review. *J Vis.* (2011) 11:9. doi: 10.1167/11.5.9
53. Martinez-Conde S. Fixational eye movements in normal and pathological vision. *Prog Brain Res.* (2006) 154:151–76. doi: 10.1016/S0079-6123(06)54008-7

54. Martinez-Conde S, Macknik SL, Hubel DH. The role of fixational eye movements in visual perception. *Nat Rev Neurosci.* (2004) 5:229–40. doi: 10.1038/nrn1348
55. Spitschan M, Jain S, Brainard DH, Aguirre GK. Opponent melanopsin and S-cone signals in the human pupillary light response. *Proc Natl Acad Sci USA.* (2014) 111:15568–72. doi: 10.1073/pnas.1400942111
56. Kronauer RE, St Hilaire MA, Rahman SA, Czeisler CA, Klerman EB. An exploration of the temporal dynamics of circadian resetting responses to short- and long-duration light exposures: cross-species consistencies and differences. *J Biol Rhythms.* (2019) 34:497–514. doi: 10.1177/0748730419862702
57. Nelson DE, Takahashi JS. Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *J Physiol.* (1991) 439:115–45. doi: 10.1113/jphysiol.1991.sp018660
58. Vidal L, Morin LP. Absence of normal photic integration in the circadian visual system: response to millisecond light flashes. *J Neurosci.* (2007) 27:3375–82. doi: 10.1523/JNEUROSCI.5496-06.2007
59. Van Den Pol AN, Cao V, Heller HC. Circadian system of mice integrates brief light stimuli. *Am J Physiol.* (1998) 275:R654–7. doi: 10.1152/ajpregu.1998.275.2.R654
60. Arvanitogiannis A, Amir S. Resetting the rat circadian clock by ultra-short light flashes. *Neurosci Lett.* (1999) 261:159–62. doi: 10.1016/S0304-3940(99)00021-X
61. Negelspach DC, Kaladchibachi S, Fernandez F. The circadian activity rhythm is reset by nanowatt pulses of ultraviolet light. *Proc Biol Sci.* (2018) 285:1288. doi: 10.1098/rspb.2018.1288
62. Kaladchibachi S, Negelspach DC, Zeitzer JM, Fernandez F. Optimization of circadian responses with shorter and shorter millisecond flashes. *Biol Lett.* (2019) 15:20190371. doi: 10.1098/rsbl.2019.0371
63. Friedman L, Zeitzer JM, Kushida C, Zhdanova I, Noda A, Lee T, et al. Scheduled bright light for treatment of insomnia in older adults. *J Am Geriatr Soc.* (2009) 57:441–52. doi: 10.1111/j.1532-5415.2008.02164.x
64. Wee R, Van Gelder RN. Sleep disturbances in young subjects with visual dysfunction. *Ophthalmology.* (2004) 111:297–302. doi: 10.1016/j.ophtha.2003.05.014
65. Terman M, Jiuan Su. Circadian rhythm phase advance with dawn simulation treatment for winter depression. *J Biol Rhythms.* (2010) 25:297–301. doi: 10.1177/0748730410374000
66. Brown TM. Using light to tell the time of day: sensory coding in the mammalian circadian visual network. *J Exp Biol.* (2016) 219:1779–92. doi: 10.1242/jeb.132167
67. Joyce DS, Spitschan M, Zeitzer JM. Integration of brief light flashes varying in intensity and duration by the human circadian system. *bioRxiv [Preprint].* (2021). doi: 10.1101/759134
68. Reid KJ, Santostasi G, Baron KG, Wilson J, Kang J, Zee PC. Timing and intensity of light correlate with body weight in adults. *PLoS ONE.* (2014) 9:e92251. doi: 10.1371/journal.pone.0092251
69. Dawson D, Campbell SS. Bright light treatment: are we keeping our subjects in the dark? *Sleep.* (1990) 13:267–71.
70. Cottaris NP, Jiang H, Ding X, Wandell BA, Brainard DH. A computational-observer model of spatial contrast sensitivity: effects of wave-front-based optics, cone-mosaic structure, and inference engine. *J Vis.* (2019) 19:8. doi: 10.1167/19.4.8
71. Cottaris NP, Wandell BA, Rieke F, Brainard DH. A computational observer model of spatial contrast sensitivity: effects of photocurrent encoding, fixational eye movements, and inference engine. *J Vis.* (2020) 20:17. doi: 10.1167/jov.20.7.17
72. Farrell JE, Jiang H, Winawer J, Brainard DH, Wandell BA. 27.2: distinguished paper: modeling visible differences: the computational observer model. *SID Symp Digest Technical Pap.* (2014) 45:352–6. doi: 10.1002/j.2168-0159.2014.tb00095.x
73. Watson AB. Temporal sensitivity. In: Boff KR, Kaufman L, Thomas JP, editors. *Handbook of Perception and Human Performance.* New York, NY: Wiley (1986).
74. Schlangen LJM, Price LLA. The lighting environment, its metrology, and non-visual responses. *Front Neurol.* (2021) 12:624861. doi: 10.3389/fneur.2021.624861
75. Münch M, Wirz-Justice A, Brown SA, Kantermann T, Martiny K, Stefani O, et al. The role of daylight for humans: gaps in current knowledge. *Clocks Sleep.* (2020) 2:61–85. doi: 10.3390/clockssleep2010008
76. Knoop M, Stefani O, Bueno B, Matusiak B, Hobday R, Wirz-Justice A, et al. Daylight: what makes the difference? *Light Res Technol.* (2019) 52:423–42. doi: 10.1177/1477153519869758

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