NEUROIMAGING APPROACHES TO THE STUDY OF COGNITIVE AGING

EDITED BY: Ronald Cohen, Clinton B. Wright, Adam J. Woods, Gene E. Alexander and Kristina Visscher

PUBLISHED IN: Frontiers in Aging Neuroscience

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-901-4
DOI 10.3389/978-2-88963-901-4

About Frontiers

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

Frontiers Journal Series

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

Dedication to Quality

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

What are Frontiers Research Topics?

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

NEUROIMAGING APPROACHES TO THE STUDY OF COGNITIVE AGING

Topic Editors:
Ronald Cohen, University of Florida, United States
Clinton B. Wright, National Institute of Neurological Disorders and Stroke (NINDS), United States
Adam J. Woods, University of Florida, United States
Gene E. Alexander, University of Arizona, United States
Kristina Visscher, University of Alabama at Birmingham, United States

Citation: Cohen, R., Wright, C. B., Woods, A. J., Alexander, G. E., Visscher, K., eds. (2020). Neuroimaging Approaches to the Study of Cognitive Aging. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-901-4

Table of Contents

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 02 May 2016
doi: 10.3389/fnagi.2016.00094

Cognitively Engaging Activity Is Associated with Greater Cortical and Subcortical Volumes

Talia R. Seider[1,2], Robert A. Fieo[1], Andrew O'Shea[1], Eric C. Porges[1], Adam J. Woods[1] and Ronald A. Cohen[1]*

[1] Center for Cognitive Aging and Memory, Department of Aging and Geriatric Research, Institute on Aging, University of Florida, Gainesville, FL, USA, [2] Department of Clinical and Health Psychology, University of Florida, Gainesville, FL, USA

As the population ages and dementia becomes a growing healthcare concern, it is increasingly important to identify targets for intervention to delay or attenuate cognitive decline. Research has shown that the most successful interventions aim at altering lifestyle factors. Thus, this study examined how involvement in physical, cognitive, and social activity is related to brain structure in older adults. Sixty-five adults (mean age = 71.4 years, standard deviation = 8.9) received the Community Healthy Activities Model Program for Seniors (CHAMPS), a questionnaire that polls everyday activities in which older adults may be involved, and also underwent structural magnetic resonance imaging. Stepwise regression with backward selection was used to predict weekly time spent in either social, cognitive, light physical, or heavy physical activity from the volume of one of the cortical or subcortical regions of interest (corrected by intracranial volume) as well as age, education, and gender as control variables. Regressions revealed that more time spent in cognitive activity was associated with greater volumes of all brain regions studied: total cortex ($\beta = 0.289$, $p = 0.014$), frontal ($\beta = 0.276$, $p = 0.019$), parietal ($\beta = 0.305$, $p = 0.009$), temporal ($\beta = 0.275$, $p = 0.020$), and occipital ($\beta = 0.256$, $p = 0.030$) lobes, and thalamus ($\beta = 0.310$, $p = 0.010$), caudate ($\beta = 0.233$, $p = 0.049$), hippocampus ($\beta = 0.286$, $p = 0.017$), and amygdala ($\beta = 0.336$, $p = 0.004$). These effects remained even after accounting for the positive association between cognitive activity and education. No other activity variable was associated with brain volumes. Results indicate that time spent in cognitively engaging activity is associated with greater cortical and subcortical brain volume. Findings suggest that interventions aimed at increasing levels of cognitive activity may delay cognitive consequences of aging and decrease the risk of developing dementia.

Keywords: cognitive aging, cognitive activity, healthy aging, brain volume, MRI, gray matter, social activity, physical activity

OPEN ACCESS

Edited by:
Michael Hornberger, University of East Anglia, UK

Reviewed by:
Agnes Lacreuse, University of Massachusetts, USA
Judith Machts, Otto-von-Guericke University Magdeburg, Germany

****Correspondence:***
Talia R. Seider
tseider@phhp.ufl.edu

Received: *21 January 2016*
Accepted: *14 April 2016*
Published: *02 May 2016*

Citation:
Seider TR, Fieo RA, O'Shea A, Porges EC, Woods AJ and Cohen RA (2016) Cognitively Engaging Activity Is Associated with Greater Cortical and Subcortical Volumes. Front. Aging Neurosci. 8:94. doi: 10.3389/fnagi.2016.00094

INTRODUCTION

Older age is the primary risk factor for neurodegenerative diseases such as Alzheimer's disease (AD). As the size and proportion of the population over age 65 increases, the number of people with dementia is expected to increase substantially, raising healthcare costs and caregiver burden (Alzheimer's Association, 2015). Thus, it is becoming increasingly important to identify targets for intervention with the aims of delaying or attenuating cognitive decline.

While several pharmaceuticals exist to delay cognitive decline, research has shown that the best results come from interventions aimed at altering lifestyle factors (Williams et al., 2010; Imtiaz et al., 2014). Greater self-reported levels of engagement in cognitive, social, and physical activity have frequently been associated with higher cognitive functioning scores (Barnes et al., 2004; Newson and Kemps, 2005; McGue and Christensen, 2007; Vemuri et al., 2012; Opdebeeck et al., 2016). Furthermore, physical activity and cognitive engagement are among the only factors consistently associated with decreased risk for AD and cognitive decline (Fratiglioni et al., 2004; Kramer et al., 2004; Hertzog et al., 2009; Williams et al., 2010). Still, the mechanisms of such effects remain poorly understood.

Brain structure and function presumably mediate the link between an active lifestyle and reduced risk for cognitive decline. Observational studies have shown that increased levels of physical activity are associated with larger brain volumes, especially in frontal and hippocampal areas (Rovio et al., 2010; Bugg and Head, 2011; Doi et al., 2015), and interventional studies have shown that physical activity can increase hippocampal (Erickson et al., 2011) and frontal volumes (Colcombe et al., 2006).

Research on the association between cognitive or social engagement and brain volume is less extensive. Proxy measures of cognitive reserve, such as intellectual attainment, have been linked with greater brain volume (Stern, 2009). New learning has been shown to cause increased parietal and hippocampal size in young adults (Draganski et al., 2006), and cognitive training has been associated with increased hippocampal volume and preserved white matter integrity in older adults (Engvig et al., 2014). Greater frequency of cognitive leisure activities has been associated with larger gray matter volume in frontal and limbic regions (Schultz et al., 2015), while higher scores on measures combining cognitive and social activities have been related to more normal-appearing white matter (Gow et al., 2012a) and reduced hippocampal decline over time (Valenzuela et al., 2008). More self-reported social engagement has also been associated with greater temporal and occipital gray matter volume (James et al., 2012), and an intervention study reported that increase in social activity was associated with increased total brain volume (Mortimer et al., 2012). Still, other research has shown no relationship between cognitive or social activity and volumetric data (Foubert-Samier et al., 2012; Vaughan et al., 2014; Van der Vegt, 2015), and the link between these lifestyle factors and regional cerebral volumes remains understudied.

The purpose of this study was to examine how physical, cognitive, and social activity is related to brain structure. Levels of engagement in everyday activities was measured via self-report. Based on previous research, we generally expected higher self-reported levels of physical, social, and cognitive activity to be associated with greater volumes, especially in frontal and limbic regions for physical activity, temporal and occipital regions for social activity, and parietal, frontal, and limbic regions for cognitive activity.

MATERIALS AND METHODS

Participants

Sixty-five community dwelling individuals in the Gainesville and North Florida region were recruited to complete a magnetic resonance imaging (MRI) scan and a cognitive assessment, including the Montreal Cognitive Assessment (MoCA), a brief screen of cognitive functioning. Exclusion criteria included history of head injury with loss of consciousness greater than 20 min, neurologic condition such as dementia, epilepsy, or stroke, major psychiatric illness such as schizophrenia or bipolar disorder, inability to undergo MRI, and MoCA score less than 20. Sample demographics and characteristics are listed in **Table 1**. Participants had a mean age of 71, they were generally well educated with a mean education of 17 years, slightly more than half were females, they were mostly Caucasian, and they were generally cognitively intact with a mean MoCA score of 26. The study was approved by University of Florida Institutional Review Board and written informed consent was obtained from all study participants.

Activity Assessment

The Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire was developed as part of an intervention study aimed at increasing participation in physical activities in community dwelling elderly. It was designed to measure current levels of energy expenditure by taking a poll of everyday activities in which older adults may be involved (Stewart et al., 1997). Participants were asked whether or not they engaged in a particular activity during a typical week in the past month. If they had, they were asked to fill in the number of times they engaged in the activity per week and mark the total number of hours spent in the activity per week. Total hours were grouped into 6 integer values such that 1 indicated less than 1 h was spent engaged in that activity per week, 2 indicated 1 – 2½ h spent, 3 indicated 3 – 4½ h, 4 indicated 5 – 6½ h, 5 indicated 7 – 8½ h, and 6 indicated 9 or more hours (**Figure 1**). Participants are also allowed to fill in "other" and record an activity that was not listed in the questionnaire.

Physical activities were divided into light and moderate-heavy groups based on the ratio of work metabolic rate to resting metabolic rate (MET) adjusted for older adults (Ainsworth et al., 1993) as was done in the original CHAMPS research (Stewart et al., 2001). Light physical activities were those with a MET

TABLE 1 | Sample characteristics ($N = 65$).

	Mean (SD)	Range
Age (years)	71.4 (8.9)	48–85
Education (years)	16.8 (2.5)	12–20
% male	43.1	
% Caucasian	95.4	
MoCA	26.0 (2.7)	20–30

MoCA, Montreal Cognitive Assessment.

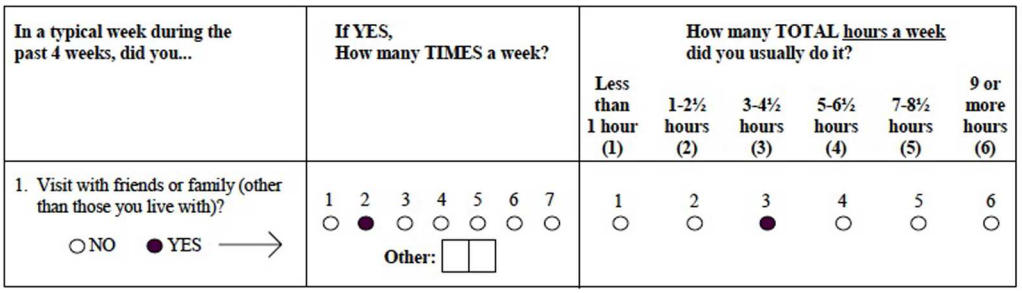


FIGURE 1 | Sample Community Healthy Activities Model Program for Seniors (CHAMPS) test item.

score below 30 and included conditioning training, yoga or tai chi, leisurely walking, walking to do errands, light gardening, light house work, golfing using a cart, and aerobic dancing. Moderate-heavy physical activities were those with a MET score equal to or above 30 and included sports, light or heavy strength training, swimming gently or fast, water exercises, working on aerobic machines, bicycling, fast walking, uphill walking or hiking, jogging or running, working on machinery (car, lawn mower, etc.), heavy gardening, heavy housework, singles or doubles tennis, golfing without use of a cart, dancing (such as square, folk, line, or ballroom), and skating (ice, roller, or in-line). Social activities were any for which the majority of time spent likely involved interpersonal interaction. These included visiting with family or friends, going to a senior center, volunteering, church-related activities, participating in clubs or groups, playing cards or board games with others, and shooting pool or billiards. Cognitive activities were those for which the majority of time spent was likely cognitively engaging rather than interpersonal. These included using a computer, doing arts or crafts, attending a concert, movie, lecture, or sport event, playing a musical instrument, and reading. If the "other" option was filled in, the activity described was placed in the most appropriate group. The integer measures (1 through 6) representing amount of time spent weekly in each activity were summed to create totals for light and moderate-heavy physical activities, social activities, and cognitive activities.

Magnetic Resonance Imaging

Acquisition

Magnetic resonance imaging data were acquired using a Philips Achieva 3.0 Tesla scanner (Achieva; Philips Electronics, Amsterdam, The Netherlands) at the McKnight Brain Institute (University of Florida, Gainesville, FL, USA) with a standard 32-channel receive-only head coil. High-resolution 3D T1-weighted MPRAGE scans were performed. Scans were acquired in a sagittal orientation with parameters as follows: voxel size = 1 mm isotropic; 1 mm slice thickness; TE = 3.2 ms; TR = 7.0 ms; FOV = 240 × 240; Number of slices = 170.

Analysis

T1-weighted MRIs were automatically segmented and volumes were calculated using FreeSurfer software, version 5.3.0, available at http://surfer.nmr.mgh.harvard.edu/ (Fischl et al., 2002). Following preprocessing, all results underwent quality control to confirm correct detection of gray and white matter. Any errors in segmentation were corrected manually and the T1 images were re-processed through FreeSurfer. Seventy one percentages of cases had some form of manual edit. These consisted of adding control points to extend the white matter boundary (55%), removing voxels from the brain mask (29%), and editing the white matter mask (6%). Some subjects had edits from more than one category, such as both control points and brain mask edits. Previous research has shown that this semi-automated procedure yields accurate and reliable results when compared to manual segmentation (Fischl et al., 2002; Jovicich et al., 2009; Morey et al., 2009) and histological measures (Morey et al., 2009). Automatically parcellated FreeSurfer gray matter regions of interest (ROIs) were based on the Desikan-Killiany atlas. Intracranial volume (ICV) was calculated based on the talairach transform (Buckner et al., 2004). ROIs for the left and right hemisphere were summed and corrected for ICV (by dividing ROI volume by ICV and multiplying by 100) to create bilateral, normalized ROIs. Some of these were then summed to create volumes for the four major lobes of the brain. Specifically, the frontal ROI consisted of the caudal and rostral anterior cingulate cortices, the caudal and rostral middle frontal cortices, the lateral and medial orbitofrontal cortices, the pars orbitalis, the superior frontal cortex, and the frontal pole; the parietal ROI consisted of the precuneus, the inferior and superior parietal lobules, and the supramarginal gyrus; the temporal ROI consisted of the entorhinal cortex, the inferior, middle, and superior temporal regions, the transverse temporal region, and the temporal pole; and the occipital ROI consisted of the lateral occipital region, the lingual and fusiform gyri, the pericalcarine region, and the cuneus. All four lobes were added together to create a measure of total cortical gray matter volume. Subcortical ROIs were chosen based on their relevance to cognitive and behavioral functioning and included the thalamus, caudate, hippocampus, and amygdala.

Statistical Analysis

All statistical analyses were performed using SPSS. Stepwise regression with backward selection was used to predict weekly time spent in either light physical, heavy physical, social, or cognitive activity using a cortical or subcortical ROI (corrected

by total ICV) as well as age, education, and gender as covariates. Individual regression analyses were conducted for each ROI. In stepwise regression with backward selection, all independent variables (predictors and covariates) are entered into the equation and sequentially removed based on the probability of F. The criterion used was $p \geq 0.10$. The first model for which all independent variables included explained significant variance in the dependent variable was chosen as the best model. The benefits of using this analytic method is that it allows for all variables to included, as it may be that a set of variables has better predictive validity than the subset, but it also removes those that may be falsely lowering the contribution of significant predictors by overlapping in variance explained. Thus, the final model efficiently explains the variance in the dependent variable and better identifies the unique contribution of the predictors.

RESULTS

Table 2 displays the percentage of ICV for cortical and subcortical regions in this sample. In regards to activity measures, scores do not refer directly to number of hours, but rather to ordinal measures reflecting roughly 1.5-h increments (**Figure 1**). Though it cannot be determined exactly how much time was spent in each of the activity categories, it appears that participants divided their time roughly evenly amongst light physical (mean = 8.7, SD = 5.1), heavy physical (mean = 8.4, SD = 5.7), social (mean = 10.5, SD = 6.0), and cognitive (mean = 11.3, SD = 4.5) activities.

Cognitive activity was the only outcome significantly associated with gray matter volume. **Table 3** lists these final, best-fitting models. Final models revealed a positive association with education ($p < 0.001$) and all cortical and subcortical ROIs examined ($ps < 0.05$). **Figure 2** depicts the relationships between cognitive activity and brain volumes, controlling for education. In each regression, variables excluded were age and sex.

In contrast, there were no significant associations observed between the volumes of these brain regions and engagement in light physical, heavy physical, or social activities as reported on the CHAMPS. More heavy physical activity was associated with younger age and male gender, whereas other activities were not associated with age or sex. Supplemental material includes these results.

TABLE 2 | Gray matter regions of interest presented as percentages of total intracranial volume.

Region of Interest	Mean (SD)	Range
Frontal lobe	7.83 (1.19)	6.18–11.52
Parietal lobe	5.77 (0.82)	4.34–8.35
Temporal lobe	4.75 (0.72)	3.48–6.94
Occipital lobe	4.43 (0.73)	3.43–6.33
Total cortex	22.78 (3.35)	18.14–32.43
Thalamus	0.91 (0.15)	0.71–1.35
Caudate	0.47 (0.08)	0.37–0.71
Hippocampus	0.54 (0.13)	0.30–1.01
Amygdala	0.22 (0.05)	0.13–0.37

DISCUSSION

In this study, we found that greater time dedicated to cognitively engaging activities was associated with greater cortical and subcortical brain volume. We did not find significant associations with physical or social activity level and volumetric data. Cognitive engagement activities represent one of three potential proxies for the construct of cognitive reserve. Epidemiologic-based support for the association between leisure and cognitive status has been accumulating for nearly a quarter of a century (Christensen and Mackinnon, 1993). In an effort to clarify causal attributions or directionality, other epidemiologic work has used sophisticated dynamic change models to show that *change* in cognitive leisure participation can result in higher scores on cognitive ability measures (Mitchell et al., 2012). Such evidence has sparked experimental investigations, with clinical trials demonstrating improved cognition over short periods (3–6 months) for those participating in cognitive leisure activities compared to controls (Stine-Morrow et al., 2008).

More recently, investigators have begun to examine imaging and volumetric evidence, showing that one's level of cognitive reserve is associated with brain volume. For instance, it has been shown that a more active cognitive lifestyle is associated with greater frontal and parietal brain volume in healthy older adults (Stine-Morrow et al., 2008). Our finding that higher cognitive engagement is associated with greater hippocampal volume was also reported in a longitudinal study (Valenzuela et al., 2008). Valenzuela et al. (2008) used the Lifetime of Experiences Questionnaire (LEQ), in which sample activities included creative arts, reading, writing, and socializing. The investigators reported a significant association between total LEQ and average hippocampal volume, controlling for age, gender, hypertension, and ICV. High LEQ individuals experienced an average loss of 3.6% of hippocampal volume over a 3-year period, while low LEQ individuals exhibited more than twice this volumetric loss (8.3%). It is important to note that these studies used a composite measure to establish these associations, which, in addition to cognitive engagement activities, also included education and job complexity. Yet cognitive engagement or cognitive leisure activities show unique contributions to cognitive health, independent of the variance attributed to education and occupational status (Stern, 2009; Foubert-Samier et al., 2012). Indeed, in the current investigation, the relationship between more involvement in cognitively engaging leisure activities and greater brain volumes was not explained by education.

Findings from the current study are similar to those of another observational study in which greater frequency of cognitive leisure activities (playing games like cards, checkers, crosswords, or other puzzles) was related to better cognitive performance and reduced brain atrophy (Schultz et al., 2015). Schultz et al. (2015) reported that higher activity scores were associated with greater gray matter volumes in several ROIs including the hippocampus, posterior cingulate, anterior

TABLE 3 | Best-fitting models predicting cognitive activity from gray matter region of interest, age, sex, and education.

Model/ROI	Variable	β	p	R^2	Model p	Excluded Variables
Frontal lobe	Frontal lobe	0.276	0.019*	0.247	<0.001*	Age
	Education	0.493	<0.001*			Sex
Parietal lobe	Parietal lobe	0.305	0.009*	0.262	<0.001*	Age
	Education	0.505	<0.001*			Sex
Temporal lobe	Temporal lobe	0.275	0.020*	0.246	<0.001*	Age
	Education	0.501	<0.001*			Sex
Occipital lobe	Occipital lobe	0.256	0.030*	0.237	<0.001*	Age
	Education	0.490	<0.001*			Sex
Total cortex	Total cortex	0.289	0.014*	0.253	<0.001*	Age
	Education	0.502	<0.001*			Sex
Thalamus	Thalamus	0.310	0.010*	0.261	<0.001*	Age
	Education	0.526	<0.001*			Sex
Caudate	Caudate	0.233	0.049*	0.227	<0.001*	Age
	Education	0.482	<0.001*			Sex
Hippocampus	Hippocampus	0.286	0.017*	0.250	<0.001*	Age
	Education	0.513	<0.001*			Sex
Amygdala	Amygdala	0.336	0.004*	0.280	<0.001*	Age
	Education	0.519	<0.001*			Sex

*ROI, region of interest. *$p < 0.05$.*

cingulate, and middle frontal gyrus. However, our findings differ from a longitudinal study that found no relationship between cognitive activity and MRI measures of whole brain volumes of gray and white matter (Vaughan et al., 2014). One potential reason for a lack of association in the Vaughan et al. (2014) study may be related to the items included in their 6-item measurement of cognitive activities. Like in the present study, they included reading and craft activities, but they also included group activities, social activities, and watching television. It is possible that group and social activities present with the local dependence, or relatedness; local independence, or unrelatedness, of items is a modern test theory assumption that, when violated, negatively impacts construct validity. Furthermore, television use has been shown to be negatively correlated with other cognitive activity items (Gow et al., 2012b; Fieo et al., 2014). Television time has also been associated with less frequent engagement in social and physical recreation (Hu et al., 2003) and increased risk for dementia (Rundek and Bennett, 2006). These factors may have reduced the power for detecting associations in the Vaughan et al. (2014) study. The current findings, combined with prior research in cognitive leisure activity, reinforce the relationship between hippocampal and cortical volumes and cognitive activity, consistent with our hypothesis.

It is somewhat surprising that heavy physical activity was not associated with volumetric data, as studies examining exercise effects on regional brain volume typically implicate frontal and hippocampal brain areas (Colcombe et al., 2006; Pereira et al., 2007; Erickson et al., 2011). Part of this discrepancy undoubtedly comes from the differences between intervention and observational studies. Intervention studies inherently involve introducing something novel in the lives of research participants. It may be that the amount of novelty an activity offers is more important than the type of activity itself in impacting cognitive and neurological functioning (Bielak, 2010). Thus, when intervention studies implement a physical activity regimen in sedentary adults (Colcombe et al., 2006), the novelty of the activity and significant change in lifestyle may very well impact brain structure.

Part of the discrepancy between the current findings and results from previous physical activity studies may be due to differences in methodology for measuring physical activity. The CHAMPS questionnaire asked participants to retrospectively indicate their levels of physical activity in a variety of class categories for a typical week over the previous month. Given the number of parameters that had to be remembered, it is possible that participants gave unreliable reports of their own activity (LaPorte et al., 1985). Indeed, Buchman et al. (2008) showed that objectively measured physical activity was associated with cognitive function, whereas self-reported daily physical activity in the same group was not. However, an evaluation of the CHAMPS questionnaire revealed a moderately strong correlation between self-reported engagement in moderate physical activity and activity objectively measured by a waist monitor ($r = 0.48$) (Harada et al., 2001). Furthermore, other self-report studies have shown associations between physical activity levels and cortical volumes (Erickson et al., 2010; Bugg and Head, 2011; Gow et al., 2012a; Benedict et al., 2013). As such, it is unlikely that the lack of association is entirely explained by the self-report nature of the physical activity measurement.

Our findings are consistent with Gow et al. (2012a), who measured baseline physical activity via self-report at age 70 years and administered MRIs at age 73. They found physical activity to be associated with white matter volume, but not gray matter volume. Thus, lack of findings in the

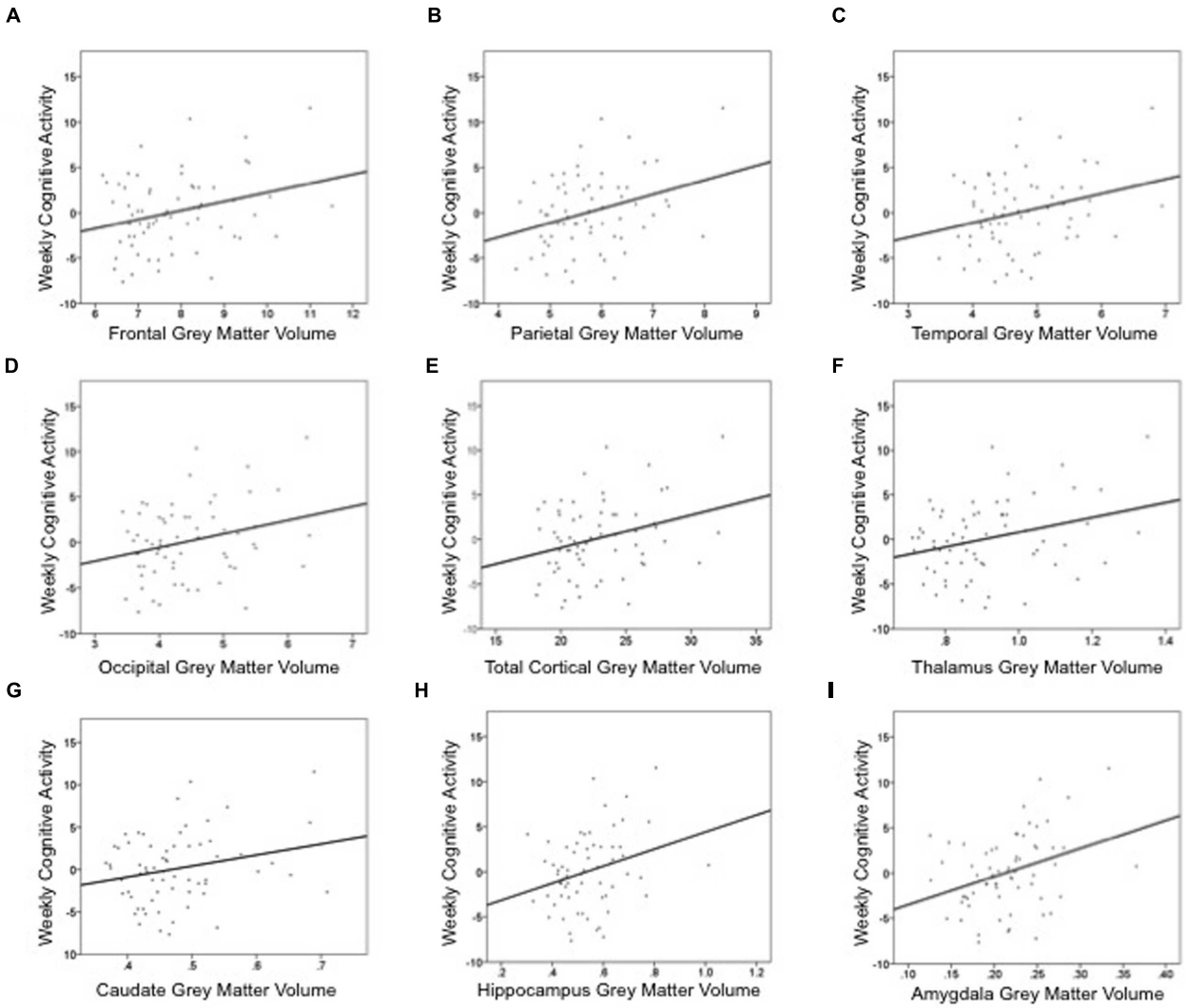


FIGURE 2 | Summed measures of weekly time spent in cognitively engaging activity are positively associated cortical and subcortical gray matter volumes: (A) frontal lobe, (B) parietal lobe, (C) temporal lobe, (D) occipital lobe, (E) total cortex, (F) thalamus, (G) caudate, (H) hippocampus, and (I) amygdala. These graphs depict the relationship between gray matter volume and cognitive activity, controlling for education. Thus, the *y*-axis is the unstandardized residual after regressing the effects of education out of cognitive activity. Gray matter volume is a percentage of intracranial volume.

present study may reflect the fact that only gray matter volume was measured. Given that the current findings are inconsistent with a large body of research on the impact of physical activity on brain volumes, we do not feel comfortable rejecting our hypothesis that greater physical activity is associated with greater volumes, particularly in frontal and limbic regions.

Social activity is more tenuously linked to brain volume than are physical and cognitive activity, and the relationship was not supported in the current investigation. Greater social involvement has been associated with greater normal-appearing white matter (Gow et al., 2012a), total brain (Mortimer et al., 2012), and gray matter (James et al., 2012) volumes. Yet methodological differences may explain discrepancies between prior results and our own.

Gow et al. (2012a) combined cognitive and social leisure activities when measuring the link between activity and brain structure. While greater cognitive and social activity was related to more normal-appearing white matter, these activities were not found to be associated with gray matter volume, consistent with the present data. Whether social activity has a stronger relationship to white matter than to gray matter volume deserves further investigation.

Mortimer et al. (2012) performed an intervention study and found that their social interaction groups showed significant increases in total brain volume over the study period. The fact that this study was an intervention may explain why their results differ from the current study, since, as previously discussed, the amount of novelty provided by an intervention may be a significant driver of results (Bielak, 2010). The intervention

group met three times per week at a neighborhood community center, the participants decided on their own to organize and select topics of conversation, and the discussions were described as extremely lively. Thus, lifestyle was significantly impacted in these participants.

James et al. (2012) demonstrated that higher social engagement was associated with greater total brain volume and total gray matter volume, as well as greater temporal and occipital gray matter lobar volumes. Yet methodological differences might also explain the discrepancies between their results and our own. The James et al. study included a question more related to physical than social activity in their eight-item measure of social engagement, as they asked participants how many times in the past week/month they had done any indoor or outdoor recreational activity like bowling, working out, fishing, hiking, boating, swimming, or golfing. Given that physical activity has a more robust association to gray matter volume than does social activity, responses to this question may have been a strong driver of results. Another important difference is in the study samples; the James sample was, for the most part, comprised of former lead workers who were recruited for a study of lead exposure and cognitive function. They were less socially engaged than population-based controls, who comprised 12% of the sample. Not only were the participants in the current study recruited from the same community population, but the sample size was significantly smaller compared to the James et al. sample of 348. Thus, differences in population and sample size may have given James et al. more statistical power to detect any relationship between the measured activities and gray matter volume.

Our null findings for social activity are similar to those of Foubert-Samier et al. (2012), who assessed activity in midlife and in retirement. They asked participants whether or not they engaged in a number of activities during midlife (when they worked) and currently (in retirement) and assigned one point for each activity. Social activity in both midlife and retirement was related to better semantic verbal fluency, but unrelated to total brain, gray matter, or white matter volumes.

Current findings are also consistent with Van der Vegt (2015), who found no association between social activity with family and friends and brain volume (Van der Vegt, 2015). In that study, participants were asked two questions: "In general, how often do you have contact with your family members (including telephone calls or letters)?" and, "In general, how often do you have contact with your friends or well-known acquaintances (including telephone calls or letters)?" Interestingly, factor analytic methods have shown that social activity can be presented as a bi-factor model: social-private and social-public (Jopp and Hertzog, 2010). Additionally, social-private was more highly correlated with cognitive functioning measures. As such, social-private may have a stronger relationship with brain volume than social private, and combining separable constructs may negatively impact predictive validity. The association between different types of social activity and volumetric data of cortical and subcortical regions warrants further study. Nevertheless, considering prior research and the results of the current study, we cannot support our hypothesis that social activity is associated with greater gray matter volumes in temporal and occipital regions.

As mentioned previously, novelty may be an important driving factor in the relationship between leisure activities that are engaging, cognitively or otherwise, and larger brain volumes. Animal models have shown that, in aged animals, environmental enrichment attenuates the age-related changes in cortical thickness (Mora et al., 2007). A defining feature of enrichment in animals is often novelty. For instance, repeatedly substituting and replacing the objects in the home cages creates a wide range of opportunities for enhanced cognitive stimulation, formation of tuned spatial maps, and proficient detection of novelty (Petrosini et al., 2009). In humans, one theoretical explanation for how novelty exerts a protective effect in older adults relates to non-adaptive "routinization" (Tournier et al., 2012). That is, as older adults continue to accumulate age-related insults (e.g., medical comorbidities or muscle related fatigue), some individuals may seek out more controlled, stable, predictive environments, thus limiting exposure to novel environments or experiences. Evidence in support of this can be found in a Bergua et al. (2006) study, demonstrating that preferences for routine were positively correlated with cognitive decline over 3 years. Additional support can be observed in the finding that greater variety of participation in cognitively stimulating activities was associated a ∼10% lower risk of cognitive impairment, regardless of how challenging these tasks were (Carlson et al., 2012). As such, though novel environments and situations may be more difficult to navigate as people age, they may be the most protective against neurological decline.

Limitations

The most common grouping scheme for investigating the efficacy of leisure activities has been the broad domains of physical, social activities, and other cognitive leisure activities, which is what we followed for this investigation. However, with such broad domains, many activities cannot be assigned unambiguously. Volunteering work, for instance, is likely to have a strong social component, but may in some instances entail more non-verbal cognitive or physical effort. Ideally, large item/activity pools would allow for the formation of more distinct, unidimensional activity constructs, for example, further delineating social activities into social-private and social-public (Jopp and Hertzog, 2010). Furthermore, historically, the evidence in observational studies, including the present study, has been limited to an examination of frequency of participation. However, contemporary conversations have moved beyond questioning the frequency of participation, placing greater emphasis on novelty/variety and cognitive challenge (Bielak, 2010; Chan et al., 2014). Finally, as previously mentioned, the accuracy of measurement in self-report questionnaires is questionable, as participants may have subjective biases and may, intentionally or unintentionally, misrepresent their objective activity levels (Erickson et al., 2012). Nevertheless, CHAMPS has several strengths, including robust psychometric properties (Harada et al., 2001; Wilcox et al., 2009), sensitivity to change

(Stewart et al., 1997), and correlations with objective measures of physical activity and functioning (King et al., 2000; Harada et al., 2001; Stewart et al., 2001).

CONCLUSION

Results of this study emphasize the relationship between volumetric data and the independent contribution of cognitively engaging activities. This process is of considerable value if we consider that cognitive leisure activities show unique contributions to cognitive health, independent of the variance attributed to other cognitive reserve proxies (e.g., education and occupational status) (Stern, 2009; Foubert-Samier et al., 2012). Results of the present study show that more time spent engaged in cognitive activity is associated with greater brain volume. Importantly, these lifestyle variables are modifiable, suggesting that interventions aimed at increasing levels of cognitive activity may delay onset or decrease risk of developing dementia. In fact, intervention studies have shown positive neurological effects of cognitive interventions. Still, the field of cognitive aging will benefit from more research investigating interventions aimed at increasing everyday cognitively engaging activities as well as the role of other biomarkers such as inflammatory agents and genetic factors in influencing brain structure and function.

AUTHOR CONTRIBUTIONS

TS was involved in the conception and writing of the manuscript and conducted statistical analyses. RF was involved in the writing of the manuscript. AO conducted volumetric analyses that were used in the manuscript. EP, AW, and RC were involved in the conception of the study, and RC was involved in the conception of the manuscript and data analysis. All authors contributed intellectual content and approved the version to be published.

ACKNOWLEDGMENT

This work was supported by an endowment from the McKnight Brain Research Foundation to the University of Florida Center for Cognitive Aging and Memory. This work was also supported by T32 AG020499 (TS).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2016.00094

REFERENCES

Ainsworth, B. E., Haskell, W. L., Leon, A. S., Jacobs, D. R. Jr., Montoye, H. J., Sallis, J. F., et al. (1993). Compendium of physical activities: classification of energy costs of human physical activities. *Med. Sci. Sports Exerc.* 25, 71–80. doi: 10.1249/00005768-199301000-00011

Alzheimer's Association (2015). Alzheimer's disease facts and figures. *Alzheimer's Dement.* 11, 332–384.

Barnes, L. L., Mendes de Leon, C. F., Wilson, R. S., Bienias, J. L., and Evans, D. A. (2004). Social resources and cognitive decline in a population of older African Americans and whites. *Neurology* 63, 2322–2326. doi: 10.1212/01.WNL.0000147473.04043.B3

Benedict, C., Brooks, S. J., Kullberg, J., Nordenskjold, R., Burgos, J., Le Greves, M., et al. (2013). Association between physical activity and brain health in older adults. *Neurobiol. Aging* 34, 83–90. doi: 10.1016/j.neurobiolaging.2012.04.013

Bergua, V., Fabrigoule, C., Barberger-Gateau, P., Dartigues, J. F., Swendsen, J., and Bouisson, J. (2006). Preferences for routines in older people: associations with cognitive and psychological vulnerability. *Int. J. Geriatr. Psychiatry* 21, 990–998. doi: 10.1002/gps.1597

Bielak, A. A. (2010). How can we not 'lose it' if we still don't understand how to 'use it'? Unanswered questions about the influence of activity participation on cognitive performance in older age–a mini-review. *Gerontology* 56, 507–519. doi: 10.1159/000264918

Buchman, A. S., Wilson, R. S., and Bennett, D. A. (2008). Total daily activity is associated with cognition in older persons. *Am. J. Geriatr. Psychiatry* 16, 697–701. doi: 10.1097/JGP.0b013e31817945f6

Buckner, R. L., Head, D., Parker, J., Fotenos, A. F., Marcus, D., Morris, J. C., et al. (2004). A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *Neuroimage* 23, 724–738. doi: 10.1016/j.neuroimage.2004.06.018

Bugg, J. M., and Head, D. (2011). Exercise moderates age-related atrophy of the medial temporal lobe. *Neurobiol. Aging* 32, 506–514. doi: 10.1016/j.neurobiolaging.2009.03.008

Carlson, M. C., Parisi, J. M., Xia, J., Xue, Q. L., Rebok, G. W., Bandeen-Roche, K., et al. (2012). Lifestyle activities and memory: variety may be the spice of life. The women's health and aging study II. *J. Int. Neuropsychol. Soc.* 18, 286–294. doi: 10.1017/S135561771100169X

Chan, M. Y., Haber, S., Drew, L. M., and Park, D. C. (2014). Training older adults to use tablet computers: does it enhance cognitive function? *Gerontologist* doi: 10.1093/geront/gnu057 [Epub ahead of print].

Christensen, H., and Mackinnon, A. (1993). The association between mental, social and physical activity and cognitive performance in young and old subjects. *Age Ageing* 22, 175–182. doi: 10.1093/ageing/22.3.175

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., et al. (2006). Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. A Biol. Sci. Med. Sci.* 61, 1166–1170. doi: 10.1093/gerona/61.11.1166

Doi, T., Makizako, H., Shimada, H., Tsutsumimoto, K., Hotta, R., Nakakubo, S., et al. (2015). Objectively measured physical activity, brain atrophy, and white matter lesions in older adults with mild cognitive impairment. *Exp. Gerontol.* 62, 1–6. doi: 10.1016/j.exger.2014.12.011

Draganski, B., Gaser, C., Kempermann, G., Kuhn, H. G., Winkler, J., Buchel, C., et al. (2006). Temporal and spatial dynamics of brain structure changes during extensive learning. *J. Neurosci.* 26, 6314–6317. doi: 10.1523/JNEUROSCI.4628-05.2006

Engvig, A., Fjell, A. M., Westlye, L. T., Skaane, N. V., Dale, A. M., Holland, D., et al. (2014). Effects of cognitive training on gray matter volumes in memory clinic patients with subjective memory impairment. *J. Alzheimers Dis.* 41, 779–791. doi: 10.3233/JAD-131889

Erickson, K. I., Raji, C. A., Lopez, O. L., Becker, J. T., Rosano, C., Newman, A. B., et al. (2010). Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study. *Neurology* 75, 1415–1422. doi: 10.1212/WNL.0b013e3181f88359

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3017–3022. doi: 10.1073/pnas.1015950108

Erickson, K. I., Weinstein, A. M., and Lopez, O. L. (2012). Physical activity, brain plasticity, and Alzheimer's disease. *Arch. Med. Res.* 43, 615–621. doi: 10.1016/j.arcmed.2012.09.008

Fieo, R., Manly, J. J., Schupf, N., and Stern, Y. (2014). Functional status in the young-old: establishing a working prototype of an extended-instrumental activities of daily living scale. *J. Gerontol. A Biol. Sci. Med. Sci.* 69, 766–772. doi: 10.1093/gerona/glt167

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. doi: 10.1016/S0896-6273(02)00569-X

Foubert-Samier, A., Catheline, G., Amieva, H., Dilharreguy, B., Helmer, C., Allard, M., et al. (2012). Education, occupation, leisure activities, and brain reserve: a population-based study. *Neurobiol. Aging* 33, 423.e15–423.e25. doi: 10.1016/j.neurobiolaging.2010.09.023

Fratiglioni, L., Paillard-Borg, S., and Winblad, B. (2004). An active and socially integrated lifestyle in late life might protect against dementia. *Lancet Neurol.* 3, 343–353. doi: 10.1016/S1474-4422(04)00767-7

Gow, A. J., Bastin, M. E., Munoz Maniega, S., Valdes Hernandez, M. C., Morris, Z., Murray, C., et al. (2012a). Neuroprotective lifestyles and the aging brain: activity, atrophy, and white matter integrity. *Neurology* 79, 1802–1808. doi: 10.1212/WNL.0b013e3182703fd2

Gow, A. J., Corley, J., Starr, J. M., and Deary, I. J. (2012b). Reverse causation in activity-cognitive ability associations: the Lothian Birth Cohort 1936. *Psychol. Aging* 27, 250–255. doi: 10.1037/a0024144

Harada, N. D., Chiu, V., King, A. C., and Stewart, A. L. (2001). An evaluation of three self-report physical activity instruments for older adults. *Med. Sci. Sports Exerc.* 33, 962–970. doi: 10.1097/00005768-200106000-00016

Hertzog, C., Kramer, A. F., Wilson, R. S., and Lindenberger, U. (2009). Enrichment effects on adult cognitive development: can the functional capacity of older adults be preserved and enhanced? *Psychol. Sci. Public Interest* 9, 1–65. doi: 10.1111/j.1539-6053.2009.01034.x

Hu, F. B., Li, T. Y., Colditz, G. A., Willett, W. C., and Manson, J. E. (2003). Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 289, 1785–1791. doi: 10.1001/jama.289.14.1785

Imtiaz, B., Tolppanen, A. M., Kivipelto, M., and Soininen, H. (2014). Future directions in Alzheimer's disease from risk factors to prevention. *Biochem. Pharmacol.* 88, 661–670. doi: 10.1016/j.bcp.2014.01.003

James, B. D., Glass, T. A., Caffo, B., Bobb, J. F., Davatzikos, C., Yousem, D., et al. (2012). Association of social engagement with brain volumes assessed by structural MRI. *J. Aging Res.* 2012, 512714. doi: 10.1155/2012/512714

Jopp, D. S., and Hertzog, C. (2010). Assessing adult leisure activities: an extension of a self-report activity questionnaire. *Psychol. Assess.* 22, 108–120. doi: 10.1037/a0017662

Jovicich, J., Czanner, S., Han, X., Salat, D., van der Kouwe, A., Quinn, B., et al. (2009). MRI-derived measurements of human subcortical, ventricular and intracranial brain volumes: reliability effects of scan sessions, acquisition sequences, data analyses, scanner upgrade, scanner vendors and field strengths. *Neuroimage* 46, 177–192. doi: 10.1016/j.neuroimage.2009.02.010

King, A. C., Pruitt, L. A., Phillips, W., Oka, R., Rodenburg, A., and Haskell, W. L. (2000). Comparative effects of two physical activity programs on measured and perceived physical functioning and other health-related quality of life outcomes in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 55, M74–M83. doi: 10.1093/gerona/55.2.M74

Kramer, A. F., Bherer, L., Colcombe, S. J., Dong, W., and Greenough, W. T. (2004). Environmental influences on cognitive and brain plasticity during aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 59, M940–M957. doi: 10.1093/gerona/59.9.M940

LaPorte, R. E., Montoye, H. J., and Caspersen, C. J. (1985). Assessment of physical activity in epidemiologic research: problems and prospects. *Public Health Rep.* 100, 131–146.

McGue, M., and Christensen, K. (2007). Social activity and healthy aging: a study of aging Danish twins. *Twin Res. Hum. Genet.* 10, 255–265. doi: 10.1375/twin.10.2.255

Mitchell, M. B., Cimino, C. R., Benitez, A., Brown, C. L., Gibbons, L. E., Kennison, R. F., et al. (2012). Cognitively stimulating activities: effects on cognition across four studies with up to 21 years of longitudinal data. *J. Aging Res.* 2012, 461592. doi: 10.1155/2012/461592

Mora, F., Segovia, G., and del Arco, A. (2007). Aging, plasticity and environmental enrichment: structural changes and neurotransmitter dynamics in several areas of the brain. *Brain Res. Rev.* 55, 78–88. doi: 10.1016/j.brainresrev.2007.03.011

Morey, R. A., Petty, C. M., Xu, Y., Hayes, J. P., Wagner, H. R. II, Lewis, D. V., et al. (2009). A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *Neuroimage* 45, 855–866. doi: 10.1016/j.neuroimage.2008.12.033

Mortimer, J. A., Ding, D., Borenstein, A. R., DeCarli, C., Guo, Q., Wu, Y., et al. (2012). Changes in brain volume and cognition in a randomized trial of exercise and social interaction in a community-based sample of non-demented Chinese elders. *J. Alzheimers Dis.* 30, 757–766. doi: 10.3233/JAD-2012-120079

Newson, R. S., and Kemps, E. B. (2005). General lifestyle activities as a predictor of current cognition and cognitive change in older adults: a cross-sectional and longitudinal examination. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 60, 113–120. doi: 10.1093/geronb/60.3.P113

Opdebeeck, C., Martyr, A., and Clare, L. (2016). Cognitive reserve and cognitive function in healthy older people: a meta-analysis. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 23, 40–60. doi: 10.1080/13825585.2015.1041450

Pereira, A. C., Huddleston, D. E., Brickman, A. M., Sosunov, A. A., Hen, R., McKhann, G. M., et al. (2007). An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5638–5643. doi: 10.1073/pnas.0611721104

Petrosini, L., De Bartolo, P., Foti, F., Gelfo, F., Cutuli, D., Leggio, M. G., et al. (2009). On whether the environmental enrichment may provide cognitive and brain reserves. *Brain Res. Rev.* 61, 221–239. doi: 10.1016/j.brainresrev.2009.07.002

Rovio, S., Spulber, G., Nieminen, L. J., Niskanen, E., Winblad, B., Tuomilehto, J., et al. (2010). The effect of midlife physical activity on structural brain changes in the elderly. *Neurobiol. Aging* 31, 1927–1936. doi: 10.1016/j.neurobiolaging.2008.10.007

Rundek, T., and Bennett, D. A. (2006). Cognitive leisure activities, but not watching TV, for future brain benefits. *Neurology* 66, 794–795. doi: 10.1212/01.wnl.0000209497.38834.d7

Schultz, S. A., Larson, J., Oh, J., Koscik, R., Dowling, M. N., Gallagher, C. L., et al. (2015). Participation in cognitively-stimulating activities is associated with brain structure and cognitive function in preclinical Alzheimer's disease. *Brain Imaging Behav.* 9, 729–736. doi: 10.1007/s11682-014-9329-5

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia* 47, 2015–2028. doi: 10.1016/j.neuropsychologia.2009.03.004

Stewart, A. L., Mills, K. M., King, A. C., Haskell, W. L., Gillis, D., and Ritter, P. L. (2001). CHAMPS physical activity questionnaire for older adults: outcomes for interventions. *Med. Sci. Sports Exerc.* 33, 1126–1141. doi: 10.1097/00005768-200107000-00010

Stewart, A. L., Mills, K. M., Sepsis, P. G., King, A. C., McLellan, B. Y., Roitz, K., et al. (1997). Evaluation of CHAMPS, a physical activity promotion program for older adults. *Ann. Behav. Med.* 19, 353–361. doi: 10.1007/BF02895154

Stine-Morrow, E. A., Parisi, J. M., Morrow, D. G., and Park, D. C. (2008). The effects of an engaged lifestyle on cognitive vitality: a field experiment. *Psychol. Aging* 23, 778–786. doi: 10.1037/a0014341

Tournier, I., Mathey, S., and Postal, V. (2012). The association between routinization and cognitive resources in later life. *Int. J. Aging Hum. Dev.* 74, 143–161. doi: 10.2190/AG.74.2.c

Valenzuela, M. J., Sachdev, P., Wen, W., Chen, X., and Brodaty, H. (2008). Lifespan mental activity predicts diminished rate of hippocampal atrophy. *PLoS ONE* 3:e2598. doi: 10.1371/journal.pone.0002598

Van der Vegt, E. (2015). *Social Activity and Brain Volume, White Matter Lesions and Cognitive Function. Are Low Levels of Social Activity Associated with Brain Abnormalities Observed on MRI and with Poor Cognitive Function?* Masters thesis, Utrecht University, Utrecht.

Vaughan, L., Erickson, K. I., Espeland, M. A., Smith, J. C., Tindle, H. A., and Rapp, S. R. (2014). Concurrent and longitudinal relationships between cognitive activity, cognitive performance, and brain volume in older adult

women. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 69, 826–836. doi: 10.1093/geronb/gbu109

Vemuri, P., Lesnick, T. G., Przybelski, S. A., Knopman, D. S., Roberts, R. O., Lowe, V. J., et al. (2012). Effect of lifestyle activities on Alzheimer disease biomarkers and cognition. *Ann. Neurol.* 72, 730–738. doi: 10.1002/ana.23665

Wilcox, S., Dowda, M., Dunn, A., Ory, M. G., Rheaume, C., and King, A. C. (2009). Predictors of increased physical activity in the Active for Life program. *Prev. Chronic Dis.* 6, A25.

Williams, J. W., Plassman, B. L., Burke, J., and Benjamin, S. (2010). Preventing Alzheimer's disease and cognitive decline. *Evid. Rep. Technol. Assess. (Full Rep.)* 193, 1–727.

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 27 May 2016
doi: 10.3389/fnagi.2016.00122

Age-Related Differences in Associative Learning of Landmarks and Heading Directions in a Virtual Navigation Task

*Jimmy Y. Zhong and Scott D. Moffat**

School of Psychology, Georgia Institute of Technology, Atlanta, GA, USA

Previous studies have showed that spatial memory declines with age but have not clarified the relevance of different landmark cues for specifying heading directions among different age groups. This study examined differences between younger, middle-aged and older adults in route learning and memory tasks after they navigated a virtual maze that contained: (a) critical landmarks that were located at decision points (i.e., intersections) and (b) non-critical landmarks that were located at non-decision points (i.e., the sides of the route). Participants were given a recognition memory test for critical and non-critical landmarks and also given a landmark-direction associative learning task. Compared to younger adults, older adults committed more navigation errors during route learning and were poorer at associating the correct heading directions with both critical and non-critical landmarks. Notably, older adults exhibited a landmark-direction associative memory deficit at decision points; this was the first finding to show that an associative memory deficit exist among older adults in a navigational context for landmarks that are pertinent for reaching a goal, and suggest that older adults may expend more cognitive resources on the encoding of landmark/object features than on the binding of landmark and directional information. This study is also the first to show that older adults did not have a tendency to process non-critical landmarks, which were regarded as distractors/irrelevant cues for specifying the directions to reach the goal, to an equivalent or larger extent than younger adults. We explain this finding in view of the low number of non-critical cues in our virtual maze (relative to a real-world urban environment) that might not have evoked older adults' usual tendency toward processing or encoding distractors. We explain the age differences in navigational and cognitive performance with regards to functional and structural changes in the hippocampus and parahippocampus, and recommend further investigations into the functional connectivity between the prefrontal cortex and hippocampus for a better understanding of the landmark-direction associative learning among the elderly. Finally, it is hoped that the current behavioral findings will facilitate efforts to identify the neural markers of Alzheimer's disease, a disease that commonly involves navigational deficits.

Keywords: age-related differences, spatial navigation, route learning, landmark recognition, associative learning, associative memory deficit

OPEN ACCESS

Edited by:
Ronald Cohen,
University of Florida, USA

Reviewed by:
Vidyaramanan Ganesan,
Rowan University, USA
Umesh Gangishetti,
Emory University, USA

***Correspondence:**
Scott D. Moffat
scott.moffat@psych.gatech.edu

Received: *21 March 2016*
Accepted: *12 May 2016*
Published: *27 May 2016*

Citation:
Zhong JY and Moffat SD (2016) Age-Related Differences in Associative Learning of Landmarks and Heading Directions in a Virtual Navigation Task. Front. Aging Neurosci. 8:122. doi: 10.3389/fnagi.2016.00122

INTRODUCTION

Age-related decline in spatial navigation skills, which refers to the ability to learn and navigate in large-scale spaces, has been documented by many studies in both real-world (e.g., Kirasic, 1991; Wilkniss et al., 1997) and virtual environments (VE; e.g., Moffat et al., 2001; Driscoll et al., 2003; Head and Isom, 2010; Harris and Wolbers, 2012, 2014). Behavioral assessments of age-related deficits in navigational ability generally showed that non-demented elderly adults are less proficient than younger adults at learning novel routes (Wilkniss et al., 1997; Moffat et al., 2001), forming and utilizing an allocentric cognitive map for navigating three-dimensional environments (Moffat and Resnick, 2002; Iaria et al., 2009; Wiener et al., 2013), path integration (Mahmood et al., 2009; Adamo et al., 2012; Harris and Wolbers, 2012) and associating landmarks to specific locations or places (Newman and Kaszniak, 2000; Head and Isom, 2010).

Despite these developments, there has been relatively little research examining age-related differences in the associative learning of landmarks and heading directions during route navigation. Utilizing a virtual maze, Head and Isom (2010) showed that younger adults outperformed older adults on a test of landmark-direction knowledge that required the recall of heading directions leading to the finishing point in the maze. The authors suggested that an associative memory deficit (Naveh-Benjamin, 2000; Old and Naveh-Benjamin, 2008) explained older adults' poorer binding of landmark and direction information. However, the specific details of this potential age-related deficit in associative memory require further investigation as memory for direction-relevant landmarks (i.e., landmarks located at intersections) and direction-irrelevant landmarks (i.e., landmarks located at the sides of one-way passageways) was not assessed separately. Thus, the extent to which younger and older adults differ in the representations of landmark-direction associations for landmarks located at different navigation sites remains unknown.

The investigation of landmarks with regards to differences in their navigational or directional relevance is important because studies have shown that not all landmarks in the environment are perceived and processed in the same fashion. Landmarks that are situated at turns and intersections on a map have been shown to be described more often by people when giving directions of travel to different places (Ward et al., 1986; Blades and Medlicott, 1992) and rated as more important for wayfinding (Lipman, 1991; Daniel and Denis, 1998) than landmarks that are located at the periphery of a route. Assessments of landmark knowledge have also shown that landmarks that are adjacent to correct turns (i.e., turns which lead to the goal point) are better recalled than landmarks that are adjacent to incorrect turns (i.e., turns leading into dead ends) and located by the sides of straight pathways (Cohen and Schuepfer, 1980; Jansen-Osmann, 2002; Jansen-Osmann and Wiedenbauer, 2004; Jansen et al., 2010). Furthermore, in the learning of a route in a virtual maze, Janzen (2006) showed that landmarks/objects located at intersections and turning points (i.e., decision points) were recognized faster than landmarks located at the sides of straight route segments (i.e., non-decision points), and suggested that the differences in recognition time could be attributed to differences in the mental representations associated with the two types of landmarks for navigation purposes.

Previous studies have not examined age group differences in the use of different types of landmark cues for specifying heading directions. Therefore, in this study, we examined and compared navigational and cognitive task performance between younger, middle-aged and older adults in a virtual maze that contained two types of landmark cues: (i) *critical* cues located at decision points (i.e., intersections) where one has to decide on the proper direction to take from several directional options; and (ii) *non-critical* cues located at non-decision points (i.e., along one-way passageways) where deliberate decisions about left- and right-turning directions are not required. In contrast to the critical landmarks which were directly pertinent for learning the right turns to make in order to reach the goal, we regard non-critical landmarks as navigationally irrelevant or even distracting cues that did not facilitate directional decision-making toward the goal. As numerous studies have demonstrated older adults to be prone toward the processing (e.g., Connelly et al., 1991; Duchek et al., 1998; May, 1999; McGinnis, 2012) and preservation of distracting lexical and semantic information compared to younger adults (e.g., Bell et al., 2008; Campbell et al., 2010; Amer and Hasher, 2014), we were further interested to investigate whether older adults would exhibit the same distractor processing tendency in a navigational context, leading to the prediction that older adults would encode/recognize more non-critical landmarks than younger adults. In addition, we included middle-aged adults for our age-group comparisons as Jansen et al. (2010) have shown that they may differ from younger and older adults in terms of route learning and cognitive map formation.

MATERIALS AND METHODS

Participants

A total of 114 participants (58 younger, 29 middle-aged and 27 older adults) participated in this study. The young adults (aged 18–38) were recruited from the psychology research volunteer pool at Wayne State University and from the community. The middle-aged (aged 51–64) and older adults (aged 65–90) were recruited using newspaper advertisements and notices distributed at older adult community centers and events. Based on self-reports of medical history following screening, all participants were in generally good health (i.e., no current history of coronary heart disease, cancer and dementia) and free of any medications that could potentially influence their cognitive performance. None of them suffered from any existing psychiatric or neurological disorders. All of them scored from 27 to 30 on the Mini-Mental State Examination (MMSE) (Folstein et al., 1975), a range that has been shown to offer high classification sensitivity and specificity for ruling out dementia (O'Bryant et al., 2008). With the exception of age and computer gaming experience (see below), older and younger participants were well-matched on demographic characteristics and mental

status. Informed consent for this study was approved by the institutional review board at Wayne State University. **Table 1** provides a summary of the demographic information for the sample.

Procedures

Assessment of Level of Computer Experience

As the elderly participants may have less experience using computers than the younger adults, all participants completed a computer experience questionnaire (CEQ) prior to being tested in the VE. This questionnaire contains three items that asked participants to rate the amount of experience they had using a computer, playing computer games and playing computer games that involve VEs. The scores from each of these items (each rated from 0 to 7) were summed to give a composite computer experience rating for each participant (maximum rating = 21) (see **Table 1**).

Pre-Test Training

Before being formally tested, the participants underwent navigation training in a VE. This was done during an initial phase of experimenter instruction, followed by a phase of free exploration of the VE using the joystick. After demonstrating their ability to guide themselves to targets designated by the experimenter, the participants were administered a joystick control speed test. On this test, they navigated a long winding corridor as quickly and accurately as possible until they reached a trophy at the finishing point. The criterion for demonstrating competency in the use of the joystick was completing the task within 120 s. Following the joystick control speed test, the participants conducted navigation in a practice maze over three trials. This maze was similar to the ensuing test maze except that it was of a simpler design.

Virtual Maze Learning Task

The maze learning task was designed using a modified version of Unreal Tournament 2003 and the software package Unreal Editor 3.0 (Epic Games, Inc., Cary, NC, USA). The task was presented on a 21-inch flat panel LCD monitor situated approximately 25 inches in front of the participants. The maze was presented from a first-person perspective and comprised of textured alleys and intersections (see **Figure 1**). There were four intersections at which the participants had to decide about their turning directions into the adjoining alleys. Some of these alleys led to the goal point (marked by the presence of a trophy) while others led to dead ends (for an overhead view, see **Figure 2**). Four intersections were created from pilot testing and showed that they were sufficiently challenging for the older participants. To facilitate maze learning, landmarks in the form of variegated wall textures were positioned at the intersections and along certain alleys. The participants traversed the maze on five consecutive trials after being instructed to locate the goal point as quickly and as accurately as possible and to try to remember the route to the goal over consecutive trials. They navigated the maze using a Logitech WingMan joystick. Aside from being instructed to learn the route to the goal point, they were not given any instruction about the follow-up cognitive tests to be performed. The absence of such instruction allowed for incidental learning of the landmarks for all participants. This ensured that any age effect from the follow-up cognitive tasks would not be overly affected by pre-existing age differences in the cognitive processing of environmental features that could be enlarged through specific instructions of intentional learning (see Old and Naveh-Benjamin, 2008).

The scoring of the maze learning task was automatically done by a program that was specifically developed for recording each participant's coordinate position and heading bearing in degrees every 20 ms. Based on this record, an analysis of navigation errors was conducted. A navigation error was scored as any deviation from the correct route into an alley that led to a dead end or a journey down a correct route in the wrong direction. An error was noted as soon as the participant crossed the threshold of an intersection into a dead-end alley (i.e., they did not have to reach the end of the alley) and every time they crossed the threshold of an intersection on the correct route but traveling in the wrong direction.

TABLE 1 | Demographic characteristics of participants.

	Younger	Middle aged	Older
Age range	18 – 38	51 – 64	65 – 90
Age[a]	20.33 (3.77)	58.79 (4.33)	71.37 (5.45)
% Females	51.7%	44.8%	63.0%
Education[a]	12.90 (1.00)	14.45 (2.17)	14.39 (2.84)
MMSE	29.29 (0.90)	29.41 (0.91)	28.93 (1.11)
CEQ[a]	14.17 (2.85)	11.38 (3.14)	9.15 (3.57)

Data presented in Mean (±SD). Education: education of participant in years. MMSE, Mini-Mental State Examination. CEQ, Computer Experience Questionnaire.
[a]*Variables on which a significant age group difference was present ($p < 0.05$).*

Post-Navigation Cognitive Tasks

Card rotations test

This is a pen-and-paper test of mental rotation with 20 target figures presented with eight other alternative illustrations of it on its right (Ekstrom et al., 1976). Among these eight alternative figures, some are rotated versions of the target figure while others are mirror images of the target figure. For each of these figures, the participant must decide whether it appears to be the same as (i.e., rotated in the picture plane) or different from (i.e., mirror image) the target figure. The test has two parts and the dependent measure was the total number of correct items minus the total number of incorrect items summed over these two parts. The maximum score attainable is 160.

Landmark recognition memory task

This task assessed the ability to recognize previously encountered landmarks in the virtual maze. It comprised of 48 trials showing the eight landmarks from the maze and eight "foil" landmarks that served as distractors. Each landmark was enlarged to the same scale and presented at the center of a gray background. Each landmark was presented three times in a randomized fashion leading to 48 trials. This repetition of landmark displays was

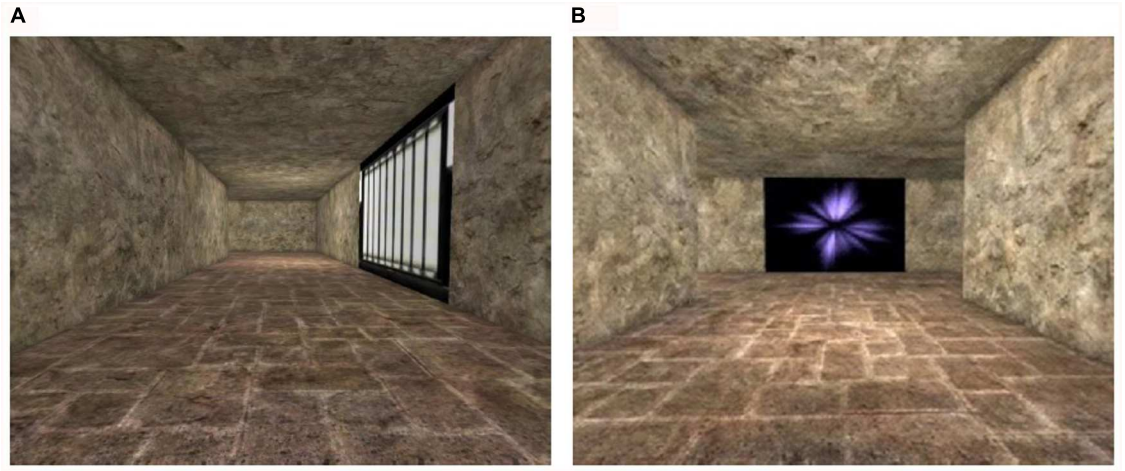


FIGURE 1 | First-person views of a one-way alley (i.e., non-decision point) (A) and an intersection (i.e., decision point) (B) within the virtual maze. Landmarks in the form of wall textures with varied colors and patterns were found at each intersection and along certain alleys. Participants were instructed to reach the goal point as quickly and as accurately as possible over five trials.

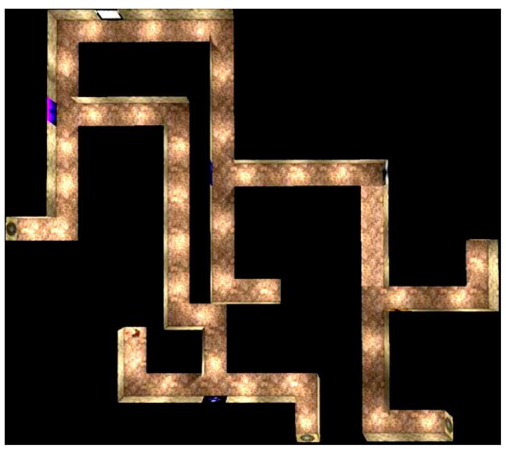


FIGURE 2 | Overhead view of the virtual maze showing the start and ending points. There were four intersections or decision points. While making the correct turn at each intersection led one on the right path to the goal point, making an erroneous turn at each intersection led to a dead-end.

made due to the fact that there were only four intersections in the maze, which were established in order to ensure that maze learning occurred at an appropriate level of difficulty for older adults. This practice of redisplaying pictures of landmarks or objects for recognition purpose has also been espoused by other researchers (e.g., Janzen, 2006). Consequently, 24 trials presented the landmarks that were found in the maze. On each trial, the participants were instructed that they must decide on whether they had seen the landmark in the maze by saying either 'yes' or 'no.' The experimenter tested every participant individually and recorded the verbal response on each trial. There was no time limit and the participants were instructed to respond as accurately as they could. The dependent measures recorded from each participant pertained respectively to the number of correctly recognized landmarks at decision and non-decision points in the maze and the number of correctly rejected foil landmarks. These recorded measures were converted to sensitivity index scores (*d'*) (Stanislaw and Todorov, 1999) that represented participants' ability to discern between previously seen and distractor landmarks. They were computed for each participant by subtracting the proportion of foil landmarks that were incorrectly recognized ('false alarms') from the proportion of correctly recognized landmarks in the maze ('hits'). Due to the number of trials showing critical and non-critical landmarks respectively (12) being less than that showing foil landmarks (24), all of the percentage scores were converted to *z*-scores to ensure standardized comparisons. For the two sets of *d'* scores reflecting the corrected hits of critical and non-critical landmarks were computed based on: $d' = Z$ (hits) – Z (false alarms) (see Macmillan and Creelman, 2005).

Landmark-direction association task

This task comprised 20 trials that required making decisions about the direction of travel in the virtual maze. A first-person still-photo of a particular location with a landmark appeared on each trial and the participant had to make a decision about the correct direction to take by pressing one of the arrow keys on the keyboard. Participants were asked "If you found yourself in this place in the maze, which direction would you travel?" On 12 trials, views of intersections were presented together with three directional arrows and the subject responded by indicating (i) forward, left, or right; (ii) backward, left, or right; (iii) left, forward, or backward; (iv) right, forward, or backward depending on the view. The participants decided on the directions by pressing arrow keys on the keyboard that corresponded to the relevant arrows on screen. On the remaining eight trials, views of

the unidirectional alleys were presented together with two directional arrows in each trial and participants made their decisions with regards to three combinations of directional arrows: (i) forward or backward; (ii) left or backward; (iii) right or backward. These eight views were organized around four distinct landmarks and regarded as non-critical decision points for making directional decisions. Altogether, these 20 views were presented to participants in a randomized trial sequence. No time limit was imposed and the participants were instructed to respond as accurately as they could on each trial. The experimenter tested every participant individually and recorded the response on each trial. The dependent measures recorded from each participant pertained to the number of correct directional decisions made at the critical and non-critical decision points respectively.

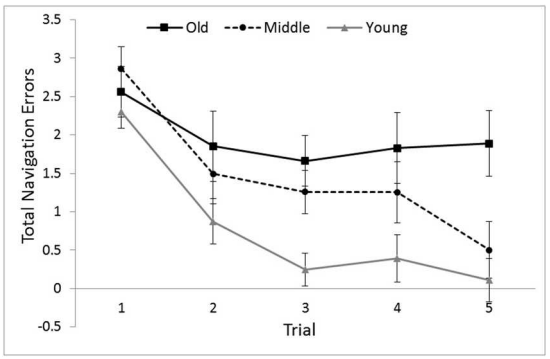


FIGURE 3 | Total number of navigation errors committed as a function of Age Group and Trial. Adjusted means and *SE*s are shown after controlling for the covariate effect of computer experience. Younger adults committed fewer errors overall. Error bars indicate ± 1 *SE*.

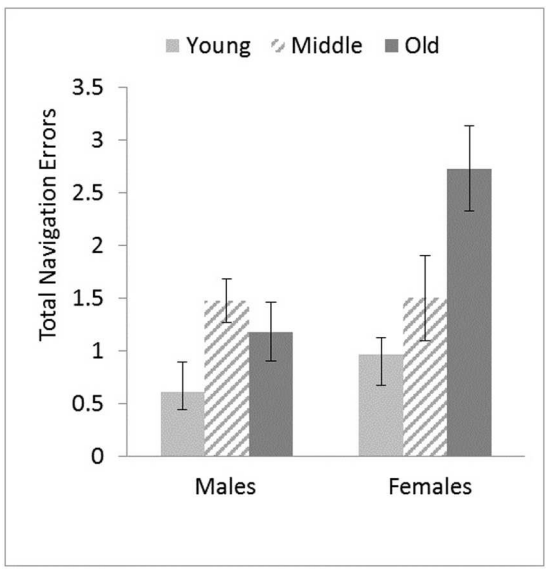


FIGURE 4 | Total number of navigation errors committed as a function of Age Group and Gender. Adjusted means and *SE*s are shown after controlling for the covariate effect of computer experience. Older female adults committed the highest number of errors overall. Error bars indicate ± 1 *SE*.

RESULTS

Virtual Maze Learning

To examine the potential gender and age group differences from the learning of the maze over the five trials, a mixed-model ANCOVA was performed with Age Group and Gender as the independent variables, navigation errors on each Trial as the within-subjects factor and computer experience as the covariate. There was a significant main effect of age, $F(2,107) = 6.36$, $p = 0.002$, $\eta^2 = 0.106$. *Post hoc* Bonferroni pairwise comparisons showed that older adults committed more errors than younger adults ($p = 0.002$) and marginally more than middle-aged adults ($p = 0.061$). There was also a significant main effect of Trial, $F(4,104) = 17.04$, $p < 0.001$, $\eta^2 = 0.396$ (see **Figure 3**) but no significant interaction between Trial and Age Group, $F(8,210) = 1.16$, $p = 0.324$, $\eta^2 = 0.042$. *Post hoc* Bonferroni pairwise comparisons showed that the number of errors committed on the first trial was significantly greater than that committed on any subsequent trial ($p < 0.001$). Moreover, there was a significant main effect of Gender, $F(1,107) = 6.59$, $p = 0.012$, $\eta^2 = 0.058$, with female adults committing more navigation errors ($M = 1.72$, $SD = 1.31$) than males ($M = 1.09$, $SD = 1.30$). Gender interacted significantly with Age Group, $F(1,107) = 4.08$, $p = 0.020$, $\eta^2 = 0.078$ and with both Age Group and Trial, $F(8,210) = 2.82$, $p = 0.006$, $\eta^2 = 0.097$.

The Gender × Age Group interaction was attributed to female adults committing more errors than male adults in both younger, $F(1,55) = 6.89$, $p = 0.011$, $\eta^2 = 0.111$ and older age groups, $F(1,24) = 4.51$, $p = 0.044$, $\eta^2 = 0.158$, (see **Figure 4**). *Post hoc* Bonferroni comparisons showed that younger females committed significantly fewer errors than both middle-aged ($p = 0.05$) and older females ($p = 0.003$) whereas younger males committed significantly fewer errors than middle-aged males only ($p = 0.010$).

As for the three-way interaction of Gender with Age Group and Trial, it resulted from the Trial × Age Group interaction being significant among the female adults, $F(8,108) = 2.19$, $p = 0.034$, $\eta^2 = 0.139$ (see **Figure 5A**), but non-significant among the male adults, $F(8,96) = 1.33$, $p = 0.238$, $\eta^2 = 0.100$ (see **Figure 5B**). Older females exhibited a learning slope that was relatively flat across the trials ($M = 2.73$, $SD = 0.63$), with no pairs of trials differing significantly in navigation errors based on an alpha of 0.01 (Bonferroni corrected). This slope was in direct contrast to the learning slopes of the other groups of females and male adults, all of which exhibited a distinctive downward trend across the trials.

Memory for Landmarks and Landmark-Direction Associations

As the initial maze learning trials were self-directed and self-paced, there were individual differences in the frequency with which each participant encountered a landmark. That is, people

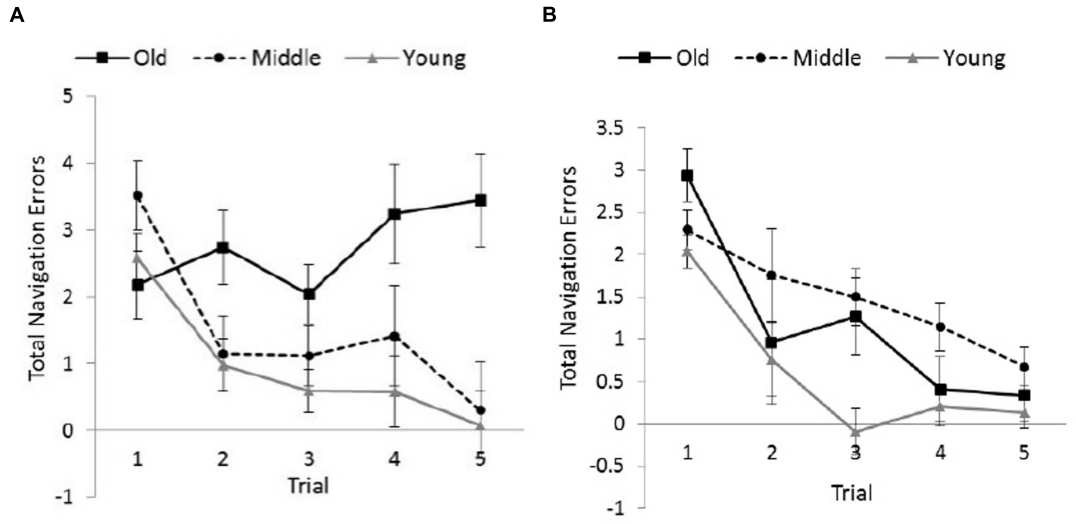


FIGURE 5 | Total number of navigation errors committed by female (A) and male participants (B) as a function of Age Group and Trial. Adjusted means and *SE*s are shown after controlling for the covariate effect of computer experience. Compared to other groups, older females showed little improvement across trials. Error bars indicate ± 1 *SE*.

who made more errors and spent more time in the maze would see the landmarks more frequently and from a variety of different viewpoints. In the analyses of the performance on the tests of landmark recognition memory and landmark-direction association that followed maze learning, an additional measure of the total number of viewing events was computed. As the age groups were found to differ on this measure, $F(2,111) = 16.23$, $p < 0.001$, $\eta^2 = 0.226$, with older adults having significantly more landmark views ($M = 15.44$, $SD = 6.03$) than both middle-aged ($M = 11.31$, 6.03) ($p = 0.031$) and younger adults ($M = 7.55$, $SD = 6.03$) ($p < 0.001$) s, this measure was added to the univariate analyses as a covariate to ensure that age differences on the cognitive task measures were not confounded by age differences in the extent of visual exposure to the different cues.

Landmark Recognition Memory

To analyze the performance on the landmark recognition memory test, a mixed-model ANCOVA was performed with Age Group and Gender as the independent variables, *d'* scores from the correct recognition of critical and non-critical landmark cues (located at decision and non-decision points respectively) as the repeated dependent measure and total viewing events as the covariate. There was a significant main effect of age, $F(2,107) = 4.43$, $p = 0.014$, $\eta^2 = 0.045$, with younger adults attaining higher *d'* scores than both middle-aged and older adults. There was no significance difference between the two sets of landmark recognition *d'* scores, $F(1,107) = 1.99 \times 10^{-4}$, $p = 0.989$, $\eta^2 < 0.001$. The interaction between Age Group and Landmark Cue Type was significant, $F(2,107) = 3.15$, $p = 0.047$, $\eta^2 = 0.056$. This interaction was due to the age groups differing significantly in non-critical landmark recognition, $F(2,107) = 5.75$, $p = 0.004$, $\eta^2 = 0.095$, but not in critical landmark recognition, $F(2,107) = 2.92$, $p = 0.058$, $\eta^2 = 0.050$ (see **Figure 6**). *Post hoc* Bonferroni comparisons showed that younger adults attained significantly higher *d'* scores than older adults ($p = 0.003$) in the recognition of non-critical landmark cues. Gender did not produce any significant main effect and did not interact significantly with Age Group and/or Landmark Cue Type, $Fs < 2.09$, $ps > 0.12$.

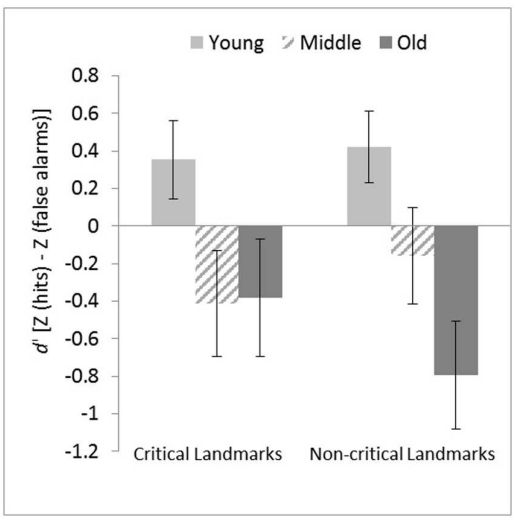


FIGURE 6 | *d'* scores on the Landmark Recognition Memory Task from all participants as a function of age group and landmark cue type. Adjusted means and *SE*s are shown after controlling for the covariate effect of total viewing events. Error bars indicate ± 1 *SE*.

Landmark-Direction Associative Learning

To analyze the performance on the landmark-direction association task, a mixed-model ANCOVA was performed with Age Group and Gender as the independent variables, percentage accuracy scores from the two types of landmark cues (shown at decision and non-decision points respectively) as the repeated dependent measure and total viewing events as the covariate. In view of the difference in chance-level performance between critical (chance = 33.33%) and non-critical landmark scenes (chance = 50%) that elicited a discrepancy in mean performance values, we converted all the percentage accuracy scores from each landmark condition to *z*-scores to render a more precise portrayal of the pattern of within-subjects differences across the age groups.

The results showed a significant main effect of age, $F(2,107) = 7.54$, $p < 0.001$, $\eta^2 = 0.123$, after controlling for the significant covariate effect of total viewing events, $F(1,107) = 5.44$, $p = 0.022$, $\eta^2 = 0.048$. *Post hoc* Bonferroni pairwise comparisons showed that younger adults attained significantly higher percentage accuracy scores ($M = 76.29$, $SD = 15.31$; $Z = 0.30$) than both middle-aged ($M = 71.14$, $SD = 14.49$; $Z = 0.04$) ($p = 0.047$) and older adults ($M = 61.32$, $SD = 15.69$; $Z = -0.48$) ($p = 0.001$). There was no significant interaction between Age Group and Landmark Cue Type, $F(2,107) = 2.35$, $p = 1.00$, $\eta^2 = 0.042$. Gender did not produce any significant main effect and did not interact significantly with Age Group and/or Landmark Cue Type, Fs < 1.0, ps > 0.66.

Card Rotation

An ANOVA with Age Group and Gender as the independent variables and card rotation accuracy as the dependent variable showed a significant main effect of age, $F(2,108) = 7.27$, $p = 0.001$, $\eta^2 = 0.119$. *Post hoc* comparisons using Tukey *HSD* showed that younger adults attained significantly higher scores ($M = 104.02$, $SD = 30.64$) than older adults ($M = 75.04$, $SD = 29.61$) ($p < 0.001$). Middle-aged adults' scores ($M = 88.17$, $SD = 32.49$) did not differ significantly from either group (ps > 0.05). There was also a significant main effect of gender, $F(1,108) = 7.40$, $p = 0.008$, $\eta^2 = 0.064$, with males scoring higher ($M = 102.75$, $SD = 32.24$) than females ($M = 84.68$, $SD = 31.17$). The interaction between Age Group and Gender was not significant, $F(2,108) = 2.16$, $p = 0.12$, $\eta^2 = 0.038$.

Correlational Analyses

We tested the relationships between navigation errors and landmark-direction association accuracy from each navigation point (in *z*-scores), landmark recognition discriminability of each cue type and card-rotation accuracy. For navigation errors, we used the sum of all errors over the five trials. As shown in **Table 2**, there were some common findings across the age groups: cumulative navigation errors were moderately and negatively correlated with the landmark-direction accuracy scores from decision points in each of the three groups ($-0.35 \leq r \leq -0.27$, ps < 0.10), as well as with the card rotation accuracy scores of younger [$r(58) = -0.32$, $p = 0.015$] and older adults [$r(27) = -0.65$, $p < 0.001$]. However, older adults were exceptional for having positive and moderate correlations between cumulative navigation errors and recognition *d'* scores for both critical [$r(27) = 0.39$, $p = 0.045$] and non-critical landmarks [$r(27) = 0.42$, $p = 0.031$].

DISCUSSION

This study examined the differences between younger, middle-aged and older adults with regards to their navigation performance in a virtual maze and subsequent performance in landmark recognition memory, landmark-direction associative learning and visuospatial ability. Overall, younger adults outperformed older adults in all the tasks except in critical landmark recognition. They also outperformed middle-aged adults in the recall of heading directions from decision and non-decision points and in card rotation performance. Middle-aged adults outperformed older adults on all the task measures but the differences did not reach significance. Correlations between the task measures further showed different patterns of relationships between the navigation performance and cognitive task performance measures across the age groups.

Closer examinations starting with total navigation errors showed that older adults committed significantly more errors than younger adults. These findings were consistent with previous studies that showed an age-related differences in the efficiency of route learning, particularly with regards to the planning or selection of the best route to reach a particular destination (e.g., Moffat et al., 2001; Allain et al., 2005; Salthouse and Siedlecki, 2007). Likewise, the prominent differences between younger and older adults in terms of landmark-direction association replicated previous findings which showed older adults to be relatively poorer at such associative learning (Head and Isom, 2010). Moreover, older adults' lower level of mental rotation ability (as represented by their card rotation accuracy scores) was consistent with those found in previous studies (e.g., Berg et al., 1982; Hertzog and Rypma, 1991). Card rotation accuracy scores were negatively correlated with cumulative navigation errors among the older adults (i.e., lower rotation accuracy, more navigation errors), suggesting that older adults' poorer route learning could be partly accounted by poorer mental rotation ability or spatial working memory capacity than younger adults (Hertzog and Rypma, 1991).

Younger adults' demonstration of better memory for non-critical landmarks was contrary to our prediction that older adults would recognize relatively more non-critical landmarks. It seems that older adults' predisposition toward encoding distractors (Bell et al., 2008; Campbell et al., 2010; Amer and Hasher, 2014) did not similarly apply to the encoding of irrelevant landmark cues in the navigational context of a virtual maze. However, it must be acknowledged that even though we deliberately placed irrelevant cues in the virtual maze, this environment was simpler and contained fewer cues than would be encountered in the real-world. Navigating an actual city, for

TABLE 2 | Pearson product-moment correlations between cumulative navigation errors and cognitive task measures in each age group.

	Cumulative navigation errors		
	Younger adults	**Middle-aged adults**	**Older adults**
Critical landmarks *d'*	−0.02	−0.18	0.39*
Non-critical landmarks *d'*	−0.05	−0.29	0.42*
Landmark-direction association ACC (Decision Points)	−0.27*	−0.34†	−0.35†
Landmark-direction association ACC (Non-decision Points)	−0.17	−0.08	−0.09
Card otation ACC	−0.32**	−0.08	−0.65**

†$p < 0.10$; *$p < 0.05$; **$p < 0.01$.

example, would present a plethora of navigationally irrelevant cues that may prove to be much more distracting.

In addition, a somewhat paradoxical finding was that item recognition memory for critical and non-critical landmarks was *positively* correlated with navigation errors (i.e., better recognition memory, poorer navigation performance) in the older adults. These findings suggest that older adults who attend to and encode item/object characteristics (leading to better item recognition memory) may do this at the expense of linking the objects to directions, culminating in poorer landmark-direction associative learning and poorer navigation performance.

It is also noteworthy that both middle-aged and older adults seem to experience a common deficit in associative memory for landmark-direction associations. This pattern of results is analogous to those from previous studies in which participants were instructed to utilize a particular memory strategy for binding together paired associates (e.g., for word pairs, see Naveh-Benjamin, 2000; Naveh-Benjamin et al., 2007; for name-face pairs, see Naveh-Benjamin et al., 2009). Owing to the fact that this study employed incidental learning with no instructions about strategy, this suggests that middle-aged and older adults might have adopted self-initialized strategies that led them to focus more on the object characteristics of the landmarks than on the relations of those landmarks with spatial or contextual information (i.e., heading directions, background scenes). Consequently, this might lead both age groups to have fewer cognitive resources or lower cognitive capacity than younger adults for the formation of appropriate landmark-direction associations. This interpretation is supported by previous research which showed that older adults have reduced cognitive resources for episodic or associative memory formation under conditions where they need to allocate more attentional resources to the processing of individual items instead of the relations between them (e.g., Craik and Byrd, 1982; Craik, 1983; Naveh-Benjamin et al., 2005; Naveh-Benjamin and Kilb, 2014).

Furthermore, the relatively high performance of younger adults in the landmark-direction association task could reflect their more effective use of spatial representation strategies. Such strategies generally pertain to an orientation strategy that focuses on the estimation and tracking of one's orientation relative to surrounding landmarks (Lawton, 1994, 1996; Pazzaglia and De Beni, 2001; Lawton and Kallai, 2002; Kato and Takeuchi, 2003) and an allocentric strategy that focuses on visualizing interobject relations and forming a cognitive map from an environment-centered perspective (Pazzaglia and De Beni, 2001; Hegarty et al., 2002; Rodgers et al., 2012). As the landmark-direction association task involved selecting directions based on viewpoints that were not necessarily directly encountered, a potentially more effective use of a spatial orientation strategy by younger adults might have facilitated their learning of landmark-direction pairings from multiple viewpoints, eventually enabling them to outperform both middle-aged and older adults on the task. Consistent with evidence showing that younger adults were stronger adherents of the allocentric strategy than older adults when navigating a two-choice Y-maze (Rodgers et al., 2012), younger adults most probably conducted better cognitive mapping of the spatial relations between the landmarks, leading them to commit substantially fewer navigation errors than older adults.

Another novel aspect of this study pertains to the gender effects that affected navigation performance. Older female adults—unlike the other female adults and male adults, all of whom demonstrated learning across repeated trials—were exceptional for not showing any noticeable decline in navigation errors with more trial exposure. This led to them committing substantially greater navigation errors than older males—a difference that might be attributed to gender differences in navigation strategy use (i.e., males prefer to use metric-based/spatial information whereas females prefer to use landmark information) (e.g., see Lawton, 1994, 1996; Dabbs et al., 1998; Lawton and Kallai, 2002; Saucier et al., 2002). Specifically, older females' potential use of a non-spatial landmark strategy that focuses on processing landmark/object characteristics (Dabbs et al., 1998; Saucier et al., 2002) might have deterred them from learning the spatial layout of the virtual maze, thereby leading them to maintain a relatively high level of navigation errors across the trials. Further research on relating potential gender differences in navigation strategy use and navigational performance among older adults is needed to clarify this possibility.

Along with the implications of the behavioral findings above, it is worth noting that age-differences in spatial navigation and associative learning may be underpinned by age-related differences in brain-related processes. Extant fMRI studies have demonstrated that activation in the hippocampal/parahippocampal region among non-demented older adults, when compared to younger adults, was either reduced (Meulenbroek et al., 2004; Moffat et al., 2006) or absent (Antonova et al., 2009) during the performance of navigational

tasks. Moreover, activation in the parahippocampal gyrus has been related to encoding of salient and navigationally relevant landmarks and their corresponding positions in space (Aguirre et al., 1996; Maguire et al., 1998; Janzen et al., 2007). For instance, when navigating a maze-like virtual museum, the parahippocampal gyrus was shown to have markedly higher activation in response to landmarks located at decision points compared to those located at non-decision points (Janzen and van Turennout, 2004; Janzen and Weststeijn, 2007; Janzen et al., 2007; Wegman and Janzen, 2011). These fMRI studies were complemented by structural MRI studies which further highlighted the hippocampus as one of the brain areas that exhibit shrinkage in volume with increased age (Raz et al., 2005) and a positive relationship between its volume and navigational performance (Moffat et al., 2007; Head and Isom, 2010; Schinazi et al., 2013). Cumulatively, these studies suggest that age-related changes in the functional and structural properties of the hippocampal/parahippocampal formation may have led to the age-related differences in navigational performance observed in our virtual maze task.

Likewise, in consideration of associative learning and memory, there have been studies that implicated the hippocampus as pivotal for the automatic binding of information (e.g., Eichenbaum and Bunsey, 1995; Henke et al., 1997; Wallenstein et al., 1998). These automatic binding processes, normally activated under incidental learning conditions, have also been shown to be negatively affected in older adults (e.g., see Moscovitch, 1994; Grady et al., 1995; Mitchell et al., 2000). In tasks that require the binding or combined encoding of different features, older adults have been shown to have lower activation in the hippocampus and prefrontal cortex than younger adults (e.g., Mitchell et al., 2000; Dennis et al., 2008). The prefrontal and hippocampal regions have also been proposed to be involved in strategic-effortful and automatic binding processes respectively (Moscovitch and Winocur, 1992; Moscovitch, 1994) and subsequent neurocomputational research has shown that senescent changes in the neuromodulatory mechanisms underlying the fronto-hippocampal circuitry may play a basic role in accounting for the associative deficit of older adults (e.g., see Li et al., 2001, 2005; Li and Sikström, 2002). Taken together, these developments makes it relevant for future studies to investigate the associative learning of landmark and directional information at the neural and systems levels, particularly with regards to the functional connectivity between the prefrontal cortex and the hippocampus.

Finally, by understanding the specific cognitive and neural factors that affect navigational decline in the normal aging population and comparing them to corresponding factors affecting patients with mild cognitive impairment and Alzheimer's disease (AD), we hope that a better identification of reliable markers of AD onset will emerge (Lithfous et al., 2013). Such an understanding will also be beneficial to the development of spatial navigation training (Lövdén et al., 2012) and/or pedestrian navigation aid devices (e.g., see Goodman et al., 2005) that cater to the special needs of older adults with different levels of navigational ability.

AUTHOR CONTRIBUTIONS

SM conceived and designed the experiment. JZ analyzed and the data and drafted the manuscript. JZ and SM revised the manuscript to ensure accuracy of all sections. Both authors approved the current final version for publication.

ACKNOWLEDGMENTS

We thank Mira Khouchane for assistance with data collection in this study.

REFERENCES

Adamo, D. E., Briceno, E. M., Sindone, J. A., Alexander, N. B., and Moffat, S. D. (2012). Age differences in virtual environment and real world path integration. *Front. Aging Neurosci.* 4:26. doi: 10.3389/fnagi.2012.00026

Aguirre, G. K., Detre, J. A., Alsop, D. C., and D'Esposito, M. (1996). The parahippocampus subserves topographical learning in 4man. *Cereb. Cortex* 6, 823–829. doi: 10.1093/cercor/6.6.823

Allain, P., Nicoleau, S., Pinon, K., Etcharry-Bouyx, F., Barre, J., Berrut, G., et al. (2005). Executive functioning in normal aging: a study of action planning using the Zoo Map Test. *Brain Cogn.* 57, 4–7. doi: 10.1016/j.bandc.2004.08.011

Amer, T., and Hasher, L. (2014). Conceptual processing of distractors by older but not younger adults. *Psychol. Sci.* 25, 2252–2258. doi: 10.1177/0956797614555725

Antonova, E., Parslow, D., Brammer, M., Dawson, G. R., Jackson, S. H., and Morris, R. G. (2009). Age-related neural activity during allocentric spatial memory. *Memory* 17, 125–143. doi: 10.1080/09658210802077348

Bell, R., Buchner, A., and Mund, I. (2008). Age-related differences in irrelevant-speech effects. *Psychol. Aging* 23, 377–391. doi: 10.1037/0882-7974.23.2.377

Berg, C., Hertzog, C., and Hunt, E. (1982). Age differences in the speed of mental rotation. *Dev. Psychol.* 18, 95–107. doi: 10.1037/0012-1649.18.1.95

Blades, M., and Medlicott, L. (1992). Developmental Differences in the ability to give route directions from a map. *J. Environ. Psychol.* 12, 175–185. doi: 10.1016/S0272-4944(05)80069-6

Campbell, K. L., Hasher, L., and Thomas, R. C. (2010). Hyper-binding: a unique age effect. *Psychol. Sci.* 21, 399–405. doi: 10.1177/0956797609359910

Cohen, R., and Schuepfer, T. (1980). The representation of landmarks and routes. *Child Dev.* 51, 1065–1071. doi: 10.2307/1129545

Connelly, S. L., Hasher, L., and Zacks, R. T. (1991). Age and Reading: the impact of distraction. *Psychol. Aging* 6, 533–541. doi: 10.1037/0882-7974.6.4.533

Craik, F. I. M. (1983). On the transfer of information from temporary to permanent memory. *Philos. Trans. R. Soc. B* 302, 341–359. doi: 10.1098/rstb.1983.0059

Craik, F. I. M., and Byrd, M. (1982). "Aging and cognitive deficits: the role of attentional resources," in *Advances in the Study of Communication and Affect: Aging and Cognitive Processes*, Vol. 8, eds F. I. M. Craik and S. E. Trehub (New York, NY: Plenum Press), 191–211.

Dabbs, J. M., Chang, E. L., Strong, R. A., and Milun, R. (1998). Spatial ability, navigation strategy, and geographic knowledge among men and women. *Evol. Hum. Behav.* 19, 89–98. doi: 10.1016/S1090-5138(97)00107-4

Daniel, M. P., and Denis, M. (1998). Spatial descriptions as navigational aids: a cognitive analysis of route directions. *Kognitionswissenschaft* 7, 45–52. doi: 10.1007/BF03354963

Dennis, N. A., Hayes, S. M., Prince, S. E., Madden, D. J., Huettel, S. A., and Cabeza, R. (2008). Effects of aging on the neural correlates of successful item and source memory encoding. *J. Exp. Psychol. Learn. Mem. Cogn.* 34, 791–808. doi: 10.1037/0278-7393.34.4.791

Driscoll, I., Hamilton, D. A., Petropoulos, H., Yeo, R. A., Brooks, W. M., Baumgartner, R. N., et al. (2003). The aging hippocampus: cognitive,

biochemical and structural findings. *Cereb. Cortex* 13, 1344–1351. doi: 10.1093/cercor/bhg081

Duchek, J. M., Balota, D. A., and Thessing, V. C. (1998). Inhibition of visual and conceptual information during reading in healthy aging and Alzheimer's disease. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 5, 169–181. doi: 10.1076/anec.5.3.169.616

Eichenbaum, H., and Bunsey, M. (1995). On the binding of associations in memory: clues from studies on the role of the hippocampal region in paired-associate learning. *Curr. Dir. Psychol. Sci.* 4, 19–23. doi: 10.1111/1467-8721.ep10770954

Ekstrom, R., French, J., and Harman, H. (1976). *Manual for Kit of Factor Referenced Cognitive Tests*. Princeton, NJ: Educational Testing Service.

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). Mini-mental state: a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Goodman, J., Brewster, S. A., and Gray, P. (2005). How can we best use landmarks to support older people in navigation? *Behav. Inf. Technol.* 24, 3–20. doi: 10.1080/01449290512331319021

Grady, C. L., Mcintosh, A. R., Horwitz, B., Maisog, J. M., Ungerleider, L. G., Mentis, M. J., et al. (1995). Age-related reductions in human recognition memory due to impaired encoding. *Science* 269, 218–221. doi: 10.1126/science.7618082

Harris, M. A., and Wolbers, T. (2012). Ageing effects on path integration and landmark navigation. *Hippocampus* 22, 1770–1780. doi: 10.1002/hipo.22011

Harris, M. A., and Wolbers, T. (2014). How age-related strategy switching deficits affect wayfinding in complex environments. *Neurobiol. Aging* 35, 1095–1102. doi: 10.1016/j.neurobiolaging.2013.10.086

Head, D., and Isom, M. (2010). Age effects on wayfinding and route learning skills. *Behav. Brain Res.* 209, 49–58. doi: 10.1016/j.bbr.2010.01.012

Hegarty, M., Richardson, A. E., Montello, D. R., Lovelace, K., and Subbiah, I. (2002). Development of a self-report measure of environmental spatial ability. *Intelligence* 30, 425–447. doi: 10.1016/S0160-2896(02)00116-2

Henke, K., Buck, A., Weber, B., and Wieser, H. G. (1997). Human hippocampus establishes associations in memory. *Hippocampus* 7, 249–256. doi: 10.1002/(SICI)1098-1063(1997)7:3<249::AID-HIPO1>3.0.CO;2-G

Hertzog, C., and Rypma, B. (1991). Age-differences in components of mental-rotation task-performance. *Bull. Psychon. Soc.* 29, 209–212. doi: 10.3758/BF03335237

Iaria, G., Palermo, L., Committeri, G., and Barton, J. J. S. (2009). Age differences in the formation and use of cognitive maps. *Behav. Brain Res.* 196, 187–191. doi: 10.1016/j.bbr.2008.08.040

Jansen, P., Schmelter, A., and Heil, M. (2010). Spatial knowledge acquisition in younger and elderly adults: a study in a virtual environment. *Exp. Psychol.* 57, 54–60. doi: 10.1027/1618-3169/a000007

Jansen-Osmann, P. (2002). Using desktop virtual environments to investigate the role of landmarks. *Comput. Hum. Behav.* 18, 427–436. doi: 10.1016/S0747-5632(01)00055-3

Jansen-Osmann, P., and Wiedenbauer, G. (2004). The representation of landmarks and routes in children and adults: a study in a virtual environment. *J. Environ. Psychol.* 24, 347–357. doi: 10.1016/j.jenvp.2004.08.003

Janzen, G. (2006). Memory for object location and route direction in virtual large-scale space. *Q. J. Exp. Psychol.* 59, 493–508. doi: 10.1080/02724980443000746

Janzen, G., and van Turennout, M. (2004). Selective neural representation of objects relevant for navigation. *Nat. Neurosci.* 7, 673–677. doi: 10.1038/nn1257

Janzen, G., Wagensveld, B., and van Turennout, M. (2007). Neural representation of navigational relevance is rapidly induced and long lasting. *Cereb. Cortex* 17, 975–981. doi: 10.1093/cercor/bhl008

Janzen, G., and Weststeijn, C. G. (2007). Neural representation of object location and route direction: an event-related fMRI study. *Brain Res.* 1165, 116–125. doi: 10.1016/j.brainres.2007.05.074

Kato, Y., and Takeuchi, Y. (2003). Individual differences in wayfinding strategies. *J. Environ. Psychol.* 23, 171–188. doi: 10.1016/S0272-4944(03)00011-2

Kirasic, K. C. (1991). Spatial cognition and behavior in young and elderly adults: implications for learning new environments. *Psychol. Aging* 6, 10–18. doi: 10.1037/0882-7974.6.1.10

Lawton, C. A. (1994). Gender differences in way-finding strategies: relationship to spatial ability and spatial anxiety. *Sex Roles* 30, 765–779. doi: 10.1007/Bf01544230

Lawton, C. A. (1996). Strategies for indoor wayfinding: the role of orientation. *J. Environ. Psychol.* 16, 137–145. doi: 10.1006/jevp.1996.0011

Lawton, C. A., and Kallai, J. (2002). Gender differences in wayfinding strategies and anxiety about wayfinding: a cross-cultural comparison. *Sex Roles* 47, 389–401. doi: 10.1023/A:1021668724970

Li, S. C., Lindenberger, U., and Sikstrom, S. (2001). Aging cognition: from neuromodulation to representation. *Trends Cogn. Sci.* 5, 479–486. doi: 10.1016/S1364-6613(00)01769-1

Li, S. C., Naveh-Benjamin, M., and Lindenberger, U. (2005). Aging neuromodulation impairs associative binding: a neurocomputational account. *Psychol. Sci.* 16, 445–450. doi: 10.1111/j.0956-7976.2005.01555.x

Li, S. C., and Sikström, S. (2002). Integrative neurocomputational perspectives on cognitive aging, neuromodulation, and representation. *Neurosci. Biobehav. Rev.* 26, 795–808. doi: 10.1016/S0149-7634(02)00066-0

Lipman, P. D. (1991). Age and exposure differences in acquisition of route information. *Psychol. Aging* 6, 128–133. doi: 10.1037/0882-7974.6.1.128

Lithfous, S., Dufour, A., and Despres, O. (2013). Spatial navigation in normal aging and the prodromal stage of Alzheimer's disease: insights from imaging and behavioral studies. *Ageing Res. Rev.* 12, 201–213. doi: 10.1016/j.arr.2012.04.007

Lövdén, M., Schaefer, S., Noack, H., Bodammer, N. C., Kuhn, S., Heinze, H. J., et al. (2012). Spatial navigation training protects the hippocampus against age-related changes during early and late adulthood. *Neurobiol. Aging* 33, 620.e9–620.e22. doi: 10.1016/j.neurobiolaging.2011.02.013

Macmillan, N. A., and Creelman, D. (2005). *Detection Theory: A User's Guide*, 2nd Edn. Mahwah, NJ: Erlbaum.

Maguire, E. A., Frith, C. D., Burgess, N., Donnett, J. G., and O'Keefe, J. (1998). Knowing where things are: parahippocampal involvement in encoding object locations in virtual large-scale space. *J. Cogn. Neurosci.* 10, 61–76. doi: 10.1162/089892998563789

Mahmood, O., Adamo, D., Briceno, E., and Moffat, S. D. (2009). Age differences in visual path integration. *Behav. Brain Res.* 205, 88–95. doi: 10.1016/j.bbr.2009.08.001

May, C. P. (1999). Synchrony effects in cognition: the costs and a benefit. *Psychon. Bull. Rev.* 6, 142–147. doi: 10.3758/Bf03210822

McGinnis, D. (2012). Susceptibility to distraction during reading in young, young-old, and old-old adults. *Exp. Aging Res.* 38, 370–393. doi: 10.1080/0361073x.2012.699365

Meulenbroek, O., Petersson, K. M., Voermans, N., Weber, B., and Fernandez, G. (2004). Age differences in neural correlates of route encoding and route recognition. *Neuroimage* 22, 1503–1514. doi: 10.1016/j.neuroimage.2004.04.007

Mitchell, K. J., Johnson, M. K., Raye, C. L., and D'Esposito, M. (2000). fMRI evidence of age-related hippocampal dysfunction in feature binding in working memory. *Cogn. Brain Res.* 10, 197–206. doi: 10.1016/S0926-6410(00)00029-X

Moffat, S. D., Elkins, W., and Resnick, S. M. (2006). Age differences in the neural systems supporting human allocentric spatial navigation. *Neurobiol. Aging* 27, 965–972. doi: 10.1016/j.neurobiolaging.2005.05.011

Moffat, S. D., Kennedy, K. M., Rodrigue, K. M., and Raz, N. (2007). Extrahippocampal contributions to age differences in human spatial navigation. *Cereb. Cortex* 17, 1274–1282. doi: 10.1093/cercor/bhl036

Moffat, S. D., and Resnick, S. M. (2002). Effects of age on virtual environment place navigation and allocentric cognitive mapping. *Behav. Neurosci.* 116, 851–859. doi: 10.1037/0735-7044.116.5.851

Moffat, S. D., Zonderman, A. B., and Resnick, S. M. (2001). Age differences in spatial memory in a virtual environment navigation task. *Neurobiol. Aging* 22, 787–796. doi: 10.1016/S0197-4580(01)00251-2

Moscovitch, M. (1994). Cognitive resources and dual-task interference effects at retrieval in normal people: the role of the frontal lobes and medial temporal cortex. *Neuropsychology* 8, 524–534. doi: 10.1037/0894-4105.8.4.524

Moscovitch, M., and Winocur, G. (1992). "The neuropsychology of memory and aging," in *The Handbook of Aging and Cognition*, eds F. I. M. Craik and T. A. Salthouse (Hillsdale, NJ: Erlbaum), 315–372.

Naveh-Benjamin, M. (2000). Adult age differences in memory performance: tests of an associative deficit hypothesis. *J. Exp. Psychol. Learn. Mem. Cogn.* 26, 1170–1187. doi: 10.1037/0278-7393.26.2.1170

Naveh-Benjamin, M., Brav, T. K., and Levy, O. (2007). The associative memory deficit of older adults: the role of strategy utilization. *Psychol. Aging* 22, 202–208. doi: 10.1037/0882-7974.22.1.202

Naveh-Benjamin, M., Craik, F. I. M., Guez, J., and Kreuger, S. (2005). Divided attention in younger and older adults: effects of strategy and relatedness on memory performance and secondary task costs. *J. Exp. Psychol. Learn. Mem. Cogn.* 31, 520–537. doi: 10.1037/0278-7393.31.3.520

Naveh-Benjamin, M., and Kilb, A. (2014). Age-related differences in associative memory: the role of sensory decline. *Psychol. Aging* 29, 672–683. doi: 10.1037/a0037138

Naveh-Benjamin, M., Shing, Y. L., Kilb, A., Werkle-Bergner, M., Lindenberger, U., and Li, S. C. (2009). Adult age differences in memory for name-face associations: the effects of intentional and incidental learning. *Memory* 17, 220–232. doi: 10.1080/09658210802222183

Newman, M. C., and Kaszniak, A. W. (2000). Spatial memory and aging: performance on a human analog of the Morris water maze. *Aging Neuropsychol. Cogn.* 7, 86–93. doi: 10.1076/1382-5585(200006)7:2;1-U;Ft086

O'Bryant, S. E., Humphreys, J. D., Smith, G. E., Ivnik, R. J., Graff-Radford, N. R., Petersen, R. C., et al. (2008). Detecting dementia with the mini-mental state examination in highly educated individuals. *Arch. Neurol.* 65, 963–967. doi: 10.1001/archneur.65.7.963

Old, S. R., and Naveh-Benjamin, M. (2008). Differential effects of age on item and associative measures of memory: a meta-analysis. *Psychol. Aging* 23, 104–118. doi: 10.1037/0882-7974.23.1.104

Pazzaglia, F., and De Beni, R. (2001). Strategies of processing spatial information in survey and landmark-centred individuals. *Eur. J. Cogn. Psychol.* 13, 493–508. doi: 10.1080/09541440042000124

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., et al. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb. Cortex* 15, 1676–1689. doi: 10.1093/cercor/bhi044

Rodgers, M. K., Sindone, J. A., and Moffat, S. D. (2012). Effects of age on navigation strategy. *Neurobiol. Aging* 33, 202.e15–202.e22. doi: 10.1016/j.neurobiolaging.2010.07.021

Salthouse, T. A., and Siedlecki, K. L. (2007). Efficiency of route selection as a function of adult age. *Brain Cogn.* 63, 279–286. doi: 10.1016/j.bandc.2006.09.006

Saucier, D. M., Green, S. M., Leason, J., MacFadden, A., Bell, S., and Elias, L. J. (2002). Are sex differences in navigation caused by sexually dimorphic strategies or by differences in the ability to use the strategies? *Behav. Neurosci.* 116, 403–410. doi: 10.1037/0735-7044.116.3.403

Schinazi, V. R., Nardi, D., Newcombe, N. S., Shipley, T. F., and Epstein, R. A. (2013). Hippocampal size predicts rapid learning of a cognitive map in humans. *Hippocampus* 23, 515–528. doi: 10.1002/hipo.22111

Stanislaw, H., and Todorov, N. (1999). Calculation of signal detection theory measures. *Behav. Res. Methods Instrum. Comput.* 31, 137–149. doi: 10.3758/Bf03207704

Wallenstein, G. V., Eichenbaum, H., and Hasselmo, M. E. (1998). The hippocampus as an associator of discontiguous events. *Trends Neurosci.* 21, 317–323. doi: 10.1016/S0166-2236(97)01220-4

Ward, S. L., Newcombe, N., and Overton, W. F. (1986). Turn left at the church, or 3 Miles north: a study of direction giving and sex differences. *Environ. Behav.* 18, 192–213. doi: 10.1177/0013916586182003

Wegman, J., and Janzen, G. (2011). Neural encoding of objects relevant for navigation and resting state correlations with navigational ability. *J. Cogn. Neurosci.* 23, 3841–3854. doi: 10.1162/jocn_a_00081

Wiener, J. M., de Condappa, O., Harris, M. A., and Wolbers, T. (2013). Maladaptive bias for extrahippocampal navigation strategies in aging humans. *J. Neurosci.* 33, 6012–6017. doi: 10.1523/JNEUROSCI.0717-12.2013

Wilkniss, S. M., Jones, M. G., Korol, D. L., Gold, P. E., and Manning, C. A. (1997). Age-related differences in an ecologically based study of route learning. *Psychol. Aging* 12, 372–375. doi: 10.1037/0882-7974.12.2.372

Conflict of Interest Statement: 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 Aging Neuroscience

REVIEW
published: 29 June 2016
doi: 10.3389/fnagi.2016.00158

Preclinical Magnetic Resonance Imaging and Spectroscopy Studies of Memory, Aging, and Cognitive Decline

Marcelo Febo[1] and Thomas C. Foster[2]*

[1] *Department of Psychiatry, William L. and Evelyn F. McKnight Brain Institute, University of Florida, Gainesville, FL, USA,*
[2] *Department of Neuroscience, William L. and Evelyn F. McKnight Brain Institute, University of Florida, Gainesville, FL, USA*

Neuroimaging provides for non-invasive evaluation of brain structure and activity and has been employed to suggest possible mechanisms for cognitive aging in humans. However, these imaging procedures have limits in terms of defining cellular and molecular mechanisms. In contrast, investigations of cognitive aging in animal models have mostly utilized techniques that have offered insight on synaptic, cellular, genetic, and epigenetic mechanisms affecting memory. Studies employing magnetic resonance imaging and spectroscopy (MRI and MRS, respectively) in animal models have emerged as an integrative set of techniques bridging localized cellular/molecular phenomenon and broader *in vivo* neural network alterations. MRI methods are remarkably suited to longitudinal tracking of cognitive function over extended periods permitting examination of the trajectory of structural or activity related changes. Combined with molecular and electrophysiological tools to selectively drive activity within specific brain regions, recent studies have begun to unlock the meaning of fMRI signals in terms of the role of neural plasticity and types of neural activity that generate the signals. The techniques provide a unique opportunity to causally determine how memory-relevant synaptic activity is processed and how memories may be distributed or reconsolidated over time. The present review summarizes research employing animal MRI and MRS in the study of brain function, structure, and biochemistry, with a particular focus on age-related cognitive decline.

Keywords: fMRI, DTI, magnetic resonance spectroscopy, hippocampus, memory, preclinical MRI, aging neuroscience

OPEN ACCESS

Edited by:
Kristina Visscher,
University of Alabama at Birmingham, USA

Reviewed by:
Afonso C. Silva,
NIH National Institute of Neurological Disorders and Stroke, USA
Stefano Delli Pizzi,
University "G. d'Annunzio" of Chieti-Pescara, Italy

****Correspondence:***
Marcelo Febo
febo@ufl.edu

Received: *05 March 2016*
Accepted: *16 June 2016*
Published: *29 June 2016*

Citation:
Febo M and Foster TC (2016) Preclinical Magnetic Resonance Imaging and Spectroscopy Studies of Memory, Aging, and Cognitive Decline. Front. Aging Neurosci. 8:158. doi: 10.3389/fnagi.2016.00158

INTRODUCTION

Among the various techniques in neuroscience, magnetic resonance imaging and spectroscopy are uniquely suited for longitudinal measurements; providing in-depth assessments of neural activity, tissue microstructural organization, and chemistry in the aging brain. Functional and diffusion magnetic resonance imaging (fMRI and dMRI, respectively) are among the most promising MRI modalities that may be used to investigate the relationship between regional changes in neural activity and structural connectivity. These neuroimaging methods have been employed in aging humans to suggest that variability in the decline of several cognitive processes

results from changes in defined neural circuits (O'sullivan et al., 2001; Gunning-Dixon and Raz, 2003; Salat et al., 2005; Dennis et al., 2008; Kaufmann et al., 2008; Duverne et al., 2009; Wang et al., 2009; Lighthall et al., 2014). However, cellular and synaptic mechanisms underlying regional differences in vulnerability to aging are difficult to assess in human subjects. Thus, the utility of fMRI and dMRI in studying functional and neuroanatomical correlates of the human aging brain is strengthened by animal studies that combine imaging with invasive assessments. Animal imaging approaches combining fMRI with electrophysiological recordings, direct electrical stimulation and/or optogenetic modulation of neuronal activity, may bring us closer to characterizing links between neural activity and memory formation, both in healthy aging and with cognitive impairment. Other animal imaging methods not widely used in human subjects, such as pharmacological MRI (**Box 1**), may be used to discern specific drug effects on BOLD activity in memory networks. Functional and anatomical imaging techniques find strong complementation with *in vivo* magnetic resonance spectroscopy (MRS), which describes biochemical correlates in memory regions.

The present article provides an overview of animal studies that use fMRI, dMRI, and MRS to assess functional, structural, and chemical characteristics in brain areas involved in learning and memory. Rather than providing an extensive overview of the full breadth of the animal imaging literature, the review focuses on studies that are particularly relevant to normal aging animal models, and on imaging and spectroscopy studies of temporal lobe, prefrontal cortical and striatal circuits. The medial temporal lobe episodic memory system and a prefrontal cortex and striatal executive function system are highly vulnerable to changes in structure and activity associated with cognitive decline in humans, monkeys, rats, and mice. Furthermore, there is a rich repertoire of behavioral paradigms that can be applied to study of age-related decline in memory and executive function across species (Moss et al., 2007; Nagahara et al., 2010; Zeamer et al., 2011; Alexander et al., 2012; Bizon et al., 2012; Foster et al., 2012; Holden and Gilbert, 2012; Roberson et al., 2012; Crystal and Wilson, 2015). Monkeys may have an advantage for behaviors that depend on the higher complexity of the cortex. In this regard, the anatomy of the prefrontal cortex in Old world primates is more analogous to that of humans. However, aged non-human primates may exhibit extensive neuronal loss in the prefrontal cortex, which is not evident in aging humans or other animal models (Smith et al., 2004; Burke and Barnes, 2006). Furthermore, while comparative studies have identified similarities in connectivity for auditory and visual system pathways, some connections involving the prefrontal cortex may be absent in non-human primates (Rilling, 2014), which may underlie differences in behaviors between humans and non-human primates (Stoet and Snyder, 2003). In contrast, rodents are a common model of "normal" cognitive aging, particularly for studies seeking to understand cellular and molecular mechanisms underlying age-related changes in brain structure and function. We will attempt to offer interpretations on the summarized literature and discuss how the imaging findings might be reconciled with what is known on the synaptic circuitry and mechanisms of learning and memory.

BOX 1 | *Pharmacological MRI* is a term used to describe the use of functional magnetic resonance imaging modalities to measure the BOLD response to neuropharmacologically active compounds (Chen et al., 1999; Salmeron and Stein, 2002). Studies typically employ BOLD imaging, however, the term is inclusive of arterial spin labeling, iron contrast-based cerebral blood volume measurements, and manganese enhanced MRI studies designed to screen brain activity in response to CNS drugs.

EMERGING APPROACHES TO DRIVE AND RECORD FROM MEMORY CIRCUITS DURING fMRI

Findings from fMRI experiments are perhaps the most intriguing among preclinical imaging studies because of the potential of resolving functional changes involving hippocampal and prefrontal circuits during specific stages of memory formation and reconsolidation. A major question concerns the underlying neuronal activity that generates the BOLD signal. Is the signal related to region specific neuronal discharge activity or does it reflect synaptic activity associated with afferent input and local circuits? Several studies have examined neuronal discharge activity in behaving animals. Based on visual stimulation induced changes in BOLD signal in humans and neuronal discharge activity in monkeys it was suggested that the BOLD signal is representative of neuronal firing rate (Heeger et al., 2000; Rees et al., 2000). However, in both rats and non-human primates BOLD fMRI signals correlate more closely with local field potentials (LFPs) than with multi-unit activity (MUA; Logothetis et al., 2001), although recent work in rats indicates that cerebral blood flow (CBF) correlates better with LFPs than do BOLD signals or cerebral blood volume (CBV; Herman et al., 2013). The LFPs represent relatively slow changes in membrane depolarization and hyperpolarization due to afferent input and local circuit synaptic activity. Thus, the BOLD response is largely associated with local neuronal processing of synaptic inputs, as well as excitatory and inhibitory synaptic activity of the local circuit, rather than the consequent neuronal discharge activity which represents the output computation (Logothetis and Wandell, 2004). Thus, it is possible that neuromodulatory influences that inhibit the discharge activity of principle cells can increase the BOLD response, while increased discharge activity due to GABA antagonist may not alter the BOLD response (Thomsen et al., 2004). In this regard, the BOLD signal will differ across regions due to the local excitatory/inhibitory configuration of the circuit.

Functional Imaging of Hippocampal Networks

Taking advantage of the well-defined synaptic circuitry of the hippocampal formation, Angenstein et al. (2007, 2009) have utilized direct current stimulation and neural recordings across a

series of studies to determine the relationship between local field activity and the BOLD signal, particularly in relation to its evoked spatial and temporal properties in hippocampus (Tiede et al., 2012; Angenstein, 2014; Scherf and Angenstein, 2015). Electrical stimulation of afferents and recording in specific hippocampal regions allowed this group to control input activity to the dorsal CA1/dentate region, where BOLD signals were measured. Using this technique they determined important properties of hippocampal BOLD responses in relation to the neuronal activity driving this signal. For BOLD response originating in the dentate gyrus, it seems that afferent synaptic activity of the perforant path correlates better with BOLD responses rather than the discharge response of the population of granule cells (Angenstein et al., 2007). Furthermore, the propagation of BOLD activity across interconnected hippocampal subregions is influenced by the internal processing dynamics and synaptic plasticity in this region (Angenstein et al., 2009). The induction of long-term potentiation (LTP) requires activation of *N*-methyl-D-aspartate (NMDA) receptors (Foster, 2012), thus treatment with an NMDA receptor blocker (MK801) prior to afferent stimulation blocks hippocampal network activity (Tiede et al., 2012). Hence, increased BOLD signal changes associated with the induction of LTP suggests that memory-related changes in neural activity are measurable with fMRI (Canals et al., 2008; Angenstein et al., 2013).

Here is where animal-imaging studies may provide key insights in the regulation of neural activity during memory formation and recalling specific events, as reported in human subjects (Ranganath et al., 2004; Flegal et al., 2014). Parahippocampal areas of normal healthy human subjects show greater BOLD responses during correct recall of events than with incorrect recall (Eldridge et al., 2000). In awake Rhesus macaques, correct recall of events on a serial probe recognition task was associated with increased BOLD in caudal entorhinal cortex, perirhinal cortex and hippocampus (Miyamoto et al., 2014). While this temporal lobe network in primates reflects early consolidation phases, the intraparietal sulcus plays a role in long-term retrieval processes (Miyamoto et al., 2013). The idea that synaptic activity mediates the BOLD response has important implications for interpreting the response as it relates to mechanisms for cognitive function during aging (**Figure 1**). Impaired memory encoding and retrieval is associated with decreased BOLD activity in the hippocampus and medial temporal lobe of humans (Daselaar et al., 2003; Morcom et al., 2003; Dennis et al., 2008; Salami et al., 2012; Pudas et al., 2013; Sherman et al., 2015). Conversely, an increase in frontal cortex neural activity is observed in older humans and may relate to performance of executive function tasks (Rosano et al., 2005; Turner and Spreng, 2012; Maillet and Rajah, 2014). Studies in animals suggest that the level of cell discharge activity is not dramatically altered in the hippocampus or prefrontal cortex of older cognitively impaired animals (Oler and Markus, 2000; Burke and Barnes, 2006; Wang et al., 2011; Caetano et al., 2012). Rather, cognitive decline is associated with an inability to modify cell discharge activity. In turn, modifiability of cell discharge activity depends on synaptic plasticity, and thus age-related cognitive decline is associated with a decrease in the strength of excitatory synapses and impaired synaptic plasticity (Luebke et al., 2004; Foster, 2012; Guidi et al., 2015b).

In spite of the aforementioned insights, direct electrical current stimulation presents technical challenges preventing straightforward interpretations that can link these results to human imaging work. Among these is the non-specificity of neuronal groups targeted for stimulation, a lack of control over excitatory versus inhibitory activity, and off-target antidromic activation of afferent inputs to the stimulation site that could hinder clear interpretations of fMRI data. Some of these limitations may be resolved through the use of optogenetics in conjunction with fMRI. Initial studies applying this strategy have focused on hippocampal and prefrontal cortical regions. Therefore, these types of experiments are highly relevant to characterizations of circuit adaptations in memory and normal aging. Following a seminal study using the light sensitive cation channel rhodopsin 2 (ChR2) to drive motor thalamocortical BOLD responses (Lee et al., 2010), Lee and colleagues conducted a similar opto-fMRI study centered on eliciting hippocampal activation (Duffy et al., 2015). Light stimulated excitation of dorsal CA1 pyramidal neurons increased BOLD activation in the hippocampus and its output regions in the medial septum (Duffy et al., 2015). Increasing stimulation frequencies to levels capable of triggering seizure-like after discharges elicited a greater distribution of BOLD activation to contralateral hippocampus, neocortex, and mediodorsal thalamus, an effect closely resembling optogenetically evoked BOLD activation in mouse CA1 circuitry (Takata et al., 2015). Among the implied conceptual benefits of the opto-fMRI studies of hippocampal networks is the potential for measuring how neural activity moves through subregions of hippocampal memory networks. Importantly, future studies are likely to target specific cell groups to characterize hippocampal network activity and how memory and recall mechanisms modify activity through this structure. Studies directed at understanding specific roles for neurotransmitter systems in modulating BOLD activation through their effects on LFPs and MUA are coming to fruition in non-human primates (Rauch et al., 2008; Arsenault et al., 2013; Zaldivar et al., 2014). Applying such targeted cell- and receptor-specific approaches in imaging hippocampal networks is likely to provide powerful insight into effects of aging on hippocampal activity, memory, and cognitive behaviors.

Functional Imaging of Prefrontal Networks

The prefrontal cortex plays a role in working memory and its role in normal aging is functionally distinct from that of hippocampus and amygdala. Due to the complexity of the prefrontal cortex, in terms of afferents, efferents, and local circuits, optogenetics is essential in order to specify neuronal activation patterns with memory and in aging. Optically exciting output neurons from the prelimbic area of the prefrontal cortex of awake rats has been shown to increased BOLD activation in ventral striatum, other neocortical areas, and the mediodorsal thalamus (Liang et al., 2015). It should be noted that BOLD activity in mediodorsal thalamic nucleus

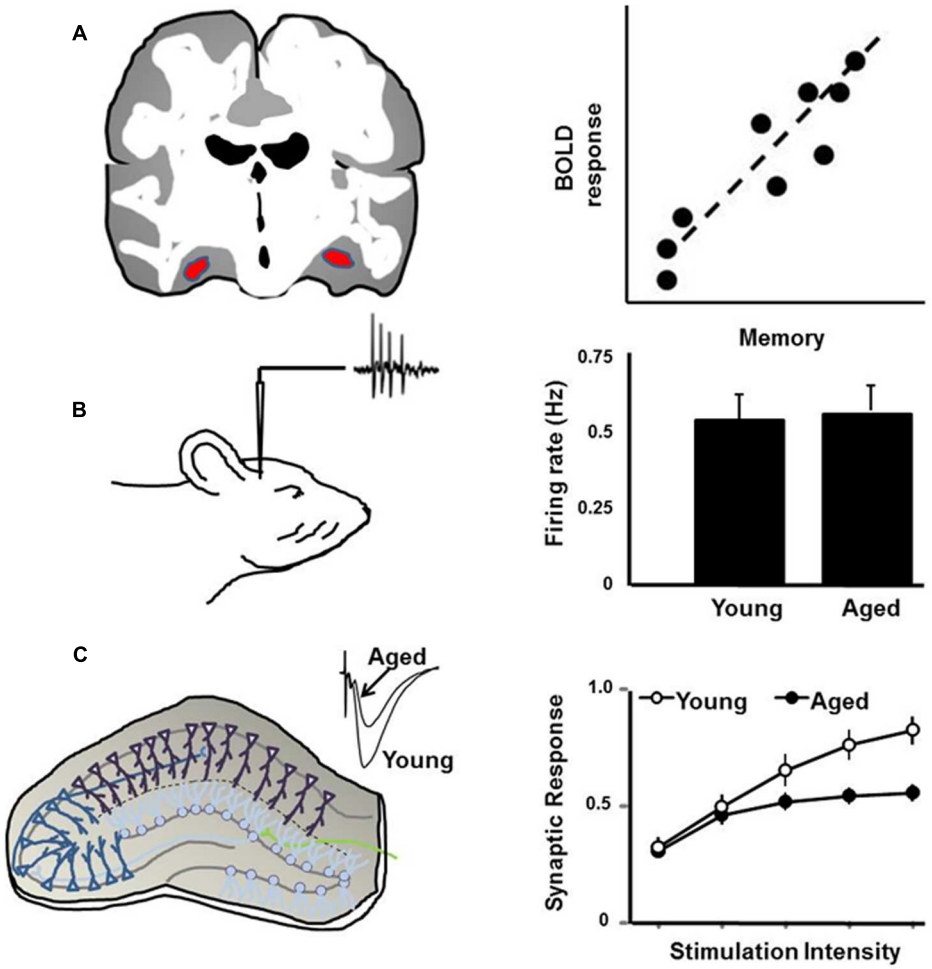


FIGURE 1 | Schematic illustration of (A) the BOLD signal, which is positively correlated with memory, such that impaired memory is associated with reduced BOLD activity in the hippocampus. (B) Discharge activity of hippocampal cells in response to environmental stimuli is not related to age or memory. Rather, cognitive impairment is associated with reduced ability to modify discharge activity. **(C)** Synaptic transmission and synaptic plasticity in the hippocampal slice is reduced with age, particularly in memory-impaired animals.

occurs with optogenetic stimulation of the hippocampus and prefrontal cortex. This region is known to be an integral part of anterior thalamic limbic circuitry involved in memory and learning (Aggleton and Brown, 1999; Aggleton et al., 2010). Interestingly, mediodorsal thalamic BOLD activation observed when driving prefrontal neurons of awake rats was not observed in rats under anesthesia. Conversely, hippocampally driven mediodorsal thalamus activation occurred under anesthetized conditions (Duffy et al., 2015). The distinct responsiveness of the mediodorsal thalamus to stimulation of these two brain areas thus appears varies according to the state of consciousness of the animals. It is possible that prefrontal cortex-to-mediodorsal thalamus activation requires an awake state whereas it does not appear to be necessary in hippocampal networks. This brings up interesting possibilities regarding the potential properties of temporal and prefrontal lobe interactions in memory networks. Mediodorsal thalamic neurons project to limbic frontal areas such as the prelimbic, orbital, insular and anterior cingulate regions (Gabbott et al., 2005). Here, they form asymmetric synaptic contacts with layer III pyramidal neurons projecting back to mediodorsal thalamus (Kuroda et al., 2004). Mediodorsal thalamic neurons also synapse onto two types of GABAergic interneurons that modulate both pyramidal cells and GABAergic interneurons, thus offering a potential network controlling and modifying thalamocortical and corticocortical activity (Kuroda et al., 2004). This medial thalamic circuitry also modulates hippocampal-to-prefrontal activity (Floresco and Grace, 2003). Driving hippocampal activity to the prefrontal cortex is modulated by stimulation of mediodorsal thalamus (Floresco and Grace, 2003). Tetanic stimulation of mediodorsal thalamus-to-prefrontal neurons potentiates ventral hippocampal-to-prefrontal activity (Floresco and Grace, 2003). Thus, the hippocampal-prefrontal circuitry shows synaptic plasticity that is under partial control by mediodorsal thalamic neurons. These, and other results, strongly suggest that mediodorsal thalamic neurons regulate the transit

of limbic activity to and from frontal cortical and hippocampal networks, and it also offers a pathway that can be targeted for further opto-fMRI studies.

The functional role of the dorsolateral area of the prefrontal cortex in working memory has been characterized using fMRI in humans and neurophysiological recordings in non-human primates (Funahashi et al., 1989; Curtis and D'Esposito, 2003; Nee and D'Esposito, 2016). Working memory tasks that engage this region elicit a BOLD activation pattern that reflects its temporary storage buffer and processing capacity (D'Esposito et al., 1999a; Rypma and D'Esposito, 1999), with a temporal neural activity profile similar to that measured electrophysiologically in non-human primates (Funahashi et al., 1989; Compte et al., 2000). It appears that with aging there is compensatory increased activation in the dorsolateral prefrontal cortex, with reduced BOLD activation in caudal sensory processing structures, such as the temporal and occipital cortices (Gigi et al., 2010; Fakhri et al., 2012). Older adults show greater BOLD activation in this prefrontal region that expands to the contralateral site when compared to young individuals (Cabeza et al., 2004). The greater BOLD activation in older versus young individuals may be related to a compensatory activation of neurons in this region during a working memory task. Interestingly, it was previously shown that the BOLD response to a high load working memory task is higher and more lateralized (to the right hemisphere of the laterodorsal prefrontal cortex) than in a low load working memory condition (Rypma and D'Esposito, 1999). More recent, work in older individuals has shown that this pattern may vary, with high cognitive load eliciting weaker activation (failure to meet demands) and low load activating the region more strongly (compensation for the functional loss; Cappell et al., 2010; Toepper et al., 2014). It is unclear if the expansion is in fact compensation in order to facilitate behavior, or is a sign of decreased specificity, and/or a sign of the impairment. Comparable studies have not been carried out in rats in order to address this matter more directly by manipulation of frontal cortical brain areas (Liang et al., 2015). Therefore, this is an area that would greatly benefit from opto-fMRI studies in rodents. During working memory tasks, the discharge activity of some cells does not increase to the same extent in aged rats (Caetano et al., 2012) and monkeys (Wang et al., 2011). In turn, the shift in discharge activity is thought to results from a shift in the balance of excitatory/inhibitory synaptic activity (Luebke et al., 2004; Guidi et al., 2015b), including the loss of dendritic spines (Dumitriu et al., 2010). If the BOLD expands in older humans, then the decline in discharge activity would not directly explain this. An alternative would be that decreased activity in the region may result in decreased lateral inhibition, permitting increased activity of other regions (thus the expansion of BOLD to other areas). Such a shift in the balance of excitatory/inhibitory synaptic activity could explain the expansion, and is an intriguing target for optogenetic manipulations in aged rats.

In summary, fMRI studies designed to activate the hippocampus of rats reveal that causally driving afferent inputs to the dentate gyrus via the perforant path increases BOLD in this region, and in downstream areas, and this appears to involve dynamic processing of synaptic activity. Variations in BOLD associated with high frequency pattern stimulation are likely due to synaptic plasticity in local circuits. These plasticity mechanisms, which can be engaged during learning or due to pathology such as epilepsy, influence the spread of neural activity to connected regions. Finally, a decrease in synaptic strength or plasticity, or a shift in the balance of excitatory/inhibitory synaptic activity may underlie changes in the BOLD response in association with cognitive aging.

IMAGING RESTING STATE NETWORKS INVOLVED IN MEMORY

Resting state fMRI is becoming a highly valuable strategy for characterizing neural circuits involved in learning and memory, especially when measures of behavioral performance on cognitive tasks are also assessed. Resting state connectivity provides information on intrinsic functional brain organization, which under baseline conditions involves correlated BOLD signals between specific subsets of brain areas (e.g., default, executive, salience networks can be assessed). Similarity in resting state functional connectivity of the hippocampus is observed between humans and animals models, including rodents, rabbits and monkeys (Becerra et al., 2011; Hutchison et al., 2011; Jonckers et al., 2011; Schroeder et al., 2016). Resting state connectivity has been examined in awake marmoset and rhesus monkeys and in anesthetized macaques. Effective connectivity (which estimates directionality of connectivity) between hippocampus and parietal cortex increases during memory retrieval in awake macaques (Miyamoto et al., 2013). Areas of the default mode network (e.g., medial and lateral parietal areas, anterior and posterior cingulate, and medial prefrontal cortex) exhibit decreases in activity during performance of goal-directed and attention-demanding tasks, and show increase functional coupling when the brain is in an "idle" mode (Raichle et al., 2001). This network appears to be active in anesthetized monkeys (Hutchison et al., 2011; Mantini et al., 2011, 2013) and rats (Upadhyay et al., 2011; Lu et al., 2012), and activity is decreased as monkeys attend to external stimuli (Mantini et al., 2011). Macaques and humans have a homologous temporal-parietal resting state network that involves parahippocampal areas, retrosplenial, posterior cingulate, superior temporal gyrus, and posterior parietal cortex, which may be involved in mnemonic processes (Vincent et al., 2010). Some of these areas are also part of the default network and this further strengthens the notion that this system is preserved across species of primates and rodents (Kojima et al., 2009; Lu et al., 2012), although a difference in the role of the striatum within the default system has been reported between human and non-human primates (Kojima et al., 2009).

Functional connectivity networks are increasingly used to assess network-level alterations associated with learning and memory and conditions of impaired cognition including aging. In rats, it has been shown that training-induced improvement in performance on a Morris water maze (MWM) task is associated with increased connectivity within hippocampal regions and between the hippocampus and other memory associated regions such as the septum, retrosplenial cortex, entorhinal cortex, and

task associate regions such as the visual and motor cortices and thalamus (Nasrallah et al., 2016). The increased connectivity between these regions was again reduced 7 days after the last MWM session, suggesting a waning of memory associated network BOLD activity (Nasrallah et al., 2016). In humans, resting state connectivity is reduced with age (Achard and Bullmore, 2007; Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Wu et al., 2011; Dennis and Thompson, 2014; Song et al., 2014; Ferreira et al., 2015; Huang et al., 2015; Li et al., 2015; Scheinost et al., 2015; La et al., 2016). Thus, one possibility is that a decrease in resting state connectivity is an indication of impaired memory formation or consolidation during aging. In a study that examined changes in resting state connectivity, older rats exhibited a postoperative impairment in cognition associated with decreased resting state connectivity, which recovered over time (Xie et al., 2013). In contrast, results obtained in middle age non-human primates showed increased connectivity strength between hippocampus and neocortical areas in animals with low memory performance scores (Koo et al., 2013). These animals showed reduced white matter integrity, suggesting that loss of memory performance with aging is associated with increased functional connectivity to compensate for structural white matter losses (Koo et al., 2013). Similarly, it should be noted that patients diagnosed with mild cognitive impairment exhibited increased connectivity between hippocampal and prefrontal regions, which the authors suggest is a result of a maladaptive reorganization of the brain (Gardini et al., 2015). It is thus possible that increased hippocampal functional connectivity reflects compensatory increases in neuronal activity in temporal lobe and neocortical networks of middle aged individuals, or individuals with milder forms of cognitive impairment. Although not yet determined, such compensatory neuronal activity might fail at later ages, or with the progression of senescent synaptic function with more advanced age and/or dementia.

In sum, experimental paradigms recruiting memory systems in normal aging may modify patterns of resting state functional connectivity across specific functional networks involving default mode and temporal lobe areas that are preserved across species (hippocampal areas in case of rats). While there is an extensive literature linking aspects of Alzheimer's disease and other forms of neurodegenerative dementia's to alterations in these networks, normal aging connectivity patterns need further investigation. Of note is the fact that functional connectivity analysis is based on statistical correlation methods and, as a result, limits the establishment of causal links to cellular and synaptic mechanisms. In spite of this limitation, future animal imaging studies should further define links between neuronal aging mechanisms and distinct functional connectivity patterns associated with impaired cognitive function.

FUNCTIONAL IMAGING AND NEUROVASCULAR COUPLING DEFICITS IN COGNITIVE AGING

Neurovascular coupling is a critical aspect of BOLD fMRI that can be impacted by cellular and molecular events altered in aging, especially as it relates to vascular mechanisms (D'Esposito et al., 1999b). This in turn could directly affect cognitive performance, even in the absence of data indicating impairments in synaptic plasticity. Cerebral metabolic rates for oxygen, arterial perfusion and blood volume changes contribute to the BOLD effect and may all be independently influenced by an aging cerebrovasculature (Mehagnoul-Schipper et al., 2002). Also, supporting cells, such as astrocytes, and the expression of vasoactive molecules, which play an important role in neurovascular coupling (Takano et al., 2006; Drake and Iadecola, 2007) may also be affected by aging mechanisms and in turn affect functional MRI results. Functional MRI alterations in the aging brain may, therefore, be influenced not only by changes in synaptic activity and strength, but may also occur as a result of changes in neurovascular coupling.

Aged rats show lower oxyhemoglobin, CBF and percent increases in BOLD signal in cortex in response to hypercapnia than young rats (Desjardins et al., 2014). These perfusion deficits worsen with age, and even more so with hypertension (Lee et al., 2011, 2014). Interestingly, deficits in response to hypercapnic challenge show a linear relationship with mild cognitive impairments in aged rats and are thought to be predictive of reduced performance on cognitive tasks (Mitschelen et al., 2009). Direct effects of aging on neurovascular uncoupling may contribute to reductions in cognitive performance, even in the absence of a change in synaptic function. Inhibiting the vasoactive signaling molecules cyclooxygenase-2, epoxygenase, and nitric oxide synthase (NOS) reduced CBF in response to whisker barrel stimulation. Reduced CBF was in turn associated with reduced performance in Y- and T-maze tasks and object recognition in the absence of altered synaptic strength (Tarantini et al., 2015). Cerebrovascular insufficiency has been shown to be associated with reduced performance on a MWM task, increased CA1 neuron damage, increased glial acidic fibrillary protein (GFAP) expression, reduced hippocampal blood flow, and increased ^{31}P-phosphomonoester, which may be an indicator of altered membrane phospholipid turnover rates (de la Torre et al., 1992). The results suggest that vascular impairments with age might lead to blunted BOLD signal responses compared to young adults and contribute to impaired cognition.

In aged animals the BOLD response is linked to several biological markers that are thought to contribute to cognitive deficits. Transcriptional profiling in vulnerable brain regions has revealed a relationship between age, cognitive function and gene expression (Prolla, 2002; Blalock et al., 2003; Loerch et al., 2008; Zeier et al., 2011). In general, aging is associated with increased expression of genes associated with the immune response and a decrease in expression of genes linked to synaptic connectivity and neural activity. Gene changes are relatively region-specific and suggest regional vulnerability to aging (Wang and Michaelis, 2010; Zeier et al., 2011). The regional specificity for an altered BOLD response suggests that the blunted BOLD signal may be due to local changes in synaptic function, metabolism, and neuroinflammation associated with these gene expression changes (Blau et al., 2012; Moreno et al., 2012; Sanganahalli et al., 2013). In support of this notion, several recent studies have combined gene manipulations with neuroimaging to understand

the relationship between transcriptional markers of aging or neurodegenerative disease and the progression brain changes (Song et al., 2004; Maheswaran et al., 2009; Moreno et al., 2012; Lewandowski et al., 2013; Pavlopoulos et al., 2013; Zerbi et al., 2014; Micotti et al., 2015; Sevgi et al., 2015). Mutation or absence of the cholesterol transporter protein apolipoprotein-ε (ApoE) is associated with deficits in functional connectivity and in CBF in the mouse hippocampus (Zerbi et al., 2014). The functional impairments are associated with increased mean diffusivity, which in turn are linked to synaptic loss and presence of pro-inflammatory cells in the region. In one study, a decline in the expression of histone binding protein RbAp48 was observed specific to the dentate gyrus of humans and mice over the course of aging (Pavlopoulos et al., 2013). Expression of a dominant-negative inhibitor of RbAp48 resulted in impaired memory and a decrease in BOLD activity within the dentate gyrus suggesting that altered histone regulation underlies cognitive impairment and/or decreased BOLD activity. The idea that age-related cognitive impairments are associated with a decrease in activation of the dentate gyrus is supported by work in a primate model of aging showing reduced CBV, which was associated with reduced expression of the neural activity marker *Arc* (Small et al., 2004). Thus, the above-cited studies appear to arrive at a consensus that age related reductions in CBF are particularly robust in the dentate gyrus. This represents a promising direction for preclinical imaging research.

IN VIVO HIPPOCAMPAL AND CORTICAL VOLUMETRIC CHANGES ASSOCIATED WITH AGING

A reduction in synaptic connectivity in the hippocampus with aging may consequently produce atrophy of this structure, impair memory functions, and this may explain not only impaired BOLD fMRI and vascular changes, but also volumetric changes in this region. There is currently a debate as to whether cognitive aging is associated with a decline in hippocampal volume in humans (Jernigan et al., 1991; Golomb et al., 1993; Sullivan et al., 1995; Raz et al., 2000), non-human primates (Shamy et al., 2006; Willette et al., 2013), and dogs (Su et al., 1998; Tapp et al., 2004; Kimotsuki et al., 2005). In aged Rhesus macaques, spatial memory was not associated with the size of the hippocampus; although, expression of the synaptic marker synaptophysin was reduced in animals with impaired memory (Haley et al., 2012). In contrast, another study indicated that gray matter areas of the prefrontal and temporal cortices of macaques show age related reductions in volume accompanied by reduced performance on delayed non-match to sample task (Alexander et al., 2008; Wisco et al., 2008). Synaptic proteins and mRNA levels, and hippocampal volumes, decline in aged-memory impaired rats, with volumes lower in aged and middle age rats compared to young rats (Smith et al., 2000; Blalock et al., 2003; Driscoll et al., 2006). The decline in hippocampal volume correlated with reduced performance on a MWM task. Similarly, transgenic mice expressing ApoE4 show greater aged related reductions in hippocampal and cortical volumes, and also suffer greater cognitive deficits than wild-type mice (Yin et al., 2011). The reduced hippocampal volume is associated with increased microglial marker iba1 and tumor necrosis factor α, suggesting a role for neuroinflammation. Finally, aged lemurs that performed poorly on shifting and discrimination tasks also show significant volumetric reductions in caudate-putamen, hippocampus, septum, and temporal, occipital and cingulate cortices (Picq et al., 2012). Volumetric reductions may be influenced by co-occurring conditions affecting the aging population. For instance, using a heart failure model, Suzuki et al. (2015) showed a significant reduction in gray matter volume in rats with coronary ligation. There is also promising evidence suggesting that physical activity may ameliorate reductions in cortical and hippocampal volumes (Fuss et al., 2014; Mariotti et al., 2014; Sumiyoshi et al., 2014).

Volumetric changes can be linked to functional changes through the use of manganese enhanced MRI (MEMRI) in animal studies (Koretsky and Silva, 2004). The paramagnetic manganese ion (Mn^{2+}) enters voltage dependent Ca^{2+} channels (VDCC), which are pervasively present on synapses across the brain. It is therefore used as a surrogate marker of synaptic activity during baseline conditions and following chronic disease states, memory tasks, or drug stimulation (Pautler et al., 2003; Pautler, 2004). Intra-synaptic sequestering and transsynaptic transport allows for the measurement of neural circuit activity-associated increases in signal intensity in high resolution T_1 weighted images. A popular application of MEMRI is to quantify rates of signal intensity change in major fiber pathways in order to indirectly estimate *in vivo* brain axonal transport rates (Bearer et al., 2007; Smith et al., 2007, 2010, 2011; Kim et al., 2011). Using this methodology, Cross et al. (2008) showed a significant decline in olfactory pathway transport rates in aged vs. young and middle aged rats. This has been demonstrated as well in mouse models of amyloidosis, tauopathy, and neurodegeneration (Serrano et al., 2008; Majid et al., 2014).

Interestingly, given its mechanism involving VDCC uptake, this imaging strategy can provide an indication of Ca^{2+} regulation (Lu et al., 2007; Berkowitz et al., 2014; Groschel et al., 2014). Dysregulation of Ca^{2+} during aging is thought to underlie changes in cell excitability (Landfield, 1988; Disterhoft et al., 1993; Foster, 2007; Thibault et al., 2007; Kumar et al., 2009; Oh and Disterhoft, 2010) and the senescence of synaptic function (Foster and Norris, 1997; Kumar et al., 2009; Foster, 2012). The MEMRI technique has been employed to demonstrate increased Ca^{2+} of sensory systems associated with an age-related impairment of sensory processing (Bissig et al., 2013; Groschel et al., 2014). For example, 13- to 18-month-old mice with significant hearing loss show greater accumulation of Mn^{2+} signal in auditory networks and the hippocampus relative to 3-month-old mice (Groschel et al., 2014). An increase in MEMRI signal intensity in the pyramidal cell layer of the CA1 is also observed in 6- to 18-month-old rats (Bissig and Berkowitz, 2014). However, it is unclear if the changes represent accumulation in active neurons, active synapses, or glial cells (Nairismagi et al., 2006; Hsu et al., 2007; Immonen et al., 2008; Eschenko et al., 2010; Perez et al., 2013; Zhang et al., 2015). For example, an increase in Mn^{2+} signal intensity in dorsal CA1 and dentate

gyrus of mice showing neurodegeneration and forebrain atrophy might be associated with increased presence of glial cells in the region (Perez et al., 2013) and an increase in the area of Mn^{2+} intensity was observed at the mossy fiber to CA3 synaptic terminal region following a learning (Zhang et al., 2015). In spite of these interesting results, a significant limitation of MEMRI is the neurotoxic effects of Mn^{2+} on dopamine neurons (Aschner et al., 2007), and its actions as a glutamatergic NMDA receptor blocker, both of which may interfere with its intended use of measuring neuronal activity and aging related changes in neuronal activity (Guilarte and Chen, 2007; Liu et al., 2013). Furthermore, because Mn^{2+} competes with Ca^{2+}, it will have effects on Ca^{2+}-dependent processes including the release of transmitter and possible synaptic plasticity (Eschenko et al., 2010). Systemic administration of Mn^{2+} may also affect overall health and chronically affect weight gain in rats (Jackson et al., 2011), thus further reducing the utility of this method for longitudinal MRI studies.

PHARMACOLOGICAL MRI OF POTENTIAL COGNITIVE MODULATORS

BOLD fMRI, arterial spin labeling, and superparamagnetic iron oxide nanoparticle based functional imaging of blood volume are also used for *in vivo* measurement of drug-induced brain activation. The use of these modalities initially started with administration of psychoactive substances in studies of drug abuse and addiction (Mandeville et al., 1998; Marota et al., 2000; Schwarz et al., 2003; Febo et al., 2004, 2005a,b), but over the last decade other applications, particularly the testing of modulators of cognitive function and mood has emerged. For instance, one of the key mechanisms involved in LTP is an increase in AMPA receptor-mediated synaptic currents through the insertion of AMPA receptors into the post-synaptic terminal (Luscher et al., 2000). Drugs that enhance AMPA mediated effects can thus be considered to be potential targets for modulating memory through a well-defined synaptic mechanism. Administration of an AMPA receptor agonist (LY404187) increased BOLD activation largely in the dorsal hippocampus and septum and this was blocked by pretreatment with the AMPA/kainite antagonist LY293558 (Jones et al., 2005). This is consistent with previous work with the same AMPA agonist compound showing increases in cerebral metabolic rates for glucose and c-fos expression in the same regions (Fowler et al., 2004). Cholinergic modulation in the brain has also been assessed using drugs that activate muscarinic and nicotinic receptors (Hoff et al., 2010, 2011; Haley et al., 2011; Bruijnzeel et al., 2014). Experiments focusing on the cholinergic system show pronounced thalamocortical activation, which is very low in studies examining AMPA receptor activation. This shows the capacity of combining pharmacology and fMRI to distinguish among these two drug classes acting through different receptor systems. Bruijnzeel et al. (2014) showed dose-dependent nicotine-induced BOLD activation of anesthetized rat brain, which was blocked by the general nicotine receptor antagonist mecamylamine (Bruijnzeel et al., 2014). Administration of the non-specific muscarinic receptor antagonist scopolamine to aged monkeys resulted in greater increases in hippocampal BOLD signal, but only in animals performing well on spatial maze task (Haley et al., 2011). This correlated with greater levels M1 but not M2 receptor density in greater performing than poorly performing animals.

In humans, an age-related decline in activity within the posterior brain regions including the hippocampus is associated with increased activity in the prefrontal cortex (Sperling, 2007; Kaufmann et al., 2008; Park and Reuter-Lorenz, 2009; Turner and Spreng, 2012; Lighthall et al., 2014; Tromp et al., 2015). Interestingly, inhibition of NMDA receptors in the hippocampus or prefrontal cortex drives activity in the prefrontal cortex (Jodo et al., 2005; Homayoun and Moghaddam, 2007). Furthermore, low level NMDA receptor blockade impairs hippocampal function and improves executive processes that depend on the prefrontal cortex (Guidi et al., 2015b). Chin et al. (2011) showed that ketamine, an amnesic/dissociative agent that blocks NMDA receptors produced robust activation of cortical regions and the hippocampus of awake rats. This effect was modulated by the glutamate metabotropic agonist LY379268 (Chin et al., 2011). More recent work has confirmed that ketamine increases functional interactions between brain regions involved in memory (hippocampus, areas of the limbic prefrontal cortex, and retrosplenium; Gass et al., 2014; Grimm et al., 2015). These studies illustrate the potential for pharmacological MRI to investigate brain wide activation in response to cognitive modulators.

DIFFUSION BRAIN IMAGING IN NORMAL AGING RATS

Compared with fMRI, diffusion MRI has been applied more extensively to the study of animal models of neurodegenerative diseases. However, we will mostly focus here on dMRI studies relevant to normal cognitive aging. In diffusion MRI, directionally applied diffusion-sensitizing magnetic field gradients tag protons in slowly moving (diffusing) water molecules (in the order of 10^{-3} $\mu m^2/s$), and thus omit water moving at faster rates (e.g., inside blood vessels, as measured in the above-cited fMRI modalities; Le Bihan et al., 2001). The fractional anisotropy (FA) index is one of the main scalars estimated from a series of diffusion-sensitized MRI images. FA has been used extensively as an indicator of underlying tissue microstructural integrity (Mori and Zhang, 2006). This value is most reliable when assessed in major white matter (WM) tracts in rodent brain at high fields, although a growing number of studies are also reporting FA for gray matter regions. FA values range from 0 to 1, with 1 indicating *high directionality* of water diffusion (anisotropic diffusion) and 0 *low directionality* (isotropic diffusion). Thus, lowest FA values are measured from cerebroventricles (because of the high mobility of unbound water molecules have in this compartment), whereas highest values are measured in WM fiber bundles, such as the corpus callosum, fimbria, internal capsule, where water molecules show a high net directionality due to the presence of highly organized barriers to diffusion formed by myelinated axonal fibers. Reductions in

myelination, increases in fluid filled inter-axonal spaces, altered cellular density, local cellular inflammatory responses, and edema can all reduce FA (Peled, 2007). Reduced FA is thought to represent WM alterations in aging and pathologies of Alzheimer's disease in humans (Bozzali et al., 2001; Teipel et al., 2010; Douaud et al., 2011, 2013). Recent evidence for a link between reduced FA and pathology was provided by studies in a mouse overexpressing microtubule associated protein tau (Sahara et al., 2014). Similar to human studies, the results demonstrate a progressive reduction of FA in WM structures of the tau-expressing mice. Changes in FA were associated with an increase in tau pathology and disorganization of unmyelinated processes. Indeed, changes in dMRI were detectable as early as 2.5 months, before the emergence of obvious overt pathology. Similar age-associated reductions in FA (and concomitant increases in diffusivity scalars, axial, radial, and mean diffusivities) have been reported in a transgenic rat model of Huntington disease (Antonsen et al., 2013), and in corpus callosum of normal aged rats (Guo et al., 2015). In spite of these correlations, the mechanistic basis for changes in FA still remains unclear.

In humans, it has been known for many years that WM content in brain shows an inverted U maturational change that peaks at middle age (45 years of age). This was first demonstrated in postmortem tissue and subsequently supported by Bartzokis et al. (2004) using MRI. The loss of WM begins early in middle-age in humans and rhesus monkeys, with a prolonged decline during aging (Makris et al., 2007; Westlye et al., 2010; Yeatman et al., 2014). In contrast, WM loss is initiated much later for chimpanzees, suggesting that older chimpanzees exhibit decreased atrophy relative to humans (Chen et al., 2013). Rodents show a age progressive change in WM similar to humans, occurring at earlier stages in the rodents lifespan, with a steep rise in FA from 0–40 days (ending at mid adolescence), and a gradual but progressive decline thereafter (Calabrese et al., 2013). Interestingly, mean diffusivity peaks earlier between 10–20 days of age and then remains stable, or shows a steady decline (Mengler et al., 2014), at least until day 80 (Calabrese et al., 2013). FA values in outer cortical layers of developing rat brain is reduced during the first 10 postnatal days (Huang et al., 2008). Therefore, diffusion MRI can distinguish between non-WM maturational changes. Thus, these represent early life maturational changes, perhaps associated with early brain development (Mengler et al., 2014). Synaptic and axonal pruning and increases in myelination of major fiber tracts account in part for these early life changes in FA and mean diffusivity (Huang et al., 2008). Compared to 3-month-old rats, 12-month-old rats show lower apparent diffusion coefficient (ADC) values in cortex (Heiland et al., 2002). ADC mapping is typically used in preclinical stroke research, with reduced gray matter ADC values indicative of early hemorrhagic events and high values indicative of progressive edematous tissue damage. Aged rats sustaining transient global ischemia also show greater reductions in ADC than young animals imaged under the same conditions (Canese et al., 1998, 2004). Hypotension-associated with a single hemorrhage event causes a greater reduction in hippocampal ADC in 18-month-old compared to 12-month-old rats (Plaschke et al., 2009). Thus, vascular events that increase in risk with age are observed to alter diffusion MRI indices of tissue integrity. While this is an area that needs further investigation, it points to the possibility of developing the diagnostic capabilities of diffusion MRI as a technique that offers tissue quantitative measures that could assess risk or vulnerability in aging brain under specific challenges. For example, age-related reduction in FA in corpus callosum is prevented in aged rats subjected to a caloric restriction (Guo et al., 2015).

PROTON MAGNETIC RESONANCE SPECTROSCOPY

A major advantage of ultra-high field imaging (7 T and above), apart from the greater signal-to-noise, is the improved capacity to resolve or separate the chemical shift peaks of various biomolecules involved in neurotransmitter metabolism in cells (Di Costanzo et al., 2003; Moser et al., 2012). MRS, particularly involving hydrogen (^{1}H) nuclei, has been used for years to assess various chemical species in brain diseases, both neuropsychiatric and neurologic conditions (Maddock and Buonocore, 2012; Rossi and Biancheri, 2013). Imaging techniques have better spatial resolution than MRS techniques, but ^{1}H-MRS offers strong complementary data because of its specificity and quantitative capabilities, which permit the assessment of tissue concentrations of distinct biologically relevant molecules and metabolic intermediates. ^{1}H-MRS can detect molecular markers for neurons and glia, transmitters, and antioxidant capacity (see **Table 1**).

For example, *N*-acetylaspartate (NAA) and myo-inositol are measured as neuronal and glial markers, respectively (Demougeot et al., 2004; Harris et al., 2015). Following experimental ischemia, a decline in NAA is observed in the most vulnerable brain regions and correlates with cell loss (Higuchi et al., 1997; Sager et al., 2001). However, there is also evidence for a decline in NAA not linked to neuronal loss (Jenkins et al., 2000). NAA is synthesize in the mitochondria and a rapid decline in NAA following ischemia may represent impaired neuronal function, which can recover during reperfusion (Demougeot et al., 2004). Indeed, minor levels of hippocampal cell loss following mild ischemia was not associated with a change in hippocampal NAA (Galisova et al., 2014).

In Alzheimer's disease, a decline in NAA is observed in hippocampus and posterior cingulate in association with a loss of synaptic markers and increased hyperphosphorylated tau, consistent with a neuronal loss. Interestingly, increased myo-inositol levels where more prominent in the cortex in association with amyloid-beta levels, suggesting activation of glial markers may precede neuronal loss (Murray et al., 2014; Wang et al., 2015). In this case, the increase in glial markers may be an early sign, possibly representing neuroinflammation. Indeed, APP/PS1 transgenic mice, an increase in myo-inositol precedes the decline in NAA and changes in both markers precede cognitive impairment (Chen et al., 2012).

In the case of normal aging in humans and in animal models of brain aging, an increase in glial markers, possibly as a sign of neuroinflammation, is observed in the absence of

TABLE 1 | Proton MRS markers relevant to aging, inflammation, neurodegeneration, and excitatory neurotransmission.

Molecule or metabolic intermediate	Marker for	Age-related change
NAA	Neuronal health	No change with normal aging, decreased in neurodegenerative disease
myo-inositol	Glia	Increased in normal aging, possibly as a sign of neuroinflammation
Acorbate, GSH	Oxidative stress	Generally decreased with age, indicating brain regions that are vulnerable to oxidative stress
Glutamate, GABA	Neurotransmitters	Region specific changes may reflect a shift in the balance of excitatory/inhibitory transmission

profound neuronal loss. Studies of ^{1}H-MRS profiles in aging humans indicates no change or a small decrease in NAA and an increase myo-inositol, consistent with an increase in glial markers (Boumezbeur et al., 2010). Similar changes are observed in ^{1}H-MRS profiles of aging non-human primates (Herndon et al., 1998; Ronen et al., 2011) and rodents (Driscoll et al., 2006; Duarte et al., 2014; Harris et al., 2014), consistent with an increase in astrocytic markers associated with chronological age. A consistent observation of aging in humans and animal models, is a persistent low level increase in serum markers of inflammation and this pro-inflammatory phenotype is thought to influence neuroinflammation, the activation glial cells, astrocytes and microglia, and may contribute to age-related cognitive decline (Rafnsson et al., 2007; Gimeno et al., 2008; Bettcher et al., 2012; Scheinert et al., 2015). Studies that employ ^{1}H-MRS and correlate brain levels of myo-inositol with measures of systemic inflammation indicate a positive relationship (Eagan et al., 2012; Schneider et al., 2012). Interestingly, just as with neurodegenerative disease and aging, not all brain regions are equally vulnerable to the effects of systemic inflammation (Semmler et al., 2005; Silverman et al., 2014; Scheinert et al., 2015) and regional markers of inflammation, including levels of myo-inositol correlate with behavioral changes (Schneider et al., 2012; Scheinert et al., 2015).

One important goal for the neurobiology of aging is to understand regional differences in neuronal vulnerability to aging (Jackson et al., 2009; Wang and Michaelis, 2010; Zeier et al., 2011). Total choline levels may reflect membrane turn over including demyelination and inflammation and brain levels increase with age in humans and animal models (Duarte et al., 2014; Harris et al., 2014). However, it is unclear whether the age-related increase in total choline is due to membrane turnover or linked to functional changes including cognitive decline (Charlton et al., 2007). Thus, animal studies could provide a rich source for hypotheses concerning the cellular and molecular constituents of total choline measures, as well as a test of regional vulnerability. Oxidative stress increases with advancing age and oxidative damage may contribute to neuronal death associated with neurodegenerative disease. Recent work indicates that the oxidation–reduction (redox) status of neurons underlies senescent physiology including impaired synaptic plasticity and the emergence of cognitive impairment (Bodhinathan et al., 2010a,b; Kumar and Foster, 2013; Lee et al., 2014; Guidi et al., 2015a). ^{1}H-MRS can be employed to examine redox status by measuring the level of antioxidant molecules, ascorbate and glutathione (GSH). GSH is mainly observed in astrocytes (Slivka et al., 1987; Keelan et al., 2001) and an increase in GHS could indicate activation of astrocytes. Alternatively, a decline in GHS could signal an increase in oxidative stress. In rats, maturation is associated with an increase in the level of GSH in the prefrontal cortex and prenatal immune activation interferes with an inability to increase GSH levels (Vernon et al., 2015). In general, aging is associated with a decline in antioxidant molecules in the brain. Moreover, the decline is regionally selective and can vary by gender (Terpstra et al., 2006; Emir et al., 2011; Duarte et al., 2014; Harris et al., 2014), which provides grist for hypotheses concerning vulnerability to aging and neurodegenerative disease.

Finally, ^{1}H-MRS can detect the level of certain transmitters. The most common measures are for glutamate, aspartate, and gamma-aminobutyric acid (GABA). Changes in transmitter levels are observed in several animal models of neurological diseases (Biedermann et al., 2012; Bagga et al., 2013; Berthet et al., 2014; Santin et al., 2014). In some cases, the animal models exhibit a good correspondence with the human condition. For example, altered levels of glutamate are observed in the prefrontal cortex of schizophrenia patients and animal models of schizophrenia (Iltis et al., 2009; Maddock and Buonocore, 2012; Puhl et al., 2015). Similarly, a decrease in prefrontal cortex glutamate is observed in depressed patients and in animal models of stress (Knox et al., 2010; Hemanth Kumar et al., 2012; Drouet et al., 2015). Examination of transmitters over time may give clues to mechanisms and how processes change over time. A decline in both glutamate and GABA show a progressive decline in animal models of Alzheimer's disease (Nilsen et al., 2012) and a differential decline in glutamate and GABA is observed during the development of temporal lobe epilepsy (van der Hel et al., 2013). An increase in glutamatergic transmission may contribute to the development of Parkinson's disease. MRS studies examining glutamate levels in the striatum of humans with Parkinson's disease do not agree possibly due to the etiology, stage of the disease, or pharmacological intervention (Kickler et al., 2007; Griffith et al., 2008; Modrego et al., 2011). Similarly, in animal models differences in glutamate levels may depend on the etiology or the animal model (Delli Pizzi et al., 2013). For example, treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine decreased glutamate levels in felines (Podell et al., 2003), no change in canines (Choi et al., 2011), and increased glutamate levels in mice (Chassain et al., 2008, 2013), Even when examined in the same animal model differences may arise associated with the power of the magnet (Kickler et al., 2009; Coune et al., 2013).

Regional differences in transmitter levels are associated with aging in humans (Grachev et al., 2001; Gao et al., 2013;

Zahr et al., 2013; Riese et al., 2015). In male rats, aspartate and glutamate exhibited a decline with in specific brain regions, which may reflect a shift in the balance of excitatory and inhibitory transmission (Harris et al., 2014). In contrast, measures of both GABA and glutamate or aspartate declined in an aging male and female mice (Duarte et al., 2014). Future studies will need to investigate these discrepancies which may include regions examined and sex differences in glutamate over the course of aging (Zahr et al., 2013), as well as variability associated with variability in cognitive decline.

CONCLUDING REMARKS

One of the key aspects of aging brain is a gradual loss of memory to the point where this affects the individuals' normal daily activities (Gauthier et al., 2006). In normal aging cognitive impairment can be progressive, and while not necessarily entailing a severe loss of neurons, as in neurodegenerative diseases, the animal literature supports alterations in neuronal activity (Kumar and Foster, 2007; Watson et al., 2012; Foster et al., 2016). Indeed, these changes may involve alterations in excitatory/inhibitory balance, changes in synaptic proteins and intracellular signaling mechanisms, and spine density and morphology (Foster et al., 1996, 2000, 2012, 2016; Foster and Norris, 1997; Foster and Dumas, 2001; Blalock et al., 2003; Foster, 2007, 2012; Kumar and Foster, 2007, 2013; Kumar et al., 2009; Dumitriu et al., 2010; Guidi et al., 2015a,b). Translating these cellular mechanisms of animal aging models to human aging is a difficult challenge and it may be possible that preclinical imaging and spectroscopy studies could serve a role in this task. We have reviewed different MRI modalities used in primate and rodent models to characterize functional activation in hippocampal and prefrontal memory networks, anatomical changes and their correlations with cognitive decline, changes in neurovascular coupling with aging, and biochemical alterations relevant to aging. Results from the different MR modalities presented here can be enhanced by combining these with invasive *in vivo* and *ex vivo* approaches to determine their relationship to changes at the synaptic, proteomic, and genetic levels, for an integrative assessment of brain aging and reduced cognitive capacity.

AUTHOR CONTRIBUTIONS

MF and TF contributed equally to the outline, drafting and editing of the present manuscript.

FUNDING

This work was supported by National Institutes of Aging Grants R01AG037984, R37AG036800, R01AG49711, R21DA038009, P50AG047266. The authors thank the William L. and Evelyn F. McKnight Brain Foundation for the support.

REFERENCES

Achard, S., and Bullmore, E. (2007). Efficiency and cost of economical brain functional networks. *PLoS Comput. Biol.* 3:e17. doi: 10.1371/journal.pcbi.0030017

Aggleton, J. P., and Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behav. Brain Sci.* 22, 425–444. discussion 444–489. doi: 10.1017/s0140525x99002034

Aggleton, J. P., O'mara, S. M., Vann, S. D., Wright, N. F., Tsanov, M., and Erichsen, J. T. (2010). Hippocampal-anterior thalamic pathways for memory: uncovering a network of direct and indirect actions. *Eur. J. Neurosci.* 31, 2292–2307. doi: 10.1111/j.1460-9568.2010.07251.x

Alexander, G. E., Chen, K., Aschenbrenner, M., Merkley, T. L., Santerre-Lemmon, L. E., Shamy, J. L., et al. (2008). Age-related regional network of magnetic resonance imaging gray matter in the rhesus macaque. *J. Neurosci.* 28, 2710–2718. doi: 10.1523/JNEUROSCI.1852-07.2008

Alexander, G. E., Ryan, L., Bowers, D., Foster, T. C., Bizon, J. L., Geldmacher, D. S., et al. (2012). Characterizing cognitive aging in humans with links to animal models. *Front. Aging Neurosci.* 4:21. doi: 10.3389/fnagi.2012.00021

Andrews-Hanna, J. R., Snyder, A. Z., Vincent, J. L., Lustig, C., Head, D., Raichle, M. E., et al. (2007). Disruption of large-scale brain systems in advanced aging. *Neuron* 56, 924–935. doi: 10.1016/j.neuron.2007.10.038

Angenstein, F. (2014). The actual intrinsic excitability of granular cells determines the ruling neurovascular coupling mechanism in the rat dentate gyrus. *J. Neurosci.* 34, 8529–8545. doi: 10.1523/JNEUROSCI.0472-14.2014

Angenstein, F., Kammerer, E., Niessen, H. G., Frey, J. U., Scheich, H., and Frey, S. (2007). Frequency-dependent activation pattern in the rat hippocampus, a simultaneous electrophysiological and fMRI study. *Neuroimage* 38, 150–163. doi: 10.1016/j.neuroimage.2007.07.022

Angenstein, F., Kammerer, E., and Scheich, H. (2009). The BOLD response in the rat hippocampus depends rather on local processing of signals than on the input or output activity. A combined functional MRI and electrophysiological study. *J. Neurosci.* 29, 2428–2439. doi: 10.1523/JNEUROSCI.5015-08.2009

Angenstein, F., Krautwald, K., Wetzel, W., and Scheich, H. (2013). Perforant pathway stimulation as a conditioned stimulus for active avoidance learning triggers BOLD responses in various target regions of the hippocampus: a combined fMRI and electrophysiological study. *Neuroimage* 75, 213–227. doi: 10.1016/j.neuroimage.2013.03.007

Antonsen, B. T., Jiang, Y., Veraart, J., Qu, H., Nguyen, H. P., Sijbers, J., et al. (2013). Altered diffusion tensor imaging measurements in aged transgenic Huntington disease rats. *Brain Struct. Funct.* 218, 767–778. doi: 10.1007/s00429-012-0427-0

Arsenault, J. T., Nelissen, K., Jarraya, B., and Vanduffel, W. (2013). Dopaminergic reward signals selectively decrease fMRI activity in primate visual cortex. *Neuron* 77, 1174–1186. doi: 10.1016/j.neuron.2013.01.008

Aschner, M., Guilarte, T. R., Schneider, J. S., and Zheng, W. (2007). Manganese: recent advances in understanding its transport and neurotoxicity. *Toxicol. Appl. Pharmacol.* 221, 131–147. doi: 10.1016/j.taap.2007.03.001

Bagga, P., Chugani, A. N., Varadarajan, K. S., and Patel, A. B. (2013). In vivo NMR studies of regional cerebral energetics in MPTP model of Parkinson's disease: recovery of cerebral metabolism with acute levodopa treatment. *J. Neurochem.* 127, 365–377. doi: 10.1111/jnc.12407

Bartzokis, G., Sultzer, D., Lu, P. H., Nuechterlein, K. H., Mintz, J., and Cummings, J. L. (2004). Heterogeneous age-related breakdown of white matter structural integrity: implications for cortical "disconnection" in aging and Alzheimer's disease. *Neurobiol. Aging* 25, 843–851. doi: 10.1016/j.neurobiolaging.2003.09.005

Bearer, E. L., Falzone, T. L., Zhang, X., Biris, O., Rasin, A., and Jacobs, R. E. (2007). Role of neuronal activity and kinesin on tract tracing by manganese-enhanced MRI (MEMRI). *Neuroimage* 37(Suppl. 1), S37–S46. doi: 10.1016/j.neuroimage.2007.04.053

Becerra, L., Pendse, G., Chang, P. C., Bishop, J., and Borsook, D. (2011). Robust reproducible resting state networks in the awake rodent brain. *PLoS ONE* 6:e25701. doi: 10.1371/journal.pone.0025701

Berkowitz, B. A., Grady, E. M., and Roberts, R. (2014). Confirming a prediction of the calcium hypothesis of photoreceptor aging in mice. *Neurobiol. Aging* 35, 1883–1891. doi: 10.1016/j.neurobiolaging.2014.02.020

Berthet, C., Xin, L., Buscemi, L., Benakis, C., Gruetter, R., Hirt, L., et al. (2014). Non-invasive diagnostic biomarkers for estimating the onset time of permanent cerebral ischemia. *J. Cereb. Blood Flow Metab.* 34, 1848–1855. doi: 10.1038/jcbfm.2014.155

Bettcher, B. M., Wilheim, R., Rigby, T., Green, R., Miller, J. W., Racine, C. A., et al. (2012). C-reactive protein is related to memory and medial temporal brain volume in older adults. *Brain Behav. Immun.* 26, 103–108. doi: 10.1016/j.bbi.2011.07.240

Biedermann, S., Weber-Fahr, W., Zheng, L., Hoyer, C., Vollmayr, B., Gass, P., et al. (2012). Increase of hippocampal glutamate after electroconvulsive treatment: a quantitative proton MR spectroscopy study at 9.4 T in an animal model of depression. *World J. Biol. Psychiatry* 13, 447–457. doi: 10.3109/15622975.2011.580778

Bissig, D., and Berkowitz, B. A. (2014). Testing the calcium hypothesis of aging in the rat hippocampus in vivo using manganese-enhanced MRI. *Neurobiol. Aging* 35, 1453–1458. doi: 10.1016/j.neurobiolaging.2013.12.019

Bissig, D., Goebel, D., and Berkowitz, B. A. (2013). Diminished vision in healthy aging is associated with increased retinal L-type voltage gated calcium channel ion influx. *PLoS ONE* 8:e56340. doi: 10.1371/journal.pone.0056340

Bizon, J. L., Foster, T. C., Alexander, G. E., and Glisky, E. L. (2012). Characterizing cognitive aging of working memory and executive function in animal models. *Front. Aging Neurosci.* 4:19. doi: 10.3389/fnagi.2012.00019

Blalock, E. M., Chen, K. C., Sharrow, K., Herman, J. P., Porter, N. M., Foster, T. C., et al. (2003). Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. *J. Neurosci.* 23, 3807–3819.

Blau, C. W., Cowley, T. R., O'sullivan, J., Grehan, B., Browne, T. C., Kelly, L., et al. (2012). The age-related deficit in LTP is associated with changes in perfusion and blood-brain barrier permeability. *Neurobiol. Aging* 33, e1023–e1035. doi: 10.1016/j.neurobiolaging.2011.09.035

Bodhinathan, K., Kumar, A., and Foster, T. C. (2010a). Intracellular redox state alters NMDA receptor response during aging through Ca2+/calmodulin-dependent protein kinase II. *J. Neurosci.* 30, 1914–1924. doi: 10.1523/JNEUROSCI.5485-09.2010

Bodhinathan, K., Kumar, A., and Foster, T. C. (2010b). Redox sensitive calcium stores underlie enhanced after hyperpolarization of aged neurons: role for ryanodine receptor mediated calcium signaling. *J. Neurophysiol.* 104, 2586–2593. doi: 10.1152/jn.00577.2010

Boumezbeur, F., Mason, G. F., De Graaf, R. A., Behar, K. L., Cline, G. W., Shulman, G. I., et al. (2010). Altered brain mitochondrial metabolism in healthy aging as assessed by in vivo magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab.* 30, 211–221. doi: 10.1038/jcbfm.2009.197

Bozzali, M., Franceschi, M., Falini, A., Pontesilli, S., Cercignani, M., Magnani, G., et al. (2001). Quantification of tissue damage in AD using diffusion tensor and magnetization transfer MRI. *Neurology* 57, 1135–1137. doi: 10.1212/WNL.57.6.1135

Bruijnzeel, A. W., Alexander, J. C., Perez, P. D., Bauzo-Rodriguez, R., Hall, G., Klausner, R., et al. (2014). Acute nicotine administration increases BOLD fMRI signal in brain regions involved in reward signaling and compulsive drug intake in rats. *Int. J. Neuropsychopharmacol.* 18:piipyu011. doi: 10.1093/ijnp/pyu011

Burke, S. N., and Barnes, C. A. (2006). Neural plasticity in the ageing brain. *Nat. Rev. Neurosci.* 7, 30–40. doi: 10.1038/nrn1809

Cabeza, R., Daselaar, S. M., Dolcos, F., Prince, S. E., Budde, M., and Nyberg, L. (2004). Task-independent and task-specific age effects on brain activity during working memory, visual attention and episodic retrieval. *Cereb. Cortex* 14, 364–375. doi: 10.1093/cercor/bhg133

Caetano, M. S., Horst, N. K., Harenberg, L., Liu, B., Arnsten, A. F., and Laubach, M. (2012). Lost in transition: aging-related changes in executive control by the medial prefrontal cortex. *J. Neurosci.* 32, 3765–3777. doi: 10.1523/JNEUROSCI.6011-11.2012

Calabrese, E., Johnson, G. A., and Watson, C. (2013). An ontology-based segmentation scheme for tracking postnatal changes in the developing rodent brain with MRI. *Neuroimage* 67, 375–384. doi: 10.1016/j.neuroimage.2012.11.037

Canals, S., Beyerlein, M., Murayama, Y., and Logothetis, N. K. (2008). Electric stimulation fMRI of the perforant pathway to the rat hippocampus. *Magn. Reson. Imaging* 26, 978–986. doi: 10.1016/j.mri.2008.02.018

Canese, R., Fortuna, S., Lorenzini, P., Podo, F., and Michalek, H. (1998). Transient global brain ischemia in young and aged rats: differences in severity and progression, but not localisation, of lesions evaluated by magnetic resonance imaging. *MAGMA* 7, 28–34. doi: 10.1007/BF02592254

Canese, R., Lorenzini, P., Fortuna, S., Volpe, M. T., Giannini, M., Podo, F., et al. (2004). Age-dependent MRI-detected lesions at early stages of transient global ischemia in rat brain. *MAGMA* 17, 109–116. doi: 10.1007/s10334-004-0072-6

Cappell, K. A., Gmeindl, L., and Reuter-Lorenz, P. A. (2010). Age differences in prefontal recruitment during verbal working memory maintenance depend on memory load. *Cortex* 46, 462–473. doi: 10.1016/j.cortex.2009.11.009

Charlton, R. A., Mcintyre, D. J., Howe, F. A., Morris, R. G., and Markus, H. S. (2007). The relationship between white matter brain metabolites and cognition in normal aging: the GENIE study. *Brain Res.* 1164, 108–116. doi: 10.1016/j.brainres.2007.06.027

Chassain, C., Bielicki, G., Carcenac, C., Ronsin, A. C., Renou, J. P., Savasta, M., et al. (2013). Does MPTP intoxication in mice induce metabolite changes in the nucleus accumbens? A (1)H nuclear MRS study. *NMR Biomed.* 26, 336–347. doi: 10.1002/nbm.2853

Chassain, C., Bielicki, G., Durand, E., Lolignier, S., Essafi, F., Traore, A., et al. (2008). Metabolic changes detected by proton magnetic resonance spectroscopy in vivo and in vitro in a murin model of Parkinson's disease, the MPTP-intoxicated mouse. *J. Neurochem.* 105, 874–882. doi: 10.1111/j.1471-4159.2007.05185.x

Chen, S. Q., Cai, Q., Shen, Y. Y., Wang, P. J., Teng, G. J., Zhang, W., et al. (2012). Age-related changes in brain metabolites and cognitive function in APP/PS1 transgenic mice. *Behav. Brain Res.* 235, 1–6. doi: 10.1016/j.bbr.2012.07.016

Chen, X., Errangi, B., Li, L., Glasser, M. F., Westlye, L. T., Fjell, A. M., et al. (2013). Brain aging in humans, chimpanzees (*Pan troglodytes*), and rhesus macaques (*Macaca mulatta*): magnetic resonance imaging studies of macro- and microstructural changes. *Neurobiol. Aging* 34, 2248–2260. doi: 10.1016/j.neurobiolaging.2013.03.028

Chen, Y. I., Brownell, A. L., Galpern, W., Isacson, O., Bogdanov, M., Beal, M. F., et al. (1999). Detection of dopaminergic cell loss and neural transplantation using pharmacological MRI, PET and behavioral assessment. *Neuroreport* 10, 2881–2886. doi: 10.1097/00001756-199909290-00001

Chin, C. L., Upadhyay, J., Marek, G. J., Baker, S. J., Zhang, M., Mezler, M., et al. (2011). Awake rat pharmacological magnetic resonance imaging as a translational pharmacodynamic biomarker: metabotropic glutamate 2/3 agonist modulation of ketamine-induced blood oxygenation level dependence signals. *J. Pharmacol. Exp. Ther.* 336, 709–715. doi: 10.1124/jpet.110.173880

Choi, C. B., Kim, S. Y., Lee, S. H., Jahng, G. H., Kim, H. Y., Choe, B. Y., et al. (2011). Assessment of metabolic changes in the striatum of a MPTP-intoxicated canine model: in vivo (1)H-MRS study of an animal model for Parkinson's disease. *Magn. Reson. Imaging* 29, 32–39. doi: 10.1016/j.mri.2010.03.043

Compte, A., Brunel, N., Goldman-Rakic, P. S., and Wang, X. J. (2000). Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. *Cereb. Cortex* 10, 910–923. doi: 10.1093/cercor/10.9.910

Coune, P. G., Craveiro, M., Gaugler, M. N., Mlynarik, V., Schneider, B. L., Aebischer, P., et al. (2013). An in vivo ultrahigh field 14.1 T (1) H-MRS study on 6-OHDA and alpha-synuclein-based rat models of Parkinson's disease: GABA as an early disease marker. *NMR Biomed.* 26, 43–50. doi: 10.1002/nbm.2817

Cross, D. J., Flexman, J. A., Anzai, Y., Maravilla, K. R., and Minoshima, S. (2008). Age-related decrease in axonal transport measured by MR imaging in vivo. *Neuroimage* 39, 915–926. doi: 10.1016/j.neuroimage.2007.08.036

Crystal, J. D., and Wilson, A. G. (2015). Prospective memory: a comparative perspective. *Behav. Process.* 112, 88–99. doi: 10.1016/j.beproc.2014.07.016

Curtis, C. E., and D'Esposito, M. (2003). Persistent activity in the prefrontal cortex during working memory. *Trends Cogn. Sci.* 7, 415–423. doi: 10.1016/S1364-6613(03)00197-9

Damoiseaux, J. S., Beckmann, C. F., Arigita, E. J., Barkhof, F., Scheltens, P., Stam, C. J., et al. (2008). Reduced resting-state brain activity in the "default network" in normal aging. *Cereb. Cortex* 18, 1856–1864. doi: 10.1093/cercor/bhm207

Daselaar, S. M., Veltman, D. J., Rombouts, S. A., Raaijmakers, J. G., and Jonker, C. (2003). Deep processing activates the medial temporal lobe in young

but not in old adults. *Neurobiol. Aging* 24, 1005–1011. doi: 10.1016/S0197-4580(03)00032-0

de la Torre, J. C., Fortin, T., Park, G. A., Butler, K. S., Kozlowski, P., Pappas, B. A., et al. (1992). Chronic cerebrovascular insufficiency induces dementia-like deficits in aged rats. *Brain Res.* 582, 186–195. doi: 10.1016/0006-8993(92)90132-S

Delli Pizzi, S., Rossi, C., Di Matteo, V., Esposito, E., Guarnieri, S., Mariggio, M. A., et al. (2013). Morphological and metabolic changes in the nigro-striatal pathway of synthetic proteasome inhibitor (PSI)-treated rats: a MRI and MRS study. *PLoS ONE* 8:e56501. doi: 10.1371/journal.pone.0056501

Demougeot, C., Marie, C., Giroud, M., and Beley, A. (2004). N-acetylaspartate: a literature review of animal research on brain ischaemia. *J. Neurochem.* 90, 776–783. doi: 10.1111/j.1471-4159.2004.02583.x

Dennis, E. L., and Thompson, P. M. (2014). Functional brain connectivity using fMRI in aging and Alzheimer's disease. *Neuropsychol. Rev.* 24, 49–62. doi: 10.1007/s11065-014-9249-6

Dennis, N. A., Hayes, S. M., Prince, S. E., Madden, D. J., Huettel, S. A., and Cabeza, R. (2008). Effects of aging on the neural correlates of successful item and source memory encoding. *J. Exp. Psychol. Learn. Mem. Cogn.* 34, 791–808. doi: 10.1037/0278-7393.34.4.791

Desjardins, M., Berti, R., Lefebvre, J., Dubeau, S., and Lesage, F. (2014). Aging-related differences in cerebral capillary blood flow in anesthetized rats. *Neurobiol. Aging* 35, 1947–1955. doi: 10.1016/j.neurobiolaging.2014.01.136

D'Esposito, M., Postle, B. R., Jonides, J., and Smith, E. E. (1999a). The neural substrate and temporal dynamics of interference effects in working memory as revealed by event-related functional MRI. *Proc. Natl. Acad. Sci. U.S.A.* 96, 7514–7519. doi: 10.1073/pnas.96.13.7514

D'Esposito, M., Zarahn, E., Aguirre, G. K., and Rypma, B. (1999b). The effect of normal aging on the coupling of neural activity to the bold hemodynamic response. *Neuroimage* 10, 6–14. doi: 10.1006/nimg.1999.0444

Di Costanzo, A., Trojsi, F., Tosetti, M., Giannatempo, G. M., Nemore, F., Piccirillo, M., et al. (2003). High-field proton MRS of human brain. *Eur. J. Radiol.* 48, 146–153. doi: 10.1016/j.ejrad.2003.08.009

Disterhoft, J. F., Moyer, J. R. Jr., Thompson, L. T., and Kowalska, M. (1993). Functional aspects of calcium-channel modulation. *Clin. Neuropharmacol.* 16(Suppl. 1), S12–S24. doi: 10.1097/00002826-199316001-00003

Douaud, G., Jbabdi, S., Behrens, T. E., Menke, R. A., Gass, A., Monsch, A. U., et al. (2011). DTI measures in crossing-fibre areas: increased diffusion anisotropy reveals early white matter alteration in MCI and mild Alzheimer's disease. *Neuroimage* 55, 880–890. doi: 10.1016/j.neuroimage.2010.12.008

Douaud, G., Menke, R. A., Gass, A., Monsch, A. U., Rao, A., Whitcher, B., et al. (2013). Brain microstructure reveals early abnormalities more than two years prior to clinical progression from mild cognitive impairment to Alzheimer's disease. *J. Neurosci.* 33, 2147–2155. doi: 10.1523/JNEUROSCI.4437-12.2013

Drake, C. T., and Iadecola, C. (2007). The role of neuronal signaling in controlling cerebral blood flow. *Brain Lang.* 102, 141–152. doi: 10.1016/j.bandl.2006.08.002

Driscoll, I., Howard, S. R., Stone, J. C., Monfils, M. H., Tomanek, B., Brooks, W. M., et al. (2006). The aging hippocampus: a multi-level analysis in the rat. *Neuroscience* 139, 1173–1185. doi: 10.1016/j.neuroscience.2006.01.040

Drouet, J. B., Fauvelle, F., Maunoir-Regimbal, S., Fidier, N., Maury, R., Peinnequin, A., et al. (2015). Differences in prefrontal cortex GABA/glutamate ratio after acute restraint stress in rats are associated with specific behavioral and neurobiological patterns. *Neuroscience* 285, 155–165. doi: 10.1016/j.neuroscience.2014.10.058

Duarte, J. M., Do, K. Q., and Gruetter, R. (2014). Longitudinal neurochemical modifications in the aging mouse brain measured in vivo by 1H magnetic resonance spectroscopy. *Neurobiol. Aging* 35, 1660–1668. doi: 10.1016/j.neurobiolaging.2014.01.135

Duffy, B. A., Choy, M., Chuapoco, M. R., Madsen, M., and Lee, J. H. (2015). MRI compatible optrodes for simultaneous LFP and optogenetic fMRI investigation of seizure-like afterdischarges. *Neuroimage* 123, 173–184. doi: 10.1016/j.neuroimage.2015.07.038

Dumitriu, D., Hao, J., Hara, Y., Kaufmann, J., Janssen, W. G., Lou, W., et al. (2010). Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. *J. Neurosci.* 30, 7507–7515. doi: 10.1523/JNEUROSCI.6410-09.2010

Duverne, S., Motamedinia, S., and Rugg, M. D. (2009). The relationship between aging, performance, and the neural correlates of successful memory encoding. *Cereb. Cortex* 19, 733–744. doi: 10.1093/cercor/bhn122

Eagan, D. E., Gonzales, M. M., Tarumi, T., Tanaka, H., Stautberg, S., and Haley, A. P. (2012). Elevated serum C-reactive protein relates to increased cerebral myoinositol levels in middle-aged adults. *Cardiovasc. Psychiatry Neurol.* 2012:120540. doi: 10.1155/2012/120540

Eldridge, L. L., Knowlton, B. J., Furmanski, C. S., Bookheimer, S. Y., and Engel, S. A. (2000). Remembering episodes: a selective role for the hippocampus during retrieval. *Nat. Neurosci.* 3, 1149–1152. doi: 10.1038/80671

Emir, U. E., Raatz, S., Mcpherson, S., Hodges, J. S., Torkelson, C., Tawfik, P., et al. (2011). Noninvasive quantification of ascorbate and glutathione concentration in the elderly human brain. *NMR Biomed.* 24, 888–894. doi: 10.1002/nbm.1646

Eschenko, O., Canals, S., Simanova, I., and Logothetis, N. K. (2010). Behavioral, electrophysiological and histopathological consequences of systemic manganese administration in MEMRI. *Magn. Reson. Imaging* 28, 1165–1174. doi: 10.1016/j.mri.2009.12.022

Fakhri, M., Sikaroodi, H., Maleki, F., Ali Oghabian, M., and Ghanaati, H. (2012). Age-related frontal hyperactivation observed across different working memory tasks: an fMRI study. *Behav. Neurol.* 25, 351–361. doi: 10.1155/2012/824049

Febo, M., Ferris, C. F., and Segarra, A. C. (2005a). Estrogen influences cocaine-induced blood oxygen level-dependent signal changes in female rats. *J. Neurosci.* 25, 1132–1136. doi: 10.1523/JNEUROSCI.3801-04.2005

Febo, M., Segarra, A. C., Nair, G., Schmidt, K., Duong, T. Q., and Ferris, C. F. (2005b). The neural consequences of repeated cocaine exposure revealed by functional MRI in awake rats. *Neuropsychopharmacology* 30, 936–943. doi: 10.1038/sj.npp.1300653

Febo, M., Segarra, A. C., Tenney, J. R., Brevard, M. E., Duong, T. Q., and Ferris, C. F. (2004). Imaging cocaine-induced changes in the mesocorticolimbic dopaminergic system of conscious rats. *J. Neurosci. Methods* 139, 167–176. doi: 10.1016/j.jneumeth.2004.04.028

Ferreira, L. K., Regina, A. C., Kovacevic, N., Martin, M. D., Santos, P. P., Carneiro, C. G., et al. (2015). Aging effects on whole-brain functional connectivity in adults free of cognitive and psychiatric disorders. *Cereb. Cortex* [Epub ahead of print].

Flegal, K. E., Marin-Gutierrez, A., Ragland, J. D., and Ranganath, C. (2014). Brain mechanisms of successful recognition through retrieval of semantic context. *J. Cogn. Neurosci.* 26, 1694–1704. doi: 10.1162/jocn_a_00587

Floresco, S. B., and Grace, A. A. (2003). Gating of hippocampal-evoked activity in prefrontal cortical neurons by inputs from the mediodorsal thalamus and ventral tegmental area. *J. Neurosci.* 23, 3930–3943.

Foster, T. C. (2007). Calcium homeostasis and modulation of synaptic plasticity in the aged brain. *Aging Cell* 6, 319–325. doi: 10.1111/j.1474-9726.2007.00283.x

Foster, T. C. (2012). Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca2+ channels in senescent synaptic plasticity. *Prog. Neurobiol.* 96, 283–303. doi: 10.1016/j.pneurobio.2012.01.007

Foster, T. C., Defazio, R. A., and Bizon, J. L. (2012). Characterizing cognitive aging of spatial and contextual memory in animal models. *Front. Aging. Neurosci.* 4:12. doi: 10.3389/fnagi.2012.00012

Foster, T. C., and Dumas, T. C. (2001). Mechanism for increased hippocampal synaptic strength following differential experience. *J. Neurophysiol.* 85, 1377–1383.

Foster, T. C., Fugger, H. N., and Cunningham, S. G. (2000). Receptor blockade reveals a correspondence between hippocampal-dependent behavior and experience-dependent synaptic enhancement. *Brain Res.* 871, 39–43. doi: 10.1016/S0006-8993(00)02379-9

Foster, T. C., Gagne, J., and Massicotte, G. (1996). Mechanism of altered synaptic strength due to experience: relation to long-term potentiation. *Brain Res.* 736, 243–250. doi: 10.1016/0006-8993(96)00707-X

Foster, T. C., Kyritsopoulos, C., and Kumar, A. (2016). Central role for NMDA receptors in redox mediated impairment of synaptic function during aging and Alzheimer's disease. *Behav. Brain Res.* doi: 10.1016/j.bbr.2016.05.012 [Epub ahead of print].

Foster, T. C., and Norris, C. M. (1997). Age-associated changes in Ca(2+)-dependent processes: relation to hippocampal synaptic plasticity. *Hippocampus* 7, 602–612. doi: 10.1002/(SICI)1098-1063(1997)7:6<602::AID-HIPO3>3.0.CO;2-G

Fowler, J. H., Whalley, K., Murray, T., O'neill, M. J., and Mcculloch, J. (2004). The AMPA receptor potentiator LY404187 increases cerebral glucose utilization and c-fos expression in the rat. *J. Cereb. Blood Flow Metab.* 24, 1098–1109. doi: 10.1097/01.WCB.0000138665.25305.7C

Funahashi, S., Bruce, C. J., and Goldman-Rakic, P. S. (1989). Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J. Neurophysiol.* 61, 331–349.

Fuss, J., Biedermann, S. V., Falfan-Melgoza, C., Auer, M. K., Zheng, L., Steinle, J., et al. (2014). Exercise boosts hippocampal volume by preventing early age-related gray matter loss. *Hippocampus* 24, 131–134. doi: 10.1002/hipo.22227

Gabbott, P. L., Warner, T. A., Jays, P. R., Salway, P., and Busby, S. J. (2005). Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *J. Comp. Neurol.* 492, 145–177. doi: 10.1002/cne.20738

Galisova, A., Baciak, L., Jozefovicova, M., Just Kukurova, I., Kebis, A., Ambrusova, K., et al. (2014). Pathophysiological rat model of vascular dementia: magnetic resonance spectroscopy, microimaging and behavioral study. *Brain Res.* 1568, 10–20. doi: 10.1016/j.brainres.2014.04.032

Gao, F., Edden, R. A., Li, M., Puts, N. A., Wang, G., Liu, C., et al. (2013). Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *Neuroimage* 78, 75–82. doi: 10.1016/j.neuroimage.2013.04.012

Gardini, S., Venneri, A., Sambataro, F., Cuetos, F., Fasano, F., Marchi, M., et al. (2015). Increased functional connectivity in the default mode network in mild cognitive impairment: a maladaptive compensatory mechanism associated with poor semantic memory performance. *J. Alzheimers Dis.* 45, 457–470. doi: 10.3233/JAD-142547

Gass, N., Schwarz, A. J., Sartorius, A., Schenker, E., Risterucci, C., Spedding, M., et al. (2014). Sub-anesthetic ketamine modulates intrinsic BOLD connectivity within the hippocampal-prefrontal circuit in the rat. *Neuropsychopharmacology* 39, 895–906. doi: 10.1038/npp.2013.290

Gauthier, S., Reisberg, B., Zaudig, M., Petersen, R. C., Ritchie, K., Broich, K., et al. (2006). Mild cognitive impairment. *Lancet* 367, 1262–1270. doi: 10.1016/S0140-6736(06)68542-5

Gigi, A., Babai, R., Penker, A., Hendler, T., and Korczyn, A. D. (2010). Prefrontal compensatory mechanism may enable normal semantic memory performance in mild cognitive impairment (MCI). *J. Neuroimaging* 20, 163–168. doi: 10.1111/j.1552-6569.2009.00386.x

Gimeno, D., Marmot, M. G., and Singh-Manoux, A. (2008). Inflammatory markers and cognitive function in middle-aged adults: the Whitehall II study. *Psychoneuroendocrinology* 33, 1322–1334. doi: 10.1016/j.psyneuen.2008.07.006

Golomb, J., De Leon, M. J., Kluger, A., George, A. E., Tarshish, C., and Ferris, S. H. (1993). Hippocampal atrophy in normal aging. An association with recent memory impairment. *Arch. Neurol.* 50, 967–973. doi: 10.1001/archneur.1993.00540090066012

Grachev, I. D., Swarnkar, A., Szeverenyi, N. M., Ramachandran, T. S., and Apkarian, A. V. (2001). Aging alters the multichemical networking profile of the human brain: an in vivo (1)H-MRS study of young versus middle-aged subjects. *J. Neurochem.* 77, 292–303. doi: 10.1046/j.1471-4159.2001.t01-1-00238.x

Griffith, H. R., Okonkwo, O. C., O'brien, T., and Hollander, J. A. (2008). Reduced brain glutamate in patients with Parkinson's disease. *NMR Biomed.* 21, 381–387. doi: 10.1002/nbm.1203

Grimm, O., Gass, N., Weber-Fahr, W., Sartorius, A., Schenker, E., Spedding, M., et al. (2015). Acute ketamine challenge increases resting state prefrontal-hippocampal connectivity in both humans and rats. *Psychopharmacology (Berl.)* 232, 4231–4241. doi: 10.1007/s00213-015-4022-y

Groschel, M., Hubert, N., Muller, S., Ernst, A., and Basta, D. (2014). Age-dependent changes of calcium related activity in the central auditory pathway. *Exp. Gerontol.* 58, 235–243. doi: 10.1016/j.exger.2014.08.014

Guidi, M., Kumar, A., and Foster, T. C. (2015a). Impaired attention and synaptic senescence of the prefrontal cortex involves redox regulation of NMDA receptors. *J. Neurosci.* 35, 3966–3977. doi: 10.1523/JNEUROSCI.3523-14.2015

Guidi, M., Rani, A., Karic, S., Severance, B., Kumar, A., and Foster, T. C. (2015b). Contribution of N-methyl-D-aspartate receptors to attention and episodic spatial memory during senescence. *Neurobiol. Learn. Mem.* 125, 36–46. doi: 10.1016/j.nlm.2015.07.015

Guilarte, T. R., and Chen, M. K. (2007). Manganese inhibits NMDA receptor channel function: implications to psychiatric and cognitive effects. *Neurotoxicology* 28, 1147–1152. doi: 10.1016/j.neuro.2007.06.005

Gunning-Dixon, F. M., and Raz, N. (2003). Neuroanatomical correlates of selected executive functions in middle-aged and older adults: a prospective MRI study. *Neuropsychologia* 41, 1929–1941. doi: 10.1016/S0028-3932(03)00129-5

Guo, J., Bakshi, V., and Lin, A. L. (2015). Early Shifts of Brain metabolism by caloric restriction preserve white matter integrity and long-term memory in aging mice. *Front. Aging Neurosci.* 7:213. doi: 10.3389/fnagi.2015.00213

Haley, G. E., Eghlidi, D. H., Kohama, S. G., Urbanski, H. F., and Raber, J. (2012). Association of microtubule associated protein-2, synaptophysin, and apolipoprotein E mRNA and protein levels with cognition and anxiety levels in aged female rhesus macaques. *Behav. Brain Res.* 232, 1–6. doi: 10.1016/j.bbr.2012.03.032

Haley, G. E., Kroenke, C., Schwartz, D., Kohama, S. G., Urbanski, H. F., and Raber, J. (2011). Hippocampal M1 receptor function associated with spatial learning and memory in aged female rhesus macaques. *Age (Dordr)* 33, 309–320. doi: 10.1007/s11357-010-9184-2

Harris, J. L., Choi, I. Y., and Brooks, W. M. (2015). Probing astrocyte metabolism in vivo: proton magnetic resonance spectroscopy in the injured and aging brain. *Front. Aging Neurosci.* 7:202. doi: 10.3389/fnagi.2015.00202

Harris, J. L., Yeh, H. W., Swerdlow, R. H., Choi, I. Y., Lee, P., and Brooks, W. M. (2014). High-field proton magnetic resonance spectroscopy reveals metabolic effects of normal brain aging. *Neurobiol. Aging* 35, 1686–1694. doi: 10.1016/j.neurobiolaging.2014.01.018

Heeger, D. J., Huk, A. C., Geisler, W. S., and Albrecht, D. G. (2000). Spikes versus BOLD: what does neuroimaging tell us about neuronal activity? *Nat. Neurosci.* 3, 631–633. doi: 10.1038/76572

Heiland, S., Sartor, K., Martin, E., Bardenheuer, H. J., and Plaschke, K. (2002). In vivo monitoring of age-related changes in rat brain using quantitative diffusion magnetic resonance imaging and magnetic resonance relaxometry. *Neurosci. Lett.* 334, 157–160. doi: 10.1016/S0304-3940(02)01073-X

Hemanth Kumar, B. S., Mishra, S. K., Rana, P., Singh, S., and Khushu, S. (2012). Neurodegenerative evidences during early onset of depression in CMS rats as detected by proton magnetic resonance spectroscopy at 7 T. *Behav. Brain Res.* 232, 53–59. doi: 10.1016/j.bbr.2012.03.011

Herman, P., Sanganahalli, B. G., Blumenfeld, H., Rothman, D. L., and Hyder, F. (2013). Quantitative basis for neuroimaging of cortical laminae with calibrated functional MRI. *Proc. Natl. Acad. Sci. U.S.A.* 110, 15115–15120. doi: 10.1073/pnas.1307154110

Herndon, J. G., Constantinidis, I., and Moss, M. B. (1998). Age-related brain changes in rhesus monkeys: a magnetic resonance spectroscopic study. *Neuroreport* 9, 2127–2130. doi: 10.1097/00001756-199806220-00040

Higuchi, T., Graham, S. H., Fernandez, E. J., Rooney, W. D., Gaspary, H. L., Weiner, M. W., et al. (1997). Effects of severe global ischemia on N-acetylaspartate and other metabolites in the rat brain. *Magn. Reson. Med.* 37, 851–857. doi: 10.1002/mrm.1910370608

Hoff, E. I., Steinbusch, H. W., Van Oostenbrugge, R. J., Garrett, L., Otte, W. M., Van Der Marel, K., et al. (2011). Alterations in the cholinergic system after frontal cortical infarction in rat brain: pharmacological magnetic resonance imaging of muscarinic receptor responsiveness and stereological analysis of cholinergic forebrain neurons. *Neurobiol. Dis.* 43, 625–634. doi: 10.1016/j.nbd.2011.05.011

Hoff, E. I., Van Oostenbrugge, R. J., Otte, W. M., Van Der Marel, K., Steinbusch, H. W., and Dijkhuizen, R. M. (2010). Pharmacological magnetic resonance imaging of muscarinic acetylcholine receptor activation in rat brain. *Neuropharmacology* 58, 1252–1257. doi: 10.1016/j.neuropharm.2010.03.007

Holden, H. M., and Gilbert, P. E. (2012). Less efficient pattern separation may contribute to age-related spatial memory deficits. *Front. Aging Neurosci.* 4:9. doi: 10.3389/fnagi.2012.00009

Homayoun, H., and Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J. Neurosci.* 27, 11496–11500. doi: 10.1523/JNEUROSCI.2213-07.2007

Hsu, Y. H., Lee, W. T., and Chang, C. (2007). Multiparametric MRI evaluation of kainic acid-induced neuronal activation in rat hippocampus. *Brain* 130, 3124–3134. doi: 10.1093/brain/awm207

Huang, C. C., Hsieh, W. J., Lee, P. L., Peng, L. N., Liu, L. K., Lee, W. J., et al. (2015). Age-related changes in resting-state networks of a large sample size of healthy elderly. *CNS Neurosci. Ther.* 21, 817–825. doi: 10.1111/cns.12396

Huang, H., Yamamoto, A., Hossain, M. A., Younes, L., and Mori, S. (2008). Quantitative cortical mapping of fractional anisotropy in developing rat brains. *J. Neurosci.* 28, 1427–1433. doi: 10.1523/JNEUROSCI.3194-07.2008

Hutchison, R. M., Leung, L. S., Mirsattari, S. M., Gati, J. S., Menon, R. S., and Everling, S. (2011). Resting-state networks in the macaque at 7 T. *Neuroimage* 56, 1546–1555. doi: 10.1016/j.neuroimage.2011.02.063

Iltis, I., Koski, D. M., Eberly, L. E., Nelson, C. D., Deelchand, D. K., Valette, J., et al. (2009). Neurochemical changes in the rat prefrontal cortex following acute phencyclidine treatment: an in vivo localized (1)H MRS study. *NMR Biomed.* 22, 737–744. doi: 10.1002/nbm.1385

Immonen, R. J., Kharatishvili, I., Sierra, A., Einula, C., Pitkanen, A., and Grohn, O. H. (2008). Manganese enhanced MRI detects mossy fiber sprouting rather than neurodegeneration, gliosis or seizure-activity in the epileptic rat hippocampus. *Neuroimage* 40, 1718–1730. doi: 10.1016/j.neuroimage.2008.01.042

Jackson, S. J., Hussey, R., Jansen, M. A., Merrifield, G. D., Marshall, I., Maclullich, A., et al. (2011). Manganese-enhanced magnetic resonance imaging (MEMRI) of rat brain after systemic administration of MnCl(2): hippocampal signal enhancement without disruption of hippocampus-dependent behavior. *Behav. Brain Res.* 216, 293–300. doi: 10.1016/j.bbr.2010.08.007

Jackson, T. C., Rani, A., Kumar, A., and Foster, T. C. (2009). Regional hippocampal differences in AKT survival signaling across the lifespan: implications for CA1 vulnerability with aging. *Cell Death. Differ.* 16, 439–448. doi: 10.1038/cdd.2008.171

Jenkins, B. G., Klivenyi, P., Kustermann, E., Andreassen, O. A., Ferrante, R. J., Rosen, B. R., et al. (2000). Nonlinear decrease over time in N-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. *J. Neurochem.* 74, 2108–2119. doi: 10.1046/j.1471-4159.2000.0742108.x

Jernigan, T. L., Archibald, S. L., Berhow, M. T., Sowell, E. R., Foster, D. S., and Hesselink, J. R. (1991). Cerebral structure on MRI, Part I: localization of age-related changes. *Biol. Psychiatry* 29, 55–67. doi: 10.1016/0006-3223(91)90210-D

Jodo, E., Suzuki, Y., Katayama, T., Hoshino, K. Y., Takeuchi, S., Niwa, S., et al. (2005). Activation of medial prefrontal cortex by phencyclidine is mediated via a hippocampo-prefrontal pathway. *Cereb. Cortex* 15, 663–669. doi: 10.1093/cercor/bhh168

Jonckers, E., Van Audekerke, J., De Visscher, G., Van Der Linden, A., and Verhoye, M. (2011). Functional connectivity fMRI of the rodent brain: comparison of functional connectivity networks in rat and mouse. *PLoS ONE* 6:e18876. doi: 10.1371/journal.pone.0018876

Jones, N., O'neill, M. J., Tricklebank, M., Libri, V., and Williams, S. C. (2005). Examining the neural targets of the AMPA receptor potentiator LY404187 in the rat brain using pharmacological magnetic resonance imaging. *Psychopharmacology (Berl.)* 180, 743–751. doi: 10.1007/s00213-005-2254-y

Kaufmann, L., Ischebeck, A., Weiss, E., Koppelstaetter, F., Siedentopf, C., Vogel, S. E., et al. (2008). An fMRI study of the numerical Stroop task in individuals with and without minimal cognitive impairment. *Cortex* 44, 1248–1255. doi: 10.1016/j.cortex.2007.11.009

Keelan, J., Allen, N. J., Antcliffe, D., Pal, S., and Duchen, M. R. (2001). Quantitative imaging of glutathione in hippocampal neurons and glia in culture using monochlorobimane. *J. Neurosci. Res.* 66, 873–884. doi: 10.1002/jnr.10085

Kickler, N., Krack, P., Fraix, V., Lebas, J. F., Lamalle, L., Durif, F., et al. (2007). Glutamate measurement in Parkinson's disease using MRS at 3 T field strength. *NMR Biomed.* 20, 757–762. doi: 10.1002/nbm.1141

Kickler, N., Lacombe, E., Chassain, C., Durif, F., Krainik, A., Farion, R., et al. (2009). Assessment of metabolic changes in the striatum of a rat model of parkinsonism: an in vivo (1)H MRS study. *NMR Biomed.* 22, 207–212. doi: 10.1002/nbm.1305

Kim, J., Choi, I. Y., Michaelis, M. L., and Lee, P. (2011). Quantitative in vivo measurement of early axonal transport deficits in a triple transgenic mouse model of Alzheimer's disease using manganese-enhanced MRI. *Neuroimage* 56, 1286–1292. doi: 10.1016/j.neuroimage.2011.02.039

Kimotsuki, T., Nagaoka, T., Yasuda, M., Tamahara, S., Matsuki, N., and Ono, K. (2005). Changes of magnetic resonance imaging on the brain in beagle dogs with aging. *J. Vet. Med. Sci.* 67, 961–967. doi: 10.1292/jvms.67.961

Knox, D., Perrine, S. A., George, S. A., Galloway, M. P., and Liberzon, I. (2010). Single prolonged stress decreases glutamate, glutamine, and creatine concentrations in the rat medial prefrontal cortex. *Neurosci. Lett.* 480, 16–20. doi: 10.1016/j.neulet.2010.05.052

Kojima, T., Onoe, H., Hikosaka, K., Tsutsui, K., Tsukada, H., and Watanabe, M. (2009). Default mode of brain activity demonstrated by positron emission tomography imaging in awake monkeys: higher rest-related than working memory-related activity in medial cortical areas. *J. Neurosci.* 29, 14463–14471. doi: 10.1523/JNEUROSCI.1786-09.2009

Koo, B. B., Oblak, A. L., Zhao, Y., Farris, C. W., Bowley, B., Rosene, D. L., et al. (2013). Hippocampal network connections account for differences in memory performance in the middle-aged rhesus monkey. *Hippocampus* 23, 1179–1188. doi: 10.1002/hipo.22156

Koretsky, A. P., and Silva, A. C. (2004). Manganese-enhanced magnetic resonance imaging (MEMRI). *NMR Biomed.* 17, 527–531. doi: 10.1002/nbm.940

Kumar, A., Bodhinathan, K., and Foster, T. C. (2009). Susceptibility to calcium dysregulation during brain aging. *Front. Aging Neurosci.* 1:2. doi: 10.3389/neuro.24.002.2009

Kumar, A., and Foster, T. C. (2007). "Neurophysiology of old neurons and synapses," in *Brain Aging: Models, Methods, and Mechanisms*, ed. D. R. Riddle (Boca Raton, FL: Taylor & Francis).

Kumar, A., and Foster, T. C. (2013). Linking redox regulation of NMDAR synaptic function to cognitive decline during aging. *J. Neurosci.* 33, 15710–15715. doi: 10.1523/JNEUROSCI.2176-13.2013

Kuroda, M., Yokofujita, J., Oda, S., and Price, J. L. (2004). Synaptic relationships between axon terminals from the mediodorsal thalamic nucleus and gamma-aminobutyric acidergic cortical cells in the prelimbic cortex of the rat. *J. Comp. Neurol.* 477, 220–234. doi: 10.1002/cne.20249

La, C., Nair, V. A., Mossahebi, P., Young, B. M., Sattin, J., Chacon, M., et al. (2016). Implication of the Slow-5 oscillations in the disruption of the default-mode network in healthy aging and stroke. *Brain Connect.* doi: 10.1089/brain.2015.0375 [Epub ahead of print],

Landfield, P. W. (1988). Hippocampal neurobiological mechanisms of age-related memory dysfunction. *Neurobiol. Aging* 9, 571–579. doi: 10.1016/S0197-4580(88)80116-7

Le Bihan, D., Mangin, J. F., Poupon, C., Clark, C. A., Pappata, S., Molko, N., et al. (2001). Diffusion tensor imaging: concepts and applications. *J. Magn. Reson. Imaging* 13, 534–546. doi: 10.1002/jmri.1076

Lee, J. H., Durand, R., Gradinaru, V., Zhang, F., Goshen, I., Kim, D. S., et al. (2010). Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature* 465, 788–792. doi: 10.1038/nature09108

Lee, T. H., Liu, H. L., Yang, S. T., Yang, J. T., Yeh, M. Y., and Lin, J. R. (2011). Effects of aging and hypertension on cerebral ischemic susceptibility: evidenced by MR diffusion-perfusion study in rat. *Exp. Neurol.* 227, 314–321. doi: 10.1016/j.expneurol.2010.12.003

Lee, W. H., Kumar, A., Rani, A., and Foster, T. C. (2014). Role of antioxidant enzymes in redox regulation of N-methyl-D-aspartate receptor function and memory in middle-aged rats. *Neurobiol. Aging* 35, 1459–1468. doi: 10.1016/j.neurobiolaging.2013.12.002

Lewandowski, N. M., Bordelon, Y., Brickman, A. M., Angulo, S., Khan, U., Muraskin, J., et al. (2013). Regional vulnerability in Huntington's disease: fMRI-guided molecular analysis in patients and a mouse model of disease. *Neurobiol. Dis.* 52, 84–93. doi: 10.1016/j.nbd.2012.11.014

Li, Y., Li, C., Wu, Q., Xu, Z., Kurata, T., Ohno, S., et al. (2015). Decreased resting-state connections within the visuospatial attention-related network in advanced aging. *Neurosci. Lett.* 597, 13–18. doi: 10.1016/j.neulet.2015.03.047

Liang, Z., Watson, G. D., Alloway, K. D., Lee, G., Neuberger, T., and Zhang, N. (2015). Mapping the functional network of medial prefrontal cortex by combining optogenetics and fMRI in awake rats. *Neuroimage* 117, 114–123. doi: 10.1016/j.neuroimage.2015.05.036

Lighthall, N. R., Huettel, S. A., and Cabeza, R. (2014). Functional compensation in the ventromedial prefrontal cortex improves memory-dependent decisions in older adults. *J. Neurosci.* 34, 15648–15657. doi: 10.1523/JNEUROSCI.2888-14.2014

Liu, Y., Zhang, R., Li, P., Huang, F., Fa, Z., Chen, L., et al. (2013). Determination of the detectable concentration of manganese used in neuronal MEMRI and its effect on cortical neurons in vitro. *Neurol. Res.* 35, 895–902. doi: 10.1179/1743132813Y.0000000226

Loerch, P. M., Lu, T., Dakin, K. A., Vann, J. M., Isaacs, A., Geula, C., et al. (2008). Evolution of the aging brain transcriptome and synaptic regulation. *PLoS ONE* 3:e3329. doi: 10.1371/journal.pone.0003329

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., and Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412, 150–157. doi: 10.1038/35084005

Logothetis, N. K., and Wandell, B. A. (2004). Interpreting the BOLD signal. *Annu. Rev. Physiol.* 66, 735–769. doi: 10.1146/annurev.physiol.66.082602.092845

Lu, H., Xi, Z. X., Gitajn, L., Rea, W., Yang, Y., and Stein, E. A. (2007). Cocaine-induced brain activation detected by dynamic manganese-enhanced magnetic resonance imaging (MEMRI). *Proc. Natl. Acad. Sci. U.S.A.* 104, 2489–2494. doi: 10.1073/pnas.0606983104

Lu, H., Zou, Q., Gu, H., Raichle, M. E., Stein, E. A., and Yang, Y. (2012). Rat brains also have a default mode network. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3979–3984. doi: 10.1073/pnas.1200506109

Luebke, J. I., Chang, Y. M., Moore, T. L., and Rosene, D. L. (2004). Normal aging results in decreased synaptic excitation and increased synaptic inhibition of layer 2/3 pyramidal cells in the monkey prefrontal cortex. *Neuroscience* 125, 277–288. doi: 10.1016/j.neuroscience.2004.01.035

Luscher, C., Nicoll, R. A., Malenka, R. C., and Muller, D. (2000). Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nat. Neurosci.* 3, 545–550. doi: 10.1038/75714

Maddock, R. J., and Buonocore, M. H. (2012). MR spectroscopic studies of the brain in psychiatric disorders. *Curr. Top. Behav. Neurosci.* 11, 199–251. doi: 10.1007/7854_2011_197

Maheswaran, S., Barjat, H., Rueckert, D., Bate, S. T., Howlett, D. R., Tilling, L., et al. (2009). Longitudinal regional brain volume changes quantified in normal aging and Alzheimer's APP x PS1 mice using MRI. *Brain Res.* 1270, 19–32. doi: 10.1016/j.brainres.2009.02.045

Maillet, D., and Rajah, M. N. (2014). Age-related differences in brain activity in the subsequent memory paradigm: a meta-analysis. *Neurosci. Biobehav. Rev.* 45, 246–257. doi: 10.1016/j.neubiorev.2014.06.006

Majid, T., Ali, Y. O., Venkitaramani, D. V., Jang, M. K., Lu, H. C., and Pautler, R. G. (2014). In vivo axonal transport deficits in a mouse model of fronto-temporal dementia. *Neuroimage Clin.* 4, 711–717. doi: 10.1016/j.nicl.2014.02.005

Makris, N., Papadimitriou, G. M., Van Der Kouwe, A., Kennedy, D. N., Hodge, S. M., Dale, A. M., et al. (2007). Frontal connections and cognitive changes in normal aging rhesus monkeys: a DTI study. *Neurobiol. Aging* 28, 1556–1567. doi: 10.1016/j.neurobiolaging.2006.07.005

Mandeville, J. B., Marota, J. J., Kosofsky, B. E., Keltner, J. R., Weissleder, R., Rosen, B. R., et al. (1998). Dynamic functional imaging of relative cerebral blood volume during rat forepaw stimulation. *Magn. Reson. Med.* 39, 615–624. doi: 10.1002/mrm.1910390415

Mantini, D., Corbetta, M., Romani, G. L., Orban, G. A., and Vanduffel, W. (2013). Evolutionarily novel functional networks in the human brain? *J. Neurosci.* 33, 3259–3275. doi: 10.1523/JNEUROSCI.4392-12.2013

Mantini, D., Gerits, A., Nelissen, K., Durand, J. B., Joly, O., Simone, L., et al. (2011). Default mode of brain function in monkeys. *J. Neurosci.* 31, 12954–12962. doi: 10.1523/JNEUROSCI.2318-11.2011

Mariotti, R., Fattoretti, P., Malatesta, M., Nicolato, E., Sandri, M., and Zancanaro, C. (2014). Forced mild physical training improves blood volume in the motor and hippocampal cortex of old mice. *J. Nutr. Health Aging* 18, 178–183. doi: 10.1007/s12603-013-0384-1

Marota, J. J., Mandeville, J. B., Weisskoff, R. M., Moskowitz, M. A., Rosen, B. R., and Kosofsky, B. E. (2000). Cocaine activation discriminates dopaminergic projections by temporal response: an fMRI study in Rat. *Neuroimage* 11, 13–23. doi: 10.1006/nimg.1999.0520

Mehagnoul-Schipper, D. J., Van Der Kallen, B. F., Colier, W. N., Van Der Sluijs, M. C., Van Erning, L. J., Thijssen, H. O., et al. (2002). Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. *Hum. Brain Mapp.* 16, 14–23. doi: 10.1002/hbm.10026.abs

Mengler, L., Khmelinskii, A., Diedenhofen, M., Po, C., Staring, M., Lelieveldt, B. P., et al. (2014). Brain maturation of the adolescent rat cortex and striatum: changes in volume and myelination. *Neuroimage* 84, 35–44. doi: 10.1016/j.neuroimage.2013.08.034

Micotti, E., Paladini, A., Balducci, C., Tolomeo, D., Frasca, A., Marizzoni, M., et al. (2015). Striatum and entorhinal cortex atrophy in AD mouse models: MRI comprehensive analysis. *Neurobiol. Aging* 36, 776–788. doi: 10.1016/j.neurobiolaging.2014.10.027

Mitschelen, M., Garteiser, P., Carnes, B. A., Farley, J. A., Doblas, S., Demoe, J. H., et al. (2009). Basal and hypercapnia-altered cerebrovascular perfusion predict mild cognitive impairment in aging rodents. *Neuroscience* 164, 918–928. doi: 10.1016/j.neuroscience.2009.08.070

Miyamoto, K., Adachi, Y., Osada, T., Watanabe, T., Kimura, H. M., Setsuie, R., et al. (2014). Dissociable memory traces within the macaque medial temporal lobe predict subsequent recognition performance. *J. Neurosci.* 34, 1988–1997. doi: 10.1523/JNEUROSCI.4048-13.2014

Miyamoto, K., Osada, T., Adachi, Y., Matsui, T., Kimura, H. M., and Miyashita, Y. (2013). Functional differentiation of memory retrieval network in macaque posterior parietal cortex. *Neuron* 77, 787–799. doi: 10.1016/j.neuron.2012.12.019

Modrego, P. J., Fayed, N., Artal, J., and Olmos, S. (2011). Correlation of findings in advanced MRI techniques with global severity scales in patients with Parkinson disease. *Acad. Radiol.* 18, 235–241. doi: 10.1016/j.acra.2010.09.022

Morcom, A. M., Good, C. D., Frackowiak, R. S., and Rugg, M. D. (2003). Age effects on the neural correlates of successful memory encoding. *Brain* 126, 213–229. doi: 10.1093/brain/awg020

Moreno, H., Burghardt, N. S., Vela-Duarte, D., Masciotti, J., Hua, F., Fenton, A. A., et al. (2012). The absence of the calcium-buffering protein calbindin is associated with faster age-related decline in hippocampal metabolism. *Hippocampus* 22, 1107–1120. doi: 10.1002/hipo.20957

Mori, S., and Zhang, J. (2006). Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron* 51, 527–539. doi: 10.1016/j.neuron.2006.08.012

Moser, E., Stahlberg, F., Ladd, M. E., and Trattnig, S. (2012). 7-T MR–from research to clinical applications? *NMR Biomed.* 25, 695–716. doi: 10.1002/nbm.1794

Moss, M. B., Moore, T. L., Schettler, S. P., Killiany, R., and Rosene, D. (2007). "Successful vs. unsuccessful aging in the rhesus monkey," in *Brain Aging: Models, Methods, and Mechanisms*, ed. D. R. Riddle (Boca Raton, FL: Taylor & Francis).

Murray, M. E., Przybelski, S. A., Lesnick, T. G., Liesinger, A. M., Spychalla, A., Zhang, B., et al. (2014). Early Alzheimer's disease neuropathology detected by proton MR spectroscopy. *J. Neurosci.* 34, 16247–16255. doi: 10.1523/JNEUROSCI.2027-14.2014

Nagahara, A. H., Bernot, T., and Tuszynski, M. H. (2010). Age-related cognitive deficits in rhesus monkeys mirror human deficits on an automated test battery. *Neurobiol. Aging* 31, 1020–1031. doi: 10.1016/j.neurobiolaging.2008.07.007

Nairismagi, J., Pitkanen, A., Narkilahti, S., Huttunen, J., Kauppinen, R. A., and Grohn, O. H. (2006). Manganese-enhanced magnetic resonance imaging of mossy fiber plasticity in vivo. *Neuroimage* 30, 130–135. doi: 10.1016/j.neuroimage.2005.09.007

Nasrallah, F. A., To, X. V., Chen, D. Y., Routtenberg, A., and Chuang, K. H. (2016). Functional connectivity MRI tracks memory networks after maze learning in rodents. *Neuroimage* 127, 196–202. doi: 10.1016/j.neuroimage.2015.08.013

Nee, D. E., and D'Esposito, M. (2016). The hierarchical organization of the lateral prefrontal cortex. *Elife* 5:e12112. doi: 10.7554/eLife.12112

Nilsen, L. H., Melo, T. M., Saether, O., Witter, M. P., and Sonnewald, U. (2012). Altered neurochemical profile in the McGill-R-Thy1-APP rat model of Alzheimer's disease: a longitudinal in vivo 1 H MRS study. *J. Neurochem.* 123, 532–541. doi: 10.1111/jnc.12003

Oh, M. M., and Disterhoft, J. F. (2010). Cellular mechanisms for altered learning in aging. *Future Neurol.* 5, 147–155. doi: 10.2217/fnl.09.74

Oler, J. A., and Markus, E. J. (2000). Age-related deficits in the ability to encode contextual change: a place cell analysis. *Hippocampus* 10, 338–350. doi: 10.1002/1098-1063(2000)10:3<338::AID-HIPO14>3.0.CO;2-Y

O'sullivan, M., Jones, D. K., Summers, P. E., Morris, R. G., Williams, S. C., and Markus, H. S. (2001). Evidence for cortical "disconnection" as a mechanism of age-related cognitive decline. *Neurology* 57, 632–638.

Park, D. C., and Reuter-Lorenz, P. (2009). The adaptive brain: aging and neurocognitive scaffolding. *Annu. Rev. Psychol.* 60, 173–196. doi: 10.1146/annurev.psych.59.103006.093656

Pautler, R. G. (2004). In vivo, trans-synaptic tract-tracing utilizing manganese-enhanced magnetic resonance imaging (MEMRI). *NMR Biomed.* 17, 595–601. doi: 10.1002/nbm.942

Pautler, R. G., Mongeau, R., and Jacobs, R. E. (2003). In vivo trans-synaptic tract tracing from the murine striatum and amygdala utilizing manganese enhanced MRI (MEMRI). *Magn. Reson Med.* 50, 33–39. doi: 10.1002/mrm.10498

Pavlopoulos, E., Jones, S., Kosmidis, S., Close, M., Kim, C., Kovalerchik, O., et al. (2013). Molecular mechanism for age-related memory loss: the

histone-binding protein RbAp48. *Sci. Transl. Med.* 5:200ra115. doi: 10.1126/scitranslmed.3006373

Peled, S. (2007). New perspectives on the sources of white matter DTI signal. *IEEE Trans. Med. Imaging* 26, 1448–1455. doi: 10.1109/TMI.2007.906787

Perez, P. D., Hall, G., Kimura, T., Ren, Y., Bailey, R. M., Lewis, J., et al. (2013). In vivo functional brain mapping in a conditional mouse model of human tauopathy (tauP301L) reveals reduced neural activity in memory formation structures. *Mol. Neurodegener.* 8:9. doi: 10.1186/1750-1326-8-9

Picq, J. L., Aujard, F., Volk, A., and Dhenain, M. (2012). Age-related cerebral atrophy in nonhuman primates predicts cognitive impairments. *Neurobiol. Aging* 33, 1096–1109. doi: 10.1016/j.neurobiolaging.2010.09.009

Plaschke, K., Frauenknecht, K., Sommer, C., and Heiland, S. (2009). A single systemic transient hypotension induces long-term changes in rats' MRI parameters and behavior: relation to aging. *Neurol. Res.* 31, 304–312. doi: 10.1179/174313209X385653

Podell, M., Hadjiconstantinou, M., Smith, M. A., and Neff, N. H. (2003). Proton magnetic resonance imaging and spectroscopy identify metabolic changes in the striatum in the MPTP feline model of parkinsonism. *Exp. Neurol.* 179, 159–166. doi: 10.1016/S0014-4886(02)00015-8

Prolla, T. A. (2002). DNA microarray analysis of the aging brain. *Chem. Senses* 27, 299–306. doi: 10.1093/chemse/27.3.299

Pudas, S., Persson, J., Josefsson, M., De Luna, X., Nilsson, L. G., and Nyberg, L. (2013). Brain characteristics of individuals resisting age-related cognitive decline over two decades. *J. Neurosci.* 33, 8668–8677. doi: 10.1523/JNEUROSCI.2900-12.2013

Puhl, M. D., Mintzopoulos, D., Jensen, J. E., Gillis, T. E., Konopaske, G. T., Kaufman, M. J., et al. (2015). In vivo magnetic resonance studies reveal neuroanatomical and neurochemical abnormalities in the serine racemase knockout mouse model of schizophrenia. *Neurobiol. Dis.* 73, 269–274. doi: 10.1016/j.nbd.2014.10.009

Rafnsson, S. B., Deary, I. J., Smith, F. B., Whiteman, M. C., Rumley, A., Lowe, G. D., et al. (2007). Cognitive decline and markers of inflammation and hemostasis: the Edinburgh Artery Study. *J. Am. Geriatr. Soc.* 55, 700–707. doi: 10.1111/j.1532-5415.2007.01158.x

Raichle, M. E., Macleod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci. U.S.A.* 98, 676–682. doi: 10.1073/pnas.98.2.676

Ranganath, C., Yonelinas, A. P., Cohen, M. X., Dy, C. J., Tom, S. M., and D'esposito, M. (2004). Dissociable correlates of recollection and familiarity within the medial temporal lobes. *Neuropsychologia* 42, 2–13. doi: 10.1016/j.neuropsychologia.2003.07.006

Rauch, A., Rainer, G., and Logothetis, N. K. (2008). The effect of a serotonin-induced dissociation between spiking and perisynaptic activity on BOLD functional MRI. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6759–6764. doi: 10.1073/pnas.0800312105

Raz, N., Williamson, A., Gunning-Dixon, F., Head, D., and Acker, J. D. (2000). Neuroanatomical and cognitive correlates of adult age differences in acquisition of a perceptual-motor skill. *Microsc. Res. Tech.* 51, 85–93. doi: 10.1002/1097-0029(20001001)51:1<85::AID-JEMT9>3.0.CO;2-0

Rees, G., Friston, K., and Koch, C. (2000). A direct quantitative relationship between the functional properties of human and macaque V5. *Nat. Neurosci.* 3, 716–723. doi: 10.1038/76673

Riese, F., Gietl, A., Zolch, N., Henning, A., O'gorman, R., Kalin, A. M., et al. (2015). Posterior cingulate gamma-aminobutyric acid and glutamate/glutamine are reduced in amnestic mild cognitive impairment and are unrelated to amyloid deposition and apolipoprotein E genotype. *Neurobiol. Aging* 36, 53–59. doi: 10.1016/j.neurobiolaging.2014.07.030

Rilling, J. K. (2014). Comparative primate neuroimaging: insights into human brain evolution. *Trends Cogn. Sci.* 18, 46–55. doi: 10.1016/j.tics.2013.09.013

Roberson, E. D., Defazio, R. A., Barnes, C. A., Alexander, G. E., Bizon, J. L., Bowers, D., et al. (2012). Challenges and opportunities for characterizing cognitive aging across species. *Front. Aging Neurosci.* 4:6. doi: 10.3389/fnagi.2012.00006

Ronen, I., Fan, X., Schettler, S., Jain, S., Murray, D., Kim, D. S., et al. (2011). Regional age-related effects in the monkey brain measured with 1H magnetic resonance spectroscopy. *Neurobiol. Aging* 32, 1138–1148. doi: 10.1016/j.neurobiolaging.2009.05.020

Rosano, C., Aizenstein, H. J., Cochran, J. L., Saxton, J. A., De Kosky, S. T., Newman, A. B., et al. (2005). Event-related functional magnetic resonance imaging investigation of executive control in very old individuals with mild cognitive impairment. *Biol. Psychiatry* 57, 761–767. doi: 10.1016/j.biopsych.2004.12.031

Rossi, A., and Biancheri, R. (2013). Magnetic resonance spectroscopy in metabolic disorders. *Neuroimaging Clin. N. A.* 23, 425–448. doi: 10.1016/j.nic.2012.12.013

Rypma, B., and D'Esposito, M. (1999). The roles of prefrontal brain regions in components of working memory: effects of memory load and individual differences. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6558–6563. doi: 10.1073/pnas.96.11.6558

Sager, T. N., Topp, S., Torup, L., Hanson, L. G., Egestad, B., and Moller, A. (2001). Evaluation of CA1 damage using single-voxel 1H-MRS and un-biased stereology: can non-invasive measures of N-acetyl-asparate following global ischemia be used as a reliable measure of neuronal damage? *Brain Res.* 892, 166–175. doi: 10.1016/S0006-8993(00)03274-1

Sahara, N., Perez, P. D., Lin, W. L., Dickson, D. W., Ren, Y., Zeng, H., et al. (2014). Age-related decline in white matter integrity in a mouse model of tauopathy: an in vivo diffusion tensor magnetic resonance imaging study. *Neurobiol. Aging* 35, 1364–1374. doi: 10.1016/j.neurobiolaging.2013.12.009

Salami, A., Eriksson, J., and Nyberg, L. (2012). Opposing effects of aging on large-scale brain systems for memory encoding and cognitive control. *J. Neurosci.* 32, 10749–10757. doi: 10.1523/JNEUROSCI.0278-12.2012

Salat, D. H., Tuch, D. S., Hevelone, N. D., Fischl, B., Corkin, S., Rosas, H. D., et al. (2005). Age-related changes in prefrontal white matter measured by diffusion tensor imaging. *Ann. N. Y. Acad. Sci.* 1064, 37–49. doi: 10.1196/annals.1340.009

Salmeron, B. J., and Stein, E. A. (2002). Pharmacological applications of magnetic resonance imaging. *Psychopharmacol. Bull.* 36, 102–129.

Sanganahalli, B. G., Herman, P., Hyder, F., and Kannurpatti, S. S. (2013). Mitochondrial functional state impacts spontaneous neocortical activity and resting state FMRI. *PLoS ONE* 8:e63317. doi: 10.1371/journal.pone.0063317

Santin, M. D., Valabregue, R., Rivals, I., Penager, R., Paquin, R., Dauphinot, L., et al. (2014). In vivo 1H MRS study in microlitre voxels in the hippocampus of a mouse model of Down syndrome at 11.7 T. *NMR Biomed.* 27, 1143–1150. doi: 10.1002/nbm.3155

Scheinert, R. B., Asokan, A., Rani, A., Kumar, A., Foster, T. C., and Ormerod, B. K. (2015). Some hormone, cytokine and chemokine levels that change across lifespan vary by cognitive status in male Fischer 344 rats. *Brain Behav. Immun.* 49, 216–232. doi: 10.1016/j.bbi.2015.06.005

Scheinost, D., Finn, E. S., Tokoglu, F., Shen, X., Papademetris, X., Hampson, M., et al. (2015). Sex differences in normal age trajectories of functional brain networks. *Hum. Brain Mapp.* 36, 1524–1535. doi: 10.1002/hbm.22720

Scherf, T., and Angenstein, F. (2015). Postsynaptic and spiking activity of pyramidal cells, the principal neurons in the rat hippocampal CA1 region, does not control the resultant BOLD response: a combined electrophysiologic and fMRI approach. *J. Cereb. Blood Flow Metab.* 35, 565–575. doi: 10.1038/jcbfm.2014.252

Schneider, P., Weber-Fahr, W., Schweinfurth, N., Ho, Y. J., Sartorius, A., Spanagel, R., et al. (2012). Central metabolite changes and activation of microglia after peripheral interleukin-2 challenge. *Brain Behav. Immun.* 26, 277–283. doi: 10.1016/j.bbi.2011.09.011

Schroeder, M. P., Weiss, C., Procissi, D., Disterhoft, J. F., and Wang, L. (2016). Intrinsic connectivity of neural networks in the awake rabbit. *Neuroimage* 129, 260–267. doi: 10.1016/j.neuroimage.2016.01.010

Schwarz, A. J., Reese, T., Gozzi, A., and Bifone, A. (2003). Functional MRI using intravascular contrast agents: detrending of the relative cerebrovascular (rCBV) time course. *Magn. Reson. Imaging* 21, 1191–1200. doi: 10.1016/j.mri.2003.08.020

Semmler, A., Okulla, T., Sastre, M., Dumitrescu-Ozimek, L., and Heneka, M. T. (2005). Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. *J. Chem. Neuroanat.* 30, 144–157. doi: 10.1016/j.jchemneu.2005.07.003

Serrano, F., Deshazer, M., Smith, K. D., Ananta, J. S., Wilson, L. J., and Pautler, R. G. (2008). Assessing transneuronal dysfunction utilizing manganese-enhanced MRI (MEMRI). *Magn. Reson. Med.* 60, 169–175. doi: 10.1002/mrm.21648

Sevgi, M., Rigoux, L., Kuhn, A. B., Mauer, J., Schilbach, L., Hess, M. E., et al. (2015). An obesity-predisposing variant of the fto gene regulates d2r-dependent reward learning. *J. Neurosci.* 35, 12584–12592. doi: 10.1523/JNEUROSCI.1589-15.2015

Shamy, J. L., Buonocore, M. H., Makaron, L. M., Amaral, D. G., Barnes, C. A., and Rapp, P. R. (2006). Hippocampal volume is preserved and fails to predict recognition memory impairment in aged rhesus monkeys (*Macaca mulatta*). *Neurobiol. Aging* 27, 1405–1415. doi: 10.1016/j.neurobiolaging.2005.07.019

Sherman, S. M., Mumford, J. A., and Schnyer, D. M. (2015). Hippocampal activity mediates the relationship between circadian activity rhythms and memory in older adults. *Neuropsychologia* 75, 617–625. doi: 10.1016/j.neuropsychologia.2015.07.020

Silverman, H. A., Dancho, M., Regnier-Golanov, A., Nasim, M., Ochani, M., Olofsson, P. S., et al. (2014). Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. *Mol. Med.* 20, 601–611. doi: 10.2119/molmed.2014.00147

Slivka, A., Mytilineou, C., and Cohen, G. (1987). Histochemical evaluation of glutathione in brain. *Brain Res.* 409, 275–284. doi: 10.1016/0006-8993(87)90712-8

Small, S. A., Chawla, M. K., Buonocore, M., Rapp, P. R., and Barnes, C. A. (2004). Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proc. Natl. Acad. Sci. U.S.A.* 101, 7181–7186. doi: 10.1073/pnas.0400285101

Smith, D. E., Rapp, P. R., Mckay, H. M., Roberts, J. A., and Tuszynski, M. H. (2004). Memory impairment in aged primates is associated with focal death of cortical neurons and atrophy of subcortical neurons. *J. Neurosci.* 24, 4373–4381. doi: 10.1523/JNEUROSCI.4289-03.2004

Smith, K. D., Kallhoff, V., Zheng, H., and Pautler, R. G. (2007). In vivo axonal transport rates decrease in a mouse model of Alzheimer's disease. *Neuroimage* 35, 1401–1408. doi: 10.1016/j.neuroimage.2007.01.046

Smith, K. D., Paylor, R., and Pautler, R. G. (2011). R-flurbiprofen improves axonal transport in the Tg2576 mouse model of Alzheimer's disease as determined by MEMRI. *Magn. Reson. Med.* 65, 1423–1429. doi: 10.1002/mrm.22733

Smith, K. D., Peethumnongsin, E., Lin, H., Zheng, H., and Pautler, R. G. (2010). Increased human wildtype tau attenuates axonal transport deficits caused by loss of APP in mouse models. *Magn. Reson. Insights* 4, 11–18.

Smith, T. D., Adams, M. M., Gallagher, M., Morrison, J. H., and Rapp, P. R. (2000). Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. *J. Neurosci.* 20, 6587–6593.

Song, J., Birn, R. M., Boly, M., Meier, T. B., Nair, V. A., Meyerand, M. E., et al. (2014). Age-related reorganizational changes in modularity and functional connectivity of human brain networks. *Brain Connect.* 4, 662–676. doi: 10.1089/brain.2014.0286

Song, S. K., Kim, J. H., Lin, S. J., Brendza, R. P., and Holtzman, D. M. (2004). Diffusion tensor imaging detects age-dependent white matter changes in a transgenic mouse model with amyloid deposition. *Neurobiol. Dis.* 15, 640–647. doi: 10.1016/j.nbd.2003.12.003

Sperling, R. (2007). Functional MRI studies of associative encoding in normal aging, mild cognitive impairment, and Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 1097, 146–155. doi: 10.1196/annals.1379.009

Stoet, G., and Snyder, L. H. (2003). Executive control and task-switching in monkeys. *Neuropsychologia* 41, 1357–1364. doi: 10.1016/S0028-3932(03)00048-4

Su, M. Y., Head, E., Brooks, W. M., Wang, Z., Muggenburg, B. A., Adam, G. E., et al. (1998). Magnetic resonance imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiol. Aging* 19, 479–485. doi: 10.1016/S0197-4580(98)00081-5

Sullivan, E. V., Marsh, L., Mathalon, D. H., Lim, K. O., and Pfefferbaum, A. (1995). Age-related decline in MRI volumes of temporal lobe gray matter but not hippocampus. *Neurobiol. Aging* 16, 591–606. doi: 10.1016/0197-4580(95)00074-O

Sumiyoshi, A., Taki, Y., Nonaka, H., Takeuchi, H., and Kawashima, R. (2014). Regional gray matter volume increases following 7days of voluntary wheel running exercise: a longitudinal VBM study in rats. *Neuroimage* 98, 82–90. doi: 10.1016/j.neuroimage.2014.04.075

Suzuki, H., Sumiyoshi, A., Matsumoto, Y., Duffy, B. A., Yoshikawa, T., Lythgoe, M. F., et al. (2015). Structural abnormality of the hippocampus associated with depressive symptoms in heart failure rats. *Neuroimage* 105, 84–92. doi: 10.1016/j.neuroimage.2014.10.040

Takano, T., Tian, G. F., Peng, W., Lou, N., Libionka, W., Han, X., et al. (2006). Astrocyte-mediated control of cerebral blood flow. *Nat. Neurosci.* 9, 260–267. doi: 10.1038/nn1623

Takata, N., Yoshida, K., Komaki, Y., Xu, M., Sakai, Y., Hikishima, K., et al. (2015). Optogenetic activation of CA1 pyramidal neurons at the dorsal and ventral hippocampus evokes distinct brain-wide responses revealed by mouse fMRI. *PLoS ONE* 10:e0121417. doi: 10.1371/journal.pone.0121417

Tapp, P. D., Siwak, C. T., Gao, F. Q., Chiou, J. Y., Black, S. E., Head, E., et al. (2004). Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. *J. Neurosci.* 24, 8205–8213. doi: 10.1523/JNEUROSCI.1339-04.2004

Tarantini, S., Hertelendy, P., Tucsek, Z., Valcarcel-Ares, M. N., Smith, N., Menyhart, A., et al. (2015). Pharmacologically-induced neurovascular uncoupling is associated with cognitive impairment in mice. *J. Cereb. Blood Flow Metab.* 35, 1871–1881. doi: 10.1038/jcbfm.2015.162

Teipel, S. J., Meindl, T., Wagner, M., Stieltjes, B., Reuter, S., Hauenstein, K. H., et al. (2010). Longitudinal changes in fiber tract integrity in healthy aging and mild cognitive impairment: a DTI follow-up study. *J. Alzheimers. Dis.* 22, 507–522. doi: 10.3233/JAD-2010-100234

Terpstra, M., Tkac, I., Rao, R., and Gruetter, R. (2006). Quantification of vitamin C in the rat brain in vivo using short echo-time 1H MRS. *Magn. Reson. Med.* 55, 979–983. doi: 10.1002/mrm.20854

Thibault, O., Gant, J. C., and Landfield, P. W. (2007). Expansion of the calcium hypothesis of brain aging and Alzheimer's disease: minding the store. *Aging Cell* 6, 307–317. doi: 10.1111/j.1474-9726.2007.00295.x

Thomsen, K., Offenhauser, N., and Lauritzen, M. (2004). Principal neuron spiking: neither necessary nor sufficient for cerebral blood flow in rat cerebellum. *J. Physiol.* 560, 181–189. doi: 10.1113/jphysiol.2004.068072

Tiede, R., Krautwald, K., Fincke, A., and Angenstein, F. (2012). NMDA-dependent mechanisms only affect the BOLD response in the rat dentate gyrus by modifying local signal processing. *J. Cereb. Blood Flow Metab.* 32, 570–584. doi: 10.1038/jcbfm.2011.182

Toepper, M., Gebhardt, H., Bauer, E., Haberkamp, A., Beblo, T., Gallhofer, B., et al. (2014). The impact of age on load-related dorsolateral prefrontal cortex activation. *Front. Aging Neurosci.* 6:9. doi: 10.3389/fnagi.2014.00009

Tromp, D., Dufour, A., Lithfous, S., Pebayle, T., and Despres, O. (2015). Episodic memory in normal aging and Alzheimer disease: insights from imaging and behavioral studies. *Ageing Res. Rev.* 24, 232–262. doi: 10.1016/j.arr.2015.08.006

Turner, G. R., and Spreng, R. N. (2012). Executive functions and neurocognitive aging: dissociable patterns of brain activity. *Neurobiol. Aging* 33, e821–e813. doi: 10.1016/j.neurobiolaging.2011.06.005

van der Hel, W. S., Van Eijsden, P., Bos, I. W., De Graaf, R. A., Behar, K. L., Van Nieuwenhuizen, O., et al. (2013). In vivo MRS and histochemistry of status epilepticus-induced hippocampal pathology in a juvenile model of temporal lobe epilepsy. *NMR Biomed.* 26, 132–140. doi: 10.1002/nbm.2828

Vernon, A. C., So, P. W., Lythgoe, D. J., Chege, W., Cooper, J. D., Williams, S. C., et al. (2015). Longitudinal in vivo maturational changes of metabolites in the prefrontal cortex of rats exposed to polyinosinic-polycytidylic acid in utero. *Eur. Neuropsychopharmacol.* 25, 2210–2220. doi: 10.1016/j.euroneuro.2015.09.022

Vincent, J. L., Kahn, I., Van Essen, D. C., and Buckner, R. L. (2010). Functional connectivity of the macaque posterior parahippocampal cortex. *J. Neurophysiol.* 103, 793–800. doi: 10.1152/jn.00546.2009

Upadhyay, J., Baker, S. J., Chandran, P., Miller, L., Lee, Y., Marek, G. J., et al. (2011). Default-mode-like network activation in awake rodents. *PLoS ONE* 6:e27839. doi: 10.1371/journal.pone.0027839

Wang, H., Tan, L., Wang, H. F., Liu, Y., Yin, R. H., Wang, W. Y., et al. (2015). Magnetic resonance spectroscopy in alzheimer's disease: systematic review and meta-analysis. *J. Alzheimers Dis.* 46, 1049–1070. doi: 10.3233/JAD-143225

Wang, M., Gamo, N. J., Yang, Y., Jin, L. E., Wang, X. J., Laubach, M., et al. (2011). Neuronal basis of age-related working memory decline. *Nature* 476, 210–213. doi: 10.1038/nature10243

Wang, T. H., Kruggel, F., and Rugg, M. D. (2009). Effects of advanced aging on the neural correlates of successful recognition memory. *Neuropsychologia* 47, 1352–1361. doi: 10.1016/j.neuropsychologia.2009.01.030

Wang, X., and Michaelis, E. K. (2010). Selective neuronal vulnerability to oxidative stress in the brain. *Front. Aging Neurosci.* 2:12. doi: 10.3389/fnagi.2010.00012

Watson, S. N., Risling, T. E., Hermann, P. M., and Wildering, W. C. (2012). Failure of delayed nonsynaptic neuronal plasticity underlies age-associated long-term associative memory impairment. *BMC Neurosci.* 13:103. doi: 10.1186/1471-2202-13-103

Westlye, L. T., Walhovd, K. B., Dale, A. M., Bjornerud, A., Due-Tonnessen, P., Engvig, A., et al. (2010). Life-span changes of the human brain white matter: diffusion tensor imaging (DTI) and volumetry. *Cereb. Cortex* 20, 2055–2068. doi: 10.1093/cercor/bhp280

Willette, A. A., Coe, C. L., Birdsill, A. C., Bendlin, B. B., Colman, R. J., Alexander, A. L., et al. (2013). Interleukin-8 and interleukin-10, brain volume and microstructure, and the influence of calorie restriction in old rhesus macaques. *Age (Dordr.)* 35, 2215–2227. doi: 10.1007/s11357-013-9518-y

Wisco, J. J., Killiany, R. J., Guttmann, C. R., Warfield, S. K., Moss, M. B., and Rosene, D. L. (2008). An MRI study of age-related white and gray matter volume changes in the rhesus monkey. *Neurobiol. Aging* 29, 1563–1575. doi: 10.1016/j.neurobiolaging.2007.03.022

Wu, J. T., Wu, H. Z., Yan, C. G., Chen, W. X., Zhang, H. Y., He, Y., et al. (2011). Aging-related changes in the default mode network and its anti-correlated networks: a resting-state fMRI study. *Neurosci. Lett.* 504, 62–67. doi: 10.1016/j.neulet.2011.08.059

Xie, P., Yu, T., Fu, X., Tu, Y., Zou, Y., Lui, S., et al. (2013). Altered functional connectivity in an aged rat model of postoperative cognitive dysfunction: a study using resting-state functional MRI. *PLoS ONE* 8:e64820. doi: 10.1371/journal.pone.0064820

Yeatman, J. D., Wandell, B. A., and Mezer, A. A. (2014). Lifespan maturation and degeneration of human brain white matter. *Nat. Commun.* 5:4932. doi: 10.1038/ncomms5932

Yin, J. X., Turner, G. H., Lin, H. J., Coons, S. W., and Shi, J. (2011). Deficits in spatial learning and memory is associated with hippocampal volume loss in aged apolipoprotein E4 mice. *J. Alzheimers Dis.* 27, 89–98. doi: 10.3233/JAD-2011-110479

Zahr, N. M., Mayer, D., Rohlfing, T., Chanraud, S., Gu, M., Sullivan, E. V., et al. (2013). In vivo glutamate measured with magnetic resonance spectroscopy: behavioral correlates in aging. *Neurobiol. Aging* 34, 1265–1276. doi: 10.1016/j.neurobiolaging.2012.09.014

Zaldivar, D., Rauch, A., Whittingstall, K., Logothetis, N. K., and Goense, J. (2014). Dopamine-induced dissociation of BOLD and neural activity in macaque visual cortex. *Curr. Biol.* 24, 2805–2811. doi: 10.1016/j.cub.2014.10.006

Zeamer, A., Decamp, E., Clark, K., and Schneider, J. S. (2011). Attention, executive functioning and memory in normal aged rhesus monkeys. *Behav. Brain Res.* 219, 23–30. doi: 10.1016/j.bbr.2010.12.021

Zeier, Z., Madorsky, I., Xu, Y., Ogle, W. O., Notterpek, L., and Foster, T. C. (2011). Gene expression in the hippocampus: regionally specific effects of aging and caloric restriction. *Mech. Ageing Dev.* 132, 8–19. doi: 10.1016/j.mad.2010.10.006

Zerbi, V., Jansen, D., Wiesmann, M., Fang, X., Broersen, L. M., Veltien, A., et al. (2014). Multinutrient diets improve cerebral perfusion and neuroprotection in a murine model of Alzheimer's disease. *Neurobiol. Aging* 35, 600–613. doi: 10.1016/j.neurobiolaging.2013.09.038

Zhang, B., Chuang, K. H., Tjio, C., Chen, W. C., Sheu, F. S., and Routtenberg, A. (2015). Spatial memory training induces morphological changes detected by manganese-enhanced MRI in the hippocampal CA3 mossy fiber terminal zone. *Neuroimage* 128, 227–237. doi: 10.1016/j.neuroimage.2015.07.084

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 13 July 2016
doi: 10.3389/fnagi.2016.00162

The Indirect Effect of Age Group on Switch Costs via Gray Matter Volume and Task-Related Brain Activity

Jason Steffener[1,2,3], Yunglin Gazes[4], Christian Habeck[4] and Yaakov Stern[4]*

[1] PERFORM Center, Concordia University, Montreal, QC, Canada, [2] Centre de Recherche de l'Institut de Gériatrie de Montréal, Montréal, QC, Canada, [3] Department of Psychology, Concordia University, Montreal, QC, Canada, [4] Cognitive Neuroscience Division, Department of Neurology and Taub Institute for Research on Alzheimer's Disease and The Aging Brain, Columbia University College of Physicians and Surgeons, New York, NY, USA

Healthy aging simultaneously affects brain structure, brain function, and cognition. These effects are often investigated in isolation ignoring any relationships between them. It is plausible that age related declines in cognitive performance are the result of age-related structural and functional changes. This straightforward idea is tested in within a conceptual research model of cognitive aging. The current study tested whether age-related declines in task-performance were explained by age-related differences in brain structure and brain function using a task-switching paradigm in 175 participants. Sixty-three young and 112 old participants underwent MRI scanning of brain structure and brain activation. The experimental task was an executive context dual task with switch costs in response time as the behavioral measure. A serial mediation model was applied voxel-wise throughout the brain testing all pathways between age group, gray matter volume, brain activation and increased switch costs, worsening performance. There were widespread age group differences in gray matter volume and brain activation. Switch costs also significantly differed by age group. There were brain regions demonstrating significant indirect effects of age group on switch costs via the pathway through gray matter volume and brain activation. These were in the bilateral precuneus, bilateral parietal cortex, the left precentral gyrus, cerebellum, fusiform, and occipital cortices. There were also significant indirect effects via the brain activation pathway after controlling for gray matter volume. These effects were in the cerebellum, occipital cortex, left precentral gyrus, bilateral supramarginal, bilateral parietal, precuneus, middle cingulate extending to medial superior frontal gyri and the left middle frontal gyri. There were no significant effects through the gray matter volume alone pathway. These results demonstrate that a large proportion of the age group effect on switch costs can be attributed to individual differences in gray matter volume and brain activation. Therefore, age-related neural effects underlying cognitive control are a complex interaction between brain structure and function. Furthermore, the analyses demonstrate the feasibility of utilizing multiple neuroimaging modalities within a conceptual research model of cognitive aging.

Keywords: gray-matter volume, task related brain activity, mediation, aging, cognitive control

OPEN ACCESS

Edited by:
Kristina Visscher,
University of Alabama at Birmingham, USA

Reviewed by:
Frini Karayanidis,
University of Newcastle, Australia
Daniel Ortuño-Sahagún,
Centro Universitario de Ciencias de la Salud, Mexico
Patrick Ragert,
University of Leipzig and Max Planck Institute for Human Cognitive and Brain Sciences, Germany

****Correspondence:***
Jason Steffener
jsteffen@uottawa.ca

Received: *15 December 2015*
Accepted: *20 June 2016*
Published: *13 July 2016*

Citation:
Steffener J, Gazes Y, Habeck C and Stern Y (2016) The Indirect Effect of Age Group on Switch Costs via Gray Matter Volume and Task-Related Brain Activity. Front. Aging Neurosci. 8:162. doi: 10.3389/fnagi.2016.00162

INTRODUCTION

Age has a multi-faceted effect on many aspects of our bodies and our cognitive abilities. Declines in cognitive control and executive function are thought to underlie other age-related declines in cognition, specifically fluid ability (Baltes, 1993). One approach to investigating cognitive control is with tasks requiring dual-task processing (Kray and Lindenberger, 2000). Engagement in multiple simultaneous tasks requires task switching and this switch hinders response time with respect to performance of either in isolation (Rogers and Monsell, 1995). The performance decline when engaged in two tasks is termed switch costs. Switch costs fall into two categories, local and global. Local costs are the trial-to-trial decrements in performance, while global costs refer to block effects, namely blocks of single task conditions compared to blocks of dual task trials. Understanding the neural origins of increasing switch costs and cognitive control is an important goal for understanding the aging process (Braver and Barch, 2002; Madden et al., 2010). Understanding the neural underpinnings of performance decline on task-switching experiments provides better insight for intervention programs aimed at maintaining and improving daily life through maintenance of cognitive control (Karayanidis et al., 2010).

Neuroimaging approaches provide insight into the effects of aging on the brain. Advancing age affects many neural measures including gray matter volume and task-related brain activation. Age-related neural declines are also related to executive functioning (Van Petten et al., 2004; Sakai et al., 2012). Integration of various age-related findings into a single conceptual research model provides a better understanding of the complexities of the aging process. This approach may shed further light on the neural underpinnings of age-related cognitive decline. Furthermore, incorporating multiple modalities of neural measures to better understand cognitive aging is a current topic of discussion in the literature (Grady, 2012; Steffener and Stern, 2012; Reuter-Lorenz and Park, 2014). The present work attempts to explain age-related differences in cognitive control with full brain voxel-wise measures of gray matter volume and brain activation.

The hypothesis of this study is that age related declines in cognitive performance are the result of age-related declines in MRI derived measures of brain structure which result in altered brain function. This straightforward hypothesis has had limited explicit testing. Previous work along the same line of inquiry has focused on linking together functional and structural connectivity (via white matter measures) and age-related changes in cognition (Gold et al., 2008; Madden et al., 2010). The current work focuses on gray matter volume and brain function and incorporates the measures into an established theoretical model of cognitive aging (Grady, 2012; Steffener and Stern, 2012; Reuter-Lorenz and Park, 2014).

The hypothesized model of this study tests whether age-related differences in switch costs are partially explained by age-related declines in gray matter volume resulting in altered brain activation resulting in greater switch-costs. The model is tested with serial mediation analyses and links together three consistent observations in the aging literature: gray matter volume decline, task-related brain activity differences, and increased switch costs. This nature of this model incorporates the main hypothesis of this work along with three other possible effects. Age may indirectly affect switch costs via gray matter volume or brain activation, while holding the other measure constant. Age may also directly affect switch costs after accounting for both brain measures. Summing the three indirect effects and the direct effects is the total effect of age on switch costs. The total effect is simply the effect size of age on switch costs in the absence of any neural measures. The statistical tests in this study imply causal relationships between variables as specified in the *a priori* theoretical model. Since the data used is cross-sectional, caution is warranted when making causal claims or interpretations. The mediation analyses test whether the data fit the conceptual research model that age effects on brain volume, alter brain activity resulting in altered switch costs.

The task employed was the executive context factor (ECF) task first discussed by Koechlin et al. (2003). This initial work with young adults demonstrated that manipulations of cognitive control included activation within increasingly rostral regions of the inferior frontal cortex. Regional age-related differences in brain activation using this task, and multivariate statistics, included middle frontal gyrus, the precentral gyrus, the anterior cingulate, and the superior parietal lobule and were shown to be related to switch costs (Gazes et al., 2012) and replicated with an independent sample (Gazes et al., 2015). For further background on switch costs we refer the reader to these referenced works.

The current study tested for indirect and direct effects of age group on global switch costs with the ECF task. Based on our previous multivariate analyses with this task and the literature (DiGirolamo et al., 2001; Madden et al., 2010), we expected the current univariate analyses to demonstrate age related differences in task related brain activation within the middle prefrontal, precentral, cingulate and parietal regions. There is an expected age-related decrease in gray matter volume throughout the brain and switch costs are expected to increase with advancing age. This study integrates full brain data into a comprehensive model of cognitive aging.

MATERIALS AND METHODS

Study Participants

One hundred and seventy-five healthy adults were scanned including 63 healthy, young participants (24 men and 39 women mean (±SD) age = 25.79 (2.70); mean (±SD) years of education = 15.46 (1.97); all right handed), and 112 healthy, old participants (4530 men and 59 women; mean (±SD) age = 65.47 (2.89); mean (±SD) years of education = 16.04 (2.64); all right handed). Participants were recruited using market-mailing procedures to equalize the recruitment process for young and old adults. Participants who responded to the mailing were telephone screened to ensure they met basic inclusion criteria (right handed, English speaking, no psychiatric or neurological disorders, normal or corrected-to-normal vision). All participants found eligible via the initial telephone screen were further screened in person with structured medical, neurological, psychiatric,

and neuropsychological evaluations to ensure that they had no neurological or psychiatric disease or cognitive impairment. The screening procedure included a detailed interview that excluded individuals with a self-reported history of major or unstable medical illness, significant neurological history (e.g., epilepsy, brain tumor, and stroke), history of head trauma with loss of consciousness for greater than 5 min or history of Axis I psychiatric disorder (American Psychiatric Association, 1994). Individuals taking psychotropic medications or medications that influenced cognition were also excluded. Global cognitive functioning was assessed with the Mattis Dementia Rating Scale, on which a score of at least 133 was required for retention in the study (Mattis, 1988). Informed consent, as approved by the Internal Review Board of the College of Physicians and Surgeons of Columbia University, was obtained in writing prior to study participation, and after the nature and risks of the study were explained. Participants were compensated for their participation in the study.

Behavioral Task

The behavioral task was derived from Experiment 2 in the task developed by Koechlin et al. (2003). This is an intrinsically cued task-switching paradigm with a no-go component where the color of each stimulus served as the task cue, see **Figure 1**. Participants were presented with a series of four conditions comprised of two single-task conditions and two identical task-switching conditions, with the duplication serving to match the number of trials for each discrimination between the single and switch-task conditions (see below). Each block was preceded by a 4.8 s instruction cue to inform the participant of the appropriate action for each stimulus. Each 33.6 s block, comprised 12 sequential letters (or trials) each presented for 1900 ms with an inter-trial time of 500 ms. Each stimulus was terminated when a response was made or when the trial deadline was reached. These trial dynamics were selected based on performance characteristics of the older adults in behavioral pilot studies, and deviated from Koechlin's briefer presentations (Koechlin et al., 2003). Participants responded to each letter with a right-hand/left-hand button press or by making no action at all.

In addition to the four active conditions, there were two 33.6 s resting conditions when no stimuli were presented and no response was required. The two resting conditions were identical, but were separately enumerated to simplify description of the Latin Square design. Each resting block presented an instruction cue ("REST") followed by a blank screen. During fMRI acquisition, each participant was given six repetitions of each of the four active and two resting conditions, for a total of 36 blocks. Conditions were presented in a 6 × 6 fully balanced Latin Square design. The fMRI data acquisition protocol requires stopping the scanner after every six blocks, typically requiring less than 30 s, resulting in the total session duration of approximately 26 min and a total of six fMRI runs with six blocks in each run.

In order to promote the scanning of participants in a stable behavioral and cognitive state, participants were pre-trained on the task and then tested on the entire paradigm in a quiet office prior to the MRI scanning session. Training consisted of giving between one and three blocks of each condition, with unlimited time to inspect the full instructions and the instruction cues preceding each block, and with auditory feedback indicating incorrect responses. Then participants were tested on the entire 6 × 6 Latin Square identical to the testing protocol described above (pre-scan phase).

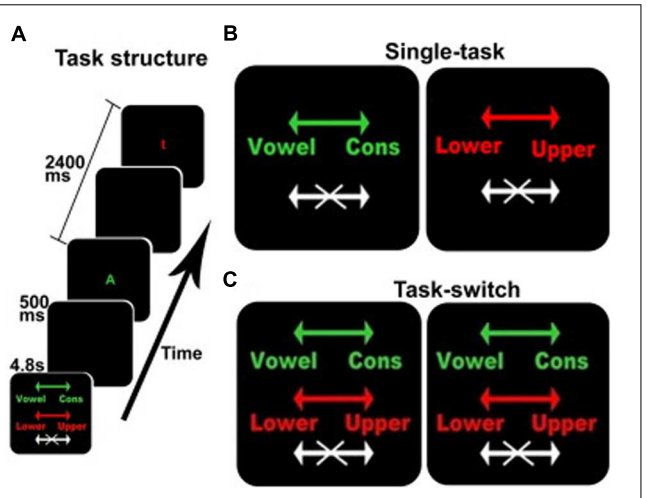


FIGURE 1 | The switching cognitive task used during fMRI scanning. **(A)** An example of the beginning of a block including the instruction screen for a task-switching block, intertrial interval, a stimulus for the vowel/consonant task, intertrial interval, and a stimulus for the upper-/lower-case task. **(B,C)** The instruction screens for the single-task and the task-switching conditions, respectively. The colors served as task-cues: green for the vowel/consonant task, red for the upper-/lower-case task, and white for no-go trials. Arrows show the response-hand assignments: left for vowel/right for consonant and left for lower-case/right for upper case.

Stimulus Presentation

Task stimuli were back-projected onto a screen located at the foot of the MRI bed using an LCD projector. Participants viewed the screen via a mirror system located in the head coil and, if needed, had vision corrected to normal using MR compatible glasses (manufactured by SafeVision, LLC. Webster Groves, MO, USA). Responses were made on a LUMItouch response system (Photon Control Company) using the index fingers. Task administration and collection of RT and accuracy data were controlled using PsyScope 5X B53 (Macwhinney et al., 1997) running on a Macintosh G3/G4 iBook. Task onset was electronically synchronized with the MRI acquisition computer. A MellonIOLabs Systems USB Button Box provided digital input–output for the response system and synchronization with the MRI acquisition computer, as well as millisecond accurate timing of responses.

MRI Data Acquisition

MRI images were acquired in a 3.0 T Philips Achieva Magnet using a standard quadrature head coil. A T1-weighted scout image was acquired to determine participant position. One hundred and sixty-five contiguous 1 mm coronal T1-weighted

images of the whole brain were acquired for each participant with an MPRAGE sequence using the following parameters: TR 6.5 ms, TE 3 ms; flip angle 8°, acquisition matrix 256 × 256 and 240 mm field of view. Six functional scan sets were acquired, each of which included collection of 111 functional images acquired using a field echo echo-planar imaging (FE–EPI) sequence TE/TR = 20 ms/2000 ms; flip angle = 72°; 112 × 112 matrix; in-plane voxel size = 2.0 mm × 2.0 mm; slice thickness = 3.0 mm (no gap); 41 transverse slices per volume. Before the initiation of the executive task, four volumes were acquired and discarded to allow transverse magnetization immediately after radiofrequency excitation to approach its steady-state value. A neuroradiologist reviewed all T1 scans for potentially clinically significant findings, such as abnormal neural structure; no clinically significant findings were identified or removed.

Image Pre-processing

All image pre-processing and statistical analyses used SPM8 (Wellcome Department of Cognitive Neurology). For each participant's EPI dataset: images were temporally shifted to correct for slice acquisition order using the first slice acquired in the TR as the reference. All EPI images were corrected for motion by realigning to the first volume of the first session. The T1-weighted (structural) image was coregistered to the first EPI volume using mutual information. This co-registered high-resolution image was used to determine the transformation into a standard space defined by the Montreal Neurologic Institute (MNI) template brain supplied with SPM8. This transformation was applied to the EPI data and re-sliced using sinc-interpolation to 2 mm × 2 mm × 2 mm. Finally, all images were spatially smoothed with an 8 mm FWHM kernel (Mikl et al., 2008).

Time-Series Analysis

First-level time-series analyses used a block-based model composed of epochs separately representing the single and switch-task conditions. Each epoch was convolved with a canonical model of the hemodynamic response function supplied with SPM8. Contrasts of the switch-task versus single task conditions were entered into the group-level mediation analysis.

Gray Matter Volume

The T1-weighted anatomical images were first segmented into three tissue types: gray matter, white matter, and cerebrospinal fluid, using the unified segmentation routines in SPM8 (Wellcome Department of Cognitive Neurology; Ashburner and Friston, 2000, 2005; Good et al., 2001b). This procedure uses a single generative model to correct for image intensity non-uniformity (bias), registration with tissue class priors, and tissue classification. The result is a classification for each voxel based on the probability that it belongs to each tissue type. Each image segment therefore contains measures of tissue densities in each voxel location. The images were spatially normalized to the study specific normalization template using 12 degrees of freedom affine transforms and non-linear warping. Once warped, the images were modulated using the Jacobian determinant, which converts the density images into measures of absolute volume at each voxel location (Good et al., 2001b). The resultant modulated, spatially normalized gray matter probability maps were spatially smoothed with an isometric Gaussian smoothing kernel of 8 mm^3 at its full-width at half-maximum (FWHM) to result in gray matter volume maps.

Covariates

As a measure of whole brain gray matter volume, the proportion of intra-cranial volume occupied by gray and white matter was calculated. Total volume of gray matter plus white matter was divided by the sum of gray, white, and CSF volume to derive the intra-cranial brain fraction (Chard et al., 2002; Fotenos et al., 2005). This measure is termed normalized whole brain volume (nWBV) and was used as a covariate in the analyses. Sex differences are well established in global brain size differences, with males having larger total brain volumes (Good et al., 2001a); therefore, sex was also included in all analyses as a covariate.

Mediation Analyses

The mediation model tested whether age related differences in global switch costs (total effects) could be partially explained by voxel-wise measures of gray matter volume and/or task related brain activation (indirect effects). This approach uses switch costs as an output measure that is assumed to be affected by brain function, brain structure, and other age-related effects. Testing for mediation involves fitting multiple linear regression models at every voxel of the brain and assessment of statistical significance using non-parametric statistics specifically designed for use with brain imaging data to correct for multiple comparisons

The mediation model used in this study includes two mediators of the relationship between age and task performance. These are structure (S) measured as gray matter volume and functional brain activity (F). The indirect effect of age on switch costs may therefore arise through four pathways (see **Figure 2**):

$A \rightarrow (P|S,F)$, the direct effect of age on performance after accounting for structure and function.

$A \rightarrow S \rightarrow (P|F)$, the indirect effect of age on performance as mediated by structure after accounting for the effects of brain function.

$A \rightarrow F \rightarrow (P|S)$, the indirect effect of age on performance as mediated by brain function after accounting for the effects of structure.

$A \rightarrow S \rightarrow F \rightarrow P$, the indirect effect of age on performance as mediated by structure and brain function.

The regression equations for testing this model are:

$$1\quad P = c \cdot A + \varepsilon_1$$
$$2\quad S = a \cdot A + \varepsilon_2$$
$$3\quad F = b \cdot S + d \cdot A + \varepsilon_3$$
$$4\quad P = e \cdot F + f \cdot S + c' \cdot A + \varepsilon_4$$

The total effect of age on performance is *c* in Eq. 1. This is the effect that the mediation model is trying to explain with measures of gray matter volume and brain activity. This total effect of age on switch costs is therefore split into the direct effect and three

indirect effects. The indirect effects are calculated by multiplying regression parameters together.

- Total effect: c
- Direct effect: c'
- Indirect effect through S: $a \cdot f$
- Indirect effect through F: $d \cdot e$
- Indirect effect through S and F: $a \cdot b \cdot e$
- Total effect = direct effect + all indirect effects:

$$c = c' + a \cdot b \cdot c + a \cdot f + d \cdot e$$

After fitting Eqs 2–4 at each voxel in the brain, the indirect effects were calculated. Testing voxel-wise significance of indirect effects used permutation inference and the 'single threshold' permutation test (Nichols and Holmes, 2002; Winkler et al., 2014) using threshold free cluster enhancement (TFCE; Smith and Nichols, 2009). This approach accounts for multiple comparisons. For this method, 2000 permutations were performed where group assignment was randomly shuffled for each permutation. All analyses used the publically available and modifiable "Process Models for Neuroimaging" toolbox[1] developed by the author JS. This toolbox implements the methods of Preacher and Hayes for use with neuroimaging data. An additional threshold was used based on the percentage of total effect of age on switch costs that was accounted for by the indirect effects. The effects had to be at least five percent of the total effect. This threshold limits results to brain regions having significant effects that are also relatively large.

RESULTS

Behavioral Results

Global switch costs for correct trials were greater for the old adults than the young when tested with linear regression of age group onto switch costs: $\beta = 0.0799$, $t(173) = 3.90$, $p < 0.001$, mean (SD) of the young: 0.22 (0.083) seconds and for the old: 0.30 (0.15) seconds. Levene's test for equality of variance demonstrated that the variance was significantly higher for the old adults, $F(1,173) = 19.79$, $p < 0.001$. After accounting for covariates of sex and nWBV this effect was still significant ($\beta = 0.067$, $t(171) = 2.21$, $p < 0.05$). The β-value here is the total effect c from Eq. 1. Analyses only focused on global switch costs since they best matched the block-design used for analysis of the fMRI data.

Brain Imaging Results

All analyses were performed in brain regions demonstrating increased task-related signal change in at least one of the groups using uncorrected two-tailed t-tests of $\alpha < 0.05$ intersected with a gray matter probability mask of 0.5. Inclusion of this mask ensures that results are limited to brain locations where there is a significantly large effect size for the brain activation measure in regions comprised largely of gray matter. This helps minimize spurious findings and provides an additional control over type I error.

[1]https://github.com/steffejr/ProcessModelsNeuroImage

Effects of age group on gray matter volume and brain activation are shown in **Figure 3**. These results represent the first step in the mediation analysis on the left hand side of **Figure 2**. They also demonstrate the magnitude and direction of the effects in the mediation analyses shown in **Figure 4**. The liberal threshold used here is to demonstrate that age group effects occur in the data. This threshold has no impact on the indirect effects reported later, which use strict correction for multiple comparisons.

Advancing age had a negative effect on gray matter volume throughout the brain. Therefore, as age increased, gray matter volume decreased, see **Figure 3A**. Advancing age was related to increased levels of task related signal change. In other words, older adults had higher levels of brain activity than younger adults, see **Figure 3B**.

Mediation Results

Mediation analyses tested the three indirect pathways between age group and global switch costs. Results were thresholded at two-tailed $\alpha < 0.05$ correcting for multiple comparisons using the TFCE method with the 'single threshold' permutation test with 2,000 permutations. The TFCE method eliminates the need for cluster threshold allowing results to be interpreted with a single threshold instead of a combination of height and cluster size thresholds. Results from the indirect effects are shown in **Figure 4** and in **Tables 1** and **2**. The tables include the proportional size of the indirect effects and the t-values for the within and between age group differences in brain activation.

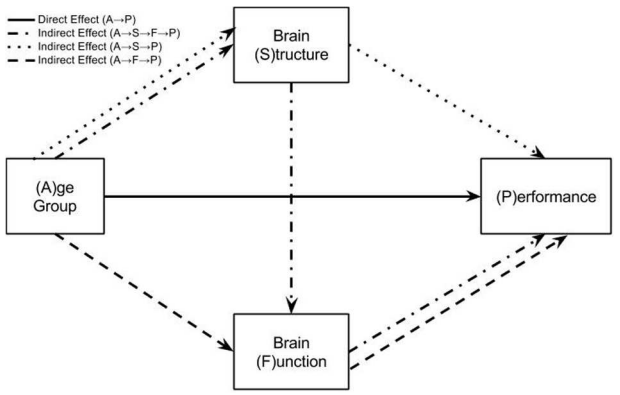


FIGURE 2 | Mediation model testing how age group differences in performance are explained by direct or indirect effects. This model represents three regression equations fit at every voxel in the brain data. The solid arrow represents the direct effect of age group on task performance. This is the effect of age group on task performance after accounting for brain structure and function in the regression model. The three broken arrows represent the three possible indirect effects from age group to task performance. The dotted line is the pathway: Age Group → Brain Structure → Performance. The dashed line is the pathway: Age Group → Brain Function → Performance. The dot-dash line is the pathway: Age Group → Brain Structure → Brain Function → Performance. The sum of the direct and three indirect effects of age group on task performance represent the total effect of age group. This total effect may also be calculated by simply regressing age group onto task performance.

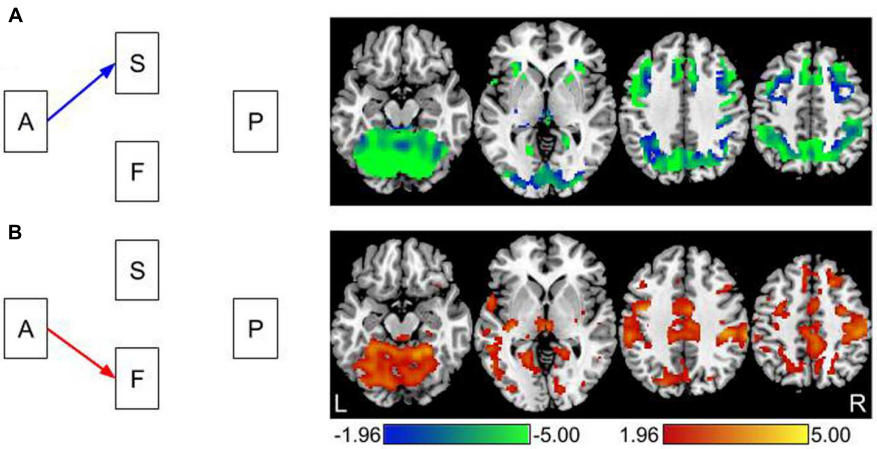


FIGURE 3 | Direct effects of age on gray matter volume and brain activity. (A) The effect of age on gray matter volume using voxel based morphometry (VBM). All effects are in the negative direction; older adults have lower gray matter volume than young adults. This is represented with a blue arrow in the path model on the left. **(B)** The effect of age on brain activation. All effects are in the positive direction; older adults have greater levels of task-related brain activation than young adults. This is represented with a red arrow in the path model on the left. Images are thresholded using a two-tailed test of $p < 0.05$, $Z > 1.96$.

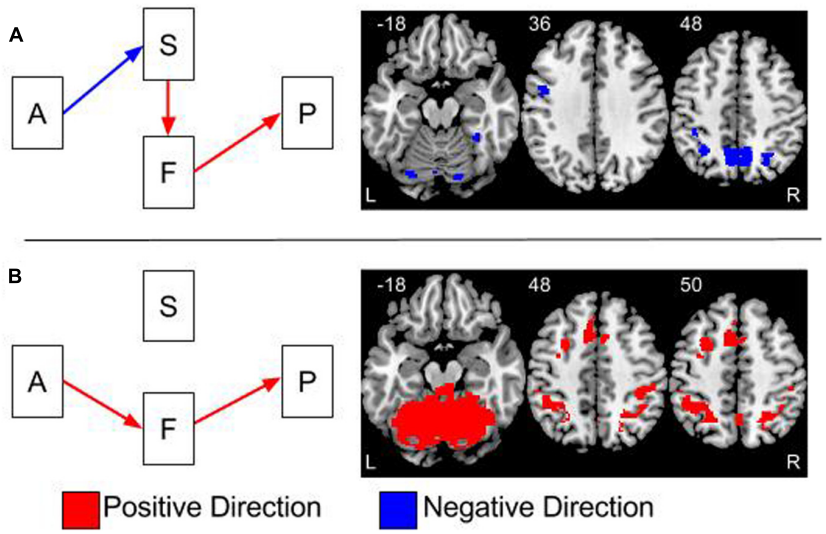


FIGURE 4 | Overlays of the two significant indirect effects in the model. (A) The indirect effect between age group and performance, via gray matter volume and brain function. **(B)** The indirect effect between age and performance via brain function. The effects in **(A,B)** are thresholded at a two-tailed alpha <0.05 corrected for multiple comparisons using 2000 permutations and threshold free cluster enhancement (tfce). The colors of the arrows on the path models represent the direction of the effects. Blue arrows represent negative effects and red arrows are positive effects. The colors of clusters of activations in the brain overlays represent the direction of the indirect effects. These indirect effects are calculated by multiplying the effects at each step in the pathway together. Therefore, the one negative and two positive effects in **(A)** result in an overall negative effect.

Mediation results were significant in two out of the three possible pathways, age to structure to function to switch costs and age to function to switch costs, see **Figure 4**. Both pathways partially explained the negative effect of age group on switch costs.

Age to Structure to Function to Switch Costs Pathway

This effect is shown in **Figure 4A** and **Table 1**. All indirect effects along this pathway were **negative**. Therefore, as age increased, gray matter volume decreased; gray matter volume and brain activation were positively related and brain activation and switch costs were also positively related. The bilateral precuneus had a significant effect and brain activity in these regions were significantly greater than zero for the old adults; however, not for the young adults. Results within the bilateral parietal cortex had highly significant brain activity for the old adults and lower, but also significantly greater than zero, brain activity for the young. Regions of the left precentral gyrus, cerebellum, fusiform, and occipital cortices also had significant indirect effects.

Age to Function to Switch Costs Pathway

This effect is shown in **Figure 4B** and **Table 2**. Results from testing this pathway were exclusively masked with the results from the age to structure to function to switch costs pathway to highlight results specific to this pathway only. Brain regions demonstrating a significant indirect effect along this pathway were all in the **positive** direction. Therefore, as age increased, brain activation increased and brain activation and switch costs were positively related. Large regions within the cerebellum and occipital cortex demonstrated this effect. Additional regions included the left precentral gyrus, bilateral supramarginal, bilateral parietal, precuneus, middle cingulate extending to medial superior frontal gyri and the left middle frontal gyri. Significant effects along this pathway are controlled for gray matter volume.

DISCUSSION

A full brain exploration identified whether the effects of age group on cognitive control, as measured with global switch costs, are related to regional gray matter volume and brain activation during the task. Results show that advanced age has a universal negative effect on gray matter volume throughout the brain. Furthermore, brain activity was always greater in the old adults compared to the young. The total effect of age on switch costs was positive: increased age was related to increased switch costs. The analyses parsed this total effect into three indirect effects through the mediating pathways of gray matter volume and brain activity, and a direct effect. The direct effect is the leftover relationship between age and switch costs after accounting for both neural variables. Therefore, the sum of the direct and indirect effects equals the total effect.

The results of this study converge on many findings from the literature relating age, neural measures, and switch costs. The current results reflect the presence of multiple age-related neural effects having relationships with task performance. At the group level, performance on the cognitive control task declined with advancing age. In all regions where the indirect effect via structure and function were significant, **Figure 4A**, they were in the same direction. Age increased, gray matter volume decreased, brain activation increased and switch costs worsened. It is possible that the increased levels of brain activity were in response to the age-related declines in gray matter volume. While a number of previous studies reported lower gray matter volume (Raz and Rodrigue, 2006; Fjell and Walhovd, 2010) and increased brain activation with aging (D'Esposito et al., 2003; Lustig et al., 2009), the current work is novel by demonstrating direct and indirect associations among age, gray matter volume, brain activation and switch costs within a single analysis.

The effect of advancing age on switch costs is positive; however, the negative indirect effect implies that as age increases, switch costs decrease. Within the statistical literature this represents inconsistent mediation or statistical suppression (MacKinnon et al., 2000). Therefore, controlling for brain function increases the negative effect of age-related declines in

TABLE 1 | Age → Gray Matter Volume → Brain Activity → Switch costs.

Region	H	BA	*x*	*y*	*z*	*k*	Y (*t*)	O (*t*)	O > Y (*t*)	%IND
Angular	L	40	−32	−50	38	166	4.22	6.52	0.40	−5.12
Sup. Parietal	L	7	−20	−62	42	–	1.97	6.60	2.03	−5.27
Inf. Parietal	L	7	−28	−60	42	–	4.43	7.25	0.78	−5.34
Angular	R	7	28	−62	46	105	2.16	5.37	1.33	−12.17
Angular	R	40	36	−54	40	–	3.17	4.79	0.72	−5.02
Precuneus	L	7	−4	−66	42	453	1.94	6.76	1.99	−6.02
Precuneus	R	–	4	−58	44	–	0.67	4.34	1.77	−5.64
Precuneus	L	–	−6	−58	44	–	1.34	5.58	2.17	−6.70
Inf. Parietal	L	40	−38	−38	46	22	0.62	3.36	1.32	−5.52
Cerebellum	L	18	−20	−78	−22	51	0.82	3.73	1.64	−6.10
Cerebellum	L	19	−28	−76	−20	–	0.93	4.60	2.25	−6.47
Cerebellum	R	18	18	−78	−18	43	1.21	4.40	1.90	−7.54
Inf. Occipital	L	19	−30	−84	−6	16	2.32	5.44	1.81	−5.52
Inf. Occipital	R	19	32	−88	−4	22	4.13	5.84	0.81	−6.52
Vermis	R	–	4	−64	−12	41	−0.32	2.95	2.07	−9.04
Fusiform	R	37	34	−40	−20	24	2.89	2.33	−0.37	−7.36
Fusiform	L	19	−38	−66	−16	10	1.56	3.84	1.70	−5.26
Fusiform	L	37	−34	−58	−16	–	0.75	5.10	2.91	−5.23
Precentral	L	–	−46	−4	38	47	−1.90	3.20	3.43	−11.07
Cerebellum	R	–	20	−56	−28	17	0.54	3.18	1.44	−6.35
Lingual	L	17	−6	−68	6	11	−0.24	2.26	1.46	−6.14
Cerebellum	L	–	−16	−60	−28	10	0.32	4.74	2.54	−6.64

Y (t), O (t), and O > Y (t) are t-values from within and between group tests for the size of the brain activation effect. %IND is the percentage, with direction, of the total effect of age on performance that this indirect effect accounts for. BA, Brodmann Areas; – refers to local maxima or locations without atlas labels, Inf, inferior; Sup, superior.

gray matter on switch-costs. In other words, if there were no brain function in any of these regions gray matter declines would have a larger effect on switch costs than they actually do. Therefore, the presence of increased brain function lessens, or suppresses, the negative effects of gray matter on task performance.

However, explaining these findings as statistical suppression does not eliminate the fact that gray matter volume and brain function are positively related. Likewise, brain function and switch costs are positively related. The directionality of these effects fits well with current theories of cognitive aging. Declining gray matter volume is thought to decrease neural efficiency and capacity (Stern, 2009; Steffener and Stern, 2012; Barulli and Stern, 2013). Decreased neural efficiency and capacity are reflected in the current data as age-related increases in switch costs due to decreased efficiency. Likewise, neural capacity, the maximal level of brain function, declines as gray matter volume declines.

In regions where the indirect effects via function alone were significant, **Figure 4B**, the results were in the positive direction. Age-related increases in switch costs were related to age-related increases in brain activity within the cerebellum. This suggests that the increased brain activity in the cerebellum is related to switch costs but independent of the effects of gray matter volume. The cerebellum was identified in previous work with this task using multivariate analyses (Gazes et al., 2012). In patients, who underwent surgical damage to the cerebellum for tumor removal, there were increased switch costs, despite normal learning of the task (Berger et al., 2005).

The cingulate and supplementary motor areas (SMA) were also highlighted by the age to function to switch costs pathway. These regions were nodes in a previous multivariate analysis of brain activation using the same task and expression of this pattern was related to switch costs (Gazes et al., 2012). Increased activation in older adults was also related to increased response time in a similar task-switching experiment (Hakun et al., 2014). In a motor switching task, increased activation in the SMA was interpreted as evidence for an alternative strategy being used by the older adults for task completion (Coxon et al., 2010). Within the current results, the young adults had non-significant levels of brain activation in these regions, while activation levels were significant for the old age group, see the Y(t), O(t), and O > Y (t) columns of **Tables 1** and **2**. This supports the idea of Coxon et al. (2010) that the medial PFC regions are used as an alternative strategy. Structural measures in this region have also been linked to cognitive performance. The gray matter volume and white matter concentration within the anterior cingulate are shown to be related to one's ability to control one's own brain activity in a study of biofeedback in young adults (Enriquez-Geppert et al., 2013). The volume of the anterior cingulate is also related to executive task performance in older adults (Elderkin-Thompson et al., 2009). These results suggest that the medial PFC is an

TABLE 2 | Age → Brain Activity → Switch costs.

Region	H	BA	*x*	*y*	*z*	*k*	Y (*t*)	O (*t*)	O > Y (*t*)	%IND
Cerebellum	R	–	12	−40	−32	8585	−0.49	3.18	2.33	11.99
Cerebellum	R	–	4	−36	−32	–	0.45	1.97	1.12	11.41
Cerebellum	L	–	−10	−36	−32	–	0.22	2.22	1.28	12.83
Mid. Occipital	L	19	−26	−86	18	529	−0.94	2.81	2.45	10.07
Mid. Occipital	L	19	−34	−84	20	–	−1.88	2.34	2.87	8.94
Mid. Occipital	L	19	−24	−80	24	–	−1.54	2.06	2.39	10.41
Precentral	L	6	−46	0	28	125	0.84	3.77	1.55	20.81
Precentral	L	6	−42	−4	34	–	−0.22	4.64	2.94	22.87
Precentral	L	6	−52	0	34	–	−0.63	2.41	1.97	26.27
Supramarginal	L	40	−36	−40	36	506	1.17	4.92	1.86	8.89
Inf. Parietal	L	40	−44	−36	36	–	0.67	3.37	1.34	8.38
Sup. Parietal	L	40	−30	−46	38	–	3.08	6.12	0.97	9.23
Supramarginal	R	40	34	−40	38	218	0.11	3.33	1.96	9.09
Supramarginal	R	40	40	−34	38	–	−1.34	2.88	2.81	11.01
Supramarginal	R	2	50	−28	40	–	−2.42	2.28	3.44	10.26
Mid. Cingulum	L	32	−6	22	40	240	3.24	6.09	1.67	23.46
Sup. Med. Frontal	L	8	−2	30	42	–	1.16	4.14	1.96	27.15
Mid. Cingulum	L	32	−6	12	44	–	1.19	4.10	2.06	25.23
Precuneus	R	7	2	−60	48	69	1.57	5.53	1.79	18.26
Precuneus	R	–	10	−56	52	–	0.96	5.26	2.52	12.79
Precuneus	L	7	−2	−56	54		−0.03	5.38	2.65	13.28
Mid. Frontal	L	6	−26	2	48	89	2.23	5.55	2.19	19.63
Mid. Frontal	L	6	−28	10	48	–	3.05	4.35	1.26	21.23
Mid. Frontal	L	–	−20	6	52	–	1.83	4.81	1.98	21.36

Y (t), O (t), and O > Y (t) are t-values from within and between group tests for the size of the brain activation effect. %IND is the percentage, with direction, of the total effect of age on performance that this direct effect accounts for. The total effect was 0.067 allowing calculation of the indirect effect size. BA, Brodmann Areas; – refers to local maxima or locations without atlas labels, Inf, inferior; Med, medial; Mid, middle; Sup, superior.

important brain region for dual task processing and the effects of age on the structure, function and structure-function interactions are complicated and require more in-depth study.

The overall results of this work demonstrate that some of the age-related effects on switch costs can be attributed to individual differences in gray matter volume and brain activation. While this finding is not surprising, the current results demonstrate that not all age effects on neural measures translate into task performance effects. Age group differences in gray matter volume and brain activation were broadly distributed to large areas of the brain as shown in **Figure 3**. The mediation results demonstrate that the age-related neural effects were only related to task performance in a relatively small subset of brain regions. Areas of the parietal cortex, precuneus, cerebellum, occipital and precentral gyrus represent locations where the combined age-related structural and functional effects appear to have some impact on switch costs.

Interestingly, the results of this study also demonstrate a subset of brain regions where the effect of age group on switch costs was mediated by brain activation after controlling for gray matter volume, **Figure 4B** and **Table 2**. This suggests that the age effect on brain activation in these regions is the result of other unmeasured processes. Interestingly, the brain regions highlighted from this pathway include multiple prefrontal cortical (PFC) regions. The pathway including gray matter volume, and controlling for brain activation, was not significant for any brain regions.

The developers of the executive context factor task used in this study posited a cascade of executive processing through the prefrontal cortex (Koechlin et al., 2003). The implicated brain regions included premotor, caudal, and rostral lateral PFC regions. The age group effects in brain activation from the current work demonstrate amplitude differences within these brain regions, **Figure 3**. Except for the precentral gyrus, these regions do not show up in the age to structure to function to switch costs mediation analysis. However, some of these regions do show up in the age to function to switch costs mediation pathway. This demonstrates that age related differences in the brain activation in these regions are related to switch costs independent of the effect of age on gray matter. This suggests the presence of a different mechanism that is causing these effects. It is plausible that these brain regions are compensating for effect of age-related declines in gray matter volume elsewhere in the brain.

To test the hypothesis that brain activation is compensatory for gray matter volume effects elsewhere in the brain, functional connectivity analyses could be integrated into mediational analyses. This interpretation of greater brain activation being compensatory is tenuous due to the declining task performance as a function of the increased brain activation. This supports the interpretation that not all additional brain activation is good for performance (see Steffener and Stern, 2012, for a review). Furthermore, usage of extra brain resources could represent a larger network of functioning nodes that increase network transit time thereby increasing response times. These interpretations reflect the importance of integrating multiple physiological measures of the aging process to understand cognitive aging (Grady, 2012). The use of mediation analysis demonstrates one approach to better understanding the neural mechanisms of cognitive aging.

The old age group had larger variability in their task performance. Their standard deviation was significantly different from the young, as per Levenes' test of equal variance. It is possible that the larger variance in the old group represents cumulative effects of individual differences in lifetime exposures. Individual differences could alter brain measures or performance (Grady, 2012) and they may affect the interrelationships between the variables (Steffener and Stern, 2012). Future directions will begin exploring the roles of individual differences in lifetime exposures to determine if they explain some of the increased variance in the older adults. A first step, will parallel our previous work by including measures of education and verbal abilities (Steffener et al., 2014). Future work will carefully and completely investigate the role of education along with genetics, leisure activities, physical activity, and nutrition.

A major concern for mediation analyses is the assumed directionality of causal inference. It was assumed that task performance is the outcome measure that is indirectly or directly affected by the neural measures. The opposite approach is also possible (Zhu et al., 2014). Although potentially contentious, we assumed that measured brain activation was influenced by measured gray matter volume. It is also plausible that long-term differences in brain activation may result in structural reorganization. Another concern in making causal inferences was outlined in Maxwell et al. (2011) and led to a debate within the statistical literature (Imai et al., 2011; Shrout, 2011; Thoemmes, 2015). The authors demonstrated cross-sectional analysis might give biased estimates of indirect effects. They further discuss that the most appropriate tests of caudal indirect effects are longitudinal models with time delays in the relationships among independent, mediating and dependent variables.

In the current work, the true longitudinal effects are unknown. We currently do not know how fMRI measures of brain activity adapt to age-related changes in gray matter volume. We also do not know whether age-related changes in brain activity would affect gray matter volume. It is also reasonable to assume that task performance is the result of neural operations occurring during the performance on that task. The current work also used tight control of multiple comparisons such that no indirect effect would be found if age was merely independently affecting the neural and performance measures. Thus, an indirect effect, while possibly biased with regard to longitudinal models, is unlikely to have arisen by chance. Interpretation of the current results as causal requires follow-up longitudinal data collection; however, the identified indirect effects between age and switch costs are valid despite the use of cross-sectional data.

These concerns are present in all mediation analyses. However, the use of mediation analyses with neuroimaging data has been increasing in recent years. Some of the first work with voxel-wise mediation comes from Wager et al. (2008) and his multilevel mediation analyses. Other recent work exploring mediating effects within aging research investigated hippocampal volume (Rodrigue et al., 2013) and white matter tract integrity (Gold et al., 2008; Voineskos et al., 2012). This trend of using imaging data to explain age-related differences in cognition and

task performance will have a great impact on the field. It provides greater insight into understanding the heterogeneity of cognitive aging and develop the understanding of the neural mechanism underlying healthy aging and the lifestyle and behavioral choices people make leading them to maintained cognition in late life.

Mediational analyses have benefits over regression and correlational studies, because they allow plausible causal directions and integrate multiple measures into a single model. Due to low computational burden, voxel-wise measures are typically used as independent variables in simple regression models. The current mediational model uses the brain measures as dependent variables predicting cognitive outputs. The high computational burden of this approach is greatly alleviated with modern computing facilities. Mediation analyses also allow straightforward and understandable testing of theories. Current theories of cognitive aging call for the integration of multiple physiological measures into a single model with hypothetical causal relationships. Mediation models explicitly test such models and allow the inclusion of moderating effects.

CONCLUSION

These overall results demonstrate that age-related effects on switch costs can be attributed to individual differences in gray matter volume and brain activation. The regional results demonstrate that some, but not all, of the age-related differences in brain activation are related to gray matter volume differences. Age-related differences in brain activation, independent of gray matter volume, may represent compensation. These additional functional resources may be employed to compensate for age-related gray matter volume declines affecting the function elsewhere in the brain. Future inter-regional explorations of structure–function–performance relationships are required to address these questions.

AUTHOR CONTRIBUTIONS

JS: conceptualized analyses and performed analyses, interpreted results, wrote manuscript. YG: interpreted results, wrote manuscript. CH: conceptualized analyses. YS: interpreted results.

FUNDING

This research was supported by grants from the National Institute on Aging (AG035061, JS, AG044467 and AG026158, AG038465, YS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The sponsors had no role in the study design, data collection, analysis or interpretation, writing of the report, or decision to submit the article for publication.

REFERENCES

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edn. Washington, DC: American Psychiatric Association.

Ashburner, J., and Friston, K. J. (2000). Voxel-based morphometry–the methods. *Neuroimage* 11(6 Pt 1), 805–821. doi: 10.1006/nimg.2000.0582

Ashburner, J., and Friston, K. J. (2005). Unified segmentation. *Neuroimage* 26, 839–851. doi: 10.1016/j.neuroimage.2005.02.018

Baltes, P. B. (1993). The aging mind: potential and limits. *Gerontologist* 33, 580–594. doi: 10.1093/geront/33.5.580

Barulli, D., and Stern, Y. (2013). Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts in cognitive reserve. *Trends Cogn. Sci.* 17, 502–509. doi: 10.1016/j.tics.2013.08.012

Berger, A., Sadeh, M., Tzur, G., Shuper, A., Kornreich, L., Inbar, D., et al. (2005). Task switching after cerebellar damage. *Neuropsychology* 19, 362–370. doi: 10.1037/0894-4105.19.3.362

Braver, T. S., and Barch, D. M. (2002). A theory of cognitive control, aging cognition, and neuromodulation. *Neurosci. Biobehav. Rev.* 26, 809–817. doi: 10.1016/S0149-7634(02)00067-2

Chard, D. T., Parker, G. J. M., Griffin, C. M. B., Thompson, A. J., and Miller, D. H. (2002). The reproducibility and sensitivity of brain tissue volume measurements derived from an SPM-based segmentation methodology. *J. Magn. Reson. Imag.* 15, 259–267. doi: 10.1002/jmri.10064

Coxon, J. P., Goble, D. J., Van Impe, A., De Vos, J., Wenderoth, N., and Swinnen, S. P. (2010). Reduced basal ganglia function when elderly switch between coordinated movement patterns. *Cereb. Cortex* 20, 2368–2379. doi: 10.1093/cercor/bhp306

D'Esposito, M., Deouell, L. Y., and Gazzaley, A. (2003). Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. *Nat. Rev. Neurosci.* 4, 863–872. doi: 10.1038/nrn1246

DiGirolamo, G. J., Kramer, A. F., Barad, V., Cepeda, N. J., Weissman, D. H., Milham, M. P., et al. (2001). General and task-specific frontal lobe recruitment in older adults during executive processes: a fMRI investigation of task-switching. *Neuroreport* 12, 2065–2071. doi: 10.1097/00001756-200107030-00054

Elderkin-Thompson, V., Hellemann, G., Pham, D., and Kumar, A. (2009). Prefrontal brain morphology and executive function in healthy and depressed elderly. *Int. J. Geriatr. Psychiatry* 24, 459–468. doi: 10.1002/gps.2137

Enriquez-Geppert, S., Huster, R. J., Scharfenort, R., Mokom, Z. N., Vosskuhl, J., Figge, C., et al. (2013). The morphology of midcingulate cortex predicts frontal-midline theta neurofeedback success. *Front. Hum. Neurosci.* 7:453. doi: 10.3389/fnhum.2013.00453

Fjell, A. M., and Walhovd, K. B. (2010). Structural brain changes in aging: courses, causes and cognitive consequences. *Rev. Neurosci.* 21, 187–221. doi: 10.1515/REVNEURO.2010.21.3.187

Fotenos, A. F., Snyder, A. Z., Girton, L. E., Morris, J. C., and Buckner, R. L. (2005). Normative estimates of cross-sectional and longitudinal brain volume decline in aging and AD. *Neurology* 64, 1032–1039. doi: 10.1212/01.WNL.0000154530.72969.11

Gazes, Y., Habeck, C., O'Shea, D., Razlighi, Q. R., Steffener, J., and Stern, Y. (2015). Functional network mediates age-related differences in reaction time: a replication and extension study. *Brain Behav.* 5:e00324. doi: 10.1002/brb3.324

Gazes, Y., Rakitin, B. C., Habeck, C., Steffener, J., and Stern, Y. (2012). Age differences of multivariate network expressions during task-switching and their associations with behavior. *Neuropsychologia* 50, 3509–3518. doi: 10.1016/j.neuropsychologia.2012.09.039

Gold, B. T., Powell, D. K., Xuan, L., Jicha, G. A., and Smith, C. D. (2008). Age-related slowing of task switching is associated with decreased integrity of frontoparietal white matter. *Neurobiol. Aging* 31, 512–522. doi: 10.1016/j.neurobiolaging.2008.04.005

Good, C. D., Johnsrude, I., Ashburner, J., Henson, R. N., Friston, K. J., and Frackowiak, R. S. (2001a). Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage* 14, 685–700. doi: 10.1006/nimg.2001.0786

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N., Friston, K. J., and Frackowiak, R. S. (2001b). A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 14(1 Pt 1), 21–36. doi: 10.1006/nimg.2001.0786

Grady, C. (2012). The cognitive neuroscience of ageing. *Nat. Rev. Neurosci.* 13, 491–505. doi: 10.1038/nrn3256

Hakun, J. G., Zhu, Z., Johnson, N. F., and Gold, B. T. (2014). Evidence for reduced efficiency and successful compensation in older adults during task switching. *Cortex* 64C, 352–362. doi: 10.1016/j.cortex.2014.12.006

Imai, K., Jo, B., and Stuart, E. A. (2011). Commentary: using potential outcomes to understand causal mediation analysis. *Multivariate Behav. Res.* 46, 861–873.

Karayanidis, F., Jamadar, S., Ruge, H., Phillips, N., Heathcote, A., and Forstmann, B. U. (2010). Advance preparation in task-switching: converging evidence from behavioral, brain activation, and model-based approaches. *Front. Psychol.* 1:25. doi: 10.3389/fpsyg.2010.00025

Koechlin, E., Ody, C., and Kouneiher, F. (2003). The architecture of cognitive control in the human prefrontal cortex. *Science* 302, 1181–1185. doi: 10.1126/science.1088545

Kray, J., and Lindenberger, U. (2000). Adult age differences in task switching. *Psychol. Aging* 15, 126–147. doi: 10.1037/0882-7974.15.1.126

Lustig, C., Shah, P., Seidler, R., and Reuter-Lorenz, P. A. (2009). Aging, training, and the brain: a review and future directions. *Neuropsychol. Rev.* 19, 504–522. doi: 10.1007/s11065-009-9119-9

MacKinnon, D. P., Krull, J. L., and Lockwood, C. M. (2000). Equivalence of the mediation, confounding and suppression effect. *Prev. Sci.* 1, 173–181. doi: 10.1023/A:1026595011371

Macwhinney, B., Cohen, J., and Provost, J. (1997). The PsyScope experiment-building system. *Spat. Vis.* 11, 99–101. doi: 10.1163/156856897X00113

Madden, D. J., Costello, M. C., Dennis, N. A., Davis, S. W., Shepler, A. M., Spaniol, J., et al. (2010). Adult age differences in functional connectivity during executive control. *Neuroimage* 52, 643–657. doi: 10.1016/j.neuroimage.2010.04.249

Mattis, S. (1988). *Dementia Rating Scale: Professional Manual*, Vol. 1. Odessa, FL: Psychological assessment resources.

Maxwell, S. E., Cole, D. A., and Mitchell, M. A. (2011). Bias in cross-sectional analyses of longitudinal mediation: partial and complete mediation under an autoregressive model. *Multivariate Behav. Res.* 46, 816–841. doi: 10.1080/00273171.2011.606716

Mikl, M., Marecek, R., Hlustík, P., Pavlicová, M., Drastich, A., Chlebus, P., et al. (2008). Effects of spatial smoothing on fMRI group inferences. *Magn. Reson. Imaging* 26, 490–503. doi: 10.1016/j.mri.2007.08.006

Nichols, T. E., and Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25. doi: 10.1002/hbm.1058

Raz, N., and Rodrigue, K. M. (2006). Differential aging of the brain: patterns, cognitive correlates and modifiers. *Neurosci. Biobehav. Rev.* 30, 730–748. doi: 10.1016/j.neubiorev.2006.07.001

Reuter-Lorenz, P. A., and Park, D. C. (2014). How does it STAC up? Revisiting the scaffolding theory of aging and cognition. *Neuropsychol. Rev.* 24, 355–370. doi: 10.1007/s11065-014-9270-9

Rodrigue, K. M., Daugherty, A. M., Haacke, E. M., and Raz, N. (2013). The role of hippocampal iron concentration and hippocampal volume in age-related differences in memory. *Cereb. Cortex* 23, 1533–1541. doi: 10.1093/cercor/bhs139

Rogers, R. D., and Monsell, S. (1995). Costs of a predictible switch between simple cognitive tasks. *J. Exp. Psychol. Gen.* 124, 207–231. doi: 10.1037/0096-3445.124.2.207

Sakai, H., Takahara, M., Honjo, N. F., Doi, S., Sadato, N., and Uchiyama, Y. (2012). Regional frontal gray matter volume associated with executive function capacity as a risk factor for vehicle crashes in normal aging adults. *PLoS ONE* 7:e45920. doi: 10.1371/journal.pone.0045920

Shrout, P. E. (2011). Commentary: mediation analysis, causal process, and cross-sectional data. *Multivariate Behav. Res.* 46, 852–860. doi: 10.1080/00273171.2011.606718

Smith, S. M., and Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 44, 83–98. doi: 10.1016/j.neuroimage.2008.03.061

Steffener, J., Barulli, D., Habeck, C., O'Shea, D., Razlighi, Q., and Stern, Y. (2014). The role of education and verbal abilities in altering the effect of age-related gray matter differences on cognition. *PLoS ONE* 9:e91196. doi: 10.1371/journal.pone.0091196

Steffener, J., and Stern, Y. (2012). Exploring the neural basis of cognitive reserve in aging. *Biochim. Biophys. Acta* 1822, 467–473. doi: 10.1016/j.bbadis.2011.09.012

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia* 47, 2015–2028. doi: 10.1016/j.neuropsychologia.2009.03.004

Thoemmes, F. (2015). Reversing arrows in mediation models does not distinguish plausible models. *Basic Appl. Soc. Psychol.* 37, 226–234. doi: 10.1080/01973533.2015.1049351

Van Petten, C., Plante, E., Davidson, P. S. R., Kuo, T. Y., Bajuscak, L., and Glisky, E. L. (2004). Memory and executive function in older adults: relationships with temporal and prefrontal gray matter volumes and white matter hyperintensities. *Neuropsychologia* 42, 1313–1335. doi: 10.1016/j.neuropsychologia.2004.02.009

Voineskos, A. N., Rajji, T. K., Lobaugh, N. J., Miranda, D., Shenton, M. E., Kennedy, J. L., et al. (2012). Age-related decline in white matter tract integrity and cognitive performance: a DTI tractography and structural equation modeling study. *Neurobiol. Aging* 33, 21–34. doi: 10.1016/j.neurobiolaging.2010.02.009

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., and Ochsner, K. N. (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron* 59, 1037–1050. doi: 10.1016/j.neuron.2008.09.006

Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., and Nichols, T. E. (2014). Permutation inference for the general linear model. *Neuroimage* 92, 381–397. doi: 10.1016/j.neuroimage.2014.01.060

Zhu, Z., Hakun, J. G., Johnson, N. F., and Gold, B. T. (2014). Age-related increases in right frontal activation during task switching are mediated by reaction time and white matter microstructure. *Neuroscience* 278, 51–61. doi: 10.1016/j.neuroscience.2014.07.076

Conflict of Interest Statement: 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 Aging Neuroscience

METHODS
published: 19 July 2016
doi: 10.3389/fnagi.2016.00176

Statistical Approaches for the Study of Cognitive and Brain Aging

Huaihou Chen[1,2], Bingxin Zhao[1], Guanqun Cao[3], Eric C. Proges[2], Andrew O'Shea[2], Adam J. Woods[2] and Ronald A. Cohen[2]*

[1] Department of Biostatistics, University of Florida, Gainesville, FL, USA, [2] Department of Aging and Geriatric Research, Center for Cognitive Aging and Memory, Institute on Aging, McKnight Brain Institute, University of Florida, Gainesville, FL, USA, [3] Department of Mathematics and Statistics, Auburn University, Auburn, AL, USA

Neuroimaging studies of cognitive and brain aging often yield massive datasets that create many analytic and statistical challenges. In this paper, we discuss and address several limitations in the existing work. (1) Linear models are often used to model the age effects on neuroimaging markers, which may be inadequate in capturing the potential nonlinear age effects. (2) Marginal correlations are often used in brain network analysis, which are not efficient in characterizing a complex brain network. (3) Due to the challenge of high-dimensionality, only a small subset of the regional neuroimaging markers is considered in a prediction model, which could miss important regional markers. To overcome those obstacles, we introduce several advanced statistical methods for analyzing data from cognitive and brain aging studies. Specifically, we introduce semiparametric models for modeling age effects, graphical models for brain network analysis, and penalized regression methods for selecting the most important markers in predicting cognitive outcomes. We illustrate these methods using the healthy aging data from the Active Brain Study.

Keywords: semiparametric model, graphical model, penalized regression methods, structural covariance, functional connectivity

OPEN ACCESS

Edited by:
Rodrigo Orlando Kuljiš, University of Miami School of Medicine, USA

Reviewed by:
Vassiliki Nikoletopoulou, Institute of Molecular Biology and Biotechnology, Greece
Alex Zhavoronkov, The Biogerontology Research Foundation, UK

****Correspondence:***
Huaihou Chen
huaihouchen@ufl.edu

Received: *07 March 2016*
Accepted: *04 July 2016*
Published: *19 July 2016*

Citation:
Chen H, Zhao B, Cao G, Proges EC, O'Shea A, Woods AJ and Cohen RA (2016) Statistical Approaches for the Study of Cognitive and Brain Aging. Front. Aging Neurosci. 8:176. doi: 10.3389/fnagi.2016.00176

INTRODUCTION

Multimodal neuroimaging collected in cognitive aging studies provides a noninvasive way to investigate brain changes in structure, function, and metabolism as people age, and thus helps us to understand age-related cognitive changes. However, the high-dimensionality and complex structure of those multimodal neuroimaging data raise statistical challenges. Additionally, the age range is large in aging studies and very often the age effects may not be linear in the large age interval. For instance, participant's age ranges from 50 to 90 in the Active Brain Study, a successful aging cohort. To efficiently analyze those data, there is a strong need for introduction of advanced statistical methods. We will elaborate on the limitations of several existing methods and introduce three advanced statistical methods in sequence.

First, age is a complex variable and often has a nonlinear effect on the outcomes of interest. In developmental studies, flexible semiparametric models have been well used, because it is well-known that growth curves are nonlinear. However, in aging studies, linear or quadratic models are often used to characterize age-related changes. Although a majority of aging research treats aging as a linear process (constant rate of change) and linear models are often considered the gold standard method for evaluating aging effects, this approach may not be the most effective method for representing the complexity of aging data. For instance, Raz et al. (2010) used linear

mixed effects models to characterize the age-related brain structural changes in a longitudinal neuroimaging study with 76 participants whose age ranges from 49 to 85. Similarly, Resnick et al. (2003) also used linear mixed effects models to show the age-related brain structural changes in the longitudinal Baltimore study. However, as noted in Fjell et al. (2010), Gogtay et al. (2004), and Thompson et al. (2011); brain structure may show complex age-related nonlinear changes, and could be misspecified by a linear or quadratic model. We have shown that misspecified linear models can result in biased estimates and low powers in statistical tests (Chen et al., 2012). As a nonparametric method, a spline model is recommended for its flexibility and robustness. To accurately model the age trajectories of the neuroimaging markers, we will introduce a spline-based semiparametric model in the methods section and illustrate these methods using the structural neuroimaging data from the Active Brain Study in the example section. The semiparametric model excels at determining rates of global and regional brain atrophy and identifying vulnerable regions of interest (ROIs) susceptible to aging.

Second, marginal correlations are often used in brain network analyses. For example, structural covariance was studied in Mechelli et al. (2005) and Alexander-Bloch et al. (2013), which may be related to structural and functional connectivity. There is also a large literature on Pearson correlation based functional connectivity analysis, where the correlation between two functional magnetic resonance imaging (fMRI) time series [that is the blood-oxygen-level dependent (BOLD) signal] is computed. However, marginal correlation between two brain ROIs is indirect and weak in the sense that all the components in a system are correlated to some degree. Two regions can be indirectly associated with each other due to their correlation with a third region. Moreover, when the number of ROIs is large, the sample covariance/correlation matrix is unstable, as the number of parameters increases quadratically with the number of ROIs. Alternatively, graphical models are attractive for inferring brain connectivity due to their advantages over conventional marginal correlation based analysis (Lauritzen, 1996; Yuan and Lin, 2007; Koller and Friedman, 2009). Graphical models can generate either partial correlations or a binary undirected graph. Sparse penalty is used to regularize the loglikelihood function and make the solution robust. Partial correlation is a desirable measure, as it quantifies the conditional association between two ROIs given the rest of ROIs. Partial correlation can be interpreted as the adjusted correlation. Preliminary applications to neuroimaging data can be found in Salvador et al. (2005), Valdés-Sosa et al. (2005), and Smith (2012). In the methods section, we will introduce two graphical methods for brain network analysis. We will apply these methods to the cortical thickness data from the Active Brain Study for building cortical networks.

Third, the high dimensional neuroimaging markers may provide informative early signs of age-related cognitive and functional decline. For example, brain atrophy in the basal ganglia, hippocampus, and prefrontal areas often precedes the clinical diagnosis of cognitive impairment (Amieva et al., 2005; Grober et al., 2008; Jedynak et al., 2012). It is of great interest to select the informative neuroimaging markers for predicting cognitive decline. However, the high-dimensionality of the neuroimaging markers posit challenges on how to efficiently pick up the informative subset of the markers. Traditional backward or forward variable selection methods are computationally inefficient given the large number of neuroimaging markers. Also neuroimaging markers are often highly correlated with each other. The unpenalized least square based estimates often suffer from high variability or instability (that is with large variance). Moreover, when the number of neuroimaging markers is larger than the sample size, the design matrix is singular and not invertible, and thus there is no unique estimate. In contrast, penalized regression methods can lead to stable solutions and are computationally efficient by using advanced algorithms (Tibshirani, 1996; Fan and Li, 2001; Zou, 2006; Meinshausen and Bühlmann, 2010). Penalized regression methods can simultaneously select and estimate the effects of the predictors. The variable selection is achieved by the sparsity penalty. In the methods section, we will introduce penalized regression methods for selecting the optimal subset of neuroimaging biomarkers for predicting cognitive outcomes. We will illustrate those methods using structural neuroimaging and cognitive data from the Active Brain Study.

The rest of the paper is structured as follows. In the methods section, we introduce the three sets of methods including the spline-based semiparametric model, graphical models, and penalized regression methods. In the examples section, we apply those methods to the data from the Active brain study. We end our paper with general discussions.

METHODS

Semiparametric Models and Methods

We first introduce some notations. Let n be the number of subjects and let R be the number of ROIs. For the ith participant, denote t_i as the age, denote Y_{ir} as the structural/metabolic imaging markers [for instance, volume, fractional anisotropy (FA), myo-inositol (MI)] at the rth ROI, and denote $\boldsymbol{Z}_i$ as other predictors such as education and sex that we want to study. To accurately and efficiently model the age effects, we introduce the following semiparametric model (1) for neuroimaging markers in cross-sectional studies.

$$Y_{ir} = \mu_r(t_i) + \boldsymbol{Z}_i\boldsymbol{\beta}_r + \epsilon_{ir}, \quad i = 1, \cdots, n, \quad r = 1, \cdots, R, \quad (1)$$

where $\mu_r(t)$ is the unspecified aging trajectory for the older people at the rth ROI evaluated at age t, and $\boldsymbol{\beta}_r$ are the regression coefficients of the other predictors at the ROI. The measurement errors ϵ_{ir} are assumed to be independently and identically distributed and follow a normal distribution $N(0, \sigma_r^2)$ with mean zero and variance σ_r^2. Model (1) consists of both the nonparametric part $\mu_r(t)$ and the parametric part $\boldsymbol{Z}\boldsymbol{\beta}_r$, and thus it is called semiparametric model. The semiparametric model is a parsimonious way to both capture the potential nonlinear age trajectory and investigate the effects of other predictors. Notably, the traditional linear model is a special case of model (1), where the function $\mu_r(t)$ is specified as a linear function $\beta_{0r} + \beta_{1r}t$. Extension of model (1) to longitudinal data

case is straightforward, which can be accomplished by either introducing subject-specific random effects or using generalized least square methods (Wood, 2006; Wu and Zhang, 2006).

For estimation, we use spline basis functions to approximate the unspecified function $\mu_r(t)$. Particularly, we assume $\mu_r(t) = B(t)\boldsymbol{\theta}_r$, where $B(t)$ is a set of B-spline basis functions and $\boldsymbol{\theta}_r$ is the associated spline coefficients (de Boor, 1978). The B-spline basis functions are piecewise polynomial functions in the age interval. A smoothing penalty $\lambda \int [\mu_r''(t)]^2 dt$ is used to achieve smoothness of the fitted function $\hat{\mu}_r(t)$, where $\mu_r''(t)$ is the second derivative function of $\mu_r(t)$, and $\lambda \geq 0$ is a smoothing parameter controlling the degree of smoothness. The tuning parameter λ is crucial for the estimation and inference and is often chosen by data driven methods. By minimizing the penalized log-likelihood function, we can obtain the estimate for these parameters including $\boldsymbol{\theta}_r$ and $\boldsymbol{\beta}_r$. Compared to traditional linear model and methods, spline method offers flexible estimation of these functions. Based on the semiparametric model (1), we will be able to more accurately delineate the aging trajectories and their derivative functions and get unbiased estimates for the parametric part.

The spline-based semiparametric model and methods have been implemented in several R packages (R Core Team, 2012) including the *mgcv* package (Wood, 2006). The *gam* function in *mgcv* can output the estimates and inferential results for both the parametric and nonparametric parts. Specifically, for the parametric part, estimates of the regression coefficients and *p*-values are provided which is similar to a linear regression model. For the nonparametric part, the procedure provides the estimate and pointwise confidence intervals for the estimated function and a *p*-value for testing the function as a constant. The 95% point-wise confidence interval $[\mu_r^L(t), \mu_r^U(t)]$ for $\mu_r(t)$ provides the variability at the age t in the rth ROI, in addition to the magnitude. The first derivative function of $\mu_r(t)$ indicate the rate of brain atrophy, where in the linear case is the slope of the line. The first derivative functions are can be easily obtained using $B'(t)\theta$, where $B'(t)$ are the first derivative functions of the B-spline basis functions. Based on the first derivative functions of the aging trajectories, ROIs/markers show early atrophy/abnormality could be candidate biomarkers for early diagnosis of diseases. We adjust for multiple comparison by controlling the *false discovery rate* (FDR) (Benjamini and Hochberg, 1995; Benjamini and Heller, 2007).

Remark 1 Misspecified linear models could introduce bias for the estimates of $\mu_r(t)$ and $\boldsymbol{\beta}_r$, that is for both the nonparametric and parametric parts.

Remark 2 To achieve good approximations of these unspecified functions, enough number of basis functions should be used for the penalized splines. If the procedure leads to an oversmooth case, one can fit a regression cubic spline with fixed number of knots without penalty, thus the degree of freedom is fixed.

Remark 3 Computing time is not a concern for ROI-level data. Some statistical packages such as the *vows* have implemented massive parallel algorithm for voxel-level data (Reiss et al., 2014).

Graphical Model and Methods

We first define a graph $G = (V, E)$, where V is a set of vertices/nodes, and E is a set of edges connecting pairs of nodes in V. An adjacency matrix of a graph is a binary matrix indicating the connection between the nodes. We introduce graphical models for brain structural and functional network analysis. In the past decade, *Gaussian graphical model* (GGM) has been a hot topic in statistics as a tool for complex system analysis. The GGM has many advantages over the traditional marginal correlation based analysis including resulting in partial correlations, i.e., direct dependency/independence, and sparse networks. Let $\boldsymbol{Y}$ be an R-dimensional random variable following a multivariate Gaussian distribution $N(\boldsymbol{\mu}, \boldsymbol{G}^{-1})$ with mean $\boldsymbol{\mu}$ and covariance $\boldsymbol{G}^{-1}$. $\boldsymbol{G}$ is a precision matrix (inverse covariance), and the i, jth component of $\boldsymbol{G}$, $g_{rs} = 0$ indicates *conditional independence* between ROIs r and s given all the other ROIs $\{1, \cdots, R\}/\{r, s\}$. The partial correlation between ROIs r and s is defined as $\rho_{rs} = -g_{rs}/\sqrt{g_{rr}g_{ss}}$ (Lauritzen, 1996). We obtain a sparse graph by minimizing the following penalized loglikelihood function (Yuan and Lin, 2007):

$$\arg\min_{G \in \mathbb{G}} -\log|\boldsymbol{G}| + \frac{1}{n}\sum_{i=1}^{n}(\boldsymbol{Y}_i - \boldsymbol{\mu})^T \boldsymbol{G}(\boldsymbol{Y}_i - \boldsymbol{\mu}) + \lambda \sum_{r \neq s} |g_{rs}|, \quad (2)$$

where argmin stands for argument of the minimum, $\mathbb{G}$ is the set of $R \times R$ positive definite matrices, and $\lambda \geq 0$ is the tuning parameter chosen by a data-driven method. The lasso penalty (Tibshirani, 1996) is used to regularize the loglikelihood function and achieve a sparse solution. This method is often called graphical lasso (glasso) in the statistical literature. Along the same line, Meinshausen and Bühlmann (2006) proposed the node-wise regression based approach for obtaining a binary graph. Both the glasso and the node-wise regression methods have been implemented in the R package *huge* with computational efficient algorithms (Zhao et al., 2012). The *huge* function in the *huge* package can provide estimate for the precision matrix or adjacency matrix of an undirected graph. The stability selection method (Meinshausen and Bühlmann, 2010) is preferred for the selection of the tuning parameter, which controls the sparsity of the estimated precision/adjacency matrix. A large tuning parameter will penalize the loglikelihood function heavily and shrink the small elements in the precision matrix/regression coefficients to zero, while a smaller tuning parameter will barely penalize the loglikelihood function and thus leads to many tiny elements in the precision matrix/regression coefficients.

Once the graph is obtained, graph summary statistics such as centrality measures and clustering coefficient can be computed. For visualizing and summarizing graphical objects, the R package *igraph* provides a set of sophisticated tools (Csardi and Nepusz, 2006).

Penalized Regression Methods

To utilize high-dimensional markers for predicting cognitive outcomes, we introduce penalized regression methods for linear models. Penalized regression methods can reduce the dimensionality of the predictors by automatically selecting the optimal subset. The variable selection is achieved by a sparsity penalty such as lasso (Tibshirani, 1996), adaptive lasso (Zou, 2006), elastic net (Zou, 2006), SCAD (Fan and Li, 2001), or by stability method (Meinshausen and Bühlmann, 2010). For the

ith participant, let Y_i be the cognitive outcome, and let $\boldsymbol{X}_i$ be the stacked $p \times 1$ vector of neuroimaging markers and other covariates. We consider the following linear model and penalized method.

$$Y_i = \boldsymbol{X}_i\boldsymbol{\beta} + \epsilon_i, \quad i = 1, \cdots, n, \tag{3}$$

$$\boldsymbol{\beta} = \arg\min_{\boldsymbol{\beta}\in\mathbb{R}^p} \sum_{i=1}^{n} (Y_i - \boldsymbol{X}_i\boldsymbol{\beta})^2 + \lambda\phi(|\boldsymbol{\beta}|), \tag{4}$$

where $\boldsymbol{\beta}$ are the coefficients for neuroimaging markers and covariates, and $\phi(.)$ is a penalty function of the regression coefficients $\boldsymbol{\beta}$. By minimizing the penalized least squares (4), we can obtain the penalized estimator $\hat{\boldsymbol{\beta}}$. The sparsity penalty shrinkages those small regression coefficients to zeros, thus the procedure automatically leads to a subset of the predictors. If $\hat{\beta}_j = 0$, then the jth predictor X_j is not selected. The sparsity of the estimate is controlled by the tuning parameter $\lambda \geq 0$, which is usually chosen by data driven methods such as cross-validation or generalized cross-validation.

Thanks to the implementation of efficient algorithms, current software package can handle thousands of predictors simultaneously for a medium sample size such as $n = 80$. In general the computational time is moderate and depends on the size of the data that is the sample size n and the number of predictors p. One of the popular R package *glmnet* has implemented a few penalized methods including lasso and elastic net. The *glmnet* function in the *glmnet* package provides all the coefficient solution paths as functions of the tuning parameter λ. To get the optimal solution, the user needs to use the cross-validation method to select the optimal tuning parameter with the smallest mean squared error (MSE).

Remark 4 The penalized regression methods are applicable to generalized outcomes including binary and count data as well. For example, we can use penalized logistic regression methods to select informative neuroimaging markers in predicting risk of mild cognitive impairment (MCI).

Remark 5 Because the penalty shrinkages those regression coefficients toward to zero according to their magnitude, large differences in the original scale of those predictors can mess up the selection. Therefore, it is recommended to standardize the predictors and make all the variables in the same scale.

EXAMPLES: THE ACTIVE BRAIN STUDY

We illustrate the introduced methods using the data from the Active Brain Study. The aim of the study is to investigate the brain changes associated with age-related cognitive decline via multimodal neuroimaging. We consider $n = 114$ participants with structural imaging. Among them 68% are female. The mean age of the sample is 72.3 with the standard deviation (SD) 10. Those participants are well educated as can be seen from the mean education years = 16.2 ($SD = 2.6$). They also show high cognitive performance with mean Montreal Cognitive Assessment (MoCA) score 25.7 ($SD = 2.6$). The structural imaging was processed using standard procedures implemented in Freesurfer version 5.3 (Dale et al., 1999; Fischl et al., 2002). For a more detailed description of the Freesurfer processing methods used by our group see Szymkowicz et al. (2016). Brain volumetric indices including regional and global volumes of cortical and subcortical structures as well as cortical thickness were generated. Particularly, we used the anatomical cortical parcellation in Desikan et al. (2006), which generated 34 ROIs in each hemisphere. Similarly, the subcortical segmentation of a brain volume is based on the existence of an atlas containing probabilistic information on the location of structures (Fischl et al., 2002).

Aging-Related Trajectories of Brain Regional Volumes and Areas

We are interested in delineating the aging trajectories for the regional volumes and areas, while adjusting for sex, education, and the total intracranial volume (ICV). For normalization purpose, the regional volumes are divided by the ICV. To check the nonlinearity of the age trajectories of the regional volumes and areas, we first applied the loess (locally weighted scatterplot smoothing) method using the R function *loess*, which is a popular exploratory tool for checking nonlinear pattern. As a lot of ROIs show nonlinear age trajectories of brain regional volumes, we fit a semiparametric model for the normalized volume at each ROI with nonparametric age trajectory and parametric effects for sex and education using the *gam* function in the *mgcv* package. Similarly, we fit a semiparametric model for the area at each ROI with nonparametric age trajectory and parametric effects for sex, education and ICV. Penalized cubic B-splines with 10 basis functions are used to fit the age trajectories. The restricted maximum likelihood (REML, Reiss and Todd Ogden, 2009) method is used to select the tuning parameters. For comparison, we also fit linear and quadratic models for the age trajectories. The quadratic age term is centered to achieve robustness. Alternatively, orthogonal polynomial model can be used to avoid multicollinearity problem.

We choose the normalized volume of the lateral ventricle and putamen for illustration. **Figure 1** shows the estimated age trajectories (the solid lines) using different methods, and the 95% pointwise confidence intervals (the shaded area) for the B-spline fits. The lateral ventricle displays considerable expansion especially after age 70, while the putamen shows a large amount of decline especially before age 75. Both the lateral ventricle expansion and the putamen volume shrinkage indicate brain atrophy as people age. We notice that both the loess and the semiparametric fits indicate nonlinear age patterns. As displayed in **Figure 1**, linear models are not flexible enough to capture the nonlinear age trajectories. Linear models assume the rate of age-related change is constant as people age, which may not be true for all the ROIs. The deviation of the linear fits from the semiparmetric model fitted curves are large in the two ends and the middle part of the interval, that is less than 60, greater than 80, and around 70. The quadratic age trajectories show agreement with the B-spline fits around the middle of the age interval [60, 80], but not in the two ends

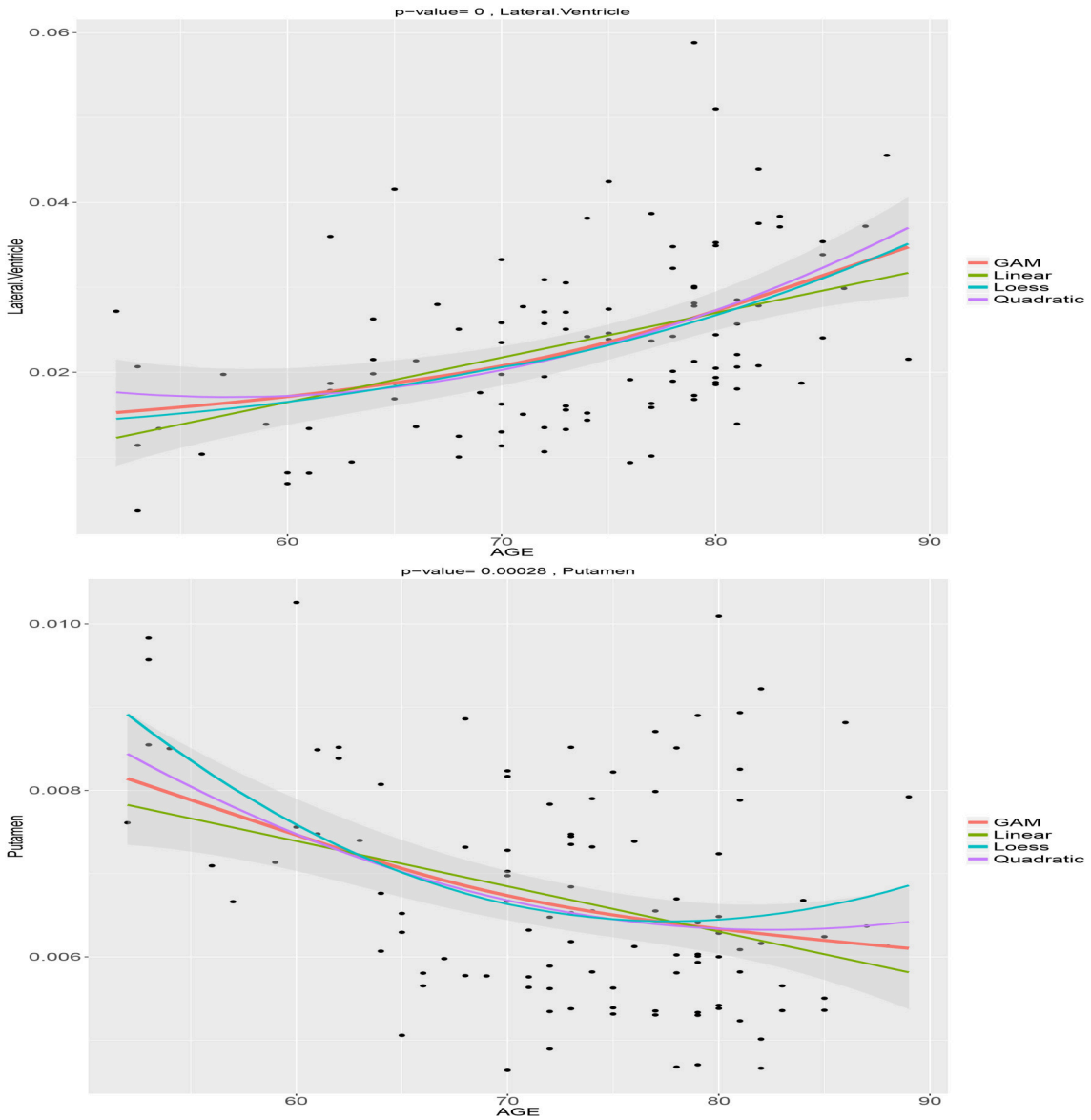


FIGURE 1 | Age trajectories of the normalized lateral ventricle and putamen volumes using the semiparametric model, loess fit, linear, and quadratic regression models.

either. The loess fits are exploratory without adjusting for sex and education. Interestingly the B-spline fits agree with the loess fit for the lateral ventricle volume but not the putamen volume.

Overall, age has significant effects on almost all of the cortical and subcortical regional volumes in both hemispheres after FDR correction. Particularly, the cortical frontal, temporal, parietal, occipital, cingulate lobes are significantly impacted by aging except the left caudal anterior cingulate, bilateral entorhinal, pericalcarine, and frontal pole. The insula shows a marginally significant age effect. The ventricle, subcortical regions, and corpus callosum are significant except for the bilateral caudate. Our findings are consistent with the literature that as people age, the brain regional volumes shrink, while the ventricle system and CSF considerably expand. We also observe that older females tend to have less brain atrophy compared to older males after FDR correction. Education does not have a significant effect on any of those regional volumes after FDR correction. Additionally, age shows similar effects on the cortical regional areas. However, after adjusting for the ICV, neither sex nor education has an effect on the cortical regional areas.

In summary, the linear/quadratic model due to its parametric nature, may not be flexible enough to capture age-related brain changes as people age. A nonparametric/semiparametric model should be used if there is a convincingly nonlinear pattern as suggested by an exploratory loess fit.

Cortical Thickness Based Cortical Network

Structural covariance has been used in the literature for studying cortical networks and patterns of neurodegeneration (Mechelli et al., 2005; Alexander-Bloch et al., 2013). Here, we are interested in applying graphical models to investigate the cortical network using the cortical thickness data from the Active Brain Study. We consider the cortical thickness at 34 ROIs in each of the hemispheres. For summary purpose, we group the 68 cortical ROIs into six lobes including the frontal, temporal, parietal, occipital, cingulate, and insula. We first compute the marginal Pearson correlation for the structural covariance/correlation. We then use the *huge* function to obtain the partial correlation (based on the precision matrix) and a binary undirected graph (or equivalently the adjacency matrix) using the glasso and node-wise regression respectively. The tuning parameters are selected by the stability method (Liu et al., 2010; Meinshausen and Bühlmann, 2010).

The results are summarized in **Figure 2**. The top two patterns in **Figure 2** display the thresholded marginal/partial correlation map for the 68 cortical ROIs (34 ROIs per hemisphere). The bottom two patterns display the undirected graph and the frontal subgraph plotted using function in the *igraph* package. The marginal correlation map is cut by 0.3. The marginal correlation and partial correlation show very different patterns. The range of the marginal correlation is much larger compared to the partial correlation. The two graphical methods share some similarity. The left and right correlation are strong even conditional on all the other ROIs. There are both inter- and intra-hemisphere correlation. Based on the bottom adjacency matrix plot, we observe that the frontal ROIs tend to be conditionally correlated (see also the bottom right panel in **Figure 2**). Other graph summary statistics can be easily calculated using functions in the *igraph* package such as degree of centrality.

In summary, the marginal correlation and partial correlation map often show different patterns. The interpretation of the two are also different. The marginal correlation between two ROIs does not account for the involvement of other ROIs, while the partial correlation between two ROIs adjusts for other ROIs. Due to the lasso penalty, the partial correlation map and the adjacency matrix are sparse that is some of the partial correlations/elements of the adjacency matrix are estimated to be zeros.

Predicting MoCA Using Brain Regional Volumes

In this section, we aim to select informative brain regional volumes in predicting the cognitive outcome MoCA. We first normalize regional volumes via dividing by the estimated intracranial volume (ICV), then standardize the variables by subtracting the sample mean and divided by sample standard deviation to make the variables comparable. The predictors we consider include the cortical and subcortical regional volumes, age, sex, and education. We used the *glmnet* function in the R package *glmnet* with both lasso and elastic net penalties. We choose the tuning parameters by 10-fold cross-validation.

Table 1 summarizes the selected variables and their coefficients using both penalties. The selected variables include regional volumes from the frontal and temporal lobes, subcortical regions, and demographic variables. Compared to the elastic net penalty, the lasso penalty tends to choose a small subset of correlated predictors. For example, the left pars opercularis (Brodmann area 44) was selected but not the right one. Consistent with the findings in the literature, we found that volumes of subcortical and cortical ROIs including the left accumbens, middle temporal, pars opercularis, temporal pole, right entorhinal, medial-orbito-frontal, pars opercularis are positively associated with MoCA.

The *glmnet* function can output the whole solution path. **Figure 3** displays the whole solution path for all the coefficients as functions of the logarithm tuning parameter λ for the lasso penalty. The vertical line corresponds to the optimal λ selected by cross-validation. When the tuning parameter λ is small (that is with less penalization), the magnitudes of the coefficients are large and the variability is large. The traditional least square estimate is similar to the small penalization case, which is not stable. As the tuning parameter increases, the variability of the coefficients declines. The regularization achieves the small variance at the cost of introducing bias. The cross-validation criterion selects the tuning parameter by balancing the variance and bias.

To check the performance of the selected subset of the regional volumes, we refit a model with the volume of the selected ROIs and compare to the model with only age, sex, and education. The selected regional volumes from the elastic net penalty explains additional 19% variance in MoCA, where R^2 increases from 16 to 35%. We conduct an ANOVA test to compare the two models, where the p-value is less than 0.001.

DISCUSSIONS

Misspecified linear models are not uncommon in the literature, which may lead to biased results and misleading conclusions. Based on our previous neuroimaging analysis (Chen et al., 2014, 2015), linear models may not always be appropriate for characterizing the age-related brain changes, although it is the default method due to its simplicity. In practice, cautions need to be raised for the potential nonlinear age-related changes. To minimize the potential bias, we introduced the spline-based semiparametric models, which are more flexible and able to capture the underlying age trends in the data. Notably a linear model is a special case of the semiparametric model. When the underlying trend is linear, semiparametric model agrees with the linear model. Semiparametric methods have been implemented in many statistical softwares such as R. One of the popular implementations is the *gam* function in the *mgcv* package. The *gam* function provides the estimated curves and inferential results for both the parametric and the nonparametric parts. Extension of the basic semiparametric model (1) has been extensively studied in the past few decades in the statistical literature. More sophisticated models such as varying coefficient models and additive models have also been developed (Wood, 2006; Wu and Zhang, 2006). All these semiparametric methods are scalable and applicable to voxel level data as well. The

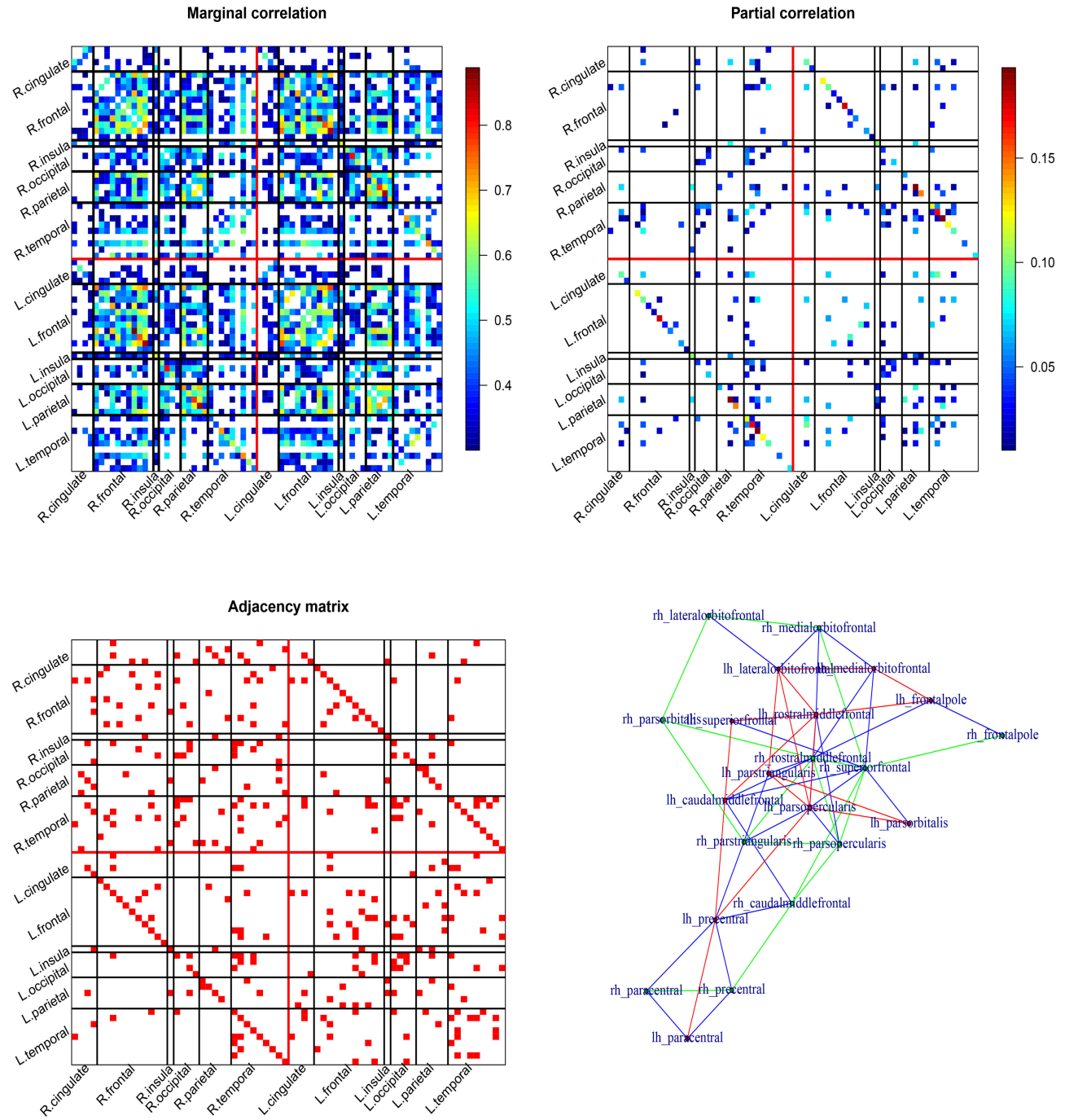


FIGURE 2 | Cortical thickness based cortical network. The top two patterns are Pearson's correlation map (left) and partial correlation map (from the Glasso; right) for the cortical network. The bottom two patterns are adjacency matrix of the undirected graph (from the node-wise regression; left) and graphical map of the frontal lobe (right).

R package *vows* has implemented semiparametric models for voxel-level data. A parallel algorithm is implemented to speed up the computational procedure.

In the neuroimaging literature, network analysis provides a systematic way to study the brain structural and functional changes. The use of network analysis is a remarkable progress from the pairwise relationship between ROIs. However, researchers often compute the marginal correlation then threshold the correlation matrix to obtain the graph/network. It is well known that sample covariance/correlation matrix is highly instable when the number of ROIs is large. The pairwise nature of the marginal correlations hurts and limits the interpretation of

TABLE 1 | Selected brain regional volumes and covariates in predicting MoCA.

	lh.middle.temporal (temporal lobe)	lh.pars.opercularis (frontal lobe)	lh.pars.orbitalis (frontal lobe)	lh.temporal.pole (temporal lobe)	rh.entorhinal (temporal lobe)	rh.medial.orbito.frontal (frontal lobe)
Elastic-net	0.047	0.345	−0.320	0.145	0.188	0.048
LASSO	0.004	0.433	−0.343	0.154	0.193	0.005
	rh.pars.opercularis (frontal lobe)	**lh.Accumbens**	**Age**	**Sex (F vs. M)**	**Education**	
Elastic-net	0.031	0.309	−0.231	0.331	0.177	
LASSO	-	0.358	−0.225	0.329	0.177	

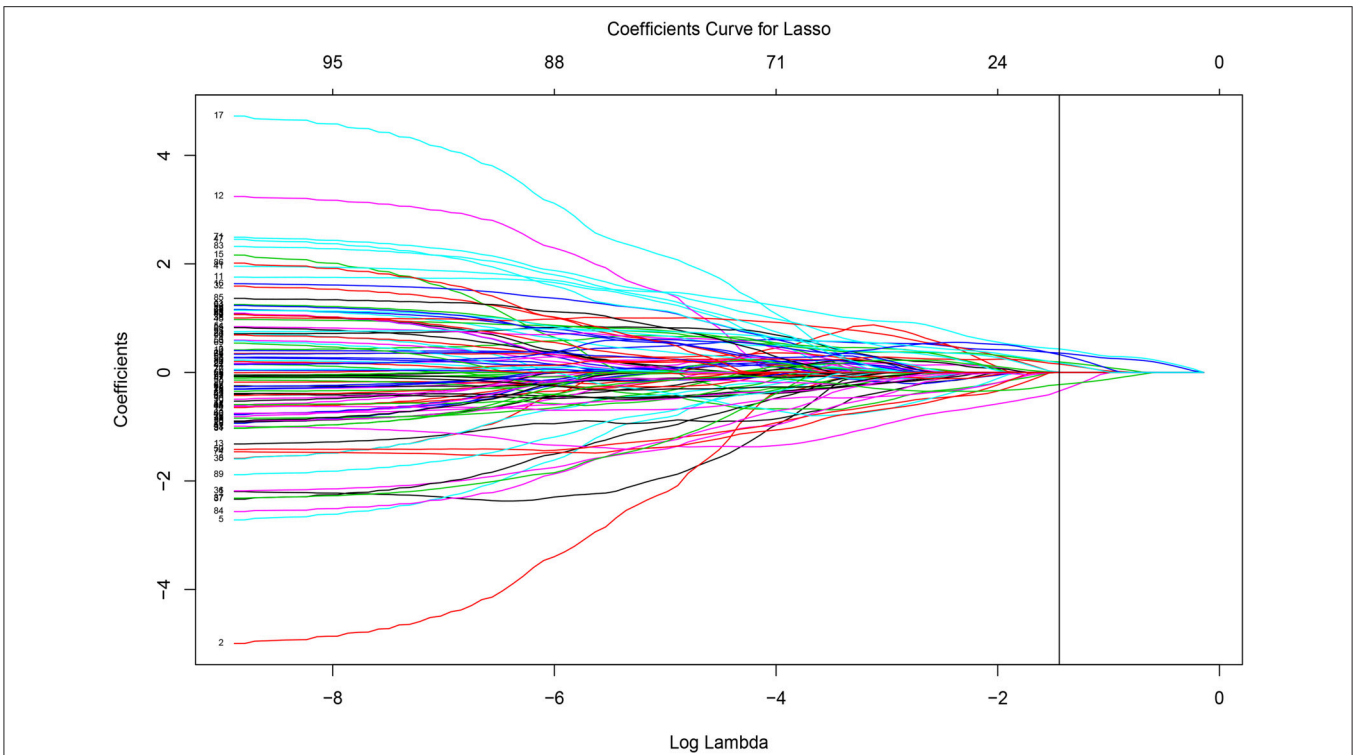


FIGURE 3 | Solution paths from the penalized regression with lasso penalty.

the subsequent network based results. To address the limitation of the marginal correlation, we introduced two Gaussian graphical models, which can generate either partial correlations or an undirected graph. Under the multivariate Gaussian assumption, a zero partial correlation for two ROIs given all the other ROIs is equivalent to conditional independence between the two ROIs. Similarly, for an undirected Gaussian graph, the edges indicate conditional dependence between ROIs. We illustrated the graphical models using the cortical thickness data, where the generated cortical networks may be related to the cortical structural connectivity. The application of graphical model to fMRI for investigating functional connectivity is straightforward, but need to be modified to account for the temporal correlation within each time series. Besides partial correlation in the time domain, there is a few works on correlation measure in the frequency domain such as the total independence (Wen et al., 2012) and partial correlation for multivariate time series (Fried and Didelez, 2005).

For testing the brain network differences between groups such as young vs. older, there are three levels of tests including the edge-level, node-level, and subgraph-level (Nichols and Holmes, 2002; Kim et al., 2014, 2015; Narayan and Allen, 2016). The edge-level testing approach first tests the group differences at the edges one by one, then applies multiple correction for the *p*-values such as FDR correction. The node-level testing method investigates the group differences in graph summary statistics at each node such as degree of centrality. The subgraph-level testing aims to detect either topologically connected cluster difference (Zalesky et al., 2010) or differences in graph overall

metrics such as clustering coefficient. The three levels of testing approaches provide complementary ways of testing the brain network differences.

Efficiently and accurately predicting cognitive decline is a central topic in cognitive and brain aging studies. In practice, very often an *a priori* subset of neuroimaging biomarkers are used to predict cognitive outcomes, which are based on the predetermined hypothesis. Hypothesis-driven methods are a recommended way to conduct research that can generate reproducible results. However, by chance, we may miss important neuroimaging markers that could indeed be predictive for cognitive decline. We introduced penalized regression methods for incorporating a large amount of neuroimaging biomarkers in predicting cognitive outcomes, where the number of predictors can be close to or even larger than the number of subjects. These data-driven methods can simultaneously estimate the regression coefficients and select a subset of the high-dimensional predictors. We illustrated those methods using the brain regional volumes in predicting MoCA outcomes. Moreover, these methods are applicable to categorical cognitive impairment outcomes such as a variable with three nominal levels: normal, mild cognitive impairment, and dementia. In addition to the penalized regression methods, some machine learning type of methods such as penalized support vector machine (SVM) can be used for building prediction/classification rule based on high dimensional neuroimaging biomarkers (Zhu et al., 2004; Zhang et al., 2006; Wu and Liu, 2007; Robinson et al., 2015). For long term followup longitudinal studies, penalized mixed effects model can be used to improve the prediction accuracy by incorporating both the individual trajectories and baseline or longitudinal neuroimaging biomarkers (Bondell et al., 2010; Ibrahim et al., 2011).

Neuroimaging data collected in studies of cognitive and brain aging raise statistical and analytic challenges due to the high dimensionality and complex structure. Fortunately, advanced statistical methods developed in the past few decades for high dimensional data and complex structured data could be applied for leveraging the multimodal neuroimaging analysis. These approaches provide a good starting point for analyzing such data. However, there is a strong need for developing new statistical methods that are specific to the multimodal neuroimaging analyses in cognitive and brain aging studies.

AUTHOR CONTRIBUTIONS

The first two authors HC and BZ conducted the analysis and initiated the paper, while the other five coauthors GC, EP, AO, AW, and RC contributed significant components for the presentations of the models and methods and the general discussions. The last two authors AW and RC are the PIs of the Active Brain Study.

ACKNOWLEDGMENTS

This work was supported by the McKnight Brain Research Foundation, the Center for Cognitive Aging and Memory at the University of Florida, and the Claude D. Pepper Center at the University of Florida (P30 AG028740). GC research was supported in part by the Simons Foundation under grant #354917.

REFERENCES

Alexander-Bloch, A., Giedd, J. N., and Bullmore, E. (2013). Imaging structural co-variance between human brain regions. *Nat. Rev. Neurosci.* 14, 322–336. doi: 10.1038/nrn3465

Amieva, H., Jacqmin-Gadda, H., Orgogozo, J.-M., Le Carret, N., Helmer, C., Letenneur, L., et al. (2005). The 9 year cognitive decline before dementia of the Alzheimer type: a prospective population-based study. *Brain* 128, 1093–1101. doi: 10.1093/brain/awh451

Benjamini, Y., and Heller, R. (2007). False discovery rates for spatial signals. *J. Am. Stat. Assoc.* 102, 1272–1281. doi: 10.1198/016214507000000941

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57, 289–300.

Bondell, H. D., Krishna, A., and Ghosh, S. K. (2010). Joint variable selection for fixed and random effects in linear mixed-effects models. *Biometrics* 66, 1069–1077. doi: 10.1111/j.1541-0420.2010.01391.x

Chen, H., Kelly, C., Castellanos, F. X., He, Y., Zuo, X.-N., and Reiss, P. T. (2015). Quantile rank maps: A new tool for understanding individual brain development. *NeuroImage* 111, 454–463. doi: 10.1016/j.neuroimage.2014.12.082

Chen, H., Paik, M. C., Dhamoon, M. S., Moon, Y. P., Willey, J., Sacco, R. L., et al. (2012). Semiparametric model for the dichotomized functional outcome after stroke: the northern Manhattan study. *Comput. Stat. Data Anal.* 56, 2598–2608. doi: 10.1016/j.csda.2012.02.001

Chen, H., Reiss, P. T., and Tarpey, T. (2014). Optimally weighted l2 distance for functional data. *Biometrics* 70, 516–525. doi: 10.1111/biom.12161

Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research. *InterJ. Comp. Syst.* 1695, 1–9.

Dale, A. M., Fischl, B., and Sereno, M. I. (1999). Cortical surface-based analysis: I. segmentation and surface reconstruction. *NeuroImage* 9, 179–194. doi: 10.1006/nimg.1998.0395

de Boor, C. (1978). *A Practical Guide to Splines*. New York, NY: Springer.

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31, 968–980. doi: 10.1016/j.neuroimage.2006.01.021

Fan, J., and Li, R. (2001). Variable selection via nonconcave penalized likelihood and its oracle properties. *J. Am. Stat. Assoc.* 96, 1348–1360. doi: 10.1198/016214501753382273

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. doi: 10.1016/S0896-6273(02)00569-X

Fjell, A. M., Walhovd, K. B., Westlye, L. T., Østby, Y., Tamnes, C. K., Jernigan, T. L., et al. (2010). When does brain aging accelerate? dangers of quadratic fits in cross-sectional studies. *NeuroImage* 50, 1376–1383. doi: 10.1016/j.neuroimage.2010.01.061

Fried, R., and Didelez, V. (2005). Latent variable analysis and partial correlation graphs for multivariate time series. *Stat. Probabil. Lett.* 73, 287–296. doi: 10.1016/j.spl.2005.04.002

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., et al. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8174–8179. doi: 10.1073/pnas.0402680101

Grober, E., Hall, C. B., Lipton, R. B., Zonderman, A. B., Resnick, S. M., and Kawas, C. (2008). Memory impairment, executive dysfunction, and intellectual decline

in preclinical Alzheimer's disease. *J. Int. Neuropsychol. Soc.* 14, 266–278. doi: 10.1017/S1355617708080302

Ibrahim, J. G., Zhu, H., Garcia, R. I., and Guo, R. (2011). Fixed and random effects selection in mixed effects models. *Biometrics* 67, 495–503. doi: 10.1111/j.1541-0420.2010.01463.x

Jedynak, B. M., Lang, A., Liu, B., Katz, E., Zhang, Y., Wyman, B. T., et al. (2012). A computational neurodegenerative disease progression score: method and results with the alzheimer's disease neuroimaging initiative cohort. *NeuroImage* 63, 1478–1486. doi: 10.1016/j.neuroimage.2012.07.059

Kim, J., Wozniak, J. R., Mueller, B. A., and Pan, W. (2015). Testing group differences in brain functional connectivity: using correlations or partial correlations? *Brain Connect.* 5, 214–231. doi: 10.1089/brain.2014.0319

Kim, J., Wozniak, J. R., Mueller, B. A., Shen, X., and Pan, W. (2014). Comparison of statistical tests for group differences in brain functional networks. *NeuroImage* 101, 681–694. doi: 10.1016/j.neuroimage.2014.07.031

Koller, D., and Friedman, N. (2009). *Probabilistic Graphical Models: Principles and Techniques*. Cambridge, MA: MIT press.

Lauritzen, S. L. (1996). *Graphical Models*. Oxford, UK: Oxford University Press.

Liu, H., Roeder, K., and Wasserman, L. (2010). "Stability approach to regularization selection (stars) for high dimensional graphical models," in *Advances in Neural Information Processing Systems,* Vol. 23, eds J. D. Lafferty, C. K. I. Williams, J. Shawe-Taylor, R. S. Zemel, and A. Culotta (New York, NY: Curran Associates, Inc.), 1432–1440.

Mechelli, A., Friston, K. J., Frackowiak, R. S., and Price, C. J. (2005). Structural covariance in the human cortex. *J. Neurosci.* 25, 8303–8310. doi: 10.1523/JNEUROSCI.0357-05.2005

Meinshausen, N., and Bühlmann, P. (2006). High-dimensional graphs and variable selection with the lasso. *Ann. Stat.* 34, 1436–1462. doi: 10.1214/009053606000000281

Meinshausen, N., and Bühlmann, P. (2010). Stability selection. *J. R. Stat. Soc. Ser. B* 72, 417–473. doi: 10.1111/j.1467-9868.2010.00740.x

Narayan, M. and Allen, G. I. (2016). Mixed effects models for resampled network statistics improves statistical power to find differences in multi-subject functional connectivity. *Front. neurosci.* 10:108. doi: 10.3389/fnins.2016.00108

Nichols, T. E., and Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25. doi: 10.1002/hbm.1058

R Core Team (2012). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. ISBN 3-900051-07-0.

Raz, N., Ghisletta, P., Rodrigue, K. M., Kennedy, K. M., and Lindenberger, U. (2010). Trajectories of brain aging in middle-aged and older adults: regional and individual differences. *NeuroImage* 51, 501–511. doi: 10.1016/j.neuroimage.2010.03.020

Reiss, P. T., Huang, L., Chen, Y.-H., Huo, L., Tarpey, T., and Mennes, M. (2014). Massively parallel nonparametric regression, with an application to developmental brain mapping. *J. Comput. Graph. Stat.* 23, 232–248. doi: 10.1080/10618600.2012.733549

Reiss, P. T., and Todd Ogden, R. (2009). Smoothing parameter selection for a class of semiparametric linear models. *J. R. Stat. Soc. Ser. B* 71, 505–523. doi: 10.1111/j.1467-9868.2008.00695.x

Resnick, S. M., Pham, D. L., Kraut, M. A., Zonderman, A. B., and Davatzikos, C. (2003). Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *J. Neurosci.* 23, 3295–3301.

Robinson, M. E., O'Shea, A. M., Craggs, J. G., Price, D. D., Letzen, J. E., and Staud, R. (2015). Comparison of machine classification algorithms for fibromyalgia: neuroimages versus self-report. *J. Pain* 16, 472–477. doi: 10.1016/j.jpain.2015.02.002

Salvador, R., Suckling, J., Schwarzbauer, C., and Bullmore, E. (2005). Undirected graphs of frequency-dependent functional connectivity in whole brain networks. *Philos. Trans. R. Soc. B Biol. Sci.* 360, 937–946. doi: 10.1098/rstb.2005.1645

Smith, S. M. (2012). The future of fmri connectivity. *NeuroImage* 62, 1257–1266. doi: 10.1016/j.neuroimage.2012.01.022

Szymkowicz, S. M., McLaren, M. E., Kirton, J. W., O'Shea, A., Woods, A. J., Manini, T. M., et al. (2016). Depressive symptom severity is associated with increased cortical thickness in older adults. *Int. J. Geriatr. Psychiatry.* 31, 325–333. doi: 10.1002/gps.4324

Thompson, W. K., Hallmayer, J., and O'Hara, R. (2011). Design considerations for characterizing psychiatric trajectories across the lifespan: application to effects of APOE-e4 on cerebral cortical thickness in Alzheimer's disease. *Am. J. Psychiatry* 168, 894–903. doi: 10.1176/appi.ajp.2011.10111690

Tibshirani, R. (1996). Regression shrinkage and selection via the lasso. *J. R. Stat. Soc. Ser. B* 58, 267–288.

Valdés-Sosa, P. A., Sánchez-Bornot, J. M., Lage-Castellanos, A., Vega-Hernández, M., Bosch-Bayard, J., Melie-García, L., et al. (2005). Estimating brain functional connectivity with sparse multivariate autoregression. *Philos. Trans. R. Soc. B Biol. Sci.* 360, 969–981. doi: 10.1098/rstb.2005.1654

Wen, X., Mo, J., and Ding, M. (2012). Exploring resting-state functional connectivity with total interdependence. *NeuroImage* 60, 1587–1595. doi: 10.1016/j.neuroimage.2012.01.079

Wood, S. (2006). *Generalized Additive Models: An Introduction with R*. Boca Raton, FL: CRC Press.

Wu, H., and Zhang, J.-T. (2006). *Nonparametric Regression Methods for Longitudinal Data Analysis: Mixed-Effects Modeling Approaches*, New York, NY: John Wiley & Sons.

Wu, Y., and Liu, Y. (2007). Robust truncated hinge loss support vector machines. *J. Am. Stat. Assoc.* 102, 974–983. doi: 10.1198/016214507000000617

Yuan, M., and Lin, Y. (2007). Model selection and estimation in the gaussian graphical model. *Biometrika* 94, 19–35. doi: 10.1093/biomet/asm018

Zalesky, A., Fornito, A., and Bullmore, E. T. (2010). Network-based statistic: identifying differences in brain networks. *NeuroImage* 53, 1197–1207. doi: 10.1016/j.neuroimage.2010.06.041

Zhang, H. H., Ahn, J., Lin, X., and Park, C. (2006). Gene selection using support vector machines with non-convex penalty. *Bioinformatics* 22, 88–95. doi: 10.1093/bioinformatics/bti736

Zhao, T., Liu, H., Roeder, K., Lafferty, J., and Wasserman, L. (2012). The huge package for high-dimensional undirected graph estimation in R. *J. Mach. Learn. Res.* 13, 1059–1062.

Zhu, J., Rosset, S., Hastie, T., and Tibshirani, R. (2004). 1-norm support vector machines. *Adv. Neural Inform. Process. Syst.* 16, 49–56.

Zou, H. (2006). The adaptive lasso and its oracle properties. *J. Am. Stat. Assoc.* 101, 1418–1429. doi: 10.1198/016214506000000735

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 29 August 2016
doi: 10.3389/fnagi.2016.00204

Cognitive Decline and Reorganization of Functional Connectivity in Healthy Aging: The Pivotal Role of the Salience Network in the Prediction of Age and Cognitive Performances

Valentina La Corte[1,2,3]†, Marco Sperduti[1,2†], Caroline Malherbe[4,5,6], François Vialatte[7], Stéphanie Lion[4], Thierry Gallarda[2,8], Catherine Oppenheim[4] and Pascale Piolino[1,2,3,9]*

[1] Laboratory of Memory and Cognition, Institute of Psychology, University Paris Descartes, Paris, France, [2] INSERM UMR S894, Center of Psychiatry and Neurosciences, University Paris Descartes, Paris, France, [3] IDEX 'Dynamique du Vieillir', Sorbonne Paris Cité, University Paris Diderot, Paris, France, [4] INSERM U894, Center of Psychiatry and Neurosciences, Department of Radiology, University Paris Descartes, Paris, France, [5] Department of Computational Neuroscience, University Medical Center Eppendorf, Hamburg, Germany, [6] Clinic and Polyclinic of Neurology, University Medical Center Eppendorf, Hamburg, Germany, [7] Brain Plasticity Unit, CNRS U8249, ESPCI ParisTech, Paris, France, [8] Laboratory of Physiopathology of Psychiatric Diseases, Center of Psychiatry and Neurosciences, Paris, France, [9] University Institute of France, IUF, Paris, France

Normal aging is related to a decline in specific cognitive processes, in particular in executive functions and memory. In recent years a growing number of studies have focused on changes in brain functional connectivity related to cognitive aging. A common finding is the decreased connectivity within multiple resting state networks, including the default mode network (DMN) and the salience network. In this study, we measured resting state activity using fMRI and explored whether cognitive decline is related to altered functional connectivity. To this end we used a machine learning approach to classify young and old participants from functional connectivity data. The originality of the approach consists in the prediction of the performance and age of the subjects based on functional connectivity by using a machine learning approach. Our findings showed that the connectivity profile between specific networks predicts both the age of the subjects and their cognitive abilities. In particular, we report that the connectivity profiles between the salience and visual networks, and the salience and the anterior part of the DMN, were the features that best predicted the age. Moreover, independently of the age of the subject, connectivity between the salience network and various specific networks (i.e., visual, frontal) predicted episodic memory skills either based on a standard assessment or on an autobiographical memory task, and short-term memory binding. Finally, the connectivity between the salience and the frontal networks predicted inhibition and updating performance, but this link was no longer significant after removing the effect of age. Our findings confirm the crucial role of episodic memory and executive functions in cognitive aging and suggest a pivotal role of the salience network in neural reorganization in aging.

Keywords: resting state, rs-fMRI, episodic memory, autobiographical memory, executive functions, functional connectivity, machine learning, aging

OPEN ACCESS

Edited by:
Ronald Cohen,
University of Florida, USA

Reviewed by:
Claire O'Callaghan,
University of Cambridge, UK
Mihai Moldovan,
University of Copenhagen, Denmark

****Correspondence:***
Valentina La Corte
valentina.lacorte@gmail.com

† These authors are co-first authors.

Received: *15 March 2016*
Accepted: *09 August 2016*
Published: *29 August 2016*

Citation:
La Corte V, Sperduti M, Malherbe C, Vialatte F, Lion S, Gallarda T, Oppenheim C and Piolino P (2016) Cognitive Decline and Reorganization of Functional Connectivity in Healthy Aging: The Pivotal Role of the Salience Network in the Prediction of Age and Cognitive Performances. Front. Aging Neurosci. 8:204. doi: 10.3389/fnagi.2016.00204

INTRODUCTION

The cognitive and neural changes accompanying healthy aging are a crucial topic in cognitive neuroscience. The age-related cognitive decline has emerged as a major societal concern given the increase in the elderly population. Nevertheless, not all cognitive domains are equally affected by age, and not all cognitive processes show age-related decline. There is compelling evidence that executive functions and memory are the most severely impaired cognitive domains in this population (Salthouse et al., 2003).

Executive functions are seen as high-level cognitive processes responsible for flexible and adaptive behavior (Miyake et al., 2000). Thus, they play a fundamental role in dealing with complex situations in everyday life. Moreover, they largely contribute to the effective functioning of other cognitive processes, such as memory. Notably, some authors have proposed that the central deficit responsible for the general cognitive decline in aging is linked to inefficient executive functioning (West, 1996; Salthouse et al., 2003). At the neural level, this decline may be accounted by the functional and structural reorganization of the frontal lobes with aging (Moscovitch and Winocur, 1995; Cabeza, 2002; Grady et al., 2005; Fjell and Walhovd, 2010).

The cognitive domain that has received the greatest attention in normal aging is memory. Many older adults complain of increased memory lapses as they age and a major focus of research has been trying to distinguish memory decline attributable to normal aging from that related to pathological aging, in particular in Alzheimer's disease.

Within the framework of long-term memory, dissociation between spared semantic memory (i.e., general knowledge about the world, words and concept) and impaired episodic memory (i.e., memory for personally experienced events that occurred in a particular place at a specific time) has been reported in aging.

The episodic memory decline in older adults may result from a parallel impairment of strategic control processes involved in encoding and memory retrieval. Accordingly, several studies using laboratory tests of episodic memory have highlighted a reduction in the use of effortful encoding strategies, which are mainly related to prefrontal brain regions (Hara and Naveh-Benjamin, 2015). In the same line, considerable evidence points to deficits in effortful retrieval in older adults. In particular several studies have shown impaired free recall along with normal cued recall or recognition (Ward and Maylor, 2005).

These findings show that memory decline in cognitive aging is strongly related to executive functions.

Moreover, a large number of studies have investigated cognitive aging changes in episodic performance via autobiographical memory, which is defined as the memory for personal experiences that underlies the personal identity and the temporal continuity of an individual (Conway and Pleydell-Pearce, 2000). A distinction between an episodic and a semantic component has also been proposed in this domain. The former refers to memory for personal events situated in a specific spatiotemporal context, while the latter refers to general knowledge about one's own past and about oneself. Again, dissociation between spared semantic and impaired episodic autobiographical memory has been documented in the elderly (Levine et al., 2002; Piolino et al., 2002, 2006; St. Jacques and Levine, 2007; Martinelli et al., 2013a). The deficit of the episodic component of autobiographical memory has been linked to a reduced availability of executive resources (Conway and Pleydell-Pearce, 2000; Conway, 2005; Piolino et al., 2010; Coste et al., 2011), and to a reduced recruitment of the underlying brain structures (Martinelli et al., 2013b).

Functional magnetic imaging (fMRI) has been widely used in order to link age-related cognitive decline with patterns of altered brain function. A consistent finding in the fMRI literature is that healthy old adults present higher brain activation in a wide range of cognitive tasks (Cabeza, 2002). On the other hand some studies have highlighted a reduced brain activity in cognitive aging (Damoiseaux et al., 2008). More recently, an increasing number of investigations have focused on the study of the relationship between cognitive functions and functional connectivity mainly derived from resting state fMRI (rs-fMRI). rs-fMRI is the study of the interregional correlation of spontaneous fluctuation in brain activity while subjects are not engaged in any specific cognitive task. It represents a promising candidate for studying the complex neural organization underlying cognition and its modification due to different conditions (normal aging, psychiatric and neurodegenerative disorders) without task-specific confounds.

The use of rs-fMRI to study functional connectivity has allowed the identification of a set of networks named resting state networks (RSNs). These networks are commonly identified across young healthy subjects (Damoiseaux et al., 2006) and have shown high reproducibility (Guo et al., 2012).

The most widely studied RSN is the default mode network (DMN), composed of regions that are deactivated during the performance of goal-directed tasks and that show high levels of activity at rest. Buckner et al. (2008) defined the core regions associated with the brain's default network: the ventral/dorsal medial prefrontal cortex (PFC), the posterior cingulate and retrosplenial cortex, the inferior parietal lobule and the hippocampal formation (including the entorhinal cortex and parahippocampal cortex).

Beside the DMN, other networks of intrinsic brain connectivity have been described in healthy populations. These findings indicate that the human brain has a network-based organization even at rest. In recent years, a consistent number of investigations have focused on the salience network (Menon, 2015; Metzler-Baddeley et al., 2016). The salience network is an intrinsically connected large-scale network anchored in the anterior insula and the dorsal anterior cingulate cortex. With the anterior insula as its dynamic hub, the salience network contributes to a variety of complex brain functions through the integration of sensory, emotional and cognitive information (Menon, 2015).

Recently, a direct link between inter-individual variability in functional connectivity measured at rest in specific networks and cognitive functions has been documented. For example, Cole et al. (2012) reported that the global connectivity of the lateral prefrontal cortex (LPFC) predicted individual differences

in fluid intelligence. A correlation between the strength of the connectivity between the two major nodes of the DMN, the ventral medial prefrontal cortex (vMPFC) and the posterior cingulate cortex (PCC), and working memory abilities (Hampson et al., 2006), or episodic memory performances (Tambini et al., 2010) has been reported.

This approach has proved fruitful in describing the neural reorganization in aging. Several studies have reported reduced connectivity between the two major nodes of the DMN, the vMPFC and the PCC (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Balsters et al., 2013; Mevel et al., 2013). Other networks with reduced connectivity are the fronto-parietal attentional (Andrews-Hanna et al., 2007; Balsters et al., 2013; Vergun et al., 2013), the sensorimotor (Meier et al., 2012) and the salience networks (Meier et al., 2012; Onoda et al., 2012). In particular, the connectivity profile in the salience network has been shown to be the best feature to classify young and old participants using a machine learning approach (Meier et al., 2012), and that internetwork connectivity between the salience and the visual and the auditory networks is reduced in aging (Onoda et al., 2012).

Moreover, a direct link between reduced network connectivity and impaired cognitive functions has been reported in aging. In particular, decreased connectivity between the anterior and the posterior node of the DMN correlated with a composite measure of memory (Andrews-Hanna et al., 2007). Concerning autobiographical memory, a correlation has been reported between the strength of connectivity between the posterior node of the DMN and middle temporal structures, comprising the hippocampus, and an episodic autobiographical fluency, and between semantic autobiographical fluency and the connectivity between the anterior node of the DMN and the ventral anterior cingulate cortex (Mevel et al., 2013). Additionally, reduced connectivity within the DMN and the salience network has been related to a decline in executive functions in aging (Damoiseaux et al., 2008; Onoda et al., 2012). Taken together, these findings highlight the pertinence of using rs-fMRI to explain the complex neuronal reorganization linked to the cognitive decline observed in aging (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Onoda et al., 2012; Sala-Llonch et al., 2015).

The principal aim of this study was to further characterize the brain functional reorganization related to cognitive aging in order to shed light on the network reorganization related to cognitive decline in older adults, in particular linked to episodic memory and executive functions.

The originality of the study consisted in using a machine-learning approach to predict age and cognitive performance from functional connectivity patterns. Gottlieb (2012) recently proposed that a closer integration of machine learning in cognitive neuroscience has the potential to answer fundamental questions about cognitive functions. Such an approach has already proven its validity in recent investigations of neuropsychological features in neurology or psychiatry (Costafreda et al., 2011; Quintana et al., 2012). Developed from a connectionist approach, this modeling strategy has several advantages over computationalist methods: it can be easily applied to multi-modal data analysis, and in addition it is not constrained by *a priori* assumptions or abstractions on the data. The model is built using the input feature vectors (e.g., multimodal recordings of cognitive tasks) and matching this vector with expected outputs (e.g., prediction of cognitive variables). Once the model has been built, it is then confronted to a new independent test dataset to estimate its validity.

Therefore, we used multivariate statistical techniques to classify young and old participants using a machine learning approach (Meier et al., 2012). We hypothesized that aging would disrupt not only DMN but also the salience network (Onoda et al., 2012) and that this pattern of modifications at the functional level would be related to cognitive changes in particular in episodic memory and executive functions.

MATERIALS AND METHODS

Subjects

Twenty-seven healthy participants, 17 young adults (YA: nine females, mean age 28.75 $\pm$ 4.62) and 10 old adults (OA: four females, mean age 70 $\pm$ 5.01) took part in the study. These participants represent a subgroup of an fMRI activation study whose data have already been published elsewhere (Martinelli et al., 2013b). All participants gave their informed written consent and the study was approved by the local ethics committee of Sainte Anne Hospital (CPP Ile de France 3 n°2687). All subjects were right-handed (according to the Edinburgh Handedness Inventory; Oldfield, 1971), and native French speakers. Medical, demographic, and psychometric data were obtained prior to the scanning session. All participants were unmedicated, living at home and rigorously screened for uncontrolled hypertension and cerebrovascular risk factors. Exclusion criteria included presence of a history of alcohol or substance abuse, head trauma, major disease affecting brain function, neuropsychiatric disorders (tested with the Mini-International Neuropsychiatric Interview, Sheehan et al., 1998), depression [tested with the Geriatric Depression Scale, Yesavage et al., 1983, cut-off score $>$ 10; YA: 2.65 $\pm$ 2.67; OA: 3.4 $\pm$ 2.91; student t-test: $t(25) = 0.68$, $p = 0.5$], abnormal general cognitive functioning as assessed by the Mattis scale (Mattis, 1976, cut-off score $<$ 136; young adults: 142.50 $\pm$ 1.26 and old adults: 139.90 $\pm$ 3.04). The two groups were matched according to their verbal abilities and crystallized intelligence as assessed by the Mill Hill test [Deltour, 1993; percentile score for YA: 54.38 $\pm$ 26.83 and OA: 53.83 $\pm$ 30.82, student t-test: $t(25) = 0.04$, $p = 0.97$].

Procedure

The whole experimental session comprised three phases (pre-scanning, scanning, post-scanning).

During the first phase (pre-scanning interview), participants were tested for exclusion and inclusion criteria, they underwent a medical examination, neuropsychological assessment and completed the Taste and Interest Questionnaire (TIQ) that was employed to create personal cues used for the autobiographical memory tasks during the scanning and post-scanning sessions. During the scanning session, participants were first trained in the autobiographical task outside the scanner, and then a

high-resolution 3D structural image was acquired as well as a resting state functional session. Subsequently they participated in an activation protocol during which they performed the autobiographical task from personal cues (other than those used for training). After the fMRI protocol, during the post-scanning session (debriefing) subjects were asked to re-evoke their autobiographical memories from the same cues seen under the scan. Here we will mainly focus on the measures of neuropsychological assessment and autobiographical memories at debriefing and the rs-fMRI (for details on the activation protocol results see Martinelli et al., 2013c).

Behavioral Measures

Autobiographical Memory

In the pre-scanning interview, exclusion and inclusion criteria were verified by means of a clinical assessment and psychometric tests, and then neuropsychological tests and the TIQ were submitted to subjects. The aim of the TIQ was mainly to collect information so as to create personalized specific event cues for each participant. Twenty-four activities or interests for episodic autobiographical memory (EAM) were selected from the TIQ (for a complete description of personal cue elaboration see Martinelli et al., 2013b; Sperduti et al., 2013).

The participants were first invited to take part in a training session before the fMRI scanning. Participants received detailed explanations on the nature of the task and participated in a brief simulation of the experiment on a laptop. For the two EAM tasks (mental retrieval under the scanner and aloud retrieval at debriefing) we gave the following explanations:

- EAM was defined as a memory of a single event that occurred at a specific time and place, of short duration, lasting less than 24 h. Participants were instructed to mentally relive personal episodes prompted by cues and to try to retrieve spatiotemporal, affective and perceptual details (such as time, location, perceptions, feelings, scenery, and people present in the scene) (e.g., "a unique memory linked to a trip to New York").

After the scanning session, in order to score the memories retrieved in the scanner, participants were asked to recall each memory again. EAMs were rated for richness and specificity on standard scales (Levine et al., 2002; Piolino et al., 2009; Martinelli et al., 2013a). More precisely, the presence of a sense of remembering with recall of specific spatial and temporal details, and other contextual and phenomenological details in each evocation was noted (1 point per type of detail, maximum 4; e.g., "I remembered my visit to the Palace of Tokyo in Paris, in August 2009, as if I was still there. I was with Chiara in a room at the exhibition on the first floor in the dark to see the TV reports and talk with other visitors, it was 6 pm and still very warm, but it was worth it!, after that we went to the restaurant of the outdoor museum on the bank of the Seine..."). For each participant we computed a global ratio of specificity (**EAM** score) totaling up the sum of spatiotemporal, other contextual and phenomenological details divided by the sum depending on the number of recalls.

Episodic Memory

The Free and Cued Selective Reminding test (FCRT) was used to assess episodic memory capacities (Grober and Buschke, 1987; French version Van der Linden et al., 2004).

Different studies have shown the validity of this tool to discriminate healthy old adults from prodromal Alzheimer's disease (AD) patients (Lemos et al., 2015; Papp et al., 2015).

The test begins with a study phase designed to control attention and cognitive processing to identify memory impairment that it is not secondary to other cognitive deficits. During the encoding phase, subjects have to identify words in response to category cues (fruits, clothing, etc.). In the test phase, subjects are asked to recall the items they learned (free recall). The category cues are used to prompt recall of items not retrieved by free recall to generate a score called cued recall. We calculated the sum of free and cued recall termed **EPI total recall**.

Executive Functions

For the assessment of the executive functions, we used the Trail Making Test (TMT; Reitan, 1958) and verbal fluency (Cardebat et al., 1990) as a measure of behavioral and cognitive flexibility respectively. For the TMT we computed the difference in execution time between part B and part A (**TMTB-A** score). Concerning the verbal fluency we added the total number of words for the lexical (number of words starting with the letter P) and the semantic (number of words belonging to the semantic category "animals") fluency (**FLU** score).

As a measure of inhibition we used the Victoria STROOP test (Stroop, 1935). In particular we computed the difference between the time of denomination of the interference part and the denomination part (**INHIB** score). For up-dating (**UP-D** score) in working memory we used the running span (Quinette et al., 2003). For visuo-spatial working memory we used a battery assessing the visuo-spatial span (**VSS** score) forward and backward task (sum of the two spans), and the short-term binding (**STB** score) ability using a visuo-spatial binding task (Picard et al., 2012).

fMRI Data Acquisition

All data were acquired with a 3 T scanner (Discovery MR 750, General Electric Healthcare). The anatomical scan used an inversion recovery 3-D T1-weighted gradient-echo sequence of images (TE = 4.3 ms, TR = 11.2 ms, TI = 400 ms, matrix = 384 × 384, slice thickness = 1.2 mm). Functional resting state images were acquired using a gradient echo echoplanar (EPI) sequence (TE = 30 ms, TR = 2000 ms, flip angle = 90°, matrix = 64 × 64, slice thickness = 3 mm, 42 contiguous sections). The functional scan lasted 5 min.

fMRI Data Analysis

Extraction of Networks and Regions of Interest

We extracted resting state networks from an independent set of resting state data available on the 1000 Functional Connectome Project[1]. This dataset contains functional scans of 86 subjects (45 females, age 19–85 years) acquired with a 3T scanner

[1]http://www.nitrc.org/frs/shownotes.php?release_id=916

with the following parameters: TR = 2 s, 23 slices, acquisition type = sequential ascending.

All data were processed using SPM5 software (Statistical Parametric Mapping 5, Welcome Department of Cognitive Neurology, UK[2]). Standard pre-processing procedures were applied to functional data. EPI volumes were corrected for slice timing, subject's rigid motion and spatially smoothed using an isotropic Gaussian kernel filter of 5 mm full-width half-maximum.

After preprocessing, resting state networks were extracted using group spatial independent component analysis (sICA) as implemented in the Network Detection using ICA (NEDICA) software (Perlbarg et al., 2008). Networks were first extracted for each subject in her/his native space. The spatial ICs obtained for each subject were then normalized in the MNI standard space and clustered into classes representative of the population. To do so we used a hierarchical clustering algorithm (Hartigan, 1975). All normalized spatial maps in each class were then averaged and thresholded at $p < 0.05$ using t-test statistics corrected for multiple comparisons using a false discovery rate (FDR) approach (Genovese et al., 2002). Threshold maps were visually inspected to select maps exhibiting a known spatial organization. These maps are referred to as functional networks (for a similar procedure, see Malherbe et al., 2014).

Then, regions of interest were extracted from the functional networks obtained by selecting the maxima of connectivity peaks of the group functional networks. All regions of interest were defined as a sphere of 10 voxels in the Montreal Neurological Institute space (voxel size: 3.5 mm × 3.5 mm × 3.5 mm). These regions of interest were then used to extract the time course of our functional resting state data after applying the same preprocessing steps described above. The mean time series were calculated across all voxels within each region of interest in the MNI space, for each subject. The motion parameters, as well as signals from white matter and CSF and linear and quadratic drifts were then used as covariates of no interest in a general linear model for the mean time courses in each region considered in the analysis. Regions were then grouped in networks. For each network, the time course was obtained by meaning all time courses of implicated regions. Finally, a correlation matrix of the average time course between each pair of networks was computed (see Table in Appendix 1). This measure was used in the following steps of data analysis.

Machine Learning Analyses

In the present study, using a machine learning approach, we performed two analyses: supervised feature selection, and supervised regression. All the analyses were performed after volume correction: all variables were orthogonalized according to the cortical volume.

Feature selection was performed using the orthogonal forward regression (OFR) algorithm (Chen et al., 1989), which was used to select the best network activities to predict either the age of a subject, or one of the cognitive variables. All the descriptors were considered as vectors (one fMRI was considered as one vector), and we analyzed iteratively the best set of features to model one expected output at a time. Given the feature vectors $f_i, i \in [1..N]$ and the output vector $\mathbf{\Omega}$, the OFR feature selection approach follows three steps:

(I) All descriptors are ranked according to their distance to the output. The distance is computed as the cosine of the angle θ between the vector and the output: $\theta = \cos(f_i, \mathbf{\Omega})$.
(II) The descriptor with the lowest absolute angle (maximum cosine) is ranked first. All remaining descriptors and the output are projected into the null-space of the best descriptor.
(III) The selected descriptor is stored and removed from the set, and the algorithm iterates on the remaining orthogonalized features.

In order to control for the relevance of the selected features, we used a probe variables approach (Stoppiglia et al., 2003). We inserted in the feature set 100 randomly drawn vectors. The rank distribution of these probes indicated the risk of a descriptor containing information that could be explained by chance. We fixed a threshold of 5% of probes in our investigation, and selected only descriptors above that threshold. The variables were analyzed after volumetric correction (the correction was performed by orthogonalizing all variables to the null-space of the volumetry measure).

Supervised regression was performed using multilayer feedforward artificial neural networks. Multilayer perceptrons are universal approximators: when good care is taken to control their complexity, they can provide better fitting than classical polynomial regressions (Haykin, 2009). We used a 2-layer perceptron, with a non-linear (sigmoid) hidden layer and a linear output. The network inputs were the two best features selected using OFR. Regression was performed using a second order gradient descent approach, with the Levenberg–Marquart algorithm (Pujol, 2007). Performances were estimated using a leave-one-out approach:

(I) One sample was taken out of the database.
(II) The network was then trained on the remaining samples, and afterwards tested on the excluded sample.
(III) The same estimation was performed iteratively for all samples of the database. The overall classification of the excluded samples is the leave-one-out error, which is a good estimate of the generalization error.

We tuned the network complexity by manipulating the number of hidden units (from 0 to 5), according to the leave-one-out error (Dreyfus, 2005).

Data analyses were performed using Matlab 2013a (Mathworks®).

RESULTS

Behavioral Results

We performed independent sample t-tests on all the measures of interest. We found significant differences for all measures in favor

[2]www.fil.ion.ucl.ac.uk/spm

of better performance in young adults than in older adults, except for FLU [$t(25) = 1.71$, $p = 0.1$]: EAM [$t(25) = 7.56$, $p < 0.001$], EPI [$t(25) = 3.34$, $p < 0.01$], TMTB-A [$t(25) = 4.24$, $p < 0.001$], INHIB [$t(25) = 6.72$, $p < 0.001$], UP-D [$t(25) = 2.41$, $p < 0.05$], VSS [$t(25) = 4.57$, $p < 0.001$], and STB [$t(25) = 3.07$, $p < 0.01$].

fMRI Results

Clustering

The best silhouette was obtained using six clusters. The within-cluster mean of distance to centroid was below 0.25 for all clusters except for cluster 6. Cluster 1 is particularly large and contains 16 networks (**Table 1**). Using the distance matrices between the cluster elements, we extracted the cluster hierarchy (**Figure 1**), which shows three blocks in these six clusters, composed of cluster 3, cluster 5, and the remaining clusters.

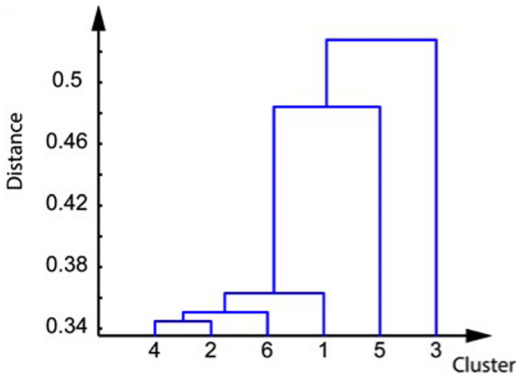


FIGURE 1 | Dendrogram of the clusters, computed from their cosine distance matrices.

Prediction of Age

The best two pairs of networks to predict age according to the OFR algorithm were sal-vis and sal-dmfr, corresponding to clusters 2 and 6. These two clusters belong to the same block on the dendrogram. A Pearson correlation R^2 of 0.61 was obtained ($p = 2.07{*}10^{-5}$) using these two variables. The best neural network architecture selected had four hidden units. A leave-one-out mean-squared error of 0.06 was achieved for the age prediction, corresponding to a generalization error of 6.69 $\pm$ 5.3 years (the prediction error reached 9.10^{-7} on the training set) (**Figure 2**). Given the pivotal role of the salience network in predicting age and cognitive variables, we conducted *post hoc* analyses on the within network connectivity. Interestingly, we found that the connectivity between the cingulate gyrus and insula (which are the main hubs of the salience network) was reduced in the group of old adults ($t = -2.52$, $p < 0.05$). Moreover the connectivity between these two brain regions showed a negative correlation with age in the old adults ($R = -0.7$, $p < 0.05$), but not in the young adults group ($R = -0.03$, $p = 0.9$). Nevertheless, when accounting for within network connectivity, we were still able to significantly predict the age from the between network connectivity between the sal-dmnf and the sal-vis ($R = 0.368$, $p = 4.06$ E-03).

Prediction of Cognitive Variables

Seven cognitive variables were successfully predicted from rs-fMRI pairs of networks (see **Table 2**): EPI total, STB, EAM, FLU, TMT B-A, and VSS Scores. The best prediction was obtained with EPI total, which had the highest linear correlation p-values and a very low leave-one-out generalization error (6%). Moreover, three of these variables were regressed efficiently using a neural network, with a satisfactory leave-one-out error (below 15%).

TABLE 1 | Clusters of pairs of networks (cosine distance measure), ordered according to their homogeneity and dimension.

clusters	1	2	3	4	5	6
homogeneity	0.18	0.18	0.18	0.20	0.21	0.35
networks	Lvattfr-dmfr	Mot-sal	Mot-dmps	Dmfr-dmps	Lvattfr-lvattps	Mot-dmfr
	Lvattfr-dmps	Mot-lvattfr	Mot-vis	Dmfr-dmtemp	Lvattfr-rvattfr	Mot-dmtemp
	Lvattfr-dmtemp	Mot-lvattps	Dmfr-vis	Dmps-dmtemp	Lvattfr-rvattps	Mot-front
	Lvattfr-front	Mot-rvattfr	Dmps-vis	Dmps-front	Lvattps-rvattfr	Sal-lvattfr
	Lvattps-dmfr	Mot-rvattps	Dmtemp-vis	Dmtemp-front	Rvattfr-rvattps	Sal-lvattps
	Lvattps-dmps	Sal-vis	Front-vis		Dmfr-front	Sal-rvattfr
	Lvattps-dmtemp	Lvattfr-vis				Sal-rvattps
	Lvattps-front	Lvattps-rvattps				Sal-dmfr
	Rvattfr-dmfr	Lvattps-vis				Sal-dmps
	Rvattfr-dmps	Rvattfr-vis				Sal-dmtemp
	Rvattfr-dmtemp					Sal-front
	Rvattfr-front					
	Rvattps-dmfr					
	Rvattps-dmps					
	Rvattps-dmtemp					
	Rvattps-front					

Lvattfr, left ventral attentional frontal; Lvattps, left ventral attentional posterior; Rvattfr, right ventral attentional frontal; Rvattps, right ventral attentional posterior; dmfr, default mode frontal; dmps, default mode posterior; dmtemps, default mode temporal; front, frontal; Mot, motor; Sal, salience; vis, visual.

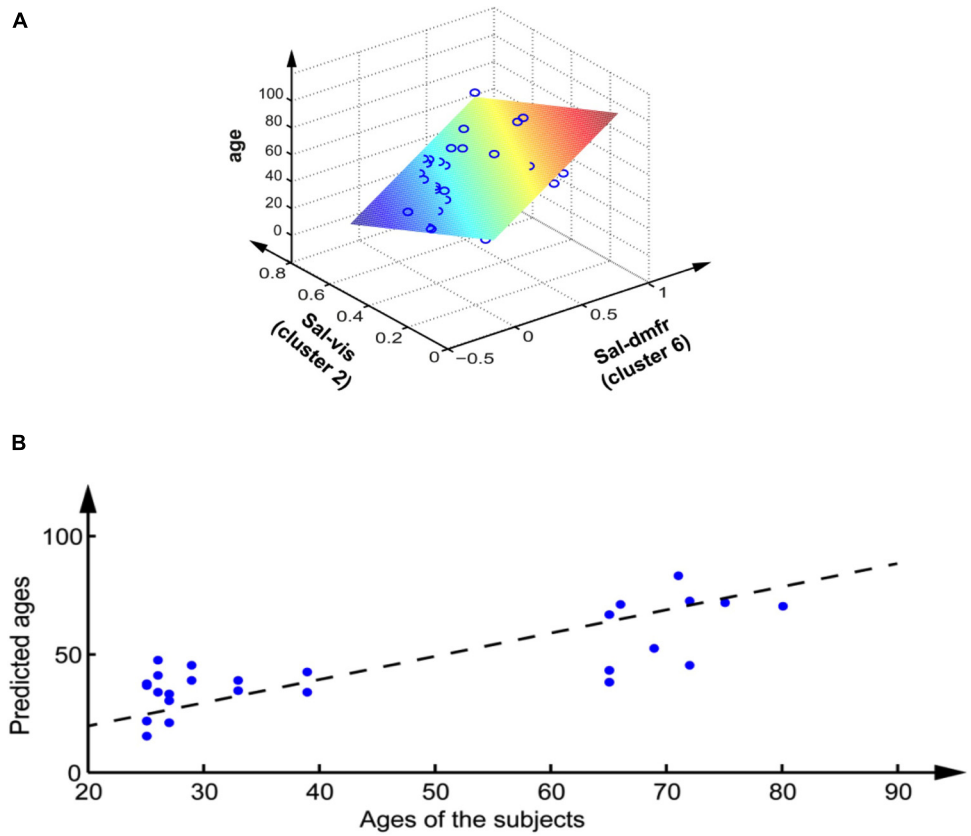


FIGURE 2 | Prediction of the age of the subjects from their rs-fMRI inter network activity. (A) Linear regression, each circle represents a subject, the colored plane represents the linear regression. **(B)** Non-linear regression based on the multilayer perceptron, on the variables after volume correction, on the leave-one-out validation set. Each dot represents a subject; the dashed line represents the optimum.

TABLE 2 | Cognitive variables prediction from fMRI pairs of networks.

Variable	Networks	Clusters	Significance	Corrected significance	Learning error
EPI total	Sal-front	6, 2	R^2 0.59	R^2 0.46	0.06
	Mot-lvattps		$p = 3.1*10\text{-}5$	$p = 7.9*10\text{-}4$	
STB	Sal-dmps	6, 2	R^2 0.31	R^2 0.44	0.14
	Rvattps-vis		$p = 1.3*10\text{-}2$	$p = 1.2*10\text{-}3$	
EAM	Sal-dmtemp	6, 2	R^2 0.60	R^2 0.30	0.14
	Sal-vis		$p = 2.4*10\text{-}5$	$p = 0.015$	
FLU	Rvattfr-dmfr	1, 5	R^2 0.44	R^2 0.35	0.21
	Lvattps-Rvattfr		$p = 1.3*10\text{-}3$	$p = 6.6*10\text{-}3$	
TMT B-A	Lvattps-Rvattfr	5, 6	R^2 0.49	R^2 0.38	0.21
	Sal-rvattps		$p = 4.4*10\text{-}4$	$p = 3.9*10\text{-}3$	
VSS	Lvattps-Rvattfr	5, 6	R^2 0.38	R^2 0.33	0.29
	Sal-rvattps		$p = 4.3*10\text{-}3$	$p = 9.9*10\text{-}3$	
INHIB	Sal-front	6	R^2 0.56	R^2 0.22	0.11
			$p = 8.9*10\text{-}5$	$p = 0.053$	
UP-D	Sal-dmtmp	6	R^2 0.34	R^2 0.16	0.19
	Sal-rvattps		$p = 8.3*10\text{-}3$	$p = 0.14$	

The network column indicates the two best pairs of networks selected using OFR algorithm. The cluster column indicates the corresponding clusters. The significance column indicates Pearson correlation results using the selected networks. Corrected significance is the result of Pearson correlation analysis after correction by the age variable. Learning error is the leave-one-out generalization error on the normalized output. Grey color indicated satisfactory leave-one-out error (below 15%). EPI total, total score episodic memory; STB, short term binding; EAM, episodic autobiographical memory; FLU, verbal fluency; TMTB-A, difference of time execution between the part B and the part A; VSS, visuo-spatial span; INHIB, difference between the time of denomination of the interference part and the denomination part; UP-D, updating in working memory.

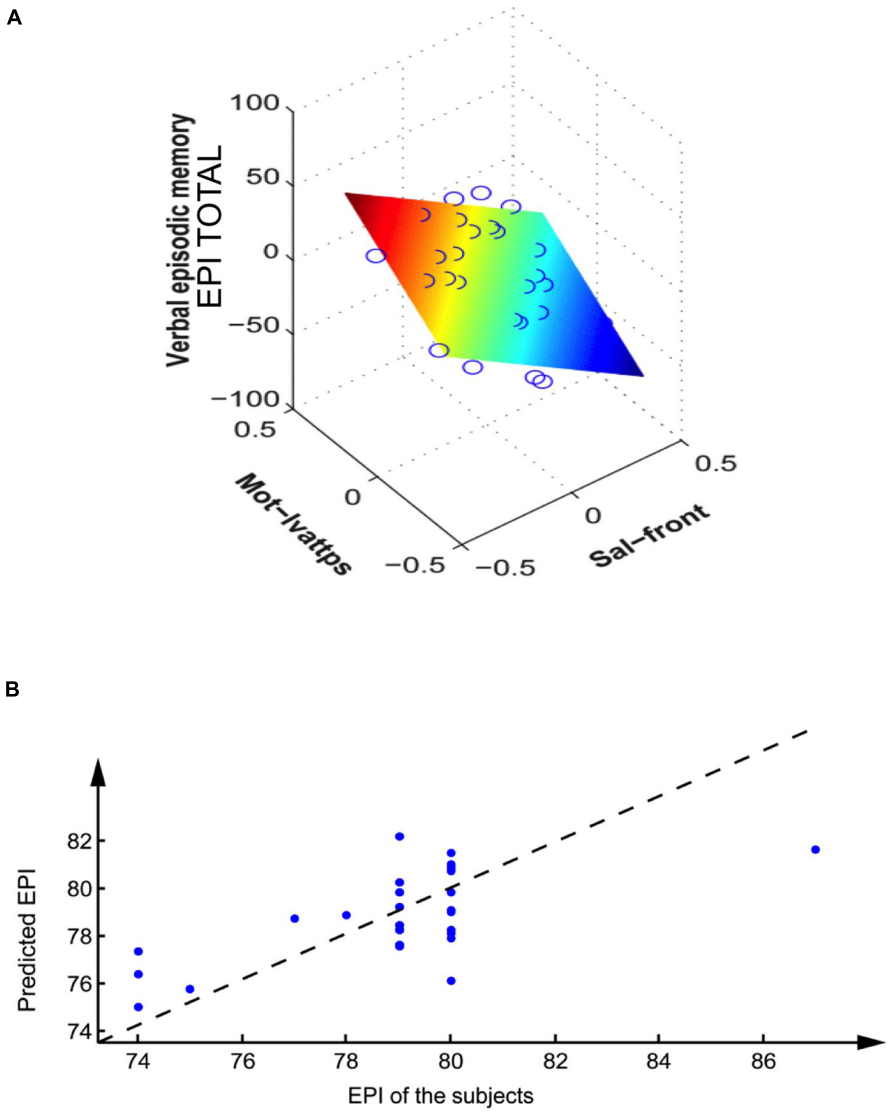


FIGURE 3 | Prediction of EPI total (verbal episodic memory) of the subjects from their rs-fMRI inter network activity. (A) Linear regression on the corrected variables (correction by volume and age). Each circle represents a subject, the colored plane represents the linear regression. **(B)** Non-linear regression based on the multilayer perceptron, on the variables after volume and age correction, on the leave-one-out validation set. Each dot represents a subject; the dashed line represents the optimum (obtained with a linear perceptron without hidden units).

These three variables (EPI total, STB, EAM) were predicted from the same clusters (clusters 6 and 2), which are the same as the age predicting clusters. The four remaining variables had poorer leave-one-out errors (above 20%). Three variables (FLU, TMT B-A, VSS) had cluster 5 in common. Within those three, the last two variables (TMT B-A, and VSS) shared the exact same networks (belonging to cluster 5 and cluster 6). Two other variables showed the same network (cluster 6), but did not show significant correlations after age correction ($p > 0.05$): INHIB score, UP-D score (**Table 2**; **Figure 3**).

Furthermore by combining the measures of distances and the variable predictions, it was possible to draw a general graph of the relationships between the cognitive activation clusters and the predicted variables independently of age (**Figure 4**). On that graph, we can identify three functional blocks (clusters 2 + 6; clusters 6 + 5, clusters 5 + 1). One can see the central importance of cluster 6, which is involved in all but one variable prediction (verbal fluency FLU) and was identified as a general cluster of cognitive decline: this cluster also predicts age, and in addition most cognitive variables when excluding age effects. The functional block of verbal fluency FLU is the only one that is not related to cluster 6. Cluster number 4 was not associated directly to any of the investigated variables (but as it is close to clusters 2 and 6, it could nevertheless be used as a replacement cluster for the prediction of the variables Age, EPI total, STB, and EAM).

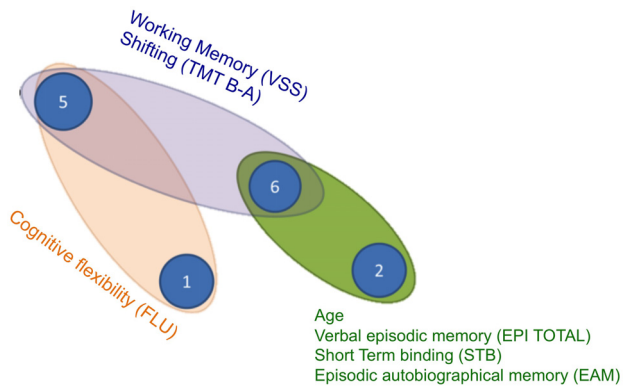


FIGURE 4 | Cognitive network of clusters. The distances between each cluster represents the dendrogram distance already illustrated in **Figure 1**. Each ellipse indicates groups of variables successfully predicted by pairs of the same activation networks. EPI total, total score verbal episodic memory; STB, short term binding; EAM, episodic autobiographical memory; FLU, verbal fluency; TMTB-A, difference of time execution between the part B and the part A; VSS, visuo-spatial span.

DISCUSSION

In this work, we showed that the connectivity profile between specific RSN networks predicts both the age of the subjects and their cognitive abilities. The originality of the study consisted in using a machine-learning approach to predict age and cognitive performance from the functional connectivity patterns characterized in the brain. In particular we reported that the connectivity between the salience and visual networks, and the salience and the anterior part of the DMN, were the best features in predicting the age of the subjects. Moreover, connectivity between the salience and different specific networks predicted the episodic performance (Sal-front), the short-term binding (Sal-dmps) and the episodic autobiographical score (Sal-dmtemp, Sal-vis), independently of the age of the subject. Finally, the connectivity between the salience and the frontal networks predicted inhibition and updating performance, but this correlation was no longer significant after removing the effect of age.

Our findings suggest a pivotal role of the salience network in the neural reorganization in aging. The connectivity profile of this network was not only the best feature to predict age, but was also involved in the prediction of several cognitive functions, such as verbal episodic memory, short-term binding and episodic autobiographical memory. Nevertheless, these scores were also predicted independently of the age of the subject, thus the variability in the strength of connectivity between these networks seems more linked to the variability in cognitive functions *per se* than to the effect of aging. On the contrary, the prediction of inhibitory and updating performances dropped when age was taken into account, suggesting that the connectivity profile of the network predicting inhibition and updating in working memory is particularly sensitive to the effect of aging. These findings are in line with studies that have related cognitive deficits in the elderly to a reduction in inhibitory control (Hasher et al., 1999). The central role of the salience network reported here is coherent with recent findings showing that the connectivity profile of this network was one of the best predictors of age (Meier et al., 2012), and that the connectivity between the salience and the visual networks and the salience and the temporal networks was correlated with age (Onoda et al., 2012). In accordance with the latter study we showed that one of the best features predicting age was the connectivity between the salience and the visual network. On the contrary, while Onoda et al. (2012) did not report robust alteration of the default mode with age, we found that another feature involved in age prediction was the connectivity between the salience and the anterior portion of the default mode. The salience network, composed of the anterior cingulate cortex (ACC) and the insula, is thought to code behaviorally relevant information (Seeley et al., 2007). One recent proposal is that this network, in particular the insular cortex, may promote the dynamic switch between other large scale networks (e.g., the default mode and the central executive network) in order to ensure adaptive behavior *via* flexible cognitive control mechanisms (Sridharan et al., 2008; Menon and Uddin, 2010). Recent studies have reported an altered salience network in normal aging. In particular He et al. (2014) showed that functional and structural impairment of the salience network may occur early in normal aging and that functional disconnection between this network and the central executive network and the DMN may also be associated with normal aging and Alzheimer's disease.

Moreover, the connectivity between the salience network and specific networks predicted different cognitive functions. In particular, the connectivity with the frontal networks predicted episodic memory performance. This finding is in line with the role of the frontal cortex in both encoding and retrieval of episodic memory (e.g., Spaniol et al., 2009). Concerning episodic autobiographical memory, we reported that performance was predicted by the connectivity between the salience network and the temporal component of the DMN, comprising the hippocampus. The role of the hippocampus in episodic autobiographical memory is well established (see for example two meta-analyses: Svoboda et al., 2006; Martinelli et al., 2013c). A recent investigation by Grady et al. (2015) pointed out that the salience network is also engaged during recall failures. In particular these findings suggest that the dedifferentiation of functional connectivity within the salience network across memory conditions and the reduction in functional coupling between it and the PFC may indicate weak inter-network communication either while retrieval is attempted or when monitoring takes place after retrieval has failed.

In addition, supplementary results showed that the connectivity between crucial hubs of the salience network, such as cingulate gyrus and insula, was reduced in elderly subjects and that the connectivity between these two regions showed a negative correlation with age only in the old adults group. These findings highlight the involvement of the principal hubs of the Salience Network in neurocognitive aging.

Moreover, this reduction of connectivity correlated with age only in the elderly group. Nevertheless, we were still able

to predict the age of the subjects from between networks connectivity when within network connectivity was taken into account. These findings suggest that while age is accompanied by an alteration of the intrinsic dynamic of the salience network; inter networks connectivity seems to represent more robust predictors of age.

Finally, the connectivity between the posterior portion of the DMN, comprising the temporo-parietal junction (TPJ) and the precuneus/posterior cingulate cortex, and the salience network predicted short-term binding in working memory. The temporo-parietal junction, beyond attentional and social functions (Scholz et al., 2009), has been linked to working memory processes (Anticevic et al., 2010). Moreover, interestingly, a recent study reported a direct involvement of the TPJ in visual feature binding (Pollmann et al., 2014). Thus, the role of this structure seems coherent with the cognitive demands of our short-term binding task. Taken together these findings suggest that the salience network allocates the necessary cortical resources to other networks that are specialized in the task at hand. Moreover, the link between the connectivity of these networks and the corresponding cognitive functions does not seem to be particularly sensitive to aging, since correlations remain significant even after the effect of age is taken into account.

On the contrary, the correlation between the connectivity of the salience and the frontal network and inhibition performance was affected by age. The link between both the ACC, one of the nodes of the salience network, and the PFC and inhibition, especially during the Stroop task, is well documented (Laird et al., 2005; Nee et al., 2007). Moreover, a recent study showed that performance on the Stroop task was associated with the integrity of fiber tracts connecting these structures in aging, even when controlling for general processing speed (Wolf et al., 2014). Interestingly another recent study has shown that the role of the salience network changes over the life span, which may have implications for the early detection of pathophysiology in elderly populations (Archer et al., 2016).

CONCLUSION

The present study highlights the crucial role of the salience network in cognitive aging related to specific cognitive decline in particular in episodic memory and executive functions. This network is situated at the interface of the cognitive, motivational and affective system of the human brain. It plays a crucial role in identifying the most biologically and cognitively relevant endogenous and external stimuli in order to adaptively guide behavior (Menon, 2015). Indeed it can be considered as a key brain system for integrating cognition, action and feelings.

Further research on normal aging and pathological populations is needed to better characterize the role of disrupted connectivity in the preclinical phase of neurodegenerative disease. Within this context the early detection of functional connectivity abnormalities may be helpful for early diagnosis of the diseases with the aim of characterizing a pathological signature of the reorganization of brain networks in pathological aging.

AUTHOR CONTRIBUTIONS

VLC, MS, FV, and PP wrote the article. MS and SL did the neuroimaging exams. CM, FV did the data processing and data analyses. PP, TG, and CO conceptualized the experiment. All the authors contributed to the final draft of the article.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from the National Hospital Clinical Research Program NEMAUVI (to TG and PP) and the Institut Universitaire de France (to PP) and the Excellent Initiative of Sorbonne Paris Cité, IDEX "Dynamics of Aging" University Paris Diderot, Paris, France (VLC postdoctoral contract). We thank all volunteers for their participation in this study and the neuroimaging staff of the Center of Psychiatry and Neuroscience at Sainte Anne Hospital, especially A. D. Devauchelle, P. Martinelli and Marion Delhommeau for their help in the neuroimaging and neuropsychological exams.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2016.00204

REFERENCES

Andrews-Hanna, J. R., Snyder, A. Z., Vincent, J. L., Lustig, C., Head, D., Raichle, M. E., et al. (2007). Disruption of large-scale brain systems in advanced aging. *Neuron* 56, 924–935. doi: 10.1016/j.neuron.2007.10.038

Anticevic, A., Repovs, G., Shulman, G. L., and Barch, D. M. (2010). When less is more: TPJ and default network deactivation during encoding predicts working memory performance. *Neuroimage* 49, 2638–2648. doi: 10.1016/j.neuroimage.2009.11.008

Archer, J. A., Lee, A., Qiu, A., and Chen, S. A. (2016). A compréhensive analysis of connectivity and aging over adukt life span. *Brain Connect.* 6, 169–185.

Balsters, J. H., O'Connell, R. G., Galli, A., Nolan, H., Greco, E., Kilcullen, S. M., et al. (2013). Changes in resting connectivity with age: a simultaneous electroencephalogram and functional magnetic resonance imaging investigation. *Neurobiol. Aging* 34, 2194–2207. doi: 10.1016/j.neurobiolaging.2013.03.004

Buckner, R. L., Andrews-Hanna, J. R., and Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann. N. Y. Acad. Sci.* 1124, 1–38. doi: 10.1196/annals.1440.011

Cabeza, R. (2002). Hemispheric asymmetry reduction in older adults: the HAROLD model. *Psychol. Aging* 17, 85–100. doi: 10.1037/0882-7974.17.1.85

Cardebat, D., Doyon, B., Puel, M., Goulet, P., and Joanette, Y. (1990). [Formal and semantic lexical evocation in normal subjects. Performance and dynamics of production as a function of sex, age and educational level]. *Acta Neurol. Belg.* 90, 207–217.

Chen, S., Billings, S. A., and Luo, W. (1989). Orthogonal least squares methods and their application to non-linear system identification. *Int. J. Control* 50, 1873–1896. doi: 10.1080/00207178908953472

Cole, M. W., Yarkoni, T., Repovš, G., Anticevic, A., and Braver, T. S. (2012). Global connectivity of prefrontal cortex predicts cognitive control and intelligence. *J. Neurosci.* 32, 8988–8999. doi: 10.1523/JNEUROSCI.0536-12.2012

Conway, M. A. (2005). Memory and the self. *J. Mem. Lang.* 53, 594–628. doi: 10.1016/j.jml.2005.08.005

Conway, M. A., and Pleydell-Pearce, C. W. (2000). The construction of autobiographical memories in the self-memory system. *Psychol. Rev.* 107, 261–88. doi: 10.1037/0033-295X.107.2.261

Costafreda, S. G., Fu, C. H., Picchioni, M., Toulopoulou, T., McDonald, C., Kravariti, E., et al. (2011). Pattern of neural responses to verbal fluency shows diagnostic specificity for schizophrenia and bipolar disorder. *BMC Psychiatry* 11:18. doi: 10.1186/1471-244X-11-18

Coste, C., Agar, N., Petitfour, E., Quinette, P., Guillery-Girard, B., Azouvi, P., et al. (2011). Exploring the roles of the executive and short-term feature-binding functions in retrieval of retrograde autobiographical memories in severe traumatic brain injury. *Cortex* 47, 771–786. doi: 10.1016/j.cortex.2010.07.004

Damoiseaux, J. S., Beckmann, C. F., Arigita, E. S., Barkhof, F., Scheltens, P., Stam, C. J., et al. (2008). Reduced resting-state brain activity in the "default network" in normal aging. *Cereb. Cortex* 18, 1856–1864. doi: 10.1093/cercor/bhm207

Damoiseaux, J. S., Rombouts, S. A. R. B., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., et al. (2006). Consistent resting-state networks across healthy subjects. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13848–13853. doi: 10.1073/pnas.0601417103

Deltour, J. J. (1993). *Echelle de Vocabulaire de Mill Hill de J. C. Raven*. Braine-le-Château: Éditions l'Application des Techniques Modernes SPRL.

Dreyfus, G. (2005). *Neural Networks: Methodology and Applications*. Berlin: Springer-Verlag.

Fjell, A. M., and Walhovd, K. B. (2010). Structural brain changes in aging: courses, causes and cognitive consequences. *Rev. Neurosci.* 21, 187–222. doi: 10.1515/REVNEURO.2010.21.3.187

Genovese, C. R., Lazar, N. A., and Nichols, T. (2002). Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 15, 870–878. doi: 10.1006/nimg.2001.1037

Gottlieb, J. (2012). Attention, learning, and the value of information. *Neuron* 76, 281–295. doi: 10.1016/j.neuron.2012.09.034

Grady, C. L., McIntosh, A. R., and Craik, F. I. (2005). Task-related activity in prefrontal cortex and its relation to recognition memory performance in young and old adults. *Neuropsychologia* 43, 1466–1481.

Grady, C. L., St-Laurent, M., and Burianovà, H. (2015). Age differences in brain activity related to unsuccessful déclarative memory retrieval. *Brain Res.* 1612, 30–47.

Grober, E., and Buschke, H. (1987). Genuine memory deficits in dementia. *Dev. Neuropsychol.* 3, 13–36. doi: 10.1080/87565648709540361

Guo, C. C., Kurth, F., Zhou, J., Mayer, E. A., Eickhoff, S. B., Kramer, J. H., et al. (2012). One-year test–retest reliability of intrinsic connectivity network fMRI in older adults. *Neuroimage* 61, 1471–1483. doi: 10.1016/j.neuroimage.2012.03.027

Hampson, M., Driesen, N. R., Skudlarski, P., Gore, J. C., and Constable, R. T. (2006). Brain connectivity related to working memory performance. *J. Neurosci.* 26, 13338–13343. doi: 10.1523/JNEUROSCI.3408-06.2006

Hara, Y., and Naveh-Benjamin, M. (2015). The role of reduced working memory storage and processing resources in the associative memory deficit of older adults : simulation studies with younger adults. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 22, 129–154.

Hartigan, J. A. (1975). *Clustering Algorithms*. New York, NY: Wiley.

Hasher, L., Zacks, R. T., and Rahhal, T. A. (1999). Timing, instructions, and inhibitory control: some missing factors in the age, and memory debate. *Gerontology* 45, 355–357. doi: 10.1159/000022121

Haykin, S. (2009). *Neural Networks and Learning Machines,* 3rd Edn. Upper Saddle River, NJ: Pearson Education.

He, X., Qin, W., Liu, Y., Zhang, X., Duan, Y., Song, J., et al. (2014). Abnormal salience network in normal aging and in amnestic mild cognitive impairement and Alzheimer disease. *Hum. Brain Mapp. Jul.* 35, 3446–3464. doi: 10.1002/hbm.22414

Laird, A. R., McMillan, K. M., Lancaster, J. L., Kochunov, P., Turkeltaub, P. E., Pardo, J. V., et al. (2005). A comparison of label-based review and ALE meta-analysis in the stroop task. *Hum. Brain Mapp.* 25, 6–21. doi: 10.1002/hbm.20129

Lemos, R., Maroco, J., Simoes, M. R., Santiago, B., Tomas, J., and Santana, I. (2015). The free and cued sélective reminding test for predictng progression to Alzheimer's disease in patients with mild cognitive impairement: a prosective longitudinal study. *J. Neuropsychol.* doi: 10.1111/jnp.12075 [Epub ahead of print].

Levine, B., Svoboda, E., Hay, J. F., Winocur, G., and Moscovitch, M. (2002). Aging and autobiographical memory: dissociating episodic from semantic retrieval. *Psychol. Aging* 17, 677–689. doi: 10.1037/0882-7974.17.4.677

Malherbe, C., Messé, A., Bardinet, E., Pélégrini-Issac, M., Perlbarg, V., Marrelec, G., et al. (2014). Combining spatial independent component analysis with régression to identify the subcortical components of resting state FMRI functional networks. *Brain Connect* 4, 181–192.

Martinelli, P., Anssens, A., Sperduti, M., and Piolino, P. (2013a). The influence of normal aging and Alzheimer's disease in autobiographical memory highly related to the self. *Neuropsychology* 27, 69–78. doi: 10.1037/a0030453

Martinelli, P., Sperduti, M., Devauchelle, A. D., Kalenzaga, S., Gallarda, T., Lion, S., et al. (2013b). Age-related changes in the functional network underlying specific and general autobiographical memory retrieval: a pivotal role for the anterior cingulate cortex. *PLoS ONE* 8:e82385. doi: 10.1371/journal.pone.0082385

Martinelli, P., Sperduti, M., and Piolino, P. (2013c). Neural substrates of the self-memory system: new insights from a meta-analysis. *Hum. Brain Mapp.* 34, 1515–1529. doi: 10.1002/hbm.22008

Mattis, S. (1976). "Mental Status examination for organic mental syndrome in the elderly patient," in *Geriatric Psychiatry*, eds L. Bellack and T. B. Karusu (New York, NY: Grune and Stratton), 77–121.

Meier, T. B., Desphande, A. S., Vergun, S., Nair, V. A., Song, J., Biswal, B. B., et al. (2012). Support vector machine classification and characterization of age-related reorganization of functional brain networks. *Neuroimage* 60, 601–613. doi: 10.1016/j.neuroimage.2011.12.052

Menon, V. (2015). "Salience network," in *Brain Mapping: An Encyclopedic Reference*, Vol. 2, ed. A. W. Toga (Cambridge, MA: Academic Press), 597–611.

Menon, V., and Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct. Funct.* 214, 655–667. doi: 10.1007/s00429-010-0262-0

Metzler-Baddeley, C., Caeyenberghs, K., Foley, S., and Jones, D. K. (2016). Task complexity, and location specific changes of cortical thickness in exécutive and salience networks after working memory training. *Neuroimage*. 130, 48–62. doi: 10.1016/j.neuroimage.2016.01.007

Mevel, K., Landeau, B., Fouquet, M., La Joie, R., Villain, N., Mézenge, F., et al. (2013). Age effect on the default mode network, inner thoughts, and cognitive abilities. *Neurobiol. Aging* 34, 1292–1301. doi: 10.1016/j.neurobiolaging.2012.08.018

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., and Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: a latent variable analysis. *Cognit. Psychol.* 41, 49–100. doi: 10.1006/cogp.1999.0734

Moscovitch, M., and Winocur, G. (1995). Frontal lobes, memory and aging. *Ann. N. Y. Acad. Sci.* 769, 119–150. doi: 10.1111/j.1749-6632.1995.tb38135.x

Nee, D. E., Wager, T. D., and Jonides, J. (2007). Interference resolution: insights from a meta- analysis of neuroimaging tasks. *Cogn. Affect. Behav. Neurosci.* 7, 1–17. doi: 10.3758/CABN.7.1.1

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Onoda, K., Ishihara, M., and Yamaguchi, S. (2012). Decreased functional connectivity by aging is associated with cognitive decline. *J. Cogn. Neurosci.* 24, 2186–2198. doi: 10.1162/jocn_a_00269

Papp, K. V., Amariglio, R. E., Mormino, E. C., Hedden, T., Dekhytar, M., Johnson, K. A., et al. (2015). Free and cued Memory in relation to biomarker-defined abnormalities in clinically normal older adults and those at risk for Alzheimer disease. *Neuropsychologia* 73, 169–175. doi: 10.1016/j.neuropsychologia.2015.04.034

Perlbarg, V., Marrelec, G., Doyon, J., Pélégrini-Issac, M., Lehericy, S., and Benali, H. (2008). "NEDICA: detection of group functional networks in fMRI using spatial independent component analysis," in *Proceedings of the 5th IEEE International Symposium on Biomedical Imaging: From Nano to Macro, 2008* (Rome: IEEE), 1247–1250.

Picard, L., Cousin, S., Guillery-Girard, B., Eustache, F., and Piolino, P. (2012). How do the different components of episodic Memory develop ? Role of exécutive

functions and short-term feature-binding abilities. *Child Dev.* 83, 1037–1050. doi: 10.1111/j.1467-8624.2012.01736.x

Piolino, P., Coste, C., Martinelli, P., Macé, A. L., Quinette, P., Guillery-Girard, B., et al. (2010). Reduced specificity of autobiographical memory and aging: Do the executive and feature binding functions of working memory have a role? *Neuropsychologia* 48, 429–440. doi: 10.1016/j.neuropsychologia.2009.09.035

Piolino, P., Desgranges, B., Benali, K., and Eustache, F. (2002). Episodic and semantic remote autobiographical memory in ageing. *Memory* 10, 239–257. doi: 10.1080/09658210143000353

Piolino, P., Desgranges, B., Clarys, D., Guillery-Girard, B., Taconnat, L., Isingrini, M., et al. (2006). Autobiographical memory, autonoetic consciousness, and self-perspective in aging. *Psychol. Aging* 21, 510. doi: 10.1037/0882-7974.21.3.510

Piolino, P., Desgranges, B., and Eustache, F. (2009). Episodic autobiographical memories over the course of time: cognitive, neuropsychological and neuroimaging findings. *Neuropsychologia* 47, 2314–2329. doi: 10.1016/j.neuropsychologia.2009.01.020

Pollmann, S., Zinke, W., Baumgartner, F., Geringswald, F., and Hanke, M. (2014). The right temporo-parietal junction contributes to visual feature binding. *Neuroimage* 101, 289–297. doi: 10.1016/j.neuroimage.2014.07.021

Pujol, J. (2007). The solution of nonlinear inverse problems and the Levenberg-Marquardt method. *Geophysics* 72, W1–W16. doi: 10.1190/1.2732552

Quinette, P., Guillery, B., Desgranges, B., de la Sayette, V., Viader, F., and Eustache, F. (2003). Working memory and executive functions in transient global amnesia. *Brain* 126, 1917–1934. doi: 10.1093/brain/awg201

Quintana, M., Guàrdia, J., Sánchez-Benavides, G., Aguilar, M., Molinuevo, J. L., Robles, A., et al. (2012). Using artificial neural networks in clinical neuropsychology: high performance in mild cognitive impairment and Alzheimer's disease. *J. Clin. Exp. Neuropsychol.* 34, 195–208. doi: 10.1080/13803395.2011.630651

Reitan, R. M. (1958). Validity of the trail making test as an indicator of organic brain damage. *Percept. Mot. Skills* 1958, 271–276. doi: 10.2466/PMS.8.7.271-276

Sala-Llonch, R., Bartres-Faz, D., and Junqué, C. (2015). Reorganization of bran networks in aging: a review of functional connectivity studies. *Front. Psychol.* 6:663. doi: 10.3389/fpsyg.2015.00663

Salthouse, T. A., Atkinson, T. M., and Berish, D. E. (2003). Executive functioning as a potential mediator of age-related cognitive decline in normal adults. *J. Exp. Psychol. Gen.* 132, 566–594. doi: 10.1037/0096-3445.132.4.566

Scholz, J., Triantafyllou, C., Whitfield-Gabrieli, S., Brown, E. N., and Saxe, R. (2009). Distinct regions of right temporo-parietal junction are selective for theory of mind and exogenous attention. *PLoS ONE* 4:e4869. doi: 10.1371/journal.pone.0004869

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* 59, 22–33.

Spaniol, J., Davidson, P. S., Kim, A. S., Han, H., Moscovitch, M., and Grady, C. L. (2009). Event-related fMRI studies of episodic encoding and retrieval: meta-analyses using activation likelihood estimation. *Neuropsychologia* 47, 1765–1779. doi: 10.1016/j.neuropsychologia.2009.02.028

Sperduti, M., Martinelli, P., Kalenzaga, S., Devauchelle, A. D., Lion, S., Malherbe, C., et al. (2013). Don't be too strict with yourself ! Rigid négative self-representation in healthy subjects mimics the neurocognitive profile of dépression for autobiographical Memory. *Front. Behav. Neurosci.* 21:41. doi: 10.3389/fnbeh.2013.00041

Sridharan, D., Levitin, D. J., and Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12569–12574. doi: 10.1073/pnas.0800005105

St. Jacques, P. L., and Levine, B. (2007). Ageing and autobiographical memory for emotional and neutral events. *Memory* 15, 129–144. doi: 10.1080/09658210601119762

Stoppiglia, H., Dreyfus, G., Dubois, R., and Oussar, Y. (2003). Ranking a random feature for variable and feature selection. *J. Mach. Learn. Res.* 3, 1399–1414.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *J. Exp. Psychol.* 18, 643–662. doi: 10.1037/h0054651

Svoboda, E., McKinnon, M. C., and Levine, B. (2006). The functional neuroanatomy of autobiographical memory: a meta-analysis. *Neuropsychologia* 44, 2189–2208. doi: 10.1016/j.neuropsychologia.2006.05.023

Tambini, A., Ketz, N., and Davachi, L. (2010). Enhanced brain correlations during rest are related to memory for recent experiences. *Neuron* 65, 280–290. doi: 10.1016/j.neuron.2010.01.001

Van der Linden, M., Coyette, F., Poitrenaud, J., Kalafat, M., Calicis, F., Wyns, C., et al. (2004). "L'épreuve de rappel libre/rappel indicé à 16 items (RL/RI-16)," in *L'Évaluation des Troubles de la Mémoire: Présentation de Quatre Tests de Mémoire Épisodique Avec Leur Étalonnage*, eds M. Van der Linden, S. Adam, A. Agniel, et les membres du GREMEM (Marseille: SOLAL).

Vergun, S., Deshpande, A. S., Meier, T. B., Song, J., Tudorascu, D. L., Nair, V. A., et al. (2013). Characterizing functional connectivity differences in aging adults using machine learning on resting state fMRI data. *Front. Comput. Neurosci.* 7:38. doi: 10.3389/fncom.2013.00038

Ward, G., and Maylor, E. A. (2005). Age –related déficits in free recall : the rôle of rehearsal. *Q. J. Exp. Psychol. A* 58, 98–119. doi: 10.1080/02724980443000223

West, R. L. (1996). An application of prefrontal cortex function theory to cognitive aging. *Psychol. Bull.* 120, 272–292. doi: 10.1037/0033-2909.120.2.272

Wolf, D., Zschutschke, L., Scheurich, A., Schmitz, F., Lieb, K., Tuscher, O., et al. (2014). Age-related increases in Stroop interférence: délinéation of général slowing based on behavioral and whitematter analyses. *Hum. Brain Mapp.* 35, 2448–2458.

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., et al. (1983). Development and validation of a geriatric depression screening scale: a preliminary report. *J. Psychiatr. Res.* 17, 37–49. doi: 10.1016/0022-3956(82)90033-4

Conflict of Interest Statement: 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 Aging Neuroscience

REVIEW
published: 18 October 2016
doi: 10.3389/fnagi.2016.00239

Real-Time fMRI in Neuroscience Research and Its Use in Studying the Aging Brain

Mohit Rana[1,2], Andrew Q. Varan[3], Anis Davoudi[4], Ronald A. Cohen[5,6], Ranganatha Sitaram[1,2,7] and Natalie C. Ebner[3,5,6]*

[1] Department of Psychiatry and Division of Neuroscience, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile, [2] Laboratory for Brain-Machine Interfaces and Neuromodulation, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Department of Psychology, University of Florida, Gainesville, FL, USA, [4] J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA, [5] Center for Cognitive Aging and Memory, Institute on Aging, University of Florida, Gainesville, FL, USA, [6] Department of Aging and Geriatric Research, College of Medicine, University of Florida, Gainesville, FL, USA, [7] Institute for Biological and Medical Engineering, Schools of Engineering, Biology and Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

Cognitive decline is a major concern in the aging population. It is normative to experience some deterioration in cognitive abilities with advanced age such as related to memory performance, attention distraction to interference, task switching, and processing speed. However, intact cognitive functioning in old age is important for leading an independent day-to-day life. Thus, studying ways to counteract or delay the onset of cognitive decline in aging is crucial. The literature offers various explanations for the decline in cognitive performance in aging; among those are age-related gray and white matter atrophy, synaptic degeneration, blood flow reduction, neurochemical alterations, and change in connectivity patterns with advanced age. An emerging literature on neurofeedback and Brain Computer Interface (BCI) reports exciting results supporting the benefits of volitional modulation of brain activity on cognition and behavior. Neurofeedback studies based on real-time functional magnetic resonance imaging (rtfMRI) have shown behavioral changes in schizophrenia and behavioral benefits in nicotine addiction. This article integrates research on cognitive and brain aging with evidence of brain and behavioral modification due to rtfMRI neurofeedback. We offer a state-of-the-art description of the rtfMRI technique with an eye towards its application in aging. We present preliminary results of a feasibility study exploring the possibility of using rtfMRI to train older adults to volitionally control brain activity. Based on these first findings, we discuss possible implementations of rtfMRI neurofeedback as a novel technique to study and alleviate cognitive decline in healthy and pathological aging.

Keywords: real-time functional magnetic resonance imaging, aging, cognition, neurofeedback, emotion

OPEN ACCESS

Edited by:
Rodrigo Orlando Kuljiš, University of Miami School of Medicine, USA

Reviewed by:
Ramesh Kandimalla, Texas Tech University, USA
Neha Sehgal, Wisconsin Institute for Discovery, USA

****Correspondence:***
Mohit Rana
makrana85@gmail.com

Received: *30 June 2016*
Accepted: *27 September 2016*
Published: *18 October 2016*

Citation:
Rana M, Varan AQ, Davoudi A, Cohen RA, Sitaram R and Ebner NC (2016) Real-Time fMRI in Neuroscience Research and Its Use in Studying the Aging Brain. Front. Aging Neurosci. 8:239. doi: 10.3389/fnagi.2016.00239

INTRODUCTION

Age-Related Cognitive Decline and Underlying Brain Mechanisms

Given current demographic developments with adults over the age of 65 years representing the fastest growing segment of the population in the USA and other industrialized nations (Census, 2012), cognitive decline in aging is of increasing societal and economic relevance, in addition to its relevance to individual lives (Williams and Kemper, 2010). It is usual, with

interindividual variation (Ram et al., 2011), to experience some deterioration in cognitive abilities with advanced age. These age-related cognitive deficits are typically characterized by slow processing speed (Eckert et al., 2010), increased difficulty in encoding and retrieving memories (Wilckens et al., 2012), increased forgetfulness (Gazzaley et al., 2005), reduced ability to selectively attend to or ignore irrelevant information (Prakash et al., 2009), increased distraction to interference (Wais et al., 2012), and reduced task switching abilities (Buchler et al., 2008). This change in cognitive functioning constraints older adults' independence and quality of life (Logsdon et al., 2002). Thus, studying ways to counteract or delay the onset of cognitive decrement in aging is crucial.

Recent research initiatives address cognitive decline and brain aging, such as the "Healthy Brain Initiation" by the American Association of Retired Persons[1] and the Alzheimer's Association[2] in the US, and the "Healthy Brain" initiative by the Brain Foundation[3] in Australia. These initiatives have targeted the creation of standardized assessment tools and the implementation of lifestyle directives (e.g., related to nutrition and physical activity) to allow for direct comparison across research studies and to inform interventional strategies towards maintenance and promotion of cognitive functioning in older adults or delay of cognitive decline until later in life. There is also a growing market for computer-based trainings, memory tapes, and computer games offered to the lay public. These products claim enhancement of cognitive performance through training (Casel, 2002). However, most of these current approaches target training of behavioral aspects of cognitive aging without consideration of brain processes.

With the recent advancement in neuroimaging technology, and especially developments in functional magnetic resonance imaging (fMRI), understanding of functional brain changes that underlie age-related cognitive decline has tremendously increased (Li et al., 2015). For instance, aging has been shown to be associated with greater involvement of frontal and parietal regions and reduced activation of occipital regions during attention (Cabeza et al., 2004), visual perception (Davis et al., 2008), working memory (Park et al., 2003), language (Grossman et al., 2002), and emotion processing (Williams et al., 2006). These findings have been discussed in the context of proposed models of brain aging, such as the hemispheric asymmetry reduction in older adults (HAROLD; Cabeza, 2002), the posterior–anterior shift in aging (PASA; Davis et al., 2008), the compensation-related utilization of neural circuit hypothesis (CRUNCH; Reuter-Lorenz and Cappell, 2008), and the scaffolding theory of aging and cognition (STAC; Goh and Park, 2009). According to the HAROLD model, older compared to younger adults show greater bilateral brain activity in prefrontal cortex (PFC) for specific cognitive tasks. Similarly, the CRUNCH model proposes that older adults have lower neural efficiency than younger adults. That is, older compared to younger adults recruit more brain regions (e.g., frontal or bilateral brain regions) for cognitive operations. The PASA model of aging states that older adults during task engagement show increased activations of PFC coupled with decreased activation of the occipital cortex leading to a shift in brain activity pattern from the posterior part of the brain to the anterior part. Similarly, STAC suggests that an increasing use of frontal brain regions with age during cognitive processing is an indication of an adaptive brain. The theory proposes that, to counter the deterioration of neural structures and functions with age, the brain develops compensatory neural circuits to achieve a particular cognitive goal.

An independent emerging literature has generated exciting results that support the benefits of volitional modulation of activity in specific brain regions and networks on cognition and behavior. In particular, a number of studies have shown that individuals can learn to voluntarily control different components of the electroencephalographic (EEG) spectrum, resulting in specific behavioral change (Kotchoubey et al., 2001; Kubler et al., 2001; Fuchs et al., 2003; Murase et al., 2004; Birbaumer, 2006; Strehl et al., 2006). EEG based neurofeedback has advantages of high temporal resolution, affordability and portability, but it has disadvantages related to its low spatial resolution, and its inability to access deeper brain regions. It also suffers from computational complexity of the inverse problem in determining the source of activations from the surface EEG signals. However, recent developments in real-time fMRI (rtfMRI; Weiskopf, 2012; Sulzer et al., 2013; Stoeckel et al., 2014) have overcome some of the limitations of the EEG based technique due to fMRI's high spatial resolution and its capacity for whole brain coverage.

In this article, we offer a state-of-the-art description of the neurofeedback technique with a particular focus on rtfMRI in its application to cognitive aging. To the best of our knowledge, no research to date has used rtfMRI in the context of studying and counteracting cognitive decline in older adults. We start by outlining current empirical evidence on cognitive and behavioral benefits of rtfMRI in young healthy adults as well as patients. We then discuss the application of this novel technique in the context of aging research, supported by preliminary data from our group. The article concludes with a discussion of future research directions using rtfMRI and related neurofeedback training techniques, such as EEG and functional near-infrared spectroscopy (fNIRS), towards preservation of cognitive function and delay of cognitive decline in aging.

Volitional Modulation of Brain Activity via rtfMRI

Neurofeedback is a procedure by which humans or animals can learn to modulate neural activity in one or more brain region(s) (Birbaumer et al., 2013). The mechanism underlying neurofeedback learning is still not completely understood, but several different mechanisms, including, operant conditioning and skill learning, have been proposed (Sitaram et al., in press). For example, via rtfMRI neurofeedback training, volitional increase or decrease of Blood Oxygenation Level Dependent

[1]http://www.aarp.org/health/brain-health/
[2]http://www.alz.org/publichealth/2013-report/index.html
[3]http://brainfoundation.org.au/healthy-brain

(BOLD) response in a circumscribed brain area or network of regions can be attained by the subjects.

Overview of the rtfMRI System

More than a decade ago, the first fMRI based Brain Computer Interface (BCI) approach was implemented (Posse et al., 2003). **Figure 1** depicts the rtfMRI neurofeedback system. It is a closed-loop system that uses the BOLD signal from one circumscribed brain region or a network of brain regions, in real-time, to calculate and present feedback (e.g., visual, auditory, or tactile) to participants (e.g., Caria et al., 2007; Sitaram et al., 2007; Rota et al., 2009; Ruiz et al., 2013a). The rtfMRI system comprises of the following subsystems (see **Figure 1**): (A) participant, (B) signal acquisition, (C) online signal analysis, and (D) feedback.

An echo planar imaging (EPI) sequence (Bandettini et al., 1992) is used to acquire functional images of the brain (see **Figure 1B**). Online computation procedures with the data in the k-space such as distortion correction, averaging of the signal, and image reconstruction are performed on the scanner's image reconstruction computer. Once the image is reconstructed and pre-processed, it is exported to the signal analysis subsystem (see **Figure 1C**). The signal analysis subsystem is implemented using the Turbo Brain Voyager (TBV) software (Brain Innovations, Maastricht, Netherlands). TBV retrieves the reconstructed image and performs data processing that includes 3D motion correction and real-time statistical analysis using the general linear model. TBV allows the user to draw regions of interest (ROIs) on the functional images. The BOLD values pertaining to these ROIs are exported to a Matlab script (Mathworks, Natwick, MA, USA) that calculates the feedback, which is then presented to the participant inside the scanner (see **Figure 1D**).

Diverse modalities of feedback can be employed, including verbal, auditory, tactile, monetary, or a combination of these, but visual feedback has been predominantly used in research. Visual feedback of the brain activity can be provided to the participant in the form of a graphically animated thermometer with bars of the thermometer changing in proportion to the percent BOLD changes in the ROIs. The majority of rtfMRI studies reported in the literature applied continuous feedback (i.e., feedback provided to a participant within one repetition time, TR, of the EPI sequence). For example, Caria et al. (2007) showed that participants were able to self-regulate anterior insula when trained with continuous feedback with a delay of 1 TR (i.e., 1.5 s), while participants who received sham feedback did not learn to self-regulate. This finding demonstrated the importance of contingent feedback to learn to self-regulate neural activity. However, intermittent feedback (i.e., feedback provided to a participant after a number of TRs of the EPI sequence) has also been used successfully (Yoo and Jolesz, 2002; Johnson et al., 2012).

Typical Design of rtfMRI Studies

A typical rtfMRI study consists of a number of neurofeedback training sessions, in which a participant learns to regulate (increase or decrease) the BOLD signal in a particular ROI. Typically, a neurofeedback training run consists of two types of conditions, namely baseline and regulation, although there is no general rule in this regard. In the majority of current studies, participants were instructed to remain in a resting state during the baseline blocks and to find a cognitive strategy that helps them to achieve self-regulation in the regulation blocks. However, there are also studies where participants were not given any instructions to use a cognitive strategy, but were trained with just real-time feedback or reward (Shibata et al., 2011; Sepulveda et al., 2016).

Types of rtfMRI Neurofeedback Approaches

The current literature generally differentiates between three types of rtfMRI neurofeedback approaches, namely, single-ROI based neurofeedback (Caria et al., 2007), functional connectivity based neurofeedback (Liew et al., 2015) and network pattern based neurofeedback (Shibata et al., 2011). Development of these approaches occurred independent of each other and temporally overlapped. The suitable rtfMRI neurofeedback approach for a specific study is selected based on the study's hypothesis.

Single-ROI Based Neurofeedback

Single-ROI based neurofeedback approach is a more conservative rtfMRI approach than the other two approaches. In this approach, individuals learn to volitionally regulate the BOLD signal from one circumscribed brain area. Feedback is calculated as a linear combination of the signal amplitude in the target ROI (e.g., motor cortex for a motor task) and a task-unrelated reference ROI (e.g., auditory cortex for a motor task). The reference area is used to subtract the global (whole-brain) increase in the BOLD signal due to general arousal, task-unrelated factors, or BOLD fluctuations caused by head motion. An example equation (Equation 1) for calculating feedback using this approach is as follows:

$$\begin{aligned} \text{Feedback} = &(\text{ROI1}_{\text{Regulation}} - \text{ROI1}_{\text{Baseline}}) \\ &- (\text{ROI2}_{\text{Regulation}} - \text{ROI2}_{\text{Baseline}}) \end{aligned} \quad (1)$$

where ROI1 is the target brain area and ROI2 is the reference brain area.

Functional Connectivity Based Neurofeedback

In general, a brain function can hardly be conceived to involve only one single brain region (Sporns et al., 2005). Rather, the brain is considered to work by coordinating activity across distributed brain regions to execute a task. Functional connectivity is defined as the statistical dependency between two or more remote neurophysiological events (Friston, 2011). It represents the connectivity between two or more brain regions that share functional properties. There are two methods of computing functional connectivity. The first method involves estimation of statistical correlation between the BOLD time-series of two ROIs using the Pearson, sample, or population correlation coefficient. Functional connectivity neurofeedback using this first method can be calculated either by including the correlation measure in the standard ROI feedback equation (e.g., Equation 2; Ruiz et al., 2012), or by subtracting correlations of the

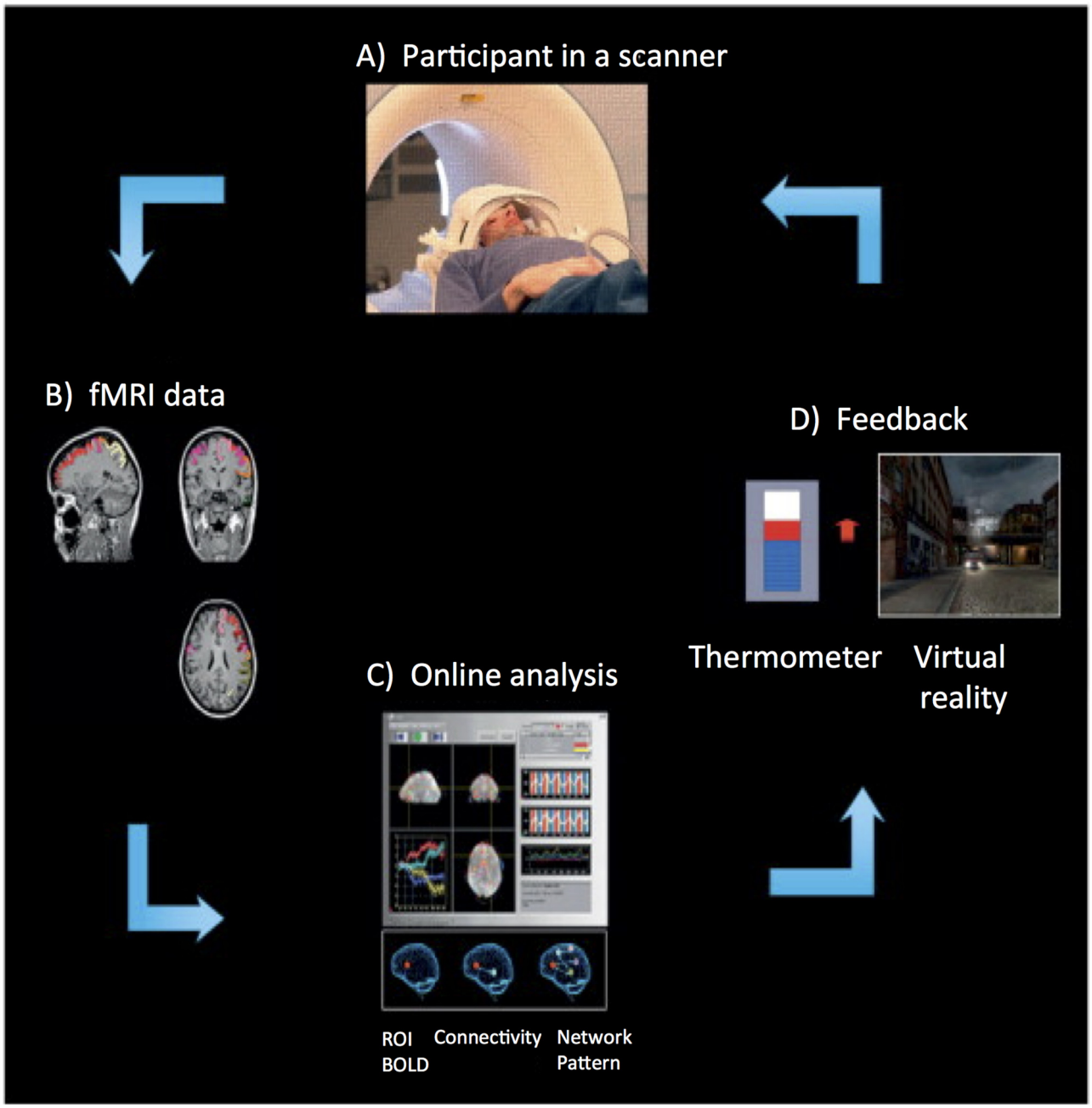


FIGURE 1 | Overview of an real-time functional magnetic resonance imaging (rtfMRI)-based neurofeedback system that comprises the following subsystems. (A) Participant in the MRI scanner. **(B)** Signal acquisition (fMRI data) using an echo planar imaging (EPI) pulse sequence. **(C)** Online analysis and computation of the neurofeedback based on the Blood Oxygenation Level Dependent (BOLD) response. **(D)** Visual feedback via the scanner projection system. This figure is adapted from Birbaumer et al. (2013). The rtfMRI system presented in this figure was developed at the Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Germany.

two ROIs in the baseline condition from those in the regulation condition (e.g., Equation 3; Liew et al., 2015).

$$\text{Feedback} = (\text{TOT\_BOLD}_{\text{Regulation}} - \text{TOT}_{\text{BOLD Baseline}}) \times (1 + \text{EC}) \quad (2)$$

$$\text{Feedback} = \text{EC}_{\text{Regulation}} - \text{EC}_{\text{Baseline}} \quad (3)$$

where TOT_BOLD is the total BOLD signal in the two ROIs (i.e., TOT_BOLD = (BOLD in ROI1 + BOLD in ROI2) and EC is the Pearson's linear correlation coefficient derived from the BOLD time-series of these two ROIs.

A sliding window of eight data points, i.e., the current time point and seven data points before the current data point from each ROI, is used to compute the correlation coefficient. The limitation of this method is that it does not provide any information about the causality or the direction of information flow, between the two ROIs. This limitation is addressed by the second method of computing functional connectivity, in that it estimates effective connectivity using Granger causality (GC; Granger, 1969) or dynamic causal modeling (DCM; Friston et al., 2003) There is evidence that use of DCM allows training of effective connectivity between two ROIs to influence the directionality of functional interactions (Koush et al., 2013).

Network Pattern Based Neurofeedback

Compared to the single-ROI based or the functional connectivity based approaches, the pattern classification approach provides greater sensitivity for detection and modulation of an entire brain network involved in a specific function (Haynes and Rees, 2006; Lewis-Peacock and Norman, 2014; Haynes, 2015). In this approach, spatial and temporal patterns of activity of multiple brain regions involved in a function are computed in real-time and presented as feedback to the participant. There are several different pattern classification techniques that have been applied to fMRI data, including Linear Discriminant Analysis (LDA; LaConte et al., 2003), Naïve Bayes (Pereira et al., 2009), Support Vector Machine (SVM; LaConte et al., 2005), Neural Networks (Hanson et al., 2004), Canonical Variates Analysis (Mourão-Miranda et al., 2006), and Fisher Linear Discriminant (Shaw et al., 2003). SVM is one of the methodologies widely used to predict the brain state based on the BOLD signal. There is evidence that SVM provides higher classification accuracy than the other methods of pattern classification (LaConte et al., 2003, 2005; Shaw et al., 2003; Strother et al., 2004; Martínez-Ramón et al., 2006). SVM is less sensitive to preprocessing steps when compared with LDA (i.e., high classification accuracy), which is useful in real-time applications (LaConte et al., 2005). SVM is a binary classification algorithm that estimates a hyper-plane in a multi-dimensional space to discriminate between two tasks (Schölkopf and Smola, 2002). BOLD signals from two different conditions (e.g., left and right hand movement imagery) are fed into the SVM algorithm and a classification model is generated based on this information. Based on this generated classification model, SVM predicts the possible condition that a particular preprocessed BOLD signal belongs to.

There are currently two approaches of performing the online pattern classification: subject-dependent classification and subject-independent classification (Rana et al., 2013). The majority of current studies used the subject-dependent classification approach, in which classification models are tailored to a specific participant's brain signals. Some studies have shown impressive results using this approach in healthy adults (Shibata et al., 2011; Sitaram et al., 2011; deBettencourt et al., 2015). However, this technique only led to very limited advancement in the field of neurorehabilitation. The main reason for this limitation is that classification models are not sufficiently generic to be used across participants due to interindividual variations in structural and functional brain characteristics, which may even be exaggerated in clinical population and may become more pronounced in aging (Meunier et al., 2014). This limitation of the subject-specific classification constitutes a hindrance for its application in patients and older adults. In contrast, the subject-independent classifier approach can be applied to healthy as well as patient populations without the need to collect subject-specific data to generate the classifier model. In addition, it can be adapted to the idiosyncrasies of individual brain size, shape, and activation patterns. Thus, the subject-independent approach has the potential to facilitate training of patients to correct and tune their abnormal brain activity towards normalcy and appears to have promise for applications in aging.

Evidence for Cognitive and Behavior Modification Using rtfMRI Neurofeedback

The rtfMRI neurofeedback approach represents a new tool for studying the relation between brain activity, cognition, and behavior. Importantly, unlike in conventional neuroimaging approaches where cognition and behavior are the independent variables and brain activity is the dependent variable, in rtfMRI, brain activity constitutes the independent variable while cognition and behavior serve as the dependent variables. Neurofeedback using rtfMRI has been used to train individuals in volitional regulation of BOLD signals in different brain regions or in connectivity among multiple regions, to determine cognitive and behavioral effects of learned self-regulation. In this endeavor, neurofeedback effects have been documented in pain modulation, in reaction times, and linguistic and emotional processing in young healthy and/or patient populations (deCharms et al., 2005; Rota et al., 2009; Ruiz et al., 2013a; Scharnowski et al., 2015).

In the early days of rtfMRI, studies were focused on evaluating effects due to self-regulation in a single, circumscribed brain area (i.e., single-ROI neurofeedback approach). This nascent field originally applied, and attempted to optimize, this more parsimonious methodology. A number of studies were conducted using volitional brain regulation of single areas such as the insula, amygdala, visual cortex, anterior cingulate, or motor cortex to understand their impact on cognition, perception, and emotion (e.g., Caria et al., 2010; Shibata et al., 2011; Paret et al., 2014; Gröne et al., 2015). For example, Posse et al. (2003) conducted the first rtfMRI study in emotion-related brain areas. Participants were trained to modulate amygdala activity using a self-inducing mood paradigm that included sad and neutral emotional states. All participants in this study were able to successfully achieve sad mood. Further, mood self-ratings were positively associated with BOLD response in amygdala. However, in this study, the self-induction task was performed in the presence of emotional faces during the entire experiment and the study lacked a control group (CG). Thus, it was not clear whether the observed correlation was due to the amygdala self-regulation or due to the presentation of emotional faces, or a combination of both. Caria et al. (2010) observed

significant modification in valence ratings related to aversive picture stimuli associated with up-regulation of anterior insula in individuals trained with contingent feedback, while the effect was not observed in individuals trained with sham feedback. This study provided further support for cognitive and behavioral modification induced by learned self-regulation and the importance of contingent feedback in neurofeedback training.

Some rtfMRI studies examined cognitive and behavioral effects using functional connectivity based neurofeedback. This was based on the rationale that a brain function works via coordination of distributed brain regions to execute a task. This development was also informed by emerging evidence that abnormal connectivity of brain areas was associated with abnormal brain functioning in neuropsychiatric disorders such as schizophrenia (Friston and Frith, 1995; Honey et al., 2005), autism (Just et al., 2007), Alzheimer's Disease (AD; Wang et al., 2007; Zhang et al., 2010), and Attention Deficit Hyperactivity Disorder (ADHD; Konrad and Eickhoff, 2010). Use of the connectivity approach was also spurred by evidence that learned volitional control of a single brain area in healthy adults lead to changes in functional connectivity across brain regions (Hamilton et al., 2011; Lee et al., 2011; Zotev et al., 2011; Ruiz et al., 2013a,b). For example, a study in schizophrenia patients found that learned self-regulation of a single brain ROI modulated brain connectivity in an entire network (Ruiz et al., 2013b), supporting the use of rtfMRI as a tool to enhance brain connectivity. However, enhancement of functional connectivity between various brain areas in this early study was observed as a by-product of single-ROI based neurofeedback training but was not the result of direct training of brain connectivity.

Thus, following up on these initial findings, studies using rtfMRI based connectivity neurofeedback demonstrated that enhancement of functional connectivity between two brain areas was possible and resulted in cognitive and behavioral modifications. Kim et al. (2015), for example, showed improved efficacy in reducing cigarette smoking via learned self-regulation of connectivity between four brain regions related to craving (i.e., anterior cingulate cortex, medial PFC, posterior cingulate cortex, and precuneus). Similarly, a study from our lab found evidence for direct enhancement of brain functional connectivity (Ruiz et al., 2012). In particular, healthy adults were trained to increase functional connectivity between inferior frontal gyrus (Broca's area) and superior temporal gyrus (Wernicke's area), which resulted in an enhanced priming effect in a semantic priming task (Sass et al., 2009).

In parallel to the other two neurofeedback approaches, the field explored the use of rtfMRI in the context of pattern classification based neurofeedback. The first study used SVM algorithm for binary classification in the context of real-time feedback on motor and cognitive states (LaConte et al., 2007). Sitaram et al. (2011) implemented a mapping technique for pattern classification of multiple emotional brain states in real-time. Shibata et al. (2011) demonstrated perceptual learning by inducing spatial patterns of activity in the primary visual cortex. In this study, pattern feedback was used to train participants to self-induce brain activity pertaining to one of three Gabor patch gratings (differing by 60° from one another), without participants' awareness of the target grating. Behavioral data collected after the neurofeedback training showed improved sensitivity to the target grating as compared to the other two gratings. This finding suggested that induction of activity patterns in the primary visual areas was sufficient for perceptual learning. A study by deBettencourt et al. (2015) further showed improved sustained attention and reduced frequency of lapses in attention using closed-loop neurofeedback. Neurofeedback was provided to participants based on their level of attention to pictures of faces and scenes in a Go-NoGo task. Task difficulty was anti-correlated with the level of attention detected by the pattern classification algorithm. Pattern classification algorithm captured a widely distributed network of brain activity associated with top-down attentional control. The same pattern of activity was enhanced by neurofeedback training, which improved participants' attentional vigilance.

The above brief review of the current literature on rtfMRI demonstrates the potential that this technique has to study cognitive and behavioral modulation via brain activation. That is, learned self-regulation of a brain region, functional connectivity of two brain areas, or a network of brain areas can serve as independent variables in determining the effect of volitional control on cognition and behavior. Our summary also highlights the promise this novel approach offers for brain-behavior interventions. Importantly, to date, this exciting new tool has not yet been applied to aging research. In the remaining sections of this article we show that the use of rtfMRI in older adults is feasible and we propose that it constitutes a powerful technique to study cognitive function and the aging brain.

Feasibility of Applying rtfMRI Neurofeedback in Aging Research

We conducted a pilot study to determine the feasibility of using rtfMRI neurofeedback in research with older adults. In particular, we examined a neurofeedback training scheme in the context of an emotion perception paradigm in a sample of eight adults aged 61 years and older. Our study was based on evidence that aging is associated with emotional changes (Ebner et al., 2006; Blanchard-Fields, 2007; Scheibe and Carstensen, 2010; Ebner and Fischer, 2014). For example, apathy increases with age and is associated with cognitive decline (Brodaty et al., 2010) and constitutes one of the central causes of suffering of close relatives (Benoit et al., 2008) leading to poor quality of life (Yeager and Hyer, 2008). Also, aging is accompanied by less effective use of some emotion-regulatory strategies (Winecoff et al., 2011; Opitz et al., 2012), and increased difficulty in the perception of emotions in others (Ruffman et al., 2008; Ebner et al., 2010). This age-related decline has the potential to negatively impact emotional well-being and quality of social relationships (Ruffman et al., 2012), putting older adults at increased risk for social isolation and reduced health (Cornwell and Waite, 2009; Norman et al.,

2011). Research suggests that alterations in brain function associated with affective processing contribute to these emotional changes in aging (Williams et al., 2006; Samanez-Larkin and Carstensen, 2011; Winecoff et al., 2011; Ebner et al., 2012).

Our study particularly focused on volitional control of the anterior insula, a region in the limbic system that is crucially involved in affective processing such as evaluation and arousal (Berntson et al., 2011). Anterior insula function appears to be impacted by aging. In particular, there is evidence of dampened anterior insula activity in older adults, with effects on affective processing (Castle et al., 2012). Previous rtfMRI studies have shown that it is possible to train young adults (Caria et al., 2007, 2010) and schizophrenia patients (Ruiz et al., 2013a) to self-regulate anterior insula with contingent feedback, modulating affective processing.

Based on this evidence, we aimed to examine whether older adults could learn to self-regulate anterior insula with contingent rtfMRI neurofeedback and whether learned self-regulation could lead to behavioral modification in this age group. We used a single-ROI rtfMRI approach to train older adults to either up-regulate anterior insula (experimental group; EG) or primary auditory cortex (CG), a brain region not specifically associated with affective processing (Pavuluri et al., 2007; Tracy and Robins, 2008). Eight older adults (mean age: 66 $\pm$ 5.18 years; five women) participated in the study. Five participants were randomly assigned to the EG and three to the CG. The experiment consisted of six rtfMRI sessions conducted over a period of a couple of weeks. In the first and the last rtfMRI session, participants engaged in a facial emotion recognition task (for similar paradigms, see Ebner and Johnson, 2009; Ebner et al., 2012). Each participant undertook 18–20 neurofeedback training runs distributed among four rtfMRI based neurofeedback training sessions.

The study took place at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility of the McKnight Brain Institute (MBI), where brain imaging was conducted on a 3.0 Tesla, 32-channel Philips whole-body human MR scanner. As depicted in **Figure 2**, an rtfMRI based neurofeedback training run consisted of alternating baseline and up-regulation blocks, each lasting 30 s. There were six up-regulation and six baseline blocks in total per run. Participants were suggested to use imagery to recall emotionally relevant experiences. Neurofeedback was provided to them visually in the form of a thermometer. During up-regulation blocks, the graphical thermometer was presented over a green background and the bars in the thermometer were changed based on the up-regulation of BOLD signal that the participant achieved relative to the BOLD signal in the preceding baseline block. Increase in the number of bars of the thermometer represented more successful up-regulation performance by the participant. During the baseline block, the feedback bar remained stationary over a blue background.

Figure 3 shows an example rtfMRI run in a single subject showing the up-regulation of BOLD response in the right and left anterior insula, overlaid on an average EPI brain image. A moderate increase in BOLD signals in both left and right insula was observed during regulation blocks as compared to baseline blocks. ROI analysis (Poldrack, 2007) was conducted using BOLD values extracted from two rectangles, each of size 5×5 voxels ($\sim 15\ mm^2 \times 15\ mm^2$).

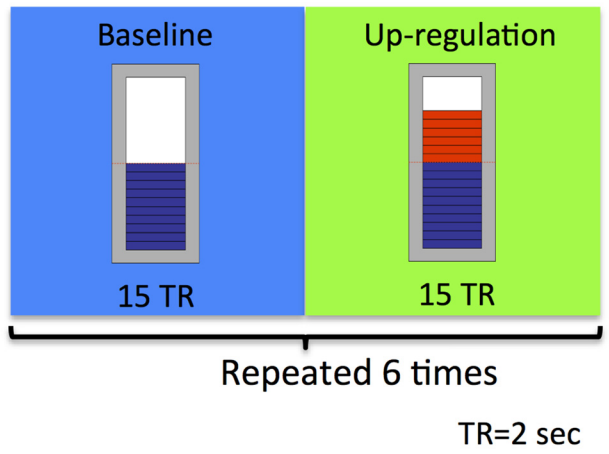


FIGURE 2 | Schematic illustration of the experimental neurofeedback paradigm used in our feasibility study. An rtfMRI-based neurofeedback training run consisted of alternating baseline and up-regulation blocks, repeated six times. Each block was 30 s long.

Figure 4 depicts results regarding older adults' ability to self-regulate a circumscribed brain area with contingent

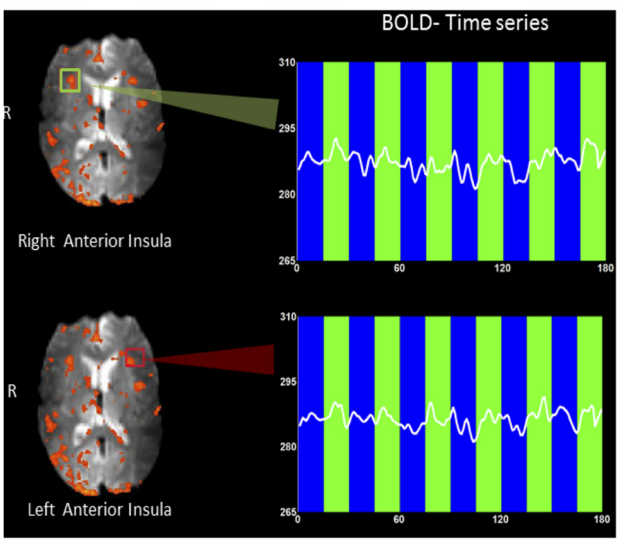


FIGURE 3 | An example rtfMRI run showing the up-regulation of BOLD response in the predetermined regions of interest (ROIs) of right (green rectangle) and left (red rectangle) anterior insula, overlaid on a mean EPI brain image. Mean BOLD signals were extracted from each of the two ROIs and are presented overlaid on baseline (blue bars) and up-regulation (green bars) blocks. A moderate increase in BOLD signal in both left and right insula (higher amplitude) was observed during regulation blocks as compared to baseline blocks.

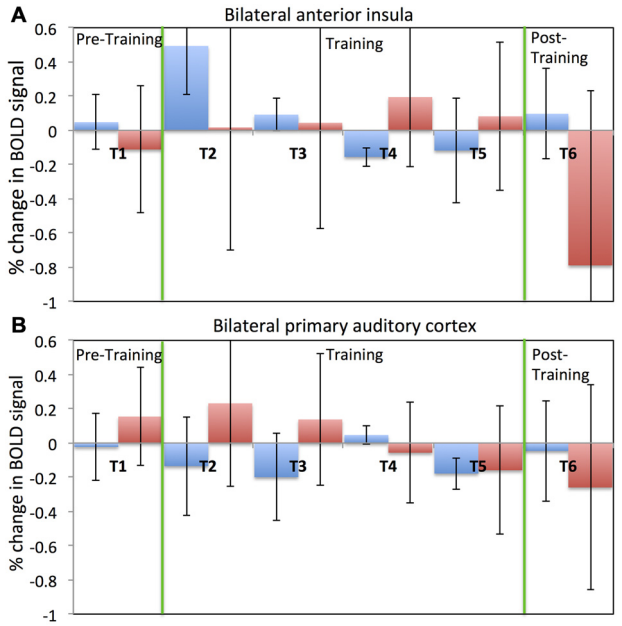


FIGURE 4 | Percentage change in BOLD signal for each run across all six fMRI sessions. Average percentage change in bilateral anterior insula cortex **(A)** for both the experimental (blue) and the control (red) groups. Average percentage change in bilateral primary auditory cortex **(B)** for both the experimental (blue) and the control (red) groups. Percentage change in the BOLD signal is calculated by comparing change in the BOLD signal during the regulation block to the previous baseline block. A positive value of the percentage change in BOLD signal indicates up-regulation of BOLD signal.

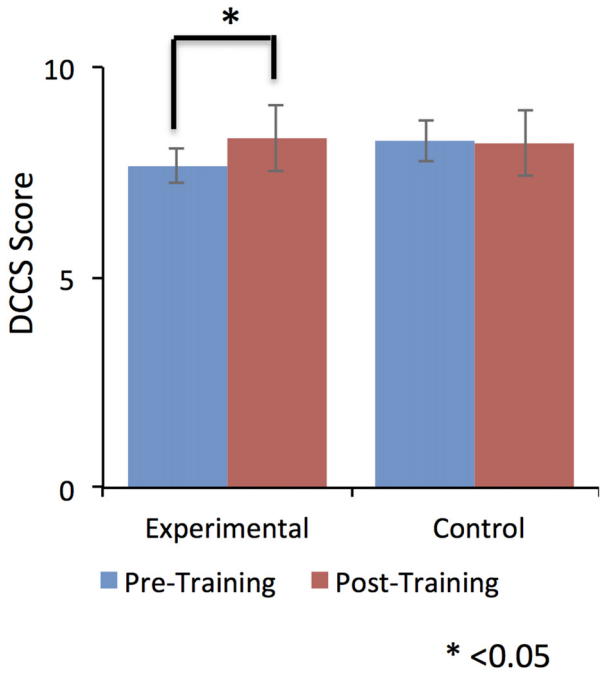


FIGURE 5 | Dimensional change card sort (DCCS) scores were significantly increased from pre-training to post-training in the experimental group (EG). No effect was observed for the control group (CG). This result suggests a positive effect of self-regulation of bilateral anterior insula on older adult's cognitive flexibility. Error bars represent the standard deviation in DCCS scores.

neurofeedback training. Participants in both the EG (see **Figure 4A**) and the CG (see **Figure 4B**) were able to up-regulate activity in anterior insula and primary auditory cortex, respectively, in the initial neurofeedback training sessions as reflected in positive values of the percentage change in the BOLD signal. Participants in the EG were able to achieve up-regulation of BOLD signal in the anterior insula for the first two training session (T2 and T3). However, their performance diminished in the fourth and fifth training session (T4 and T5). In the last session (T6), participants in the EG were able to up-regulate in six out of eight rtfMRI runs. A somewhat similar pattern of findings was observed in the CG (see **Figure 4B**): CG participants were able to up-regulate BOLD signal in the primary auditory cortex in the first two training sessions (T2 and T3). However, in later sessions, control participants were not able to maintain their performance.

Despite this dip in performance in the last two training sessions, participant in the EG (but not participants in the CG) performed relatively better in the post-training session compared to the pre-training session. Further, this self-regulation training lead to behavioral modification. In particular, there was an effect on participants' cognitive flexibility in the EG, but not in the CG. Cognitive flexibility was measured with the dimensional change card sort test (DCCS; Zelazo, 2006) from the cognitive test battery in the NIH toolbox (Heaton et al., 2014). As shown in **Figure 5**, the EG but not the CG showed a significant increase ($T = -3.9$, one tailed; $p = 0.008$) in cognitive flexibility post-training (8.31 ± 0.8) compared to pre-training (7.6 ± 0.63). This behavioral modification was specific to self-regulation training of the anterior insula, as it was not observed in the auditory cortex CG.

Possible factors that could have led to the dip in self-regulation performance observed in our study participants during the course of the training may have been related to the dual-task conflict inherent in the approach or increased fatigue as the study progressed. During neurofeedback training, to achieve self-regulation, participants have to apply a cognitive strategy to get positive feedback and simultaneously they have to evaluate the feedback presented to them. Therefore, participants have to switch between two tasks, which is cognitively demanding, particularly for older adults. Factors related to motivation and attention could also have affected task performance in the course of the study. Before every session start, participants responded to the short version of the Positive Affect and Negative Affect Scale (PANAS; Watson et al., 1988) to assess current mood. **Figure 6** depicts the average ratings for three different aspect of participants' current mood with possible relevance to the learning process, namely, novelty/motivation (comprised the adjectives interested, excited, inspired, enthusiastic, determined; see **Figure 6A**), attention (comprised the adjectives alert,

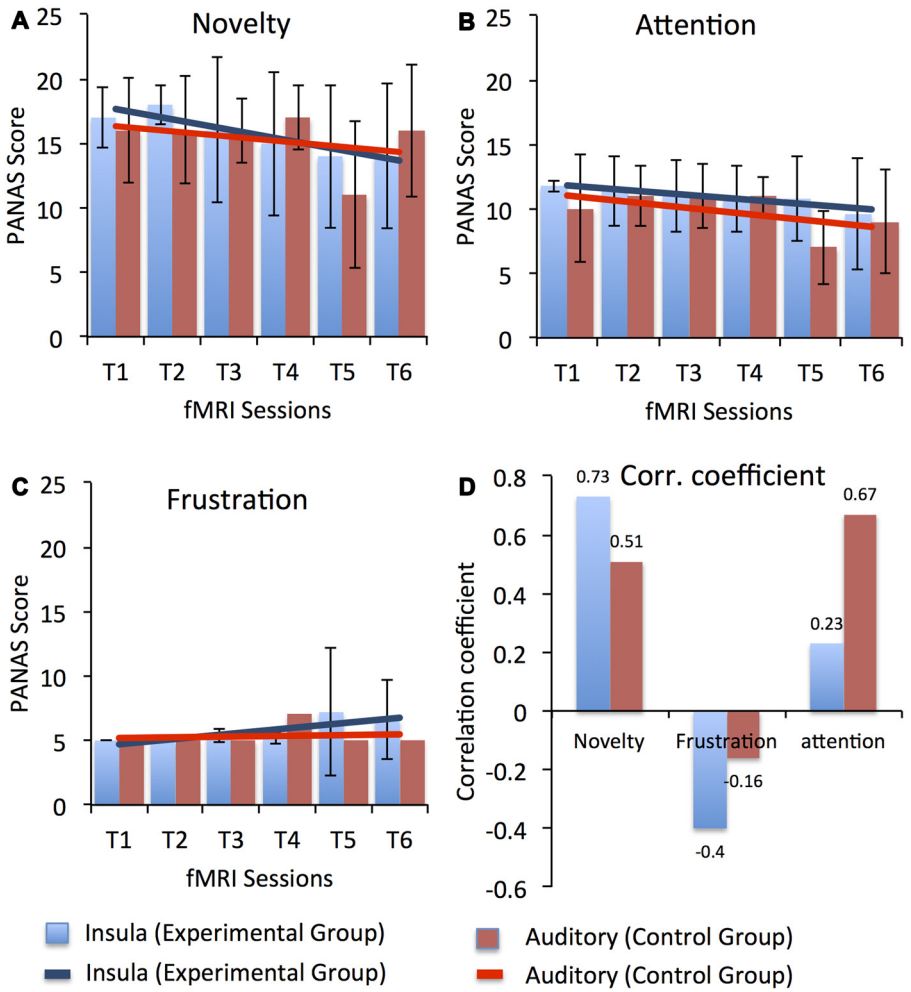


FIGURE 6 | Average Positive Affect and Negative Affect Scale (PANAS) score for three different aspect of participants' current mood with possible relevance to the learning process, namely, novelty/motivation (comprised the adjectives interested, excited, inspired, enthusiastic, determined), attention (comprised the adjectives alert, attentive, active) and frustration (comprised the adjectives distressed, downhearted, upset, frustrated, irritable) across all fMRI sessions for both the experimental (blue) and the control (red) groups. Scores for novelty, attention and frustration were calculated by averaging across the rating of respective adjectives. Error bars represent the standard deviation in the scores for novelty, attention and frustration across the group. Results from a linear regression analysis are represented by the solid blue and red lines for the experimental and the CGs, respectively. A gradual linear downward trend was observed in the ratings of novelty **(A)** and attention **(B)** across the sessions in both groups. However, a minute increase was observed in the ratings of frustration **(C)** in the later sessions for participants in both groups. High positive correlation coefficients **(D)** between up-regulation performance (i.e., % change in BOLD signal in bilateral anterior insula for the EG and in bilateral primary auditory cortex for the CG) and the PANAS scores were observed for novelty and attention, indicating that reduced motivation and attention levels might have led to reduced self-regulation performance. In contrast, a negative correlation was observed between PANAS scores for frustration and up-regulation performance in both groups, indicating that frustration did not affect participants' self-regulation performance.

attentive, active; see **Figure 6B**) and frustration (comprised the adjectives distressed, downhearted, upset, frustrated, irritable; see **Figure 6C**). A linear regression analysis showed a downward trend in the rating of novelty/motivation (EG: $y = -0.8X + 18.4$, $R^2 = 0.84$; CG: $y = -0.4X + 16.73$, $R^2 = 0.12$) and attention (EG: $y = -0.37X + 12.2$, $R^2 = 0.86$; CG: $y = -0.48X + 11.5$, $R^2 = 0.32$) for both the EG and the CG with duration in the study. Also, we observed high correlations (Pearson's correlation) between the percentage change in the BOLD signal for both target ROIs (i.e., bilateral anterior insula for EG and bilateral primary auditory cortex for CG) and the rating of novelty/motivation (EG: $r = 0.73$ and CG: $r = 0.51$) and attention (EG: $r = 0.23$ and CG: $r = 0.67$). In contrast, we observed a positive trend in the rating of frustration for both the EG ($y = 0.41X + 4.2$, $R^2 = 0.67$) and the CG ($y = 0.05X + 5.1$, $R^2 = 0.017$; see **Figure 6D**). The correlation between the percentage change in the BOLD signal for both target ROIs and the ratings of frustration, however, were negative for both the EG ($r = -0.4$) and the CG ($r = -0.16$). Thus, low motivation and reduced attention levels may have resulted in reduced self-

regulation performance as the training progressed. Supporting this explanation is our finding that participants' ability to up-regulate the anterior insula activity improved in the last session, in which the task was novel again since participants performed the self-regulation task along with the emotion perception task.

After every run, participants indicated what strategies they had used in order to control the thermometer bar. We explored these self-reports and found that the kind of strategies participants used did not differ across sessions in which they were more vs. less successful in up-regulation of brain activity. For example, participants in both groups reported the use of emotionally charged imagery during regulation blocks (e.g., thinking about a sick friend, an annoying colleague at work, spending quality time with family and friends, or engaging in hobbies). Participants did not appear to change their strategies when they experienced no up-regulation success, even though they had been instructed to change strategies if those did not result in positive feedback. During the neurofeedback training, older adults needed to assess their performance continuously based on the feedback provided to them and simultaneously change their strategy to improve up-regulation of brain activity. It is possible that lower levels of cognitive flexibility associated with age resulted in less ability of our older study participants (compared to young participants used in previous research) to switch from an unsuccessful to a more successful strategy.

Another reason for relatively less successful up-regulation in our older adults than is typically reported in the literature with young adults could be that the present study design used longer intervals between training sessions due to logistic reasons. All neurofeedback training sessions were conduct on the weekends and thus extended the total duration of the experiment to four weeks for six rtfMRI sessions. Thus, it is possible that the total amount of training that our participants received was sufficient, but that the interval between training sessions was too extended for full skill transfer.

Taken together, the results from our study support feasibility of the rtfMRI neurofeedback approach in healthy older adults. In addition to evidence of older adults' ability to volitionally up-regulate targeted brain regions, we observed improvement in cognitive flexibility scores of older adults. The potential of this novel technique in aging research will be discussed next as will be challenges that this line of work has to overcome in future applications with older populations.

Studying Healthy and Pathological Aging via rtfMRI Based Neurofeedback

There is ample evidence that alterations in structural and functional brain aging are associated with decline in cognitive function (Grady, 2012). The complexity of neural activity and cognitive functions, however, makes exact mapping between brain and behavior extraordinarily difficult, and so these relations remain largely speculative, although they are ultimately testable. Some of the proposed explanations for the decline in cognitive performance are age-related gray and white matter atrophy (Good et al., 2001), synaptic degeneration (Toth et al., 2012), low blood perfusion (Liu et al., 2012), and change in whole-brain connectivity (Ferreira et al., 2016) such as disconnectedness or dysfunctionality of brain networks (Tomasi and Volkow, 2012). As summarized above, several neurofeedback studies have shown that it is possible to obtain volitional control over a circumscribed region or networks of regions, with cognitive and behavioral effects (Shibata et al., 2011; Ghaziri et al., 2013; Ruiz et al., 2013a; Kim et al., 2015). In the rtfMRI approach, we constitute brain activity as the independent variable while cognition and behavior serve as the dependent variables. Thus, rtfMRI is a novel tool that can help us to test these proposed brain structural and functional explanations of cognitive decline in aging.

Aging is typically associated with overall decrease in gray matter. Some evidence suggests that the overall decrease in gray matter does not necessarily lead to cognitive decline; rather small decrease in gray matter in specific brain areas such as the insula (Good et al., 2001; Sowell et al., 2003), dorsolateral PFC (Grieve et al., 2005), and medial PFC (Uylings and de Brabander, 2002) may underlie age-related cognitive decline. A neurofeedback study on young adults showed significant increase in gray matter and white matter connectivity in areas related to attention (e.g., the intraparietal sulcus and the middle frontal gyrus) along with enhanced performance in visual and auditory attention after 13 weeks of neurofeedback training (Ghaziri et al., 2013). Thus, it is possible to increase gray matter in a particular brain area via neurofeedback training in young adults. The use of neurofeedback training in aging could similarly result in reduced rate of gray matter atrophy or even increase in gray matter in specific areas of the brain with possible effects on improved cognitive functioning in aging.

A number of studies have suggested that the aging brain has the potential to re-organize neural activity to compensate for anatomical and physiological change such as proposed in HAROLD (Cabeza, 2002) and PASA (Davis et al., 2008). This re-organization of brain activity is considered to be a compensatory mechanism by the aging brain to counter physiological deficit. In particular, these effects are observed in high-preforming when compared with low-preforming older adults in various domains of cognition such as episodic memory retrieval (Madden et al., 1999; Grady, 2002), episodic memory encoding (Logan and Buckner, 2001; Stebbins et al., 2002), working memory (Dixit et al., 2000; Reuter-Lorenz et al., 2000), perception (Grady et al., 1994; Grady, 2002), and inhibitory control (Nielson et al., 2002). Important questions that have not been answered yet are why some but not other older adults show this compensatory neural re-organization, and to what extent it is possible to train low-preforming older adults to use compensatory neural re-organization for performance improvement. As mentioned earlier, rtfMRI studies have demonstrated increase in connectivity between two brain areas or a network of regions using neurofeedback training (Koush

et al., 2013; Kim et al., 2015). This raises the possibility that rtfMRI based neurofeedback training could be used in older adults to enhance compensatory neural re-organization towards performance improvement. This would importantly inform current models of aging and significantly advance scientific understanding of neural mechanisms in the aging brain.

There is evidence of some qualitative similarity between cognitive decline in pathological and in healthy aging, even though pathological and healthy brain aging differ in the rate and extent of the cognitive decrements (Walhovd et al., 2014). Both AD and Parkinson's disease (PD) are characterized by memory difficulties, slowed processing speed, impaired attention, visuoperceptual/visuospatial dysfunction, and dysexecutive syndrome (Weiner et al., 2013; Todorova et al., 2014). Neuroimaging evidence further supports disturbed functional connectivity between the frontal and parietal lobes in AD patients (Wang et al., 2007; Zhang et al., 2010), and dysfunction of cortico-striatal functional connectivity in PD (Kwak et al., 2010). Neurofeedback training studies have reported improvement in memory (Berman and Frederick, 2009) and verbal comprehension (Becerra et al., 2012) in AD patients. These studies used EEG based neurofeedback. Recently rtfMRI based neurofeedback has been used to train PD patients. Patients learned to increase activity in the supplementary motor area (SMA) and subsequently improved their speed of finger tapping (Subramanian et al., 2011); but see Buyukturkoglu et al. (2013) for a contradictory finding. Thus, there is some initial evidence suggesting neurofeedback training success in pathological aging.

Challenges in Using rtfMRI Based Neurofeeback in Aging Populations

Evidence of neurofeedback success in young adults and clinical populations summarized throughout this article, combined with the feasibility data of healthy older adults from our group, suggest that rtfMRI based neurofeedback may be a potential tool to study the aging brain and to inform development of interventions to maintain cognitive function and defray cognitive decline in older adults. This exciting new approach to the study of cognitive and brain aging, however, also faces challenges.

For example, in our feasibility study participants received contingent rtfMRI neurofeedback, but were only able to maintain moderate levels of BOLD up-regulation in the anterior insula or the primary auditory cortex, respectively. As discussed, possible explanations for these moderate levels of self-regulation found in our study may come from fatigue and lack of novelty when older adults engage in training sessions that are highly cognitively demanding. Future studies need to apply conditioning paradigms like shaping to improve self-regulation success (Peterson, 2004). In shaping, small changes towards the desired behavior are rewarded, which leads to gradual change across successive trials. Thus, the new method would change the baseline BOLD value after each TR so that any gradual change towards the desired BOLD signal value is rewarded. The dual-task conflict that may underlie relatively lower self-regulation performance in older adults also needs to be addressed in future studies in the attempt to reduce the cognitive demand in neurofeedback training. One alternative could be to reduce the frequency at which feedback is presented, as this would reduce the overall cognitive load of continuous evaluation of feedback. Also, due to logistic reasons in our feasibility study, we had to conduct the neurofeedback training on the weekends. This extended the total duration of the experiment to a couple of weeks for six rtfMRI sessions, which is longer than a typical rtfMRI study. Thus, it is possible that the total amount of training that our participants received was sufficient, but that the interval between training runs was sub-optimal for learning.

The issue of slow learners, which may particularly apply to older adults, can be addressed in future research by increasing the number of training sessions, e.g., 13 weeks of neurofeedback training (Ghaziri et al., 2013). However, greater numbers of training sessions will drastically increase the cost of conducting a study to the extent where this approach would not be feasible anymore as clinical intervention given the high costs for MRI. Therefore, development of less cost-intensive neurofeedback training methods based on cheaper modalities such as EEG or fNIRS is crucial. However, each of these alternative modalities comes with a set of limitations. For example, both EEG and fNIRS cannot be used for self-regulation of deeper brain region (e.g., anterior insula, amygdala), which are particularly relevant for emotion processing and thus will limit domains of study for these techniques. A useful approach for future research is to start neurofeedback training with rtfMRI and later transition to cheaper modalities. Along these lines, a technique called "EEG Finger-Print" was developed (Meir-Hasson et al., 2014). In this approach, advanced signal processing to remove artifacts and machine learning algorithms are applied on EEG data acquired simultaneously with fMRI to find EEG features that can predict specific deeper brain activity. With this approach, an experiment can be designed in which older adults initially learn self-regulation of a circumscribed brain region or network of brain regions by using rtfMRI based neurofeedback training. During the rtfMRI training, simultaneous EEG recordings will be conducted to determine the neuroelectric components that correlate with the volitional control of the ROIs. Using the EEG Finger-Print technique, the EEG neural-correlates of volitional control of deeper brain region could be identified. The identified EEG pattern can be used to continue neurofeedback training via EEG without fMRI. This would make long neurofeedback training studies cost-effective and more flexible (i.e., portable, convenient system). The EEG Finger-Print technique could also target older adults who have implanted stents, pacemakers, or other metallic implants in their body and hence are unable to participate in MRI experiments. This would allow test of a more representative sample of older adults.

Another factor that could influence learning in older adults pertains to the way instructions are given and the type of feedback. According to Knowles' theory of adult learning (Knowles, 1984), older adults learn better if they are aware of the

background of the topic. Also, readiness to learn and motivation to attain new information are critical factors, which is in line with the current study showing that lower motivation levels reduced the performance in self-regulation. Therefore, in future studies, it will be important to instruct older adults in a way that keeps them motivated and provides them with background information pertaining to the study. For example, information can be presented to older adults in a manner that is personally relevant to them (Zurakowski et al., 2006). In this context, it is also necessary to explore new ways of providing feedback apart from traditional feedback modalities such as the use of virtual reality where feedback can be associated with certain events in the virtual world and may thus be more intuitively processed and eventually more effective.

CONCLUSION

Age-related cognitive decline is of increasing societal, political, and economic concern, and dramatically affects individual lives. Improvement of neuroimaging techniques has advanced the investigation of cognitive decline in aging with a particular focus on brain processes underlying age-related change. Although this research field has benefited greatly from recent advancements in imaging technology, there are still a number of unresolved issues such as pertaining to age-related change in brain structure and function underlying inter-individual variation of cognitive decline in aging or validation of proposed theories of aging related to loss of gray matter in certain regions of the brain as well as hypoactivation of brain areas or networks. We propose that rtfMRI neurofeedback offers a potent tool to study cognitive decline processes towards development of effective training and intervention protocols in aging. Preliminary results of our feasibility study suggest that it is possible for older individuals to volitionally control a circumscribed brain area and that neurofeedback training of anterior insula is associated with increased cognitive flexibility, supporting benefits of this technique in use with older adults. However, this nascent field faces some challenges that need to be overcome for advanced application in aging. We hope that this article will spur research in unexplored areas of cognitive aging, towards development of effective intervention programs to promote cognitive health in older adults.

AUTHOR CONTRIBUTIONS

MR: design of the work, data acquisition, fMRI analysis and writing the article. AQV: design of the work, data acquisition, behavioral analysis. AD: data acquisition, fMRI analysis. RAC: final approval of the manuscript. RS and NCE: design of the work, interpretation of results and final approval of the manuscript.

FUNDING

This work was supported by the Center for Cognitive Aging and Memory, Clinical Translational Research Program (CAM-CTRP) in the Institute on Aging, the Department of Psychology and the Department of Biomedical Engineering at University of Florida.

ACKNOWLEDGMENTS

The authors are grateful to Ian Frazier, Lily Nelson, and the research team from the Social-Cognitive and Affective Development lab for assistance in study implementation, data collection and data management.

REFERENCES

Bandettini, P. A., Wong, E. C., Hinks, R. S., Tikofsky, R. S., and Hyde, J. S. (1992). Time course EPI of human brain function during task activation. *Magn. Reson. Med.* 25, 390–397. doi: 10.1002/mrm.1910250220

Becerra, J., Fernández, T., Roca-Stappung, M., Díaz-Comas, L., Galán, L., Bosch, J., et al. (2012). Neurofeedback in healthy elderly human subjects with electroencephalographic risk for cognitive disorder. *J. Alzheimers Dis.* 28, 357–367. doi: 10.3233/JAD-2011-111055

Benoit, M., Andrieu, S., Lechowski, L., Gillette-Guyonnet, S., Robert, P. H., and Vellas, B. (2008). Apathy and depression in Alzheimer's disease are associated with functional deficit and psychotropic prescription. *Int. J. Geriatr. Psychiatry* 23, 409–414. doi: 10.1002/gps.1895

Berman, M. H., and Frederick, J. A. (2009). Efficacy of neurofeedback for executive and memory function in dementia. *Alzheimers Dement.* 5:e8. doi: 10.1016/j.jalz.2009.07.046

Berntson, G. G., Norman, G. J., Bechara, A., Bruss, J., Tranel, D., and Cacioppo, J. T. (2011). The insula and evaluative processes. *Psychol. Sci.* 22, 80–86. doi: 10.1177/0956797610391097

Birbaumer, N. (2006). Breaking the silence: brain-computer interfaces (BCI) for communication and motor control. *Psychophysiology* 43, 517–532. doi: 10.1111/j.1469-8986.2006.00456.x

Birbaumer, N., Ruiz, S., and Sitaram, R. (2013). Learned regulation of brain metabolism. *Trends Cogn. Sci.* 17, 295–302. doi: 10.1016/j.tics.2013.04.009

Blanchard-Fields, F. (2007). Everyday problem solving and emotion—An adult developmental perspective. *Curr. Dir. Psychol. Sci.* 6, 26–31. doi: 10.1111/j.1467-8721.2007.00469.x

Brodaty, H., Altendorf, A., Withall, A., and Sachdev, P. S. (2010). Mortality and institutionalization in early survivors of stroke: the effects of cognition, vascular mild cognitive impairment and vascular dementia. *J. Stroke Cerebrovasc. Dis.* 19, 485–493. doi: 10.1016/j.jstrokecerebrovasdis.2009.09.006

Buchler, N. G., Hoyer, W. J., and Cerella, J. (2008). Rules and more rules: the effects of multiple tasks, extensive training and aging on task-switching performance. *Mem. Cognit.* 36, 735–748. doi: 10.3758/mc.36.4.735

Buyukturkoglu, K., Rana, M., Ruiz, S., Hackley, S. A., Soekadar, S. R., Birbaumer, N., et al. (2013). "Volitional regulation of the supplementary motor area with fMRI-BCI neurofeedback in Parkinson's disease: a pilot study," in *6th International IEEE/EMBS Conference*, (San Diego, CA), 677–681.

Cabeza, R. (2002). Hemispheric asymmetry reduction in older adults: the HAROLD model. *Psychol. Aging* 17, 85–100. doi: 10.1037/0882-7974.17.1.85

Cabeza, R., Daselaar, S. M., Dolcos, F., Prince, S. E., Budde, M., and Nyberg, L. (2004). Task-independent and task-specific age effects on brain activity during working memory, visual attention and episodic retrieval. *Cereb. Cortex* 14, 364–375. doi: 10.1093/cercor/bhg133

Caria, A., Sitaram, R., Veit, R., Begliomini, C., and Birbaumer, N. (2010). Volitional control of anterior insula activity modulates the response to aversive stimuli. A real-time functional magnetic resonance imaging

study. *Biol. Psychiatry* 68, 425–432. doi: 10.1016/j.biopsych.2010.04.020

Caria, A., Veit, R., Sitaram, R., Lotze, M., Weiskopf, N., Grodd, W., et al. (2007). Regulation of anterior insular cortex activity using real-time fMRI. *Neuroimage* 35, 1238–1246. doi: 10.1016/j.neuroimage.2007.01.018

Casel, C. (2002). Use it or lose it: activity may be the best treatment for Aging. *JAMA* 18, 2333–2334. doi: 10.1001/jama.288.18.2333

Castle, E., Eisenberger, N. I., Seeman, T. E., Moons, W. G., Boggero, I. A., Grinblatt, M. S., et al. (2012). Neural and behavioral bases of age differences in perceptions of trust. *Proc. Natl. Acad. Sci. U S A* 109, 20848–20852. doi: 10.1073/pnas.1218518109

Census, U. S. (2012). Age and sex. Available online at: https://www.census.gov/population/age/data/2012comp.html

Cornwell, E. Y., and Waite, L. J. (2009). Social disconnectedness, perceived isolation and health among older adults. *J. Health Soc. Behav.* 50, 31–48. doi: 10.1177/002214650905000103

Davis, S. W., Dennis, N. A., Daselaar, S. M., Fleck, M. S., and Cabeza, R. (2008). Que PASA? The posterior-anterior shift in aging. *Cereb. Cortex* 18, 1201–1209. doi: 10.1093/cercor/bhm155

deBettencourt, M. T., Cohen, J. D., Lee, R. F., Norman, K. A., and Turk-Browne, N. B. (2015). Closed-loop training of attention with real-time brain imaging. *Nat. Neurosci.* 18, 470–475. doi: 10.1038/nn.3940

deCharms, R. C., Maeda, F., Glover, G. H., Ludlow, D., Pauly, J. M., Soneji, D., et al. (2005). Control over brain activation and pain learned by using real-time functional MRI. *Proc. Natl. Acad. Sci. U S A* 102, 18626–18631. doi: 10.1073/pnas.0505210102

Dixit, N. K., Gerton, B. K., Dohn, P., Meyer-Lindenberg, A., and Berman, K. F. (2000). "Age-related changes in rCBF activation during an N-Back working memory paradigm occur prior to age 50," in *Paper Presented at Human Brain Mapping Meeting*, (San Antonio, TX).

Ebner, N. C., and Fischer, H. (2014). Studying the various facets of emotional aging. *Front. Psychol.* 5:1007. doi: 10.3389/fpsyg.2014.01007

Ebner, N. C., Freund, A. M., and Baltes, P. B. (2006). Developmental changes in personal goal orientation from young to late adulthood: from striving for gains to maintenance and prevention of losses. *Psychol. Aging* 21, 664–678. doi: 10.1037/0882-7974.21.4.664

Ebner, N. C., and Johnson, M. K. (2009). Young and older emotional faces: are there age group differences in expression identification and memory? *Emotion* 9, 329–339. doi: 10.1037/a0015179

Ebner, N. C., Johnson, M. K., and Fischer, H. (2012). Neural mechanisms of reading facial emotions in young and older adults. *Front. Psychol.* 3:223. doi: 10.3389/fpsyg.2012.00223

Ebner, N. C., Riediger, M., and Lindenberger, U. (2010). FACES—a database of facial expressions in young, middle-aged and older women and men: development and validation. *Behav. Res. Methods* 42, 351–362. doi: 10.3758/BRM.42.1.351

Eckert, M. A., Keren, N. I., Roberts, D. R., Calhoun, V. D., and Harris, K. C. (2010). Age-related changes in processing speed: unique contributions of cerebellar and prefrontal cortex. *Front. Hum. Neurosci.* 4:10. doi: 10.3389/neuro.09.010.2010

Ferreira, L. K., Regina, A. C., Kovacevic, N., Martin Mda, G., Santos, P. P., Carneiro, C. G., et al. (2016). Aging effects on whole-brain functional connectivity in adults free of cognitive and psychiatric disorders. *Cereb. Cortex* 26, 3851–3865. doi: 10.1093/cercor/bhv190

Friston, K. J. (2011). Functional and effective connectivity: a review. *Brain Connect.* 1, 13–36. doi: 10.1089/brain.2011.0008

Friston, K. J., and Frith, C. D. (1995). Schizophrenia: a disconnection syndrome? *Clin. Neurosci.* 3, 89–97.

Friston, K. J., Harrison, L., and Penny, W. (2003). Dynamic causal modelling. *Neuroimage* 19, 1273–1302. doi: 10.1016/s1053-8119(03)00202-7

Fuchs, T., Birbaumer, N., Lutzenberger, W., Gruzelier, J. H., and Kaiser, J. (2003). Neurofeedback treatment for attention-deficit/hyperactivity disorder in children: a comparison with methylphenidate. *Appl. Psychophysiol. Biofeedback* 28, 1–12. doi: 10.1023/A:1022353731579

Gazzaley, A., Cooney, J. W., Rissman, J., and D'Esposito, M. (2005). Top-down suppression deficit underlies working memory impairment in normal aging. *Nat. Neurosci.* 8, 1298–1300. doi: 10.1038/nn1543

Ghaziri, J., Tucholka, A., Larue, V., Blanchette-Sylvestre, M., Reyburn, G., Gilbert, G., et al. (2013). Neurofeedback training induces changes in white and gray matter. *Clin. EEG Neurosci.* 44, 265–272. doi: 10.1177/1550059413476031

Goh, J. O., and Park, D. C. (2009). Neuroplasticity and cognitive aging: the scaffolding theory of aging and cognition. *Restor. Neurol. Neurosci.* 27, 391–403. doi: 10.3233/RNN-2009-0493

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N., Friston, K. J., and Frackowiak, R. S. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 14, 21–36. doi: 10.1006/nimg.2001.0786

Grady, C. L. (2002). Introduction to the special section on aging, cognition and neuroimaging. *Psychol. Aging* 17, 3–6. doi: 10.1037/0882-7974.17.1.3

Grady, C. (2012). The cognitive neuroscience of ageing. *Nat. Rev. Neurosci.* 13, 491–505. doi: 10.1038/nrn3256

Grady, C. L., Maisog, J. M., Horwitz, B., Ungerleider, L. G., Mentis, M. J., Salerno, J. A., et al. (1994). Age-related changes in cortical blood flow activation during visual processing of faces and location. *J. Neurosci.* 14, 1450–1462.

Granger, C. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica* 37, 424–438. doi: 10.2307/1912791

Grieve, S. M., Clark, C. R., Williams, L. M., Peduto, A. J., and Gordon, E. (2005). Preservation of limbic and paralimbic structures in aging. *Hum. Brain Mapp.* 25, 391–401. doi: 10.1002/hbm.20115

Gröne, M., Dyck, M., Koush, Y., Bergert, S., Mathiak, K. A., Alawi, E. M., et al. (2015). Upregulation of the rostral anterior cingulate cortex can alter the perception of emotions: fMRI-based neurofeedback at 3 and 7 T. *Brain Topogr.* 28, 197–207. doi: 10.1007/s10548-014-0384-4

Grossman, M., Cooke, A., DeVita, C., Alsop, D., Detre, J., Chen, W., et al. (2002). Age-related changes in working memory during sentence comprehension: an fMRI study. *Neuroimage* 15, 302–317. doi: 10.1006/nimg.2001.0971

Hamilton, J. P., Chen, G., Thomason, M. E., Schwartz, M. E., and Gotlib, I. H. (2011). Investigating neural primacy in Major Depressive Disorder: multivariate Granger causality analysis of resting-state fMRI time-series data. *Mol. Psychiatry* 16, 763–772. doi: 10.1038/mp.2010.46

Hanson, S. J., Matsuka, T., and Haxby, J. V. (2004). Combinatorial codes in ventral temporal lobe for object recognition: Haxby (2001) revisited: is there a "face" area? *Neuroimage* 23, 156–166. doi: 10.1016/j.neuroimage.2004.05.020

Haynes, J. D. (2015). A primer on pattern-based approaches to fMRI: principles, pitfalls and perspectives. *Neuron* 87, 257–270. doi: 10.1016/j.neuron.2015.05.025

Haynes, J. D., and Rees, G. (2006). Decoding mental states from brain activity in humans. *Nat. Rev. Neurosci.* 7, 523–534. doi: 10.1038/nrn1931

Heaton, R. K., Akshoomoff, N., Tulsky, D., Mungas, D., Weintraub, S., Dikmen, S., et al. (2014). Reliability and validity of composite scores from the NIH Toolbox Cognition Battery in adults. *J. Int. Neuropsychol. Soc.* 20, 588–598. doi: 10.1017/s1355617714000241

Honey, G. D., Pomarol-Clotet, E., Corlett, P. R., Honey, R. A., McKenna, P. J., Bullmore, E. T., et al. (2005). Functional dysconnectivity in schizophrenia associated with attentional modulation of motor function. *Brain* 128, 2597–2611. doi: 10.1093/brain/awh632

Johnson, K. A., Hartwell, K., LeMatty, T., Borckardt, J., Morgan, P. S., Govindarajan, K., et al. (2012). Intermittent real-time fMRI feedback is superior to continuous presentation for a motor imagery task: a pilot study. *J. Neuroimaging* 22, 58–66. doi: 10.1111/j.1552-6569.2010.00529.x

Just, M. A., Cherkassky, V. L., Keller, T. A., Kana, R. K., and Minshew, N. J. (2007). Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. *Cereb. Cortex* 17, 951–961. doi: 10.1093/cercor/bhl006

Kim, D. Y., Yoo, S. S., Tegethoff, M., Meinlschmidt, G., and Lee, J. H. (2015). The inclusion of functional connectivity information into fMRI-based neurofeedback improves its efficacy in the reduction of cigarette cravings. *J. Cogn. Neurosci.* 27, 1552–1572. doi: 10.1162/jocn_a_00802

Knowles, M. (1984). *The Adult Learner: A Neglected Species.* Houston, TX: Gulf Publishing.

Konrad, K., and Eickhoff, S. B. (2010). Is the ADHD brain wired differently? A review on structural and functional connectivity in attention deficit hyperactivity disorder. *Hum. Brain Mapp.* 31, 904–916. doi: 10.1002/hbm.21058

Kotchoubey, B., Strehl, U., Uhlmann, C., Holzapfel, S., König, M., Fröscher, W., et al. (2001). Modification of slow cortical potentials in patients with refractory epilepsy: a controlled outcome study. *Epilepsia* 42, 406–416. doi: 10.1046/j.1528-1157.2001.22200.x

Koush, Y., Rosa, M. J., Robineau, F., Heinen, K., Rieger, S. W., Weiskopf, N., et al. (2013). Connectivity-based neurofeedback: dynamic causal modeling for real-time fMRI. *Neuroimage* 81, 422–430. doi: 10.1016/j.neuroimage.2013.05.010

Kubler, A., Kotchoubey, B., Kaiser, J., Wolpaw, J. R., and Birbaumer, N. (2001). Brain-computer communication: unlocking the locked in. *Psychol. Bull.* 127, 358–375. doi: 10.1037/0033-2909.127.3.358

Kwak, Y., Peltier, S., Bohnen, N. I., Müller, M. L., Dayalu, P., and Seidler, R. D. (2010). Altered resting state cortico-striatal connectivity in mild to moderate stage Parkinson's disease. *Front. Syst. Neurosci.* 4:143. doi: 10.3389/fnsys.2010.00143

LaConte, S., Anderson, J., Muley, S., Ashe, J., Frutiger, S., Rehm, K., et al. (2003). The evaluation of preprocessing choices in single-subject BOLD fMRI using NPAIRS performance metrics. *Neuroimage* 18, 10–27. doi: 10.1006/nimg.2002.1300

LaConte, S. M., Peltier, S. J., and Hu, X. P. (2007). Real-time fMRI using brain-state classification. *Hum. Brain Mapp.* 28, 1033–1044. doi: 10.1002/hbm.20326

LaConte, S., Strother, S., Cherkassky, V., Anderson, J., and Hu, X. (2005). Support vector machines for temporal classification of block design fMRI data. *Neuroimage* 26, 317–329. doi: 10.1016/j.neuroimage.2005.01.048

Lee, S., Ruiz, S., Caria, A., Veit, R., Birbaumer, N., and Sitaram, R. (2011). Detection of cerebral reorganization induced by real-time fMRI feedback training of insula activation: a multivariate investigation. *Neurorehabil. Neural Repair* 25, 259–267. doi: 10.1177/1545968310385128

Lewis-Peacock, J. A., and Norman, K. A. (2014). Competition between items in working memory leads to forgetting. *Nat. Commun.* 5:5768. doi: 10.1038/ncomms6768

Li, H.-J., Hou, X.-H., Liu, H.-H., Yue, C.-L., Lu, G.-M., and Zuo, X.-N. (2015). Putting age-related task activation into large-scale brain networks: a meta-analysis of 114 fMRI studies on healthy aging. *Neurosci. Biobehav. Rev.* 57, 156–174. doi: 10.1016/j.neubiorev.2015.08.013

Liew, S.-L., Rana, M., Cornelsen, S., Fortunato de Barros Filho, M., Birbaumer, N., Sitaram, R., et al. (2015). Improving motor corticothalamic communication after stroke using real-time fMRI connectivity-based neurofeedback. *Neurorehabil. Neural Repair* 30, 671–675. doi: 10.1177/1545968315619699

Liu, Y., Zhu, X., Feinberg, D., Guenther, M., Gregori, J., Weiner, M. W., et al. (2012). Arterial spin labeling MRI study of age and gender effects on brain perfusion hemodynamics. *Magn. Reson. Med.* 68, 912–922. doi: 10.1002/mrm.23286

Logan, J. M., and Buckner, R. L. (2001). *Age-Related Changes in Neural Correlates of Encoding*. New York, NY: Cognitive Neuroscience Society.

Logsdon, R. G., Gibbons, L. E., McCurry, S. M., and Teri, L. (2002). Assessing quality of life in older adults with cognitive impairment. *Psychosom. Med.* 64, 510–519. doi: 10.1097/00006842-200205000-00016

Madden, D. J., Gottlob, L. R., Denny, L. L., Turkington, T. G., Provenzale, J. M., Hawk, T. C., et al. (1999). Aging and recognition memory: changes in regional cerebral blood flow associated with components of reaction time distributions. *J. Cogn. Neurosci.* 11, 511–520. doi: 10.1162/089892999563571

Martínez-Ramón, M., Koltchinskii, V., Heileman, G. L., and Posse, S. (2006). fMRI pattern classification using neuroanatomically constrained boosting. *Neuroimage* 31, 1129–1141. doi: 10.1016/j.neuroimage.2006.01.022

Meir-Hasson, Y., Kinreich, S., Podlipsky, I., Hendler, T., and Intrator, N. (2014). An EEG finger-print of fMRI deep regional activation. *Neuroimage* 102, 128–141. doi: 10.1016/j.neuroimage.2013.11.004

Meunier, D., Stamatakis, E. A., and Tyler, L. K. (2014). Age-related functional reorganization, structural changes and preserved cognition. *Neurobiol. Aging* 35, 42–54. doi: 10.1016/j.neurobiolaging.2013.07.003

Mourão-Miranda, J., Reynaud, E., McGlone, F., Calvert, G., and Brammer, M. (2006). The impact of temporal compression and space selection on SVM analysis of single-subject and multi-subject fMRI data. *Neuroimage* 33, 1055–1065. doi: 10.1016/j.neuroimage.2006.08.016

Murase, N., Duque, J., Mazzocchio, R., and Cohen, L. G. (2004). Influence of interhemispheric interactions on motor function in chronic stroke. *Ann. Neurol.* 55, 400–409. doi: 10.1002/ana.10848

Nielson, K. A., Langenecker, S. A., and Garavan, H. (2002). Differences in the functional neuroanatomy of inhibitory control across the adult life span. *Psychol. Aging* 17, 56–71. doi: 10.1037//0882-7974.17.1.56

Norman, G. J., Cacioppo, J. T., Morris, J. S., Karelina, K., Malarkey, W. B., Devries, A. C., et al. (2011). Selective influences of oxytocin on the evaluative processing of social stimuli. *J. Psychopharmacol.* 25, 1313–1319. doi: 10.1177/0269881110367452

Opitz, P. C., Rauch, L. C., Terry, D. P., and Urry, H. L. (2012). Prefrontal mediation of age differences in cognitive reappraisal. *Neurobiol. Aging* 33, 645–655. doi: 10.1016/j.neurobiolaging.2010.06.004

Paret, C., Kluetsch, R., Ruf, M., Demirakca, T., Hoesterey, S., Ende, G., et al. (2014). Down-regulation of amygdala activation with real-time fMRI neurofeedback in a healthy female sample. *Front. Behav. Neurosci.* 8:299. doi: 10.3389/fnbeh.2014.00299

Park, D. C., Welsh, R. C., Marshuetz, C., Gutchess, A. H., Mikels, J., Polk, T. A., et al. (2003). Working memory for complex scenes: age differences in frontal and hippocampal activations. *J. Cogn. Neurosci.* 15, 1122–1134. doi: 10.1162/089892903322598094

Pavuluri, M. N., O'Connor, M. M., Harral, E., and Sweeney, J. A. (2007). Affective neural circuitry during facial emotion processing in pediatric bipolar disorder. *Biol. Psychiatry* 62, 158–167. doi: 10.1016/j.biopsych.2006.07.011

Pereira, F., Mitchell, T., and Botvinick, M. (2009). Machine learning classifiers and fMRI: a tutorial overview. *Neuroimage* 45, S199–S209. doi: 10.1016/j.neuroimage.2008.11.007

Peterson, G. B. (2004). A day of great illumination: B. F. Skinner's discovery of shaping. *J. Exp. Anal. Behav.* 82, 317–328. doi: 10.1901/jeab.2004.82-317

Poldrack, R. A. (2007). Region of interest analysis for fMRI. *Soc. Cogn. Affect. Neurosci.* 2, 67–70. doi: 10.1093/scan/nsm006

Posse, S., Fitzgerald, D., Gao, K., Habel, U., Rosenberg, D., Moore, G. J., et al. (2003). Real-time fMRI of temporolimbic regions detects amygdala activation during single-trial self-induced sadness. *Neuroimage* 18, 760–768. doi: 10.1016/s1053-8119(03)00004-1

Prakash, R. S., Erickson, K. I., Colcombe, S. J., Kim, J. S., Voss, M. W., and Kramer, A. F. (2009). Age-related differences in the involvement of the prefrontal cortex in attentional control. *Brain Cogn.* 71, 328–335. doi: 10.1016/j.bandc.2009.07.005

Ram, N., Gerstorf, D., Lindenberger, U., and Smith, J. (2011). Developmental change and intraindividual variability: relating cognitive aging to cognitive plasticity, cardiovascular lability and emotional diversity. *Psychol. Aging* 26, 363–371. doi: 10.1037/a0021500

Rana, M., Gupta, N., Dalboni Da Rocha, J. L., Lee, S., and Sitaram, R. (2013). A toolbox for real-time subject-independent and subject-dependent classification of brain states from fMRI signals. *Front. Neurosci.* 7:170. doi: 10.3389/fnins.2013.00170

Reuter-Lorenz, P. A., and Cappell, K. (2008). Neurocognitive aging and the compensation hypothesis. *Curr. Dir. Psychol. Sci.* 18, 177–182. doi: 10.1111/j.1467-8721.2008.00570.x

Reuter-Lorenz, P. A., Jonides, J., Smith, E. E., Hartley, A., Miller, A., Marshuetz, C., et al. (2000). Age differences in the frontal lateralization of verbal and spatial working memory revealed by PET. *J. Cogn. Neurosci.* 12, 174–187. doi: 10.1162/089892900561814

Rota, G., Sitaram, R., Veit, R., Erb, M., Weiskopf, N., Dogil, G., et al. (2009). Self-regulation of regional cortical activity using real-time fMRI: the right inferior frontal gyrus and linguistic processing. *Hum. Brain Mapp.* 30, 1605–1614. doi: 10.1002/hbm.20621

Ruffman, T., Henry, J. D., Livingstone, V., and Phillips, L. H. (2008). A meta-analytic review of emotion recognition and aging: implications for neuropsychological models of aging. *Neurosci. Biobehav. Rev.* 32, 863–881. doi: 10.1016/j.neubiorev.2008.01.001

Ruffman, T., Taumoepeau, M., and Perkins, C. (2012). Statistical learning as a basis for social understanding in children. *Br. J. Dev. Psychol.* 30, 87–104. doi: 10.1111/j.2044-835x.2011.02045.x

Ruiz, S., Lee, S., Soekadar, S. R., Caria, A., Veit, R., Kircher, T., et al. (2013a). Acquired self-control of insula cortex modulates emotion recognition and brain network connectivity in schizophrenia. *Hum. Brain Mapp.* 34, 200–212. doi: 10.1002/hbm.21427

Ruiz, S., Birbaumer, N., and Sitaram, R. (2013b). Abnormal neural connectivity in schizophrenia and fMRI-brain-computer interface as a potential therapeutic approach. *Front. Psychiatry* 4:17. doi: 10.3389/fpsyt.2013.00017

Ruiz, S., Rana, M., Sass, K., Kircher, T., Birbaumer, N., and Sitaram, R. (2012). "Enhancement of functional brain connectivity and semantic priming using real-time fMRI neurofeedback," in *Organization for Human Brain Mapping*, (Beijing, China).

Samanez-Larkin, G. R., and Carstensen, L. L. (2011). *Socioemotional Functioning and the Aging Brain.* New York, NY: Oxford University Press.

Sass, K., Krach, S., Sachs, O., and Kircher, T. (2009). Lion—tiger—stripes: neural correlates of indirect semantic priming across processing modalities. *Neuroimage* 45, 224–236. doi: 10.1016/j.neuroimage.2008.10.014

Scharnowski, F., Veit, R., Zopf, R., Studer, P., Bock, S., Diedrichsen, J., et al. (2015). Manipulating motor performance and memory through real-time fMRI neurofeedback. *Biol. Psychol.* 108, 85–97. doi: 10.1016/j.biopsycho.2015.03.009

Scheibe, S., and Carstensen, L. L. (2010). Emotional aging: recent findings and future trends. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 65B, 135–144. doi: 10.1093/geronb/gbp132

Schölkopf, B., and Smola, A. J. (2002). *Learning with Kernels: Support Vector Machines, Regularization, Optimization and Beyond (Adaptive Computation and Machine Learning).* Cambridge: MIT Press.

Sepulveda, P., Sitaram, R., Rana, M., Montalba, C., Tejos, C., and Ruiz, S. (2016). How feedback, motor imagery and reward influence brain self-regulation using real-time fMRI. *Hum. Brain Mapp.* 37, 3153–3171. doi: 10.1002/hbm.23228

Shaw, M. E., Strother, S. C., Gavrilescu, M., Podzebenko, K., Waites, A., Watson, J., et al. (2003). Evaluating subject specific preprocessing choices in multisubject fMRI data sets using data-driven performance metrics. *Neuroimage* 19, 988–1001. doi: 10.1016/s1053-8119(03)00116-2

Shibata, K., Watanabe, T., Sasaki, Y., and Kawato, M. (2011). Perceptual learning incepted by decoded fMRI neurofeedback without stimulus presentation. *Science* 334, 1413–1415. doi: 10.1126/science.1212003

Sitaram, R., Caria, A., Veit, R., Gaber, T., Rota, G., Kuebler, A., et al. (2007). FMRI brain-computer interface: a tool for neuroscientific research and treatment. *Comput. Intell. Neurosci.* 2007:25487. doi: 10.1155/2007/25487

Sitaram, R., Lee, S., Ruiz, S., Rana, M., Veit, R., and Birbaumer, N. (2011). Real-time support vector classification and feedback of multiple emotional brain states. *Neuroimage* 56, 753–765. doi: 10.1016/j.neuroimage.2010.08.007

Sitaram, R., Ros, T., Stoeckel, L., Haller, S., Scharnowski, F., Lewis-Peacock, J., et al. (in press). Closed-loop brain training: the science of neurofeedback. *Nat. Neurosci.*

Sowell, E. R., Peterson, B. S., Thompson, P. M., Welcome, S. E., Henkenius, A. L., and Toga, A. W. (2003). Mapping cortical change across the human life span. *Nat. Neurosci.* 6, 309–315. doi: 10.1038/nn1008

Sporns, O., Tononi, G., and Kötter, R. (2005). The human connectome: a structural description of the human brain. *PLoS Comput. Biol.* 1:e42. doi: 10.1371/journal.pcbi.0010042

Stebbins, G. T., Carrillo, M. C., Dorfman, J., Dirksen, C., Desmond, J. E., Turner, D. A., et al. (2002). Aging effects on memory encoding in the frontal lobes. *Psychol. Aging* 17, 44–55. doi: 10.1037//0882-7974.17.1.44

Stoeckel, L. E., Garrison, K. A., Ghosh, S., Wighton, P., Hanlon, C. A., Gilman, J. M., et al. (2014). Optimizing real time fMRI neurofeedback for therapeutic discovery and development. *Neuroimage Clin.* 5, 245–255. doi: 10.1016/j.nicl.2014.07.002

Strehl, U., Leins, U., Goth, G., Klinger, C., Hinterberger, T., and Birbaumer, N. (2006). Self-regulation of slow cortical potentials: a new treatment for children with attention-deficit/hyperactivity disorder. *Pediatrics* 118, e1530–e1540. doi: 10.1542/peds.2005-2478

Strother, S., La Conte, S., Kai Hansen, L., Anderson, J., Zhang, J., Pulapura, S., et al. (2004). Optimizing the fMRI data-processing pipeline using prediction and reproducibility performance metrics: I. A preliminary group analysis. *Neuroimage* 23, S196–S207. doi: 10.1016/j.neuroimage.2004.07.022

Subramanian, L., Hindle, J. V., Johnston, S., Roberts, M. V., Husain, M., Goebel, R., et al. (2011). Real-time functional magnetic resonance imaging neurofeedback for treatment of Parkinson's disease. *J. Neurosci.* 31, 16309–16317. doi: 10.1523/jneurosci.3498-11.2011

Sulzer, J., Haller, S., Scharnowski, F., Weiskopf, N., Birbaumer, N., Blefari, M. L., et al. (2013). Real-time fMRI neurofeedback: progress and challenges. *Neuroimage* 76, 386–399. doi: 10.1016/j.neuroimage.2013.03.033

Todorova, A., Jenner, P., and Ray Chaudhuri, K. (2014). Non-motor Parkinson's: integral to motor Parkinson's, yet often neglected. *Pract. Neurol.* 14, 310–322. doi: 10.1136/practneurol-2013-000741

Tomasi, D., and Volkow, N. D. (2012). Aging and functional brain networks. *Mol. Psychiatry* 17, 549–558. doi: 10.1038/mp.2011.81

Toth, M. L., Melentijevic, I., Shah, L., Bhatia, A., Lu, K., Talwar, A., et al. (2012). Neurite sprouting and synapse deterioration in the aging Caenorhabditis elegans nervous system. *J. Neurosci.* 32, 8778–8790. doi: 10.1523/JNEUROSCI.1494-11.2012

Tracy, J. L., and Robins, R. W. (2008). The automaticity of emotion recognition. *Emotion* 8, 81–95. doi: 10.1037/1528-3542.8.1.81

Uylings, H. B., and de Brabander, J. M. (2002). Neuronal changes in normal human aging and Alzheimer's disease. *Brain Cogn.* 49, 268–276. doi: 10.1006/brcg.2001.1500

Walhovd, K. B., Fjell, A. M., and Espeseth, T. (2014). Cognitive decline and brain pathology in aging—need for a dimensional, lifespan and systems vulnerability view. *Scand. J. Psychol.* 55, 244–254. doi: 10.1111/sjop.12120

Wais, P. E., Martin, G. M., and Gazzaley, A. (2012). The impact of visual distraction on episodic retrieval in older adults. *Brain Res.* 1430, 78–85. doi: 10.1016/j.brainres.2011.10.048

Wang, K., Liang, M., Wang, L., Tian, L., Zhang, X., Li, K., et al. (2007). Altered functional connectivity in early Alzheimer's disease: a resting-state fMRI study. *Hum. Brain Mapp.* 28, 967–978. doi: 10.1002/hbm.20324

Watson, D., Clark, L. A., and Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063–1070. doi: 10.1037/0022-3514.54.6.1063

Weiner, M. W., Veitch, D. P., Aisen, P. S., Beckett, L. A., Cairns, N. J., Green, R. C., et al. (2013). The Alzheimer's disease neuroimaging initiative: a review of papers published since its inception. *Alzheimers Dement.* 9, e111–e194. doi: 10.1016/j.jalz.2013.05.1769

Weiskopf, N. (2012). Real-time fMRI and its application to neurofeedback. *Neuroimage* 62, 682–692. doi: 10.1016/j.neuroimage.2011.10.009

Wilckens, K. A., Erickson, K. I., and Wheeler, M. E. (2012). Age-related decline in controlled retrieval: the role of the PFC and sleep. *Neural Plast.* 2012:624795. doi: 10.1155/2012/624795

Williams, L. M., Brown, K. J., Palmer, D., Liddell, B. J., Kemp, A. H., Olivieri, G., et al. (2006). The mellow years?: neural basis of improving emotional stability over age. *J. Neurosci.* 26, 6422–6430. doi: 10.1523/JNEUROSCI.0022-06.2006

Williams, K. N., and Kemper, S. (2010). Interventions to reduce cognitive decline in aging. *J. Psychosoc. Nurs. Ment. Health Serv.* 48, 42–51. doi: 10.3928/02793695-20100331-03

Winecoff, A., Labar, K. S., Madden, D. J., Cabeza, R., and Huettel, S. A. (2011). Cognitive and neural contributors to emotion regulation in aging. *Soc. Cogn. Affect. Neurosci.* 6, 165–176. doi: 10.1093/scan/nsq030

Yeager, C. A., and Hyer, L. (2008). Apathy in dementia: relations with depression, functional competence and quality of life. *Psychol. Rep.* 102, 718–722. doi: 10.2466/pr0.102.3.718-722

Yoo, S. S., and Jolesz, F. A. (2002). Functional MRI for neurofeedback: feasibility study on a hand motor task. *Neuroreport* 13, 1377–1381. doi: 10.1097/00001756-200208070-00005

Zelazo, P. D. (2006). The dimensional change card sort (DCCS): a method of assessing executive function in children. *Nat. Protoc.* 1, 297–301. doi: 10.1038/nprot.2006.46

Zhang, H.-Y., Wang, S.-J., Liu, B., Ma, Z.-L., Yang, M., Zhang, Z.-J., et al. (2010). Resting brain connectivity: changes during the progress of Alzheimer disease. *Radiology* 256, 598–606. doi: 10.1148/radiol.10091701

Zotev, V., Krueger, F., Phillips, R., Alvarez, R. P., Simmons, W. K., Bellgowan, P., et al. (2011). Self-regulation of amygdala activation using real-time FMRI neurofeedback. *PLoS One* 6:e24522. doi: 10.1371/journal.pone.0024522

Zurakowski, T., Taylor, M., and Bradway, C. (2006). Effective teaching strategies for the older adult with urologic concerns. *Urol. Nurs.* 26, 355–360.

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 25 October 2016
doi: 10.3389/fnagi.2016.00248

Age-Dependent Cortical Thinning of Peripheral Visual Field Representations in Primary Visual Cortex

Joseph C. Griffis[1], Wesley K. Burge[1] and Kristina M. Visscher[2]**

[1] Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA, [2] Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA

The cerebral cortex changes throughout the lifespan, and the cortical gray matter in many brain regions becomes thinner with advancing age. Effects of aging on cortical thickness (CT) have been observed in many brain regions, including areas involved in basic perceptual functions such as processing visual inputs. An important property of early visual cortices is their topographic organization—the cortical structure of early visual areas forms a topographic map of retinal inputs. Primary visual cortex (V1) is considered to be the most basic cortical area in the visual processing hierarchy, and is topographically organized from posterior (central visual representation) to anterior (peripheral visual representation) along the calcarine sulcus. Some studies have reported strong age-dependent cortical thinning in portions of V1 that likely correspond to peripheral visual representations, while there is less evidence of substantial cortical thinning in central V1. However, the effect of aging on CT in V1 as a function of its topography has not been directly investigated. To address this gap in the literature, we estimated the CT of different eccentricity sectors in V1 using T1-weighted MRI scans acquired from groups of healthy younger and older adults, and then assessed whether between-group differences in V1 CT depended on cortical eccentricity. These analyses revealed age-dependent cortical thinning specific to peripheral visual field representations in anterior portions of V1, but did not provide evidence for age-dependent cortical thinning in other portions of V1. Additional analyses found similar effects when analyses were restricted to the gyral crown, sulcul depth and sulcul wall, indicating that these effects are not likely due to differences in gyral/sulcul contributions to our regions of interest (ROI). Importantly, this finding indicates that age-dependent changes in cortical structure may differ among functionally distinct zones within larger canonical cortical areas. Likely relationships to known age-related declines in visual performance are discussed to provide direction for future research in this area.

Keywords: aging, visual cortex organization, primary visual cortex (V1), cortical thickness, structural MRI

OPEN ACCESS

Edited by:
Aurel Popa-Wagner, University of Rostock, Germany

Reviewed by:
Hideyuki Sawada, Utano National Hospital, Utano, Japan
Raluca Sandu Vintilescu, University of Medicine and Pharmacy of Craiova, Romania

****Correspondence:***
Joseph C. Griffis
joegriff@uab.edu
Kristina M. Visscher
kmv@uab.edu

Received: *18 August 2016*
Accepted: *12 October 2016*
Published: *25 October 2016*

Citation:
Griffis JC, Burge WK and Visscher KM (2016) Age-Dependent Cortical Thinning of Peripheral Visual Field Representations in Primary Visual Cortex. Front. Aging Neurosci. 8:248. doi: 10.3389/fnagi.2016.00248

INTRODUCTION

Aging is associated with changes in the structural properties of the cerebral cortex. Specifically, there is substantial evidence that aging leads to decreased thickness (thinning) of the cortical gray matter (Salat et al., 2004; Fjell et al., 2009; McGinnis et al., 2011). While it is generally accepted that widespread cortical thinning occurs as people age, there is some disagreement

in the literature about the effects of aging on cortical thickness (CT) in early visual areas: several groups have reported decreased CT in primary visual cortex (V1) due to aging (Salat et al., 2004; Fjell et al., 2009; McGinnis et al., 2011), while others have not (Raz et al., 2004; Thambisetty et al., 2010; Lemaitre et al., 2012). These discrepancies might result, in part, from differences among studies in how V1 is defined. It is important to consider that cortex in V1 is organized as a topographic map of retinal inputs that progresses from foveal to peripheral retinotopic representations along the posterior-anterior dimension of the calcarine sulcus (Inoue, 1909; Fox et al., 1987; Engel et al., 1997), and it is therefore possible that different parts of this map may be differentially impacted by aging.

Importantly, portions of V1 that correspond to foveal vs. peripheral retinotopic representations differ in a variety of characteristics. These include differences in the cellular structure of retinal inputs to the lateral geniculate nucleus (LGN; Curcio and Allen, 1990; Dacey, 1993; Neitz et al., 2006), neuronal density (Collins et al., 2010), inter-regional connections (Hadjikhani and Tootell, 2000; Clavagnier et al., 2004; Palmer and Rosa, 2006), sensitivity to threatening stimuli (Bayle et al., 2009; Gomez et al., 2011), responses during cross-modal attention (Cate et al., 2009; Griffis et al., 2015), and modulation of activity by spatial attention (Roberts et al., 2007). Thus, it is reasonable to hypothesize that foveal vs. peripheral retinotopic representations in V1 might be differentially affected by processes such as aging. Indeed, several studies suggest that functional vision may decline differently for the central and peripheral fields (Drance et al., 1967; Haas et al., 1986), suggesting that age-related changes in the cortical structure of foveal representations in V1 (near the occipital pole) might be distinct from the developmental changes that occur further along the calcarine sulcus. Here, we examine how the CT of V1 at different retinotopic representations is affected by aging.

MATERIALS AND METHODS

Participants

Thirty-two healthy younger adults (age range 19–32) and 41 older adults (age range 65–87) from the Birmingham area participated in this study. All participants did not have any psychiatric or neurological diagnoses at the time of the study. Participant demographics are shown in **Table 1**. Informed consent was obtained from all participants before proceeding with the experiment. All aspects of the study were approved by the University of Alabama at Birmingham Institutional Review Board.

TABLE 1 | Participant demographics.

	Younger adults	Older adults
Number of participants	32	41
Age (Mean/SD)	25 (3.19)	71 (4.74)
Sex (% male)	53%	54%

Anatomical MRI Acquisition

For each participant, we obtained a single 3D high-resolution MPRAGE T1-weighted anatomical scan using a 3 Tesla head-only Siemens Magnetom Allegra scanner. Repetition time (TR) = 2250 ms; echo time (TE) = 2.6 ms; inversion time (T1) = 900 ms; field of view (FOV[ap, fh, rl]) = 240 mm × 256 mm × 176 mm; slice gap, 0; 1.0 mm × 1.0 mm × 1.1 mm voxel size; flip angle (FA) = 9.

Anatomical MRI Processing

Image processing (registration, segmentation and surface reconstruction) and CT estimation were performed using Freesurfer (version 5.3.0), a surface based analysis tool that estimates CT by measuring the distance between the boundary of gray/white mater and the pial surface (Dale et al., 1999; Fischl et al., 1999; Fischl and Dale, 2000). All cortical regions of interest (ROIs) were created using Freesurfer (version 5.3.0) on the cortical surface.

V1 Regions of Interest

For each hemisphere, a set of nine ROIs were defined on a flat-map of the occipital pole using the Freesurfer *fsaverage* brain as described in our previous publication (Burge et al., 2016). Each ROI was defined manually as a bar that extended across the dorsal-ventral axis of V1 and that was oriented perpendicular to the calcarine sulcus using the Freesurfer V1 label file as a guide (**Figure 1A**). The first 8 ROIs (moving anteriorly along the calcarine sulcus from the occipital pole) were each approximately 10 mm wide as estimated using the *plot_curv* function in *tksurfer*. An additional ninth ROI was also defined containing the remaining vertices in the space between the eighth ROI and the anterior-most end of the V1 label file (**Figure 1A**). While each ROI corresponded to different eccentricity sectors in V1, all of the bar ROIs contained the upper, middle and lower visual field representations in V1 (Inoue, 1909). Each of the manually defined ROIs were then transformed from the *fsaverage* space to the anatomical space of each participant, enabling consistent localization across participants. The mean surface area of the V1 bar ROIs was 309 mm^2. Using a previously published probabilistic retinotopy template (Benson et al., 2012), we estimated the mean eccentricities (in degrees visual angle) of each ROI as <1°, 1.34°, 2.2°, 4.1°, 7.3°, 14.1°, 25.5°, 40.0° and 63.3°, respectively. It should be noted that the eccentricity of the first ROI is less precisely defined because the lower limit of the probabilistic template is 1° visual angle, due to the difficulty of retinotopic mapping at the foveal confluence (Benson et al., 2012).

Controlling for Ratio of Gyrus Tissue to Sulcus Tissue

CT at the depth of sulci in the brain has been found to be, in general, less than at the gyral crowns (Fischl and Dale, 2000). To exclude the possibility that the effects observed for the previously described bar ROIs might be due to disproportionate representations of the sulcal depth or gyral crowns in a given

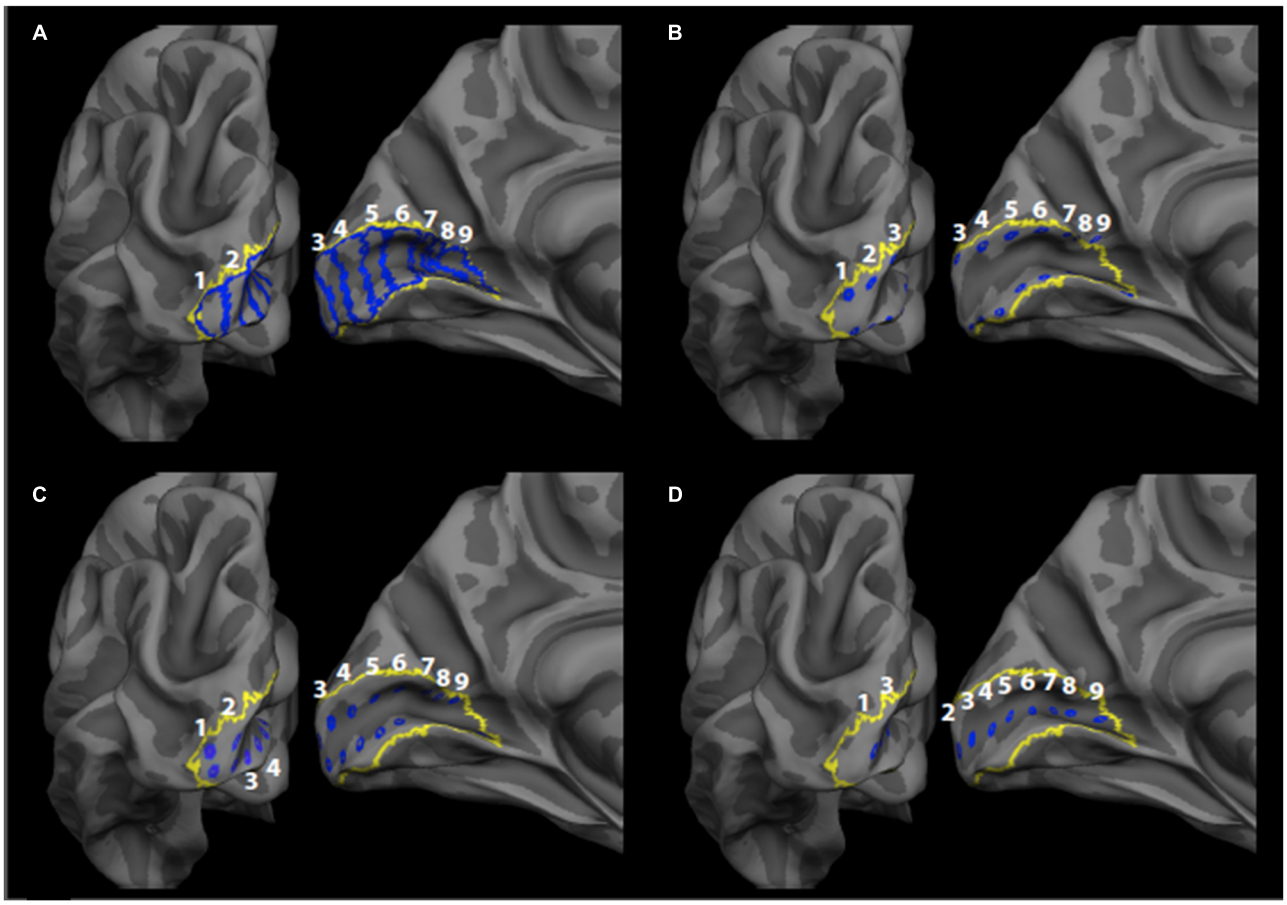


FIGURE 1 | Primary visual cortex (V1) regions of interest (ROIs). The label boundaries (blue) for the **(A)** bar ROIs, **(B)** gyrus crown ROIs, **(C)** sulcus wall ROIs, and **(D)** sulcus depth ROIs are shown within the Freesurfer V1 label boundary (yellow) on the fsaverage brain. ROIs are numbered 1–9, such that one corresponds to the most posterior ROI (most central representation) and nine corresponds to the most anterior ROI (most peripheral representation).

ROI, we created additional ROIs that allowed us to separately measure CT at each location separately. Each ROI was created as a grouping of vertices on the cortical surface (**Figures 1B–D**) within each bar ROI. The additional ROIs consisted of ROIs in the *gyrus crown* that were located on the V1 side of the V1/V2 border on both the upper and lower banks of the calcarine sulcus (**Figure 1B**), ROIs in the *sulcus wall* that were located halfway between the gyrus crown and the depth of the sulcus for both the upper and lower banks of the calcarine sulcus (**Figure 1C**), and ROIs in the *sulcus depth* (**Figure 1D**). Each ROI was defined in Freesurfer by manually selecting a vertex within the initial set of bar ROIs and creating a Freesurfer "label." Each single vertex was then dilated using the FreeSurfer "Dilate Label" function, which dilates the original vertex label to include neighboring vertices. Each label was dilated a total of three times. The area of each of these regions was roughly 20 mm^2, and each ROI was spaced roughly 10 mm apart. The ROIs were then transformed from the *fsaverage* brain to each participant's anatomical space, as described above for the bar ROIs.

Statistical Analyses

To evaluate the effects of age on CT across different eccentricities in V1, we performed mixed measures analysis of variances (ANOVAs) with a between-subjects factor of group (two levels—younger adults vs. older adults) and a within-subjects factor of eccentricity (nine levels—one for each ROI). Identical ANOVAs were performed for each set of ROIs. Interaction effects were of primary interest, as they would indicate that differences in CT between the groups depended on eccentricity. Interactions were considered significant if *p*-values for the interaction term were not greater than 0.05 (Bonferroni-Holm corrected across four ANOVAs). Sphericity violations were corrected using Greenhouse-Geisser adjustments to the number of degrees of freedom (Abdi, 2010). *Post hoc* independent samples *t*-tests were performed to identify ROIs showing significant differences between groups as follow-ups to significant interaction effects, and were considered significant if *p*-values were not greater than 0.05 (Bonferroni-Holm corrected across nine ROIs).

RESULTS

Bar ROIs

The assumption of sphericity was violated for the repeated measures factor (Greenhouse-Geisser epsilon = 0.53, $p < 0.001$). Therefore, Greenhouse-Geisser adjustment was performed on the degrees of freedom to account for this violation. The analysis revealed a significant eccentricity by group interaction effect ($F_{(4.2,298.6)} = 8.28$, corrected $p < 0.001$, partial eta-squared = 0.10), a main effect of eccentricity ($F_{(4.2,298.65)} = 189.64$, $p < 0.001$, partial eta-squared = 0.73), and a main effect of group ($F_{(1,71)} = 10.15$, $p = 0.002$, partial eta-squared = 0.13). To identify regions showing significant differences in CT between the groups, follow-up independent samples *t*-tests were performed at each ROI and corrected using the Bonferonni-Holm procedure to control the family-wise error rate at 0.05. This revealed that the older adults had significantly thinner cortex than young adults in ROI 6 ($t_{(71)} = -3.67$, corrected $p = 0.003$), ROI 7 ($t_{(71)} = -4.41$, corrected $p < 0.001$), ROI 8 ($t_{(71)} = -5.31$, corrected $p < 0.001$), and ROI 9 ($t_{(71)} = -6.03$, corrected $p < 0.001$). Results are illustrated in **Figure 2**.

Gyrus Crown ROIs

The assumption of sphericity was violated for the repeated measures factor (Greenhouse-Geisser epsilon = 0.67, $p < 0.001$). Greenhouse-Geisser adjustment was performed on the degrees of freedom to account for this violation. The analysis revealed a significant eccentricity by group interaction effect ($F_{(5.35,379.49)} = 3.35$, corrected $p = 0.01$, partial eta-squared = 0.05), a main effect of eccentricity ($F_{(5.35,379.49)} = 95.72$, $p < 0.001$, partial eta-squared = 0.57), and a main effect of group ($F_{(1,71)} = 3.82$, $p = 0.05$, partial eta-squared = 0.05). To identify regions showing significant differences in CT between the groups, follow-up independent samples *t*-tests were performed at each ROI and corrected using the Bonferonni-Holm procedure to control the family-wise error rate at 0.05. This revealed that the older adults had significantly thinner cortex than young adults in ROI 8 ($t_{(71)} = -3.30$, corrected $p = 0.01$) and ROI 9 ($t_{(71)} = -4.15$, corrected $p < 0.001$). Results are illustrated in **Figure 3**.

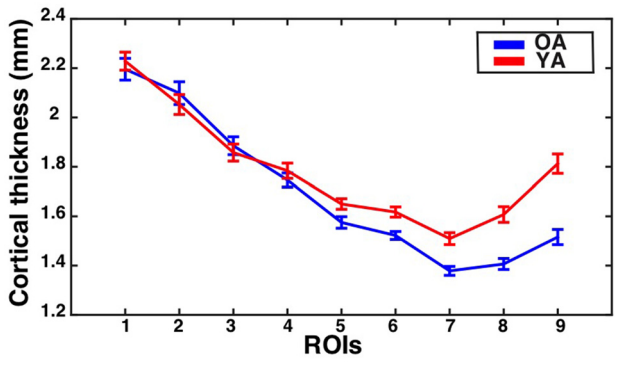


FIGURE 2 | Cortical thickness (CT) for the V1 bar ROIs as a function of eccentricity for younger and older adults. Mean ± standard error of the mean for CT is shown for each group at each bar ROI. Values for older adults are plotted in blue, while values for younger adults are plotted in red.

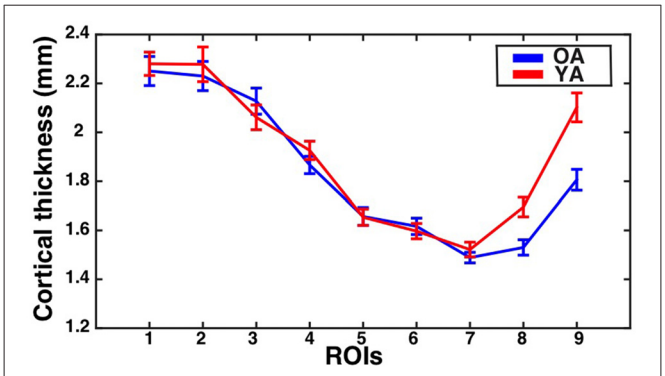


FIGURE 3 | CT for the V1 gyrus crown ROIs as a function of eccentricity for younger and older adults. Mean ± standard error of the mean for CT is shown for each group at each gyrus crown ROI. Values for older adults are plotted in blue, while values for younger adults are plotted in red.

Sulcus Depth ROIs

The assumption of sphericity was violated for the repeated measures factor (Greenhouse-Geisser epsilon = 0.75, $p < 0.001$). Greenhouse-Geisser adjustment was performed on the degrees of freedom to account for this violation. The analysis revealed a significant eccentricity by group interaction effect ($F_{(5.96,423.44)} = 2.11$, corrected $p = 0.05$, partial eta-squared = 0.03), a main effect of eccentricity ($F_{(5.96,423.44)} = 17.63$, $p < 0.001$, partial eta-squared = 0.20) and a main effect of group ($F_{(1,71)} = 7.61$, $p < 0.001$, partial eta-squared = 0.10). To identify regions showing significant differences in CT between the groups, follow-up independent samples *t*-tests were performed at each ROI and corrected using the Bonferonni-Holm procedure to control the family-wise error rate at 0.05. This revealed that the older adults had significantly thinner cortex than young adults in ROI 7 ($t_{(71)} = -4.24$, corrected $p < 0.001$). Results are illustrated in **Figure 4**.

Sulcus Wall ROIs

The assumption of sphericity was violated for the repeated measures factor (Greenhouse-Geisser epsilon = 0.67, $p < 0.001$). Greenhouse-Geisser adjustment was performed on the degrees of freedom to account for this violation. The analysis revealed a significant eccentricity by group interaction effect ($F_{(5.36,380.64)} = 5.60$, corrected $p = 0.002$, partial eta-squared = 0.07), a main effect of eccentricity ($F_{(5.36,380.64)} = 87.38$, $p < 0.001$, partial eta-squared = 0.55), and a main effect of group ($F_{(1,71)} = 7.58$, $p = 0.007$, partial eta-squared = 0.10). To identify regions showing significant differences in CT between the groups, follow-up independent samples *t*-tests were performed at each ROI and corrected using the Bonferonni-Holm procedure to control the family-wise error rate at 0.05. This revealed that the older adults had significantly thinner cortex than young adults in ROI 7 ($t_{(71)} = -3.66$,

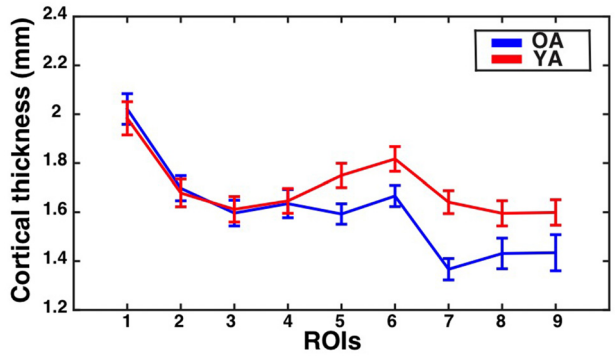


FIGURE 4 | CT for the V1 sulcus depth ROIs as a function of eccentricity for younger and older adults. Mean ± standard error of the mean for CT is shown for each group at each sulcus depth ROI. Values for older adults are plotted in blue, while values for younger adults are plotted in red.

corrected $p = 0.003$), ROI 8 ($t_{(71)} = -4.62$, corrected $p < 0.001$), and ROI 9 ($t_{(71)} = -5.49$, corrected $p < 0.001$). Results are illustrated in **Figure 5**.

DISCUSSION

In the current study, we assessed the effect of aging on CT in human V1. Specifically, we investigated whether age-dependent changes in the CT of V1 might differ among portions of V1 that represent different visual eccentricities. Our analyses revealed that while the regions of V1 corresponding to peripheral visual representations had significantly thinner cortex in older adults than in younger adults, the regions of V1 corresponding to central visual representations did not show significant differences in CT between groups. Additionally, our results indicate that these effects are unlikely to be driven by differences in gyral/sulcul contributions to central vs. peripheral eccentricity sectors. Together, our results suggest that age-dependent cortical thinning in human V1 is specific to portions that represent peripheral visual space.

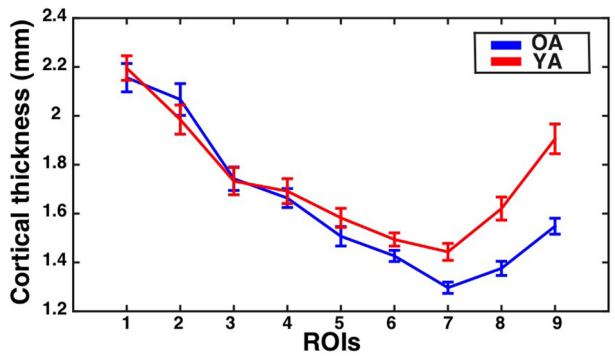


FIGURE 5 | CT for the V1 sulcus wall ROIs as a function of eccentricity for younger and older adults. Mean ± standard error of the mean for CT is shown for each group at each sulcal wall ROI. Values for older adults are plotted in blue, while values for younger adults are plotted in red.

Several previous whole-brain studies of age-related changes in CT have found consistent evidence for age-dependent thinning in early visual areas including V1 (Salat et al., 2004; Fjell et al., 2009; McGinnis et al., 2011). In contrast, a previous study using manually defined ROIs corresponding to only a portion of V1 did not find strong evidence for cortical thinning in V1 (Raz et al., 2004). Other recent studies have not found substantial age-related thinning in V1 when assessing age-related changes in the CT of V1 as a whole (Thambisetty et al., 2010; Lemaitre et al., 2012). The current article suggests an explanation of these divergent findings: age-dependent cortical thinning in V1 primarily occurs in areas corresponding to peripheral visual field representations. Here, we performed a comprehensive analysis assessing the effects of aging on CT in anatomically defined sub-regions of V1 that correspond to different representations of visual space. The results of our analyses provide evidence for age-related cortical thinning that is specific to anterior portions of the calcarine sulcus (corresponding to representations of peripheral visual space), but do not provide support for age-related cortical thinning in posterior portions of the calcarine sulcus (corresponding to representations of central visual space). These results confirm and build upon previous reports of age-dependent cortical thinning in V1 (Salat et al., 2004; Fjell et al., 2009; McGinnis et al., 2011), by indicating that age-dependent changes in cortical structure may be selective to specific, functionally distinct sub-regions of larger anatomical areas.

As mentioned previously, the other studies that have found evidence for age-dependent thinning in V1 have used whole-brain approaches that separately model the effects of aging at each surface vertex (Salat et al., 2004; Fjell et al., 2009; McGinnis et al., 2011). Interestingly, the calcarine sulcus ROI identified by Salat et al. (2004) by their whole-brain analysis as showing maximal age-dependent cortical thinning consisted primarily of anterior portions of the calcarine sulcus, consistent with our results here, though that analysis did not directly compare central to peripheral visual cortex. In contrast, the current study directly assessed whether the effects of aging on CT varied across central and peripheral parts of V1. Similarly, the strongest aging effects in visual cortex identified by the whole-brain analyses reported by McGinnis et al. (2011) were also primarily located in more anterior calcarine regions. In contrast, the V1 ROI employed by Raz et al. (2004) likely consisted primarily of the cortex within the sulcus of regions corresponding to the mid-periphery, although it is difficult to discern the precise extent of their ROI based on the description/figure provided in the report. Another recent study that analyzed the effects of aging on the CT of V1 as a whole only identified substantial aging effects when global decreases in CT were not modeled as a control for global aging effects (Lemaitre et al., 2012). The current study, in a sense, uses V1 as its own control by directly comparing aging effects across different eccentricity sectors, making our results unlikely to be influenced by global aging effects. Indeed, the results of our analyses suggest that while some age-dependent cortical thinning may be present

in middle portions of the calcarine sulcus, the most substantial thinning is observed in more anterior regions (**Figures 2**–**4**), and this effect may not be observed by studies that estimate the average effect of aging on the thickness of V1 as a whole.

While the present study did not incorporate behavioral measurements, it is worth discussing that the effective use of vision declines with healthy aging. For example, measures of low-level vision such as acuity and contrast sensitivity decline with age (Sekuler and Sekuler, 2000; Owsley, 2011). Central and peripheral fields show similar declines with age in some low level measures (Haegerstrom-Portnoy et al., 1999), but other metrics appear to show that peripheral vision may be more starkly influenced by age than central vision (Drance et al., 1967; Haas et al., 1986). While some of these declines in vision come from aging of the anterior eye, for example yellowing of the lens or decreased pupil size, age-related changes in vision also stem from a neural source (Johnson et al., 1989).

Standard acuity tests of visual function appear to underestimate the degree of difficulty that older adults have in doing everyday activities that require peripheral vision (e.g., driving; Ball et al., 1990). However, somewhat more complex vision tests appear to better predict behaviors on everyday tasks like driving (Ball et al., 1993; Owsley et al., 1998). These measures incorporate peripheral stimuli and performance appears to decline steeply with age (Ball and Owsley, 1993). Several studies using different approaches have shown that the ability to identify a stimulus in the periphery in the presence of distracting information in the center declines with age (Scialfa et al., 1987; Ball and Owsley, 1993; Haegerstrom-Portnoy et al., 1999; Sekuler et al., 2000). Thus, previously reported data show that performance on complex visual tasks using peripheral visual information decline with age. Together with the data from the current manuscript, this suggests that age-related declines in complex visual tasks using peripheral vision might result from age-related changes in the neural representations of peripheral vision.

A great deal of work examining simultaneous central and peripheral stimuli has used a task called the "Useful Field of View" Task. It is interesting to note that early descriptions of this work interpreted age-associated declines in performance in terms of declines of peripheral vision (e.g., Ball et al., 1988). This mirrors an early work which suggested that the size of the visual field shrinks with age (Drance et al., 1967; Haas et al., 1986). However, more recent articles have interpreted these declines in Useful Field of View performance as resulting from declines in Speed of Processing, the speed with which a participant can take in and process information from the full visual field (e.g., Ball et al., 2007). The two interpretations share many factors—for example, peripheral information must be processed quickly for good speed of processing performance. Our data are particularly interesting because they imply that the brain regions that are specific to peripheral information are selectively thinned through the aging process. Future work should examine the degree to which this selective thinning relates to individual differences in general visual behavior such as speed of processing as well as measures of low level vision in the peripheral vs. central field.

Participants with adult-onset central vision loss are forced to use peripheral vision for daily tasks. These participants have thicker cortex compared to normally sighted controls in peripheral parts of V1 (corresponding to the parts of the visual field with increased attentive use; Burge et al., 2016). This suggests that the thickness of cortex is use-dependent, and appears to be plastic, even in adults. The majority of attention-demanding tasks that a person performs in a typical day involve central vision: recognizing faces, tasks requiring fine hand-eye coordination, reading, etc. One explanation for our data is that a lifetime of experience attending primarily to central vision, but often ignoring peripheral vision might contribute to thinning in the parts of cortex associated with peripheral vision in older age. Further research is needed to directly test this hypothesis by investigating the relationships between the age-dependent thinning of peripheral V1 and age-dependent changes in peripheral visual function.

It is also important to note that these data were not initially collected with the explicit goal of testing age related differences. Our groups were not matched on race or education. These results should be interpreted with the cross sectional design of this study in mind. Nonetheless, the results indicate that future studies should consider the possibility of non-uniform aging effects in cortical areas such as V1 that contain distinct functional subdivisions.

AUTHOR CONTRIBUTIONS

JCG, WKB and KMV contributed to the study conceptualization/design. WKB and KMV contributed to data collection. JCG, WKB and KMV contributed to data analysis and interpretation. JCG and KMV wrote the manuscript.

ACKNOWLEDGMENTS

We would like to acknowledge assistance with data acquisition from Ryan Vaden and Dr. Abdurahman Elkhetali. We would like to acknowledge support from the McKnight Brain Research Foundation, the Dana Foundation, the UAB Center for Clinical And Translational Science UL1 TR000165, NIH NEI F31EY025920, the Vision Science Research Center P30 EY003039, and the Civitan International Research Center.

REFERENCES

Abdi, H. (2010). "The greenhouse-geisser correction," in *Encyclopedia of Research Design*, ed. N. Salkind (Thousand Oaks, CA: Sage), 544–548.

Ball, K. K., Beard, B. L., Roenker, D. L., Miller, R. L., and Griggs, D. S. (1988). Age and visual search: expanding the useful field of view. *J. Opt. Soc. Am. A* 5, 2210–2219. doi: 10.1364/josaa.5.002210

Ball, K., Edwards, J. D., and Ross, L. A. (2007). The impact of speed of processing training on cognitive and everyday functions. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 62, 19–31. doi: 10.1093/geronb/62.special_issue_1.19

Ball, K., and Owsley, C. (1993). The useful field of view test: a new technique for evaluating age-related declines in visual function. *J. Am. Optom. Assoc.* 64, 71–79.

Ball, K., Owsley, C., and Beard, B. (1990). Clinical visual perimetry underestimates peripheral field problems in older adults. *Clin. Vis. Sci.* 5, 113–125.

Ball, K., Owsley, C., Sloane, M. E., Roenker, D. L., and Bruni, J. R. (1993). Visual attention problems as a predictor of vehicle crashes in older drivers. *Invest. Ophthalmol. Vis. Sci.* 34, 3110–3123.

Bayle, D. J., Henaff, M.-A., and Krolak-Salmon, P. (2009). Unconsciously perceived fear in peripheral vision alerts the limbic system: a MEG study. *PLoS One* 4:e8207. doi: 10.1371/journal.pone.0008207

Benson, N. C., Butt, O. H., Datta, R., Radoeva, P. D., Brainard, D. H., and Aguirre, G. K. (2012). The retinotopic organization of striate cortex is well predicted by surface topology. *Curr. Biol.* 22, 2081–2085. doi: 10.1016/j.cub.2012.09.014

Burge, W., Griffis, J., Nenert, R., Elkhetali, A., DeCarlo, D., ver Hoef, L., et al. (2016). Cortical thickness in human V1 associated with central vision loss. *Sci. Rep.* 6:23268. doi: 10.1038/srep23268

Cate, A. D., Herron, T. J., Yund, E. W., Stecker, G. C., Rinne, T., Kang, X., et al. (2009). Auditory attention activates peripheral visual cortex. *PLoS One* 4:e4645. doi: 10.1371/journal.pone.0004645

Clavagnier, S., Falchier, A., and Kennedy, H. (2004). Long-distance feedback projections to area V1: implications for multisensory integration, spatial awareness and visual consciousness. *Cogn. Affect. Behav. Neurosci.* 4, 117–126. doi: 10.3758/cabn.4.2.117

Collins, C. E., Airey, D. C., Young, N. A., Leitch, D. B., and Kaas, J. H. (2010). Neuron densities vary across and within cortical areas in primates. *Proc. Natl. Acad. Sci. U S A* 107, 15927–15932. doi: 10.1073/pnas.1010356107

Curcio, C. A., and Allen, K. A. (1990). Topography of ganglion cells in human retina. *J. Comp. Neurol.* 300, 5–25. doi: 10.1002/cne.903000103

Dacey, D. M. (1993). Morphology of a small-field bistratified ganglion cell type in the macaque and human retina. *Vis. Neurosci.* 10, 1081–1098. doi: 10.1017/s0952523800010191

Dale, A. M., Fischl, B., and Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 9, 179–194. doi: 10.1006/nimg.1998.0395

Drance, S. M., Berry, V., and Hughes, A. (1967). Studies on the effects of age on the central and peripheral isopters of the visual field in normal subjects. *Am. J. Ophthalmol.* 63, 1667–1672. doi: 10.1016/0002-9394(67)93644-6

Engel, S. A., Glover, G. H., and Wandell, B. A. (1997). Retinotopic organization in human visual cortex and the spatial precision of functional MRI. *Cereb. Cortex* 7, 181–192. doi: 10.1093/cercor/7.2.181

Fischl, B., and Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U S A* 97, 11050–11055. doi: 10.1073/pnas.200033797

Fischl, B., Sereno, M. I., and Dale, A. M. (1999). Cortical surface-based analysis. II: Inflation, flattening and a surface-based coordinate system. *Neuroimage* 9, 195–207. doi: 10.1006/nimg.1998.0396

Fjell, A. M., Westlye, L. T., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., et al. (2009). High consistency of regional cortical thinning in aging across multiple samples. *Cereb. Cortex* 19, 2001–2012. doi: 10.1093/cercor/bhn232

Fox, P. T., Miezin, F. M., Allman, J. M., Van Essen, D. C., and Raichle, M. E. (1987). Retinotopic organization of human visual cortex mapped with positron-emission tomography. *J. Neurosci.* 7, 913–922.

Gomez, A., Rothkirch, M., Kaul, C., Weygandt, M., Haynes, J.-D., and Rees, G. (2011). Emotion modulates the effects of endogenous attention on retinotopic visual processing. *Neuroimage* 57, 1542–1551. doi: 10.1016/j.neuroimage.2011.05.072

Griffis, J. C., Elkhetali, A. S., Vaden, R. J., and Visscher, K. M. (2015). Distinct effects of trial-driven and task set-related control in primary visual cortex. *Neuroimage* 120, 285–297. doi: 10.1016/j.neuroimage.2015.07.005

Haas, A., Flammer, J., and Schneider, U. (1986). Influence of age on the visual fields of normal subjects. *Am. J. Ophthalmol.* 101, 199–203. doi: 10.1016/0002-9394(86)90595-7

Hadjikhani, N., and Tootell, R. B. H. (2000). Projection of rods and cones within human visual cortex. *Hum. Brain Mapp.* 9, 55–63. doi: 10.1002/(SICI)1097-0193(2000)9:1<55::AID-HBM6>3.0.CO;2-U

Haegerstrom-Portnoy, G., Schneck, M. E., and Brabyn, J. A. (1999). Seeing into old age: vision function beyond acuity. *Optom. Vis. Sci.* 76, 141–158. doi: 10.1097/00006324-199903000-00014

Inoue, T. (1909). *Die Sehstörungen Bei Schussverletzungen Der kortikalen Sehsphäre, Nach Beobachtungen an Verwundeten Der Letzten Japanischen Kriege.* Leipzig: Engelmann.

Johnson, C. A., Adams, A. J., and Lewis, R. A. (1989). Evidence for a neural basis of age-related visual field loss in normal observers. *Invest. Ophthalmol. Vis. Sci.* 30, 2056–2064.

Lemaitre, H., Goldman, A. L., Sambataro, F., Verchinski, B. A., Meyer-Lindenberg, A., Weinberger, D. R., et al. (2012). Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol. Aging* 33, 617.e1–617.e9. doi: 10.1016/j.neurobiolaging.2010.07.013

McGinnis, S., Brickhouse, M., Pascual, B., and Dickerson, B. (2011). Age-related changes in the thickness of cortical zones in humans. *Brain Topogr.* 24, 279–291. doi: 10.1007/s10548-011-0198-6

Neitz, M., Balding, S. D., McMahon, C., Sjoberg, S. A., and Neitz, J. (2006). Topography of long- and middle-wavelength sensitive cone opsin gene expression in human and Old World monkey retina. *Vis. Neurosci.* 23, 379–385. doi: 10.1017/s095252380623325x

Owsley, C. (2011). Aging and vision. *Vision Res.* 51, 1610–1622. doi: 10.1016/j.visres.2010.10.020

Owsley, C., Ball, K., McGwin, G., Sloane, M. E., Roenker, D. L., White, M. F., et al. (1998). Visual processing impairment and risk of motor vehicle crash among older adults. *JAMA* 279, 1083–1088. doi: 10.1001/jama.279.14.1083

Palmer, S. M., and Rosa, M. G. P. (2006). A distinct anatomical network of cortical areas for analysis of motion in far peripheral vision. *Eur. J. Neurosci.* 24, 2389–2405. doi: 10.1111/j.1460-9568.2006.05113.x

Raz, N., Gunning-Dixon, F., Head, D., Rodrigue, K. M., Williamson, A., and Acker, J. D. (2004). Aging, sexual dimorphism and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. *Neurobiol. Aging* 25, 377–396. doi: 10.1016/s0197-4580(03)00118-0

Roberts, M., Delicato, L. S., Herrero, J., Gieselmann, M. A., and Thiele, A. (2007). Attention alters spatial integration in macaque V1 in an eccentricity-dependent manner. *Nat. Neurosci.* 10, 1483–1491. doi: 10.1038/nn1967

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S. R., Busa, E., et al. (2004). Thinning of the cerebral cortex in aging. *Cereb. Cortex* 14, 721–730. doi: 10.1093/cercor/bhh032

Scialfa, C. T., Kline, D. W., and Lyman, B. J. (1987). Age differences in target identification as a function of retinal location and noise level: examination of the useful field of view. *Psychol. Aging* 2, 14–19. doi: 10.1037/0882-7974.2.1.14

Sekuler, A. B., Bennett, P. J., and Mamelak, M. (2000). Effects of aging on the useful field of view. *Exp. Aging Res.* 26, 103–120. doi: 10.1080/036107300243588

Sekuler, R., and Sekuler, A. B. (2000). "Vision and aging," in *Encyclopedia of Psychology*, ed. A. E. Kazdin (Washington, DC: American Psychological Association), 180–183.

Thambisetty, M., Wan, J., Carass, A., An, Y., Prince, J. L., and Resnick, S. M. (2010). Longitudinal changes in cortical thickness associated with normal aging. *Neuroimage* 52, 1215–1223. doi: 10.1016/j.neuroimage.2010.04.258

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 08 December 2016
doi: 10.3389/fnagi.2016.00298

Cognitive Aging and the Hippocampus in Older Adults

Andrew O'Shea[1], *Ronald A. Cohen*[1], *Eric C. Porges*[1], *Nicole R. Nissim*[1,2] *and Adam J. Woods*[1,2]*

[1]Center for Cognitive Aging and Memory, McKnight Brain Institute, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL, USA, [2]Department of Neuroscience, University of Florida, Gainesville, FL, USA

The hippocampus is one of the most well studied structures in the human brain. While age-related decline in hippocampal volume is well documented, most of our knowledge about hippocampal structure-function relationships was discovered in the context of neurological and neurodegenerative diseases. The relationship between cognitive aging and hippocampal structure in the absence of disease remains relatively understudied. Furthermore, the few studies that have investigated the role of the hippocampus in cognitive aging have produced contradictory results. To address these issues, we assessed 93 older adults from the general community (mean age = 71.9 ± 9.3 years) on the Montreal Cognitive Assessment (MoCA), a brief cognitive screening measure for dementia, and the NIH Toolbox-Cognitive Battery (NIHTB-CB), a computerized neurocognitive battery. High-resolution structural magnetic resonance imaging (MRI) was used to estimate hippocampal volume. Lower MoCA Total ($p = 0.01$) and NIHTB-CB Fluid Cognition ($p < 0.001$) scores were associated with decreased hippocampal volume, even while controlling for sex and years of education. Decreased hippocampal volume was significantly associated with decline in multiple NIHTB-CB subdomains, including episodic memory, working memory, processing speed and executive function. This study provides important insight into the multifaceted role of the hippocampus in cognitive aging.

Keywords: cognitive aging, hippocampus, MoCA, NIH toolbox, structural magnetic resonance imaging

OPEN ACCESS

Edited by:
Ashok Kumar,
University of Florida, USA

Reviewed by:
Michael R. Foy,
Loyola Marymount University, USA
Con Stough,
Swinburne University of Technology, Australia

***Correspondence:**
Adam J. Woods
ajwoods@ufl.phhp.edu

Received: *11 May 2016*
Accepted: *22 November 2016*
Published: *08 December 2016*

Citation:
O'Shea A, Cohen RA, Porges EC, Nissim NR and Woods AJ (2016) Cognitive Aging and the Hippocampus in Older Adults. Front. Aging Neurosci. 8:298. doi: 10.3389/fnagi.2016.00298

INTRODUCTION

From the discovery of its role in episodic memory following bilateral resection in patient "HM" to the discovery of its role in symptoms of Alzheimer's disease (AD), the hippocampus is considered a structure fundamental for human cognition (Scoville and Milner, 1957; Squire, 1992; Jack et al., 1999). In addition to its well-documented role in memory function, recent research demonstrates that the hippocampus also plays a role in executive function, processing speed, intelligence, path integration and spatial processing (Reuben et al., 2011; Papp et al., 2014; Yamamoto et al., 2014). Each of these cognitive processes is shown to decline in the context of cognitive aging, in the absence of neurodegenerative diseases or neurological injury (Salthouse, 2010). Thus, understanding how change in hippocampal structure impacts cognition in the context of aging may prove important for identifying: (a) critical neural underpinnings of the cognitive aging process; and (b) intervention targets for combating cognitive aging.

Most of our knowledge of hippocampal structure-function relationships in humans is based on the findings in various disease states or following resection of the medial temporal

lobes resulting in gross memory disturbance. Models of structure-function relationships in non-human animals have highlighted the hippocampus as a spatial map, crucial for navigation and spatial memory (O'Keefe and Dostrovsky, 1971; O'Keefe, 1979). In addition, recent functional magnetic resonance imaging (MRI) findings have provided insight into the functional role of the hippocampus in various cognitive abilities beyond episodic and spatial memory (Eldridge et al., 2000; Iaria et al., 2007; Woods et al., 2013). However, the impact of subtle changes in hippocampal structure in the context of normal aging, in the absence of neurodegenerative or other disease states, remains poorly understood.

Individuals with diagnoses of mild cognitive impairment (MCI) and dementia have smaller hippocampi than age-matched controls in numerous MRI studies (Shi et al., 2009). Hippocampal atrophy is considered a hallmark of Alzheimer's disease (AD) (Jack et al., 1999). Premorbid hippocampal volume in patients with MCI predicts future conversion to AD (Jack et al., 1999). Thus, change in the structure of the hippocampus appears to play an important role in dementia. However, hippocampal volume is also well-documented to decline in normal aging (Raz et al., 2005). Yet, the functional consequences of this age-related volumetric loss is not well characterized in the context of aging in the absence of neurodegenerative disease. While changes in cognitive scores on dementia screening and cognitive assessment measures in patients with MCI and AD are associated with smaller hippocampal volume, it is unclear whether these findings are unique to dementia/disease states or extend to more subtle variations in hippocampal structure from normal aging.

The few studies that have investigated cognitive aging and the hippocampus have produced results that contrast significantly with prior research in neurological and neurodegenerative disease (Van Petten, 2004; Paul et al., 2011; Colom et al., 2013). For example, Van Petten (2004), in a meta-analysis, reported that the relationship between hippocampal size and episodic memory were weak. These inconsistencies between aging and disease-related findings highlight the need for further investigation of the role of the hippocampus in cognitive aging. Understanding the relationship between hippocampal structure and function in cognitive aging may have predictive value for identifying persons at higher risk for future cognitive decline, cognitive frailty and conversion to MCI (Woods et al., 2013). The prevalence of older adults is expected to accelerate over the coming decades. With this shift in the age of the world population comes an increase in the number of people that will suffer from MCI and other neurodegenerative disorders. Thus, there is a pressing need to identify predictive markers of decline. However, pursuit of such markers is difficult, if not impossible, without first understanding the normal variation present in the aging brain, as well as the overall structure-function relationship between the hippocampus and different components of cognitive function.

In the current study, we sought to examine the relationship between hippocampal volume and a commonly administered dementia-screening tool and a comprehensive cognitive battery in a cohort of 93 older adults without neurological injury, neurodegenerative disease or major psychiatric illness to: (1) better understand the structure-function relationship between the hippocampus and cognitive aging; and (2) to providing a foundation for development of predictive biomarkers by characterizing the sensitivity of commonly administered MCI screening and cognitive assessment tools to age-related structural changes in the hippocampus. We specifically examined the relationship between the Montreal Cognitive Assessment (MoCA) and the NIH Toolbox Cognitive Battery (NIHTB-CB). The MoCA is a brief (10 min) screening tool for MCI (Nasreddine et al., 2005), whereas the NIH toolbox cognitive assessment is a brief comprehensive computerized cognitive battery (∼60 min) consisting of tests to assess executive function, attention, episodic memory, language, processing speed and working memory. These measures are sub-divided into two composite cognitive scores comprising cognitive abilities that change with age (fluid cognitive function) or remain stable over time (crystalized cognitive functions). The delineation of a two-factor model (a fluid factor and a crystalized factor) instead of a single general intelligence factor is valuable when studying cognitive aging due to differences in the age curves of fluid and crystalized abilities (Cattell, 1987). Mungas et al. (2014) found a two-factor solution fit the NIHTB-CB validation data better than a single general intelligence factor; however, the best fitting model was a five-factor solution comprised of the following: reading, vocabulary, episodic memory, working memory and executive function/processing speed. An extension of the two-factor, fluid and crystalized model, the Cattell-Horn-Carroll (CHC) theory of cognition extends the factors of general intelligence to nine broad abilities (fluid reasoning, comprehension-knowledge, short-term memory, visual processing, auditory processing, long-term storage and retrieval, cognitive processing speed, quantitative knowledge and reading and writing; McGrew, 2009). The CHC taxonomy may provide a more thorough description of individual domains of the NIHTB-CB. However, a single NIHTB-CB task would likely incorporate multiple factors of the CHC model, rather than representing distinct entities.

We hypothesized that older adults with smaller hippocampal volumes would evidence lower performance on both the MoCA and NIHTB fluid cognition scores. In contrast, language-based cognitive abilities (i.e., crystalized cognition) would not change as a function of hippocampal volume. Furthermore, we hypothesized that hippocampal volume would be most strongly associated with performance on the memory domain of the MoCA and NIHTB. In addition, we also predicted that smaller hippocampal volume would be associated with slower processing speed and poorer executive functions. These data would not only support the role of the hippocampus in cognition as shown in prior research on neurodegenerative disease and neurological disease states, but also extend these findings to cognitive aging. Furthermore, these data would provide a strong foundation for development of predictive hippocampal biomarkers for future decline in longitudinal cohorts by characterizing cognitive aging in the hippocampus

in the absence of neurological and neurodegenerative disease.

MATERIALS AND METHODS

Participants

Ninety-three older adults (60% female) were recruited from the north-central Florida community through newspaper advertising, fliers and community outreach. Participants had a mean age of 71.7 years (SD = 9.8 years) and an average of 16.26 years education (SD = 2.61, see **Table 1** for detailed demographics). All participants provided written informed consent prior to enrollment. All study procedures were approved by the University of Florida Institutional Review Board prior to the start of the study. Participants had the opportunity to ask the researchers any questions about study procedures prior to the start of the study. No vulnerable populations were studied. Exclusionary criteria included pre-existing neurological or psychiatric brain disorders, MRI contraindications (such as metal or medical devices inside the body not approved to be scanned at 3T), reported diagnosis of a neurodegenerative brain disease (i.e., dementia or Alzheimer's) or self-reported difficulty with thinking and memory.

Study Procedures

Participants completed a neuropsychological battery (see "Measures" Section for more details) that included the NIHTB-CB and MoCA. Neuropsychological tasks were administered at an onsite clinical research facility by trained study staff. The neuropsychological battery was completed as a single visit. Neuroimaging scanning was completed at a subsequent MRI visit.

Measures

NIH Toolbox

In this study, NIH Toolbox was used as a brief, comprehensive assessment to examine neurological and behavioral function, allowing for the study of functional changes across the lifespan. The cognitive domain measure was used which covered subdomains of: executive function and attention, episodic memory, language, processing speed and working memory. Executive function and attention was measured by NIH-Toolbox Flanker Inhibitory Control and Attention test and the Dimensional Change Card Sort test. Flanker measures the ability to inhibit visual attention to irrelevant task dimensions. The Dimensional Card Sort test was used to assess the set-shifting component of executive function. Working memory was tested by the List Sorting test. Episodic Memory was assessed by Picture Sequence memory test and the Auditory Verbal Learning (Rey) test. To test language, the Oral Reading Recognition test and the Picture Vocabulary test were used. Processing speed was assessed by the Pattern Comparison test and the Oral Symbol Digit test. The fluid cognition composite is composed of the following tasks: Dimensional Change Card Sort, Flanker, Picture Sequence Memory, List Sorting and Pattern Comparison. The crystalized cognition composite is composed of the Picture Vocabulary Test and the Oral Reading Recognition Test. The NIH toolbox cognitive battery has been shown to have high test-retest reliability, as well as high convergent validity with "gold standard" measures of crystalized and fluid cognition (Heaton et al., 2014).

TABLE 1 | Sample demographics.

	Mean	*SD*	*Range*
Age	71.69	9.45	43–85
Education	16.26	2.61	12–20
Sex distribution			
	Number	*% of sample*	
Male	37	40	
Female	56	60	

MoCA

The MoCA is a 10-min, 30-point clinical assessment of multiple cognitive functions, including orientation (6 points), attention (6 points), short-term memory recall (5 points), abstract thinking (2 points), visuospatial executive function assessed by a clock-drawing task, trails task and reproducing a geometrical figure (5 points), naming task (3 points) and language function assessed by verbal fluency test (3 points). An additional one point was added for subjects with less than/equal to 12 years in education (per guidelines of MoCA administration (Nasreddine et al., 2005)). The suggested cut-off point on the MoCA is below 26 for MCI.

Neuroimaging Acquisition

All participants were imaged in a Philips 3.0 Tesla (3T) scanner (Achieva; Philips Electronics, Amsterdam, Netherlands) at the McKnight Brain Institute (University of Florida, Gainesville, FL, USA) with a 32-channel receive-only head coil. A pillow was placed under the head to limit motion during the scan. A high-resolution 3D T1 weighted MPRAGE scan was performed. Scanning parameters consisted of: voxel size = 1 mm isotropic; 1 mm slice thickness; TE = 3.2 ms; TR = 7.0 ms; FOV = 240 × 240; Number of slices = 170; acquired in a sagittal orientation.

Neuroimaging Processing

T_1-weighted MRI scans were processed with the software FreeSurfer version 5.3. To measure hippocampal volume, the automated subcortical segmentation stream in FreeSurfer was used. The software uses Bayesian inference methods relying on prior anatomical probabilities in a labeled data set, along with *a priori* known T_1 intensity characteristics of subcortical regions, as well as T_1 intensity information from the scan being processed, in order to label discrete regions (Fischl et al., 2002). Previous research has shown this automated procedure produces accurate and reliable results, while taking a fraction of the time of the gold standard of manual segmentation (Fischl et al., 2002;

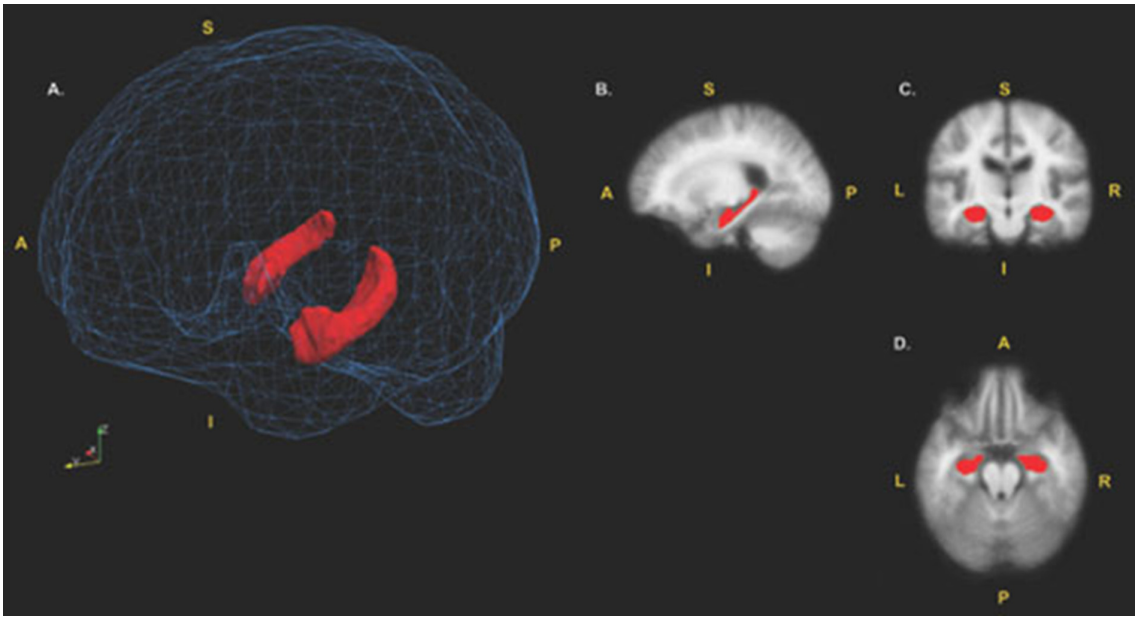


FIGURE 1 | Hippocampal region of interest (ROI). (A) surface models of the left and right hippocampi ROIs displayed in red. The hippocampi are visualized inside a wireframe mesh provided by Madan (2015). **(B)** Sagittal view (x = 105), **(C)** coronal view (z = 110) and **(D)** axial view (y = 151) of the hippocampi displayed in red. Coordinates are in MNI305 space. A, Anterior; P, Posterior; I, Inferior; S, Superior.

Jovicich et al., 2009). This makes automated segmentation well suited for large samples. Any errors in segmentation were fixed manually, and were re-processed through FreeSurfer, producing results that have been validated against manual segmentation (Morey et al., 2009) and histological measures (Cardinale et al., 2014). Whole hippocampal volume was computed as a sum of left and right hemisphere measures; this measure was then normalized in respect to total intracranial volume. All subsequent uses of the term "hippocampal volume" refer to the normalized value. See **Figure 1** for a visual depiction of the hippocampal region of interest (ROI; mesh provided by Madan, 2015).

Statistical Analyses

Neuroimaging data was analyzed using a ROI approach predicting hippocampal volume. MoCA and NIHTB-CB composite scores were used as predictor variables. Descriptive statistics and inter-measure correlations can be found in **Tables 2**, **3**. Hippocampal volume was normalized using estimated total intracranial volume, to control for differences in head size. Covariates of sex and education years were included in all models. A secondary set of analyses was aimed at examining the sub-scales of MoCA and NIH toolbox fluid cognition composite to determine whether a sub-scale was driving the relationship in the total score. Due to the characteristics of MoCA, certain sub-scales did not lend themselves to further analyses. Naming, Language, Abstraction and Orientation sections were excluded due to a restriction of range in observation (i.e., a 1 point scale) and/or a lack of variability. Two subjects were excluded as outliers because they had values greater than 2.5 the standard deviation from the mean (1 hippocampal volume outlier; 1 crystallized cognition outlier).

TABLE 2 | Descriptive statistics.

Measure	*Mean*	*SD*	*Range*
NIH Toolbox crystalized cognition	127.54	11.05	100–154
NIH Toolbox fluid cognition	96.21	9.72	80–133
MoCA	25.74	2.53	20–30

SD, Standard Deviation.

TABLE 3 | Correlation matrix.

	MoCA	Crystal	Fluid
MoCA	1	0.37**	0.42**
Crystal	0.37**	1	0.28**
Fluid	0.42**	0.28**	1

Crystal, NIH Toolbox crystallized cognition; Fluid, NIH Toolbox fluid cognition. $^{**}p < 0.01$.

RESULTS

NIH Toolbox

Relationship Between NIH Toolbox Fluid Cognition and Neuroimaging Measures

There was a significant positive linear relationship between hippocampal volume and fluid cognition composite score (t = 3.3, p = 0.001, partial η^2 = 0.11; see **Figure 2**) while controlling for sex and years of education (full model ($F_{(3,89)}$ = 3.81, p = 0.013, r^2 = 0.11).

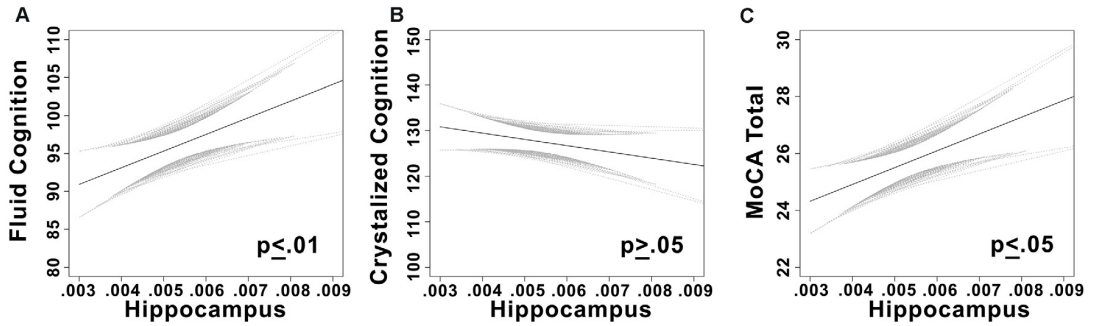


FIGURE 2 | (A) Plots the linear relationship between fluid cognition and hippocampal volume. **(B)** Plots the linear relationship between crystalized cognition and hippocampal volume. **(C)** Plots the linear relationship between Montreal Cognitive Assessment (MoCA) total score and hippocampal volume. Confidence bands are 95% confidence intervals of the regression line.

Relationship Between NIH Toolbox Crystallized Cognition and Neuroimaging Measures

As expected, no relationship was observed between hippocampal volume and crystallized cognition while controlling for covariates ($t = -0.21$, $p = 0.84$). Univariate models were nonsignificant as well; neither composite nor individual sub-scales of crystallized cognition were significantly related to hippocampal volume (p's > 0.05).

Relationship Between NIH Toolbox Sub-Scales and Neuroimaging Measures

Five linear models were analyzed using each sub-scale of the NIH toolbox fluid cognition composite while controlling for sex and years of education. Dimensional Change Card Sorting ($p = 0.027$, partial $\eta^2 = 0.05$, observed power = 0.60), Picture sequence memory ($p = 0.001$, partial $\eta^2 = 0.11$, observed power = 0.90), List Sorting ($p = 0.04$, partial $\eta^2 = 0.05$, observed power = 0.54) and Pattern comparison ($p = 0.002$, partial $\eta^2 = 0.11$, observed power = 0.89) were predicted by hippocampal volume. The attention domain flanker task was not significantly ($p > 0.25$) related to hippocampal volume. The strongest predictor of hippocampal volume was the pattern comparison task, which is in the processing speed domain. The episodic memory (picture sequence memory task) domain and the working memory domain (list sorting task) were both significantly related to hippocampal volume. Two supplemental tasks, the symbol digit search and Rey verbal learning were also analyzed (these tasks do not factor into the fluid cognition composite score, but were including in our NIH-Toolbox cognitive module). Rey verbal learning ($p = 0.008$, partial $\eta^2 = 0.08$, observed power = 0.77) and symbol digit search ($p = 0.073$, partial $\eta^2 = 0.04$, observed power = 0.44) scores showed a positive relationship with hippocampal volume. See **Figure 3** for a summary.

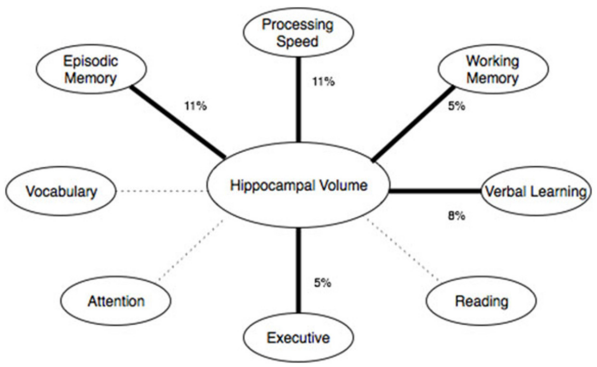


FIGURE 3 | Shows the cognitive domains of the NIH toolbox and their relationship with hippocampal volume. Thick, solid lines represent significant ($p < 0.05$) effects. Dashed lines represent non-significant effects. The percentage represents the effect size (percent variance explained by the predictor variable).

MoCA

Relationship Between MoCA and Neuroimaging Measures

There was a significant positive linear relationship between hippocampal volume and total MoCA score while controlling for sex and years of education ($t = 2.36$, $p = 0.02$, partial $\eta^2 = 0.06$).

Relationship Between MoCA Subscales and Neuroimaging Measures

Three subscales of the MoCA were analyzed individually: delayed recall, attention and visual-spatial/executive. As hypothesized, delayed recall was associated with hippocampal volume ($t = 1.96$, $p = 0.052$). No associations were found between attention ($t = 0.75$, $p = 0.454$) or visual-spatial/executive ($t = 0\ 0.88$, $p = 0.382$).

DISCUSSION

Hippocampal volume predicted cognitive performance on both the MoCA and NIH toolbox fluid cognition composite score in a community sample of 93 older adults without clinical history of MCI, neurodegenerative disease, neurological injury or self-reported memory problems. This finding supports the study hypothesis that smaller hippocampal volume is associated with poorer cognitive performance in older adults, particularly with respect to memory-related functions. A relationship in hippocampal volume was found only for fluid abilities, and not crystallized abilities such as vocabulary or reading. This

disassociation has been described in patient studies such as HM, where bilateral hippocampal resection caused profound memory disturbances while general knowledge remained intact (Scoville and Milner, 1957).

Prior literature demonstrates a strong relationship between the hippocampus and learning, memory and other fluid cognitive functions in both animals and humans (Raz et al., 1998; Petersen et al., 2000), with smaller volumes associated with poorer performance (Persson et al., 2006). However, these results have not been universal in older adults (Van Petten, 2004). Our data not only demonstrate a strong relationship between hippocampal volume and episodic memory, but also relationships with executive function, working memory and speed of processing.

Cognitive Subdomains and Hippocampal Volume

Within the MoCA and NIHTB, subtests that targeted the memory domain were significantly related to hippocampal volume. This replicates previous research showing a positive relationship between hippocampal atrophy and memory measures in non-demented subjects (Golomb et al., 1996; Persson et al., 2006). However, as mentioned, not all studies have replicated this finding. A meta-analysis by Van Petten (Van Petten, 2004) suggested that overall evidence in the literature for a positive relationship between hippocampal size and episodic memory in older adults was "surprisingly weak." In addition, a prior study investigating the relationship between hippocampal volume and MoCA failed to find such a relationship (Paul et al., 2011). While the current study and Paul et al. (2011) were similar in statistical power, imaging methods, and study inclusion/exclusion criteria, our sample was approximately 10 years older. As the relationship between hippocampal volume and memory is non-linear with age, this difference in our cohort's average age may account for the difference in our findings (Chen et al., 2016).

Regardless, this study is the first to report a significant positive relationship between hippocampal volume, MoCA and NIHTB-CB memory measures. However, caution in the interpretation of these findings is warranted. "Bigger is better" is certainly an oversimplification; smaller hippocampi have been associated with better memory in children and adolescents (Sowell et al., 2001). Furthermore, in pathological conditions, such as Fragile X syndrome, enlarged hippocampi are associated with poorer memory performance (Molnár and Kéri, 2014). The biological change associated with increased or decreased brain volume could be the result of multiple processes, which we are unable to elucidate with T1 structural MRI techniques. For example, increased volume could be the result of increased neuronal cell bodies, increases in glia or astrocytes, neuroinflammation or insufficient neuronal pruning. Nonetheless, our results demonstrate that smaller hippocampal volume is associated with decreased performance on two well validated and commonly administered measures of cognitive function in older adults, with particular sensitivity to memory function across both tasks.

Hippocampal volume was associated with performance in other cognitive domains besides memory on the NIHTB Cognitive Battery, specifically speed of processing, working memory and executive function (see **Figure 3**). In fact, the association between hippocampal volume and processing speed on the NIHTB was slightly stronger than for episodic memory. As delayed recall is not assessed by the NIH-Toolbox, it is possible that the relationship with delayed recall observed on the MoCA was not detectable from the NIH-Toolbox Cognitive Battery. Regardless, our results highlight the multifaceted role of the hippocampus in cognitive aging. The hippocampus contributes to other cognitive functions besides memory, and optimal learning and memory depends on other cognitive functions, such as working memory, processing speed and executive functioning, in addition to encoding and storage. A relationship between speed of processing and hippocampal volume has been shown in some (Tisserand et al., 2000), but not all past studies (Colom et al., 2013). An association between hippocampal volume and executive functioning was only evident on the NIH toolbox (dimension change card sorting), not for the MoCA executive-visual spatial sub-scale. Dimensional Change Card Sorting has greater cognitive demand and requires higher-order executive processes compared to the MoCA executive tasks. For example, the Dimensional Change Card Sorting task would require effort from multiple CHC factors, such as fluid reasoning, short-term memory, visual processing and reaction speed. Even though the executive tasks in NIH Toolbox and MoCA are classified as part of the same domain, performance on these tasks was not correlated ($r = 0.07$, $p > 0.05$). This supports the conclusion that these tests measure different elements of executive functioning. While the relationship between fluid cognition and hippocampal volume may seem surprising due to the traditional association of fluid abilities (particularly executive function and processing speed) and the pre-frontal cortex, previous studies have implicated hippocampal volume as a predictor of fluid ability in older adults (while no such association was found in younger adults; Reuben et al., 2011). A potential mechanism of the hippocampal association with fluid ability in older adults may relate to compensatory processes in the hippocampus as a result of the pre-frontal atrophy observed with age. Further research, particularly longitudinal studies, are needed to clarify whether the relationship between hippocampal volume and fluid ability changes throughout the lifespan, and which potential mechanisms may account for such change.

CONCLUSION

Prior research has produced controversy over the role of the hippocampus in cognitive aging, casting doubt on its role in episodic memory, as well as other domains (e.g., speed of processing, Van Petten, 2004; Colom et al., 2013). Our findings demonstrate that the hippocampus is a critical structure in cognitive aging, playing a role not only in episodic memory, but also processing speed, working memory and executive function. Whether effects of the hippocampus on domains outside of episodic memory are direct or meditational in nature remains to be seen. Our findings also demonstrate that performance on

a commonly used bedside dementia screening (MoCA) and a comprehensive cognitive battery (NIH Toolbox) are significantly related to hippocampal volume. Whereas, a prior study failed to find a relationship between MoCA and hippocampal volume in an older adult population (Paul et al., 2011), we found that our cohort, approximately 10 years senior in average age, evidenced a significant relationship. These data suggest a foundation for longitudinal research investigating hippocampal volume in older adults as a possible predictor of future decline or MCI conversion. Such data would help to elucidate issues of acute vs. progressive atrophy in the study, and further clarify potential implications for pathologies like MCI and AD. More importantly, our data provide strong evidence in support of the multifaceted role of the hippocampus in cognitive aging.

AUTHOR CONTRIBUTIONS

AO, RAC, ECP, NRN and AJW contributed text to the manuscript. AO and AJW performed data analysis. All authors provided edits and approved the final version of the manuscript.

FUNDING

AJW and AO are partially supported by the McKnight Brain Research Foundation and the University of Florida Cognitive Aging and Memory Clinical Translational Research Program. AJW is partially supported by the NIH/NCATS CTSA grant UL1 TR000064 and KL2 TR000065, NIA K01AG050707-A1 and R01AG054077. The funding sources had no involvement in the study design, collection, analysis and interpretation of the data, in the writing of the manuscript and in the decision to submit the manuscript for publication. Neuroimaging was performed at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain Institute of the University of Florida, which is supported by National Science Foundation Cooperative Agreement no. DMR-1157490 and the State of Florida.

REFERENCES

Cardinale, F., Chinnici, G., Bramerio, M., Mai, R., Sartori, I., Cossu, M., et al. (2014). Validation of FreeSurfer-estimated brain cortical thickness: comparison with histologic measurements. *Neuroinformatics* 12, 535–542. doi: 10.1007/s12021-014-9229-2

Cattell, R. B. (1987). *Intelligence: Its Structure, Growth and Action.* Amsterdam, New York, NY: North-Holland.

Chen, H., Zhao, B., Cao, G., Porges, E. C., O'Shea, A., Woods, A. J., et al. (2016). Statistical approaches for the study of cognitive and brain aging. *Front. Aging Neurosci.* 8:176. doi: 10.3389/fnagi.2016.00176

Colom, R., Stein, J. L., Rajagopalan, P., Martínez, K., Hermel, D., Wang, Y., et al. (2013). Hippocampal structure and human cognition: key role of spatial processing and evidence supporting the efficiency hypothesis in females. *Intelligence* 41, 129–140. doi: 10.1016/j.intell.2013.01.002

Eldridge, L. L., Knowlton, B. J., Furmanski, C. S., Bookheimer, S. Y., and Engel, S. A. (2000). Remembering episodes: a selective role for the hippocampus during retrieval. *Nat. Neurosci.* 3, 1149–1152. doi: 10.1038/80671

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. doi: 10.1016/S0896-6273(02)00569-X

Golomb, J., Kluger, A., de Leon, M. J., Ferris, S. H., Mittelman, M. P., Cohen, J., et al. (1996). Hippocampal formation size predicts declining memory performance in normal aging. *Neurology* 47, 810–813. doi: 10.1212/WNL.47.3.810

Heaton, R. K., Akshoomoff, N., Tulsky, D., Mungas, D., Weintraub, S., Dikmen, S., et al. (2014). Reliability and validity of composite scores from the NIH toolbox cognition battery in adults. *J. Int. Neuropsychol. Soc.* 20, 588–598. doi: 10.1017/S1355617714000241

Iaria, G., Chen, J.-K., Guariglia, C., Ptito, A., and Petrides, M. (2007). Retrosplenial and hippocampal brain regions in human navigation: complementary functional contributions to the formation and use of cognitive maps. *Eur. J. Neurosci.* 25, 890–899. doi: 10.1111/j.1460-9568.2007.05371.x

Jack, C. R. Jr., Petersen, R. C., Xu, Y. C., O'Brien, P. C., Smith, G. E., Ivnik, R. J., et al. (1999). Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology* 52, 1397–1403. doi: 10.1212/WNL.52.7.1397

Jovicich, J., Czanner, S., Han, X., Salat, D., van der Kouwe, A., Quinn, B., et al. (2009). MRI-derived measurements of human subcortical, ventricular and intracranial brain volumes: reliability effects of scan sessions, acquisition sequences, data analyses, scanner upgrade, scanner vendors and field strengths. *Neuroimage* 46, 177–192. doi: 10.1016/j.neuroimage. 2009.02.010

Madan, C. R. (2015). Creating 3D visualizations of MRI data: a brief guide. *F1000Res.* 4:466. doi: 10.12688/f1000research.6838.1

McGrew, K. S. (2009). CHC theory and the human cognitive abilities project: standing on the shoulders of the giants of psychometric intelligence research. *Intelligence* 37, 1–10. doi: 10.1016/j.intell.2008.08.004

Molnár, K., and Kéri, S. (2014). Bigger is better and worse: on the intricate relationship between hippocampal size and memory. *Neuropsychologia* 56, 73–78. doi: 10.1016/j.neuropsychologia.2014.01.001

Morey, R. A., Petty, C. M., Xu, Y., Hayes, J. P., Wagner, H. R. II., Lewis, D. V., et al. (2009). A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *Neuroimage* 45, 855–866. doi: 10.1016/j.neuroimage.2008.12.033

Mungas, D., Heaton, R., Tulsky, D., Zelazo, P. D., Slotkin, J., Blitz, D., et al. (2014). Factor structure, convergent validity and discriminant validity of the NIH toolbox cognitive health battery (NIHTB-CHB) in adults. *J. Int. Neuropsychol. Soc.* 20, 579–587. doi: 10.1017/S1355617714000307

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53, 695–699. doi: 10.1111/j.1532-5415.2005.53221.x

O'Keefe, J. (1979). A review of the hippocampal place cells. *Prog. Neurobiol.* 13, 419–439. doi: 10.1016/0301-0082(79)90005-4

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 34, 171–175. doi: 10.1016/0006-8993(71)90358-1

Papp, K. V., Kaplan, R. F., Springate, B., Moscufo, N., Wakefield, D. B., Guttmann, C. R. G., et al. (2014). Processing speed in normal aging: effects of white matter hyperintensities and hippocampal volume loss. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 21, 197–213. doi: 10.1080/13825585.2013.795513

Paul, R., Lane, E. M., Tate, D. F., Heaps, J., Romo, D. M., Akbudak, E., et al. (2011). Neuroimaging signatures and cognitive correlates of the montreal cognitive assessment screen in a nonclinical elderly sample. *Arch. Clin. Neuropsychol.* 26, 454–460. doi: 10.1093/arclin/acr017

Persson, J., Nyberg, L., Lind, J., Larsson, A., Nilsson, L.-G., Ingvar, M., et al. (2006). Structure-function correlates of cognitive decline in aging. *Cereb. Cortex* 16, 907–915. doi: 10.1093/cercor/bhj036

Petersen, R. C., Jack, C. R. Jr., Xu, Y.-C., Waring, S. C., O'Brien, P. C., Smith, G. E., et al. (2000). Memory and MRI-based hippocampal volumes in aging and AD. *Neurology* 54, 581–581. doi: 10.1212/WNL.54.3.581

Raz, N., Gunning-Dixon, F. M., Head, D., Dupuis, J. H., and Acker, J. D. (1998). Neuroanatomical correlates of cognitive aging: evidence from structural

magnetic resonance imaging. *Neuropsychology* 12, 95–114. doi: 10.1037/0894-4105.12.1.95

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., et al. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb. Cortex* 15, 1676–1689. doi: 10.1093/cercor/bhi044

Reuben, A., Brickman, A. M., Muraskin, J., Steffener, J., and Stern, Y. (2011). Hippocampal atrophy relates to fluid intelligence decline in the elderly. *J. Int. Neuropsychol. Soc.* 17, 56–61. doi: 10.1017/S135561771000127X

Salthouse, T. A. (2010). Selective review of cognitive aging. *J. Int. Neuropsychol. Soc.* 16, 754–760. doi: 10.1017/S1355617710000706

Scoville, W. B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* 20, 11–21. doi: 10.1136/jnnp.20.1.11

Shi, F., Liu, B., Zhou, Y., Yu, C., and Jiang, T. (2009). Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: meta-analyses of MRI studies. *Hippocampus* 19, 1055–1064. doi: 10.1002/hipo.20573

Sowell, E. R., Delis, D., Stiles, J., and Jernigan, T. L. (2001). Improved memory functioning and frontal lobe maturation between childhood and adolescence: a structural MRI study. *J. Int. Neuropsychol. Soc.* 7, 312–322. doi: 10.1017/s135561770173305x

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol. Rev.* 99, 195–231. doi: 10.1037//0033-295x.99.2.195

Tisserand, D. J., Visser, P. J., van Boxtel, M. P. J., and Jolles, J. (2000). The relation between global and limbic brain volumes on MRI and cognitive performance in healthy individuals across the age range. *Neurobiol. Aging* 21, 569–576. doi: 10.1016/s0197-4580(00)00133-0

Van Petten, C. (2004). Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. *Neuropsychologia* 42, 1394–1413. doi: 10.1016/j.neuropsychologia. 2004.04.006

Woods, A. J., Cohen, R. A., and Pahor, M. (2013). Cognitive frailty: frontiers and challenges. *J. Nutr. Health Aging* 17, 741–743. doi: 10.1007/s12603-013-0398-8

Yamamoto, N., Philbeck, J. W., Woods, A. J., Gajewski, D. A., Arthur, J. C., Potolicchio, S. J., et al. (2014). Medial temporal lobe roles in human path integration. *PLoS One* 9:e96583. doi: 10.1371/journal.pone.0096583

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

The handling Editor declared a shared affiliation, though no other collaboration, with the authors and states that the process nevertheless met the standards of a fair and objective review.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 04 January 2017
doi: 10.3389/fnagi.2016.00328

Frontal Structural Neural Correlates of Working Memory Performance in Older Adults

*Nicole R. Nissim[1,2], Andrew M. O'Shea[1], Vaughn Bryant[1], Eric C. Porges[1], Ronald Cohen[1] and Adam J. Woods[1,2]**

[1] Center for Cognitive Aging and Memory, McKnight Brain Institute, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL, USA, [2] Department of Neuroscience, University of Florida, Gainesville, FL, USA

Working memory is an executive memory process that allows transitional information to be held and manipulated temporarily in memory stores before being forgotten or encoded into long-term memory. Working memory is necessary for everyday decision-making and problem solving, making it a fundamental process in the daily lives of older adults. Working memory relies heavily on frontal lobe structures and is known to decline with age. The current study aimed to determine the neural correlates of decreased working memory performance in the frontal lobes by comparing cortical thickness and cortical surface area from two demographically matched groups of healthy older adults, free from cognitive impairment, with high versus low N-Back working memory performance ($N = 56$; average age $= 70.29 \pm 10.64$). High-resolution structural T1-weighted images (1 mm isotropic voxels) were obtained on a 3T Philips MRI scanner. When compared to high performers, low performers exhibited significantly decreased cortical surface area in three frontal lobe regions lateralized to the right hemisphere: medial orbital frontal gyrus, inferior frontal gyrus, and superior frontal gyrus (FDR $p < 0.05$). There were no significant differences in cortical thickness between groups, a proxy for neurodegenerative tissue loss. Our results suggest that decreases in cortical surface area (a proxy for brain structural integrity) in right frontal regions may underlie age-related decline of working memory function.

Keywords: cognitive aging, cortical surface area, cortical thickness, FreeSurfer, N-Back, Structural Magnetic Resonance Imaging

OPEN ACCESS

Edited by:
Changiz Geula, Northwestern University, USA

Reviewed by:
Neha Sehgal, Wisconsin Institute for Discovery, USA
Clinton B. Wright, University of Miami, USA

***Correspondence:**
Adam J. Woods
ajwoods@phhp.ufl.edu

Received: *04 August 2016*
Accepted: *19 December 2016*
Published: *04 January 2017*

Citation:
Nissim NR, O'Shea AM, Bryant V, Porges EC, Cohen R and Woods AJ (2017) Frontal Structural Neural Correlates of Working Memory Performance in Older Adults. Front. Aging Neurosci. 8:328. doi: 10.3389/fnagi.2016.00328

INTRODUCTION

Working memory is a vital process underlying human thought. Working memory is a limited capacity system that involves active manipulation of information currently being maintained in focal attention (Glisky, 2007). Working memory is one component of executive function that allows for transitional information to be held and manipulated temporarily in memory stores, before either being forgotten or encoded into long-term memory (Baddeley, 1992; Goldman-Rakic, 1996; Owen et al., 2005). As with other components of executive function, working memory processes rely heavily on frontal lobe structures (Courtney et al., 1998). Working memory processes guide voluntary or goal-directed behaviors including short-term maintenance of relevant information, mental manipulations, and mental organization of imminent sequence of actions (Goldman-Rakic, 1987; Boisgueheneuc et al., 2006). Working memory is necessary for everyday decision-making and problem-solving, making it a fundamental process in the lives of older adults. Activities of daily living such

as preparing meals, taking medication, paying bills, as well as organizing and planning daily routines and appointments require working memory and other components of executive function (Mograbi et al., 2014). As such, declines in working memory can lead to deficits in these domains and consequently lead to loss of independence and decreased quality of life (Klingberg, 2010; Williams and Kemper, 2010). Working memory performance can be impacted by age-related reductions in working memory capacity and is increasingly susceptible to interference in older adults. Not surprisingly, memory loss and perceived declines in memory performance are frequent complaints in older adult populations (Gazzaley et al., 2007; Kaup et al., 2014). As frontal cortices undergo the most pronounced structural decline with advanced age (Lemaitre et al., 2012) and play an important role in working memory function (Freeman et al., 2008), identifying frontal structures underlying age-related working memory decline may provide important therapeutic targets for combating cognitive aging.

The prefrontal cortex participates in cognitive features of behavior, engaging the organization of goal-directed behaviors (Fuster, 1988). Although the frontal lobe is the last brain region to mature in humans around age 25, it is also one of the first regions to structurally decline during the aging process, following the 'last in, first out' model of aging (Salat et al., 2004). Studies of brain morphometry show that the prefrontal cortex experiences the most striking reductions (Lemaitre et al., 2012). Similarly, age-related decreases in cortical surface area are greatest in frontal regions (Salat et al., 2004), while the greatest age-related volume reductions occur in the middle frontal gyrus, the superior frontal gyrus (SFG), and the frontal pole (Lemaitre et al., 2012).

The frontal lobes, and the right frontal lobe in particular, play an important role in working memory function. The ability to hold onto visuospatial information, to be fractioned into separate visual and spatial components, is thought to be principally represented within the right hemisphere (Baddeley, 2000). Prabhakaran et al. (2000) compared the retention of verbal and spatial information held in integrated or unintegrated forms using functional magnetic resonance imaging (fMRI), and found greater right frontal activation for integrated information, providing evidence for the right frontal lobe being particularly critical for retention of integrated information (Baddeley, 2000; Prabhakaran et al., 2000). Previous fMRI work studying the functional neural basis of aging and working memory have shown distinct activation patterns in older versus younger adults, and for high versus low performance rates on an N-Back working memory task (Cabeza, 2002; Dolcos et al., 2002). Positron emission tomography (PET) and fMRI studies of higher-order cognitive functions have been associated with prominent activations in the prefrontal cortex. Often, activations are sometimes lateralized, which may reflect the nature of the processes and/or the stimuli involved (Nyberg et al., 1996; Cabeza et al., 2002). Prefrontal cortex activity tends to be less asymmetrical in older than younger adults (Cabeza et al., 2002). Young high performers on working memory tasks tend to exhibit significant activation of the dorsolateral prefrontal cortex (DLPFC) lateralized to the right hemisphere. Older adults with low performance exhibit more robust right hemisphere activation than young, potentially reflecting inefficiency of activation, whereas older adults who perform at the same level as young adults exhibit bilateral activation patterns in the prefrontal cortex. This difference in activation patterns of high performing older adults compared to high performing younger adults may counteract age-related neurocognitive declines as a form of compensatory mechanism (compensation hypothesis), or it could reflect age-related deficits in recruiting specialized neural mechanisms (dedifferentiation hypothesis; Cabeza et al., 2002). While the functional pattern of working memory performance in older adults has been well explored, the age-related structural alterations in frontal cortices underlying working memory decline versus compensation remains unclear.

Older adults exhibit significant deficits in tasks that involve active manipulation, reorganization, or integration of the contents of working memory (Salthouse et al., 1989). Investigating the structural neural correlates of performance on working memory tasks in older adults is necessary to understand how working memory systems change with age. This study aimed to determine the frontal structures underlying poorer working memory performance. We hypothesized that working memory deficits would be associated with decreases in cortical surface area in right frontal brain regions in healthy older adults. We did not expect any significant changes in cortical thickness, as decreases in thickness signify neurodegenerative tissue loss (Shefer, 1973; Fischl and Dale, 2000) and our population of interest was healthy older adults. In contrast, cortical surface area serves as a proxy for gray matter structural integrity (Fischl and Dale, 2000; Salat et al., 2004; Dickstein et al., 2007; Lemaitre et al., 2012).

MATERIALS AND METHODS

Participants

We recruited healthy community dwelling older individuals in the Gainesville and North Florida region ($N = 56$, 50% female, 52 right handers). A thorough medical history questionnaire for each participant provided detailed information on health status, medication status, and allowed us to rule out the presence of age-related brain disorders. Exclusionary criteria for the study included pre-existing neurological or psychiatric brain disorders, MRI exclusions, mild cognitive impairment (MCI) or diagnosis with a neurodegenerative brain disease (i.e., dementia or Alzheimer's). The Montreal Cognitive Assessment (MoCA) was given to assess general cognitive ability and rule out possible MCI (Nasreddine et al., 2005). Additionally, the MoCA allowed us to control for differences in global cognitive function and insure our analyses were directly relevant to working memory rather than a reflection of generalized cognitive deficits. The MoCA cut off score to be an eligible participant in the study was 20. A comprehensive neuropsychological battery was performed on each participant to provide for clinical assessment of MCI status. A clinical neuropsychologist assessed participant performance on the battery to determine MCI status. No participants in this sample were clinically indicated to have MCI using this approach. Participants did not significantly differ in age, sex, education,

TABLE 1 | Demographic data and independent *t*-test statistics (means, standard deviation).

	Total sample $N = 56$	High performers $n = 29$	Low performers $n = 27$	Independent *t*-test t (p)
Age	70.29 (10.64)	68.00 (11.06)	72.74 (9.77)	−1.695 (0.096)
Sex	28F:28M	14F:15M	14F:13M	−0.263 (0.794)
Education	16.30 (2.33)	16.24 (1.88)	16.37 (2.76)	−0.203 (0.840)
MoCA total score	25.89 (2.67)	26.48 (2.69)	25.26 (2.55)	1.742 (0.087)
Intracranial volume	1443534.46 (225849.81)	1457084.28 (216839.60)	1428980.96 (238413.09)	0.462 (0.646)

MoCA score, or intracranial volume (ICV; $p > 0.05$). ICV is especially important to control for as it is closely relates to brain size (Hentschel and Kruggel, 2004; Im et al., 2008), and thus was also included as a covariate in our model to rule out the possibility of head size driving any cortical thickness or cortical surface area differences between groups. The total sample ($N = 56$) consisted of 28 female and 28 male older adults. The range for the total sample of the following covariates were: MoCA scores = 20–30, age = 44–89 years old, education = 12–20 years, and ICV = 975547.27–1988968.30. See **Table 1** for demographic means, standard deviations, and statistics for the total sample. All participants in the study underwent cognitive testing followed by an MRI scanning session where the N-Back task was performed inside the scanner. fMRI data on N-Back will be presented in a subsequent paper. N-Back performance data was used to characterize participants into high and low working memory groups (described in detail below). Prior to any study procedures, all participants provided written informed consent. The study protocol was carried out in accordance with the Declaration of Helsinki, and the University of Florida Institutional Review Board approved all procedures in this study.

N-Back Task

The N-Back task requires continuous performance in which participants are asked to monitor the identity of a series of stimuli and indicate when the currently presented stimulus is the same as the one presented *n*-trials previously (Kirchner, 1958; Owen et al., 2005). This task is known to engage working memory processes and thus was used in this study. Participants completed an N-Back practice session to ensure that all instructions were clear and that participants could accurately perform the task. All N-Back tasks were created with E-Prime version 2.0 (Psychology Software Tools Inc., Pittsburgh, PA, USA). The task was completed inside the scanner, with images projected onto a screen behind the participants' head and viewed through a mirror mounted on the head coil. Responses were made via an MRI-compatible button box, using the middle and index finger. Participants performed two runs of the N-Back, which included both 0-Back and a 2-Back version of the N-Back, totaling 15 min of functional task time. For 0-Back, participants were asked to respond by button press (with index finger) when they saw a X on the screen, and respond with another button press (with middle finger) when they saw any other letter (distractors). This task was used as an attention control. Each letter was displayed one at a time, for 700 ms, followed by a crosshair for 2300 ms. The participants could respond by button press at any point in the total 3000 ms trial interval. During the 2-Back task, participants viewed single letters (i.e., letters of identical font, color, size) on the screen with the same timing scheme as 0-Back. When a letter appeared and was the same as the letter that was presented two letters prior, participants were asked to respond to that target letter by a button press of their index finger (see **Figure 1** for visual example). All letters that did not match the 2-Back pattern were used as distractors, and participants were asked to respond by button press with their middle finger. The order of whether participants received 2-Back or 0-Back first was randomized.

N-Back Working Memory Performance Characterization

N-Back accuracy rates were collected and recorded in E-Prime v2.0 then transferred as total percent accuracy scores of both runs into SPSS. The data was then processed through SPSS version 21. All participants responded to greater than 75% of all N-back trials. A median split based on 2-Back accuracy was performed to determine high versus low performers. High performers ($N = 29$) scored 67% or above correctly on 2-Back, while low performers ($N = 27$) had an accuracy score of 66% or below. For 5 participants (three high performers, two low performers), one of their runs was lost during data collection due to technical problems. For these participants, the one run collected was used for analyses.

Working Memory Group Demographics

Behavioral data for 0-Back average accuracy was 83.71 ± 17.38% (range = 19–98%) while 2-Back average accuracy was

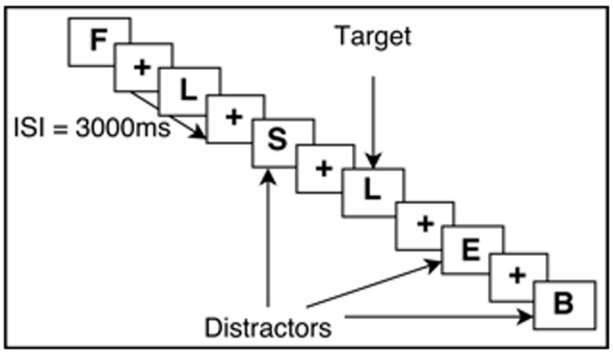


FIGURE 1 | Example of the 2-Back working memory paradigm. ISI, Interstimulus interval.

64.88 ± 16.93% (range = 20–90%) for the overall sample ($N = 56$). High and low working memory performers on the 2-Back task were determined by performing a median split of accuracy scores. Accuracy scores of 67% or above were grouped as high performers. In contrast, scores below 67%, were grouped as low performers. See **Table 2** for more detailed task performance information for high and low groups. The range for the following covariates for the low performing group was: age = 47–89, education = 12–20, MoCA = 20–30, ICV = 1001786.49–1954571.14. The range for the following covariates for the high performing group was: age = 44–85, education = 12–20, MoCA = 21–30, ICV = 975547.27–1988968.30. High versus low working memory groups did not significantly differ on the above covariates ($p > 0.05$). For high and low group demographic means, standard deviations, and test statistics, see **Table 1**.

MRI Acquisition

T1-weighted MPRAGE structural MRI scans were performed on all participants. Participants were imaged in a Philips Achieva 3.0 Tesla (3T) scanner (Philips Electronics, Amsterdam, The Netherlands) with a 32-channel receive-only head coil. Scan parameters: repetition time (TR) = 7.0 ms; echo time (TE) = 3.2 ms; flip angle = 8°; field of view = 240 mm × 240 mm × 170 mm; voxel = 1 mm × 1 mm × 1 mm. Foam padding was placed around the head to limit motion during the scan. No images exhibited evidence of motion artifact. Participants were given headphones and earplugs to minimize noise while inside the scanner.

T_1-Weighted Neuroimaging Processing

Cortical reconstruction and volumetric segmentation was performed with FreeSurfer version 5.3 image analysis suite. The technical details of these procedures are described in prior publications (Dale and Sereno, 1993; Dale et al., 1999; Fischl et al., 1999a,b, 2001, 2002, 2004a,b; Fischl and Dale, 2000; Segonne et al., 2004; Han et al., 2006; Jovicich et al., 2006). Briefly, this processing includes removal of non-brain tissue (Segonne et al., 2004), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl et al., 2002, 2004a), intensity normalization (Sled et al., 1998), tessellation of the gray matter white matter boundary, automated topology correction (Fischl et al., 2001; Segonne et al., 2007), and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale and Sereno, 1993; Dale et al., 1999; Fischl and Dale, 2000). Once the cortical models are complete, a number of deformable procedures can be performed for in further data processing and analysis including surface inflation (Fischl et al., 1999a), registration to a spherical atlas which utilized individual cortical folding patterns to match cortical geometry across subjects (Fischl et al., 1999b), parcellation of the cerebral cortex into units based on gyral and sulcal structure (Fischl et al., 2004b; Desikan et al., 2006). This method uses both intensity and continuity information from the entire three dimensional volume in segmentation and deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000). The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The maps produced are not restricted to the voxel resolution of the original data and thus are capable of detecting submillimeter differences between groups. FreeSurfer measures have been shown to be both reliable and valid. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003; Salat et al., 2004). FreeSurfer morphometric procedures have been demonstrated to show good test–retest reliability across scanner manufacturers and across field strengths (Han et al., 2006; Reuter et al., 2012). Once processed through FreeSurfer, all output was visually inspected for processing errors (e.g., mislabeling white matter, gray matter, or skull inclusions) and manually corrected for when necessary.

TABLE 2 | Average N-Back accuracy scores and standard deviation.

	High performers $n = 29$	Low performers $n = 27$	Total sample $N = 56$
2-Back	77.90% (7.04)	50.89% (12.69)	64.87% (16.93)
0-Back	90.79% (8.82)	76.11% (21.75)	83.71% (17.83)

Neuroimaging Measures: Cortical Thickness and Cortical Surface Area

The relationship between cortical surface area and cortical thickness creates a quantifiable brain volume. For example, although two objects may have the exact same volume, the shape or contours of the objects can vary considerably, exhibiting very different topography. If we consider a cube measuring 3 × 3 × 3 versus a rectangular shape measuring 3 × 9 × 1, both shapes share the same volume of 27 mm^3; this exemplifies that surface area and thickness may exhibit a very different pattern while sharing the same volume. When we consider the human brain, age-related changes in surface area versus thickness may have different implications for behavioral and cognitive processes. These two components exhibit distinct patterns of change when comparing healthy versus diseased brains (Dotson et al., 2015). Gray matter, which makes up the cortical ribbon, experiences volume loss throughout adulthood into advanced age (Scott and Thacker, 2004). Neuronal density is relatively stable throughout life; any robust decrease in neuronal density is thought to reflect a disease state (Morrison and Hof, 2002; Dickstein et al., 2007). Decrease in cortical thickness is a proxy for neuronal loss due to neurodegenerative disease (Shefer, 1973; Fischl and Dale, 2000). While changes in cortical surface area and its relationship to general cognitive function is less known (Schnack et al., 2015), cortical surface area is thought to reflect the structural integrity of gray matter (Fischl and Dale, 2000; Salat et al., 2004; Lemaitre

et al., 2012). It has been suggested that preservation in neuronal number, but loss of neuronal dendritic architecture underlies neocortical volume loss with increasing age in the absence of Alzheimer's disease (Morrison and Hof, 2002; Freeman et al., 2008). In normal healthy aging, to our best knowledge, there are no studies that have closely examined cortical surface area changes and the possible role this may play in driving age-related declines in working memory function.

Regions of Interest and Neuroimaging Statistical Analyses

Frontal lobe regions (defined as all regions anterior to the pre-central gyrus using the Desikan-Killiany parcellation, see **Table 3** for a comprehensive list of ROIs) and two control regions outside the frontal loges (left and right pericalcarine areas of the occipital cortex; i.e., V1; Desikan et al., 2006) were analyzed for both thickness and area using separate univariate general linear models with performance group (high versus low) as a fixed factor and age, sex, years of education, ICV and MoCA score as covariates using the software SPSS version 21. Control sites were included to assess the regional specificity of our frontal focused analyses. To control for multiple comparison type I error we implemented a false discovery rate (Benjamini and Hochberg, 1995) threshold of FDR < 0.05 using the software R, which is freely available for download online[1].

RESULTS

Cortical Surface Area and Thickness

Low performers exhibited significantly less surface area in three frontal lobe regions lateralized to the right hemisphere: SFG ($p_{FDR} = 0.018$; Cohen's $D = 0.81$; surface area of high performers $= 6791.59 \pm 648.62$ mm^2; low performers $= 6266.26 \pm 649.15$ mm^2), pars opercularis of the inferior frontal gyrus ($p_{FDR} = 0.024$; Cohen's $D = 0.79$; surface area of high performers $= 1361.38 \pm 183.34$ mm^2; low performers $= 1224.19 \pm 163.97$ mm^2), and medial orbital frontal gyrus [$p_{FDR} = 0.018$; Cohen's $D = 0.85$; surface area of high performers $= 1833.14 \pm (225.09)$ mm^2; low performers $= 1657.00 \pm 186.57$ mm^2]. No significant differences in cortical thickness were observed after correcting for multiple comparisons (FDR > 0.05). As a control brain region, the pericalcarine gyrus of the occipital lobe was analyzed in both hemispheres and did not significantly differ in thickness or surface area between groups. See **Figure 2** for significant surface area results and **Table 3** for all surface area and thickness results.

TABLE 3 | Surface area and thickness measures from frontal ROIs and control brain region.

Brain region	*F*-value	*P*-value	η_p^2	P-FDR
Measurement: surface area				
R Medial orbital frontal gyrus	10.99	0.002	0.183	0.018*
R Superior frontal gyrus	9.84	0.003	0.167	0.018*
R Pars opercularis	8.211	0.006	0.144	0.024*
L Rostral anterior cingulate	5.815	0.02	0.106	0.24
L Medial orbital frontal gyrus	2.553	0.117	0.05	0.448
R Pars orbitalis	2.198	0.145	0.043	0.435
L Pars orbitalis	1.831	0.182	0.036	0.448
L Superior frontal gyrus	1.669	0.202	0.033	0.448
L Frontal pole	1.308	0.258	0.026	0.448
L Rostral middle frontal gyrus	1.185	0.282	0.024	0.448
R Pars triangularis	1.118	0.295	0.022	0.708
L Caudal middle frontal gyrus	1.11	0.297	0.022	0.448
L Lateral orbital frontal gyrus	0.947	0.335	0.019	0.448
L Caudal anterior cingulate	0.943	0.336	0.019	0.448
R Frontal pole	0.784	0.38	0.016	0.76
L Pars triangularis	0.55	0.462	0.011	0.554
R Caudal anterior cingulate	0.542	0.472	0.011	0.786
L Pars opercularis	0.282	0.598	0.006	0.652
R Rostral middle frontal gyrus	0.245	0.623	0.005	0.786
R Lateral orbital frontal	0.196	0.66	0.004	0.786
R Caudal middle frontal	0.143	0.707	0.003	0.786
R Rostral anterior cingulate	0.129	0.721	0.003	0.786
L Pericalcarine (control region)	0.109	0.742	0.002	0.742
R Pericalcarine (control region)	0.007	0.935	0.000139	0.935
Measurement: thickness				
L Pars triangularis	6.075	0.017	0.11	0.204
R Medial orbital frontal gyrus	4.851	0.032	0.09	0.261
R Pars opercularis	3.312	0.075	0.063	0.261
L Rostral anterior cingulate	3.319	0.075	0.063	0.295
R Caudal anterior cingulate	3.291	0.076	0.063	0.261
R Pars orbitalis	3.055	0.087	0.059	0.261
L Medial orbital frontal gyrus	2.88	0.096	0.056	0.295
L Caudal anterior cingulate	2.642	0.11	0.051	0.295
L Frontal pole	2.458	0.123	0.048	0.295
R Superior frontal gyrus	2.165	0.148	0.042	0.355
L Pars orbitalis	1.801	0.186	0.035	0.361
R Rostral anterior cingulate	1.489	0.228	0.029	0.413
L Pericalcarine (control region)	1.453	0.234	0.029	0.361
R Pericalcarine (control region)	1.41	0.241	0.028	0.413
L Pars opercularis	1.302	0.259	0.026	0.361
L Lateral orbital frontal gyrus	1.238	0.271	0.025	0.361
R Rostral middle frontal gyrus	1.187	0.281	0.024	0.421
L Superior frontal gyrus	1.001	0.322	0.02	0.386
L Rostral middle frontal gyrus	0.722	0.4	0.015	0.436
R Frontal pole	0.687	0.411	0.014	0.538
R Pars triangularis	0.484	0.49	0.01	0.538
R Caudal middle frontal gyrus	0.474	0.494	0.01	0.538
L Caudal middle frontal gyrus	0.33	0.568	0.007	0.568
R Lateral orbital frontal gyrus	0.014	0.908	0.000278	0.908

*R, Right, L, Left, * = FDR < 0.05.*

DISCUSSION

The current study investigated the neural correlates of age-related decreases in working memory performance in frontal cortices. We found significant differences in cortical surface area for three regions of the right frontal lobe. Low working memory performers had significantly less surface area for the inferior frontal gyrus (pars opercularis), SFG, and the medial orbital frontal gyrus, when compared to high working memory

[1]https://www.r-project.org/

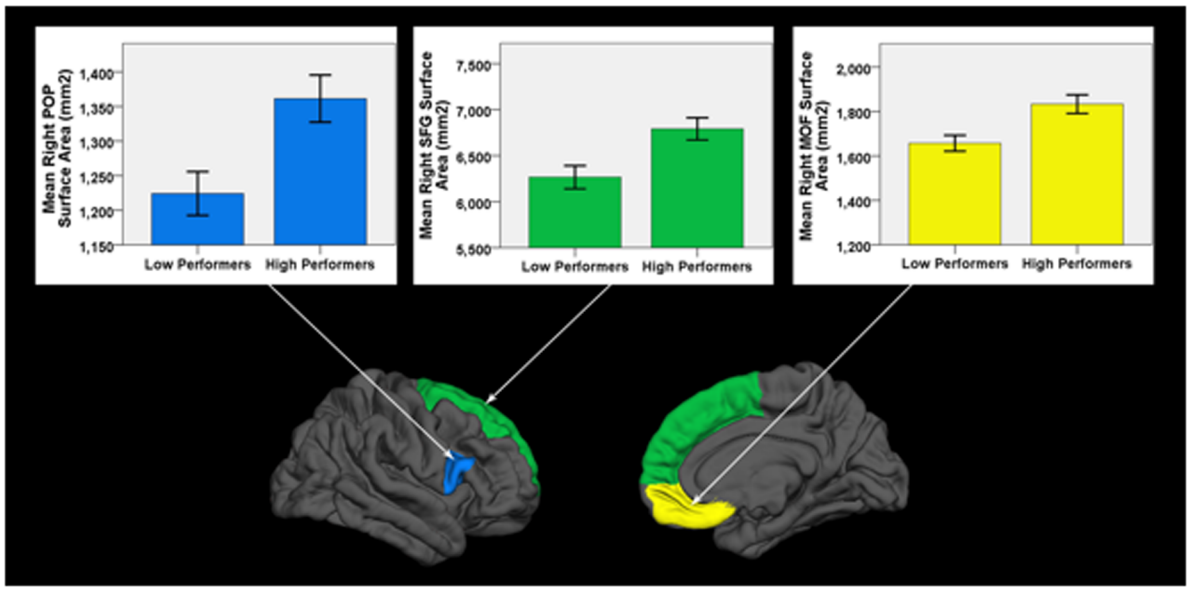


FIGURE 2 | Cortical surface differences between low versus high working memory performers. Arrows connect graphs of between group differences to the affiliated gyri ROI highlighted on a FreeSurfer brain model. POP, pars opercularis of the inferior frontal gyrus; SFG, superior frontal gyrus; MOF, medial orbital frontal gyrus. Error bars = ± 1 SE.

performers. These areas of decreased structural integrity are consistent with prior fMRI findings for functional correlates of working memory performance (Curtis and D'Esposito, 2003; Owen et al., 2005). These results are also consistent with prior research demonstrating right lateralized BOLD activation of frontal cortices in young adults with high working memory performance, but bilateral (potentially compensatory) activation of right and left frontal cortices in older adults able to maintain a high level of working memory performance (Cabeza et al., 2002). In contrast, older adults unable to maintain performance showed unilateral increase in activation of right frontal regions, perhaps consistent with less efficient neural processing (Cabeza et al., 2002). Collectively, our structural MRI findings, when considered in concert with prior functional MRI research (Cabeza et al., 2002), suggests that areas in the right prefrontal cortex are critical substrates for age-related change in working memory function. Our findings provide evidence that right lateralized structural abnormalities in inferior, superior, and medial orbital frontal gyri underlie age-related working memory decline.

Pars Opercularis of the Inferior Frontal Gyrus

The pars opercularis (BA44), a sub-region of the inferior frontal gyrus, is included in the functionally defined ventrolateral prefrontal cortex (VLPFC; Molnar-Szakacs et al., 2005). The VLPFC is consistently found to be active in working memory fMRI studies; early functional neuroimaging studies that activated this region in humans tended to emphasize the explicit retrieval of one or a few pieces of information, as well as the sequencing of responses based directly on stored information (Owen et al., 2005). Aron et al. (2004) argues that the right VLPFC plays a critical role in cognitive inhibition. Cognitive inhibition is a component of executive control that can be localized to the right inferior frontal gyrus, specifically the pars opercularis (Molnar-Szakacs et al., 2005; Falquez et al., 2014). Inhibition can be defined as the suppression of inappropriate responses (Aron et al., 2004). Cognitive inhibition could be one of a set of functions (including working memory maintenance of task sets and items, selection and manipulation of information in working memory, and conflict detection) implemented by different, possibly overlapping prefrontal cortical regions. The voluntary blocking of memory retrieval may also be dependent on this region. As more information in the environment is perceived than can accurately and appropriately be attended to, inhibition is an integral feature of the prefrontal cortex that allows irrelevant information to be inhibited enabling more important information to be processed more quickly and efficiently.

Superior Frontal Gyrus

The SFG is a large region of the prefrontal cortex, making up about 1/3 of the frontal lobe in the human brain. The SFG is thought to contribute to higher cognitive functions, and play a particularly important role in working memory (Boisgueheneuc et al., 2006). The functional anatomical region referred to as the DLPFC (BA9) overlaps structurally, in part, with SFG (Owen et al., 2005; Falquez et al., 2014).

The DLPFC plays a crucial role in terms of working memory. It has been established as a crucial node that supports working memory processes. Neurophysiological unit recordings of the DLPFC in monkeys have shown persistent sustained levels of neuronal firing during retention intervals of delayed response tasks (Curtis and D'Esposito, 2003). Sustained activity in the

DLPFC is thought to provide a bridge between the stimulus cue and its contingent response (i.e., goal-directed behavior) in a working memory task. Goldman-Rakic (1987) has shown that lesions in the DLPFC impair the ability to maintain sensory representations on-line that are no longer present in the external environment. Studies of patients with SFG lesions show global impairments in working memory tasks with impairments present months to years post-lesion, indicating that the SFG may be a key component in the working memory network (Boisgueheneuc et al., 2006).

Medial Orbital Frontal Cortex

The orbitofrontal cortex in primates is situated ventrally and frontally in the brain (Kringelbach and Rolls, 2004), and can be further divided into distinct areas. Two major subdivisions have been cytoarchitecturally and functionally identified: the lateral orbitofrontal cortex and medial orbitofrontal cortex (MOF). The medial orbital frontal cortex surface includes BA14 (Rolls, 2004).

The MOF receives input from all sensory modalities. Accumulating evidence from fMRI implicates the orbitofrontal cortex as a necessary component in working memory, demonstrating activity in this area while coordinating multiple working memory operations (Wager and Smith, 2003; Owen et al., 2005; Barbey et al., 2011). Studies of human brain lesion patients with damage to orbitofrontal cortex have shown specific behavioral outcome deficits on components central to working memory (Barbey et al., 2011). Orbitofrontal damage was associated with deficits on working memory tasks involving coordination of maintenance, manipulation, and monitoring processes (e.g., N-Back task). However, this association was not seen on neuropsychological tests of working memory maintenance (digit/spatial span forward) or manipulation (digit/spatial span backward and letter-number sequencing; Barbey et al., 2011).

LIMITATIONS

Although not significantly different on age, the low performers tended to be older than high performers. If the sample size increased, it is possible this could impact the overall results as structural brain changes increase in older age. Even still, age was used in our models as a covariate to account for any numerical differences in age between groups. It is also possible that the clinical assessment of MCI by the study neuropsychologist did not capture participants in the earliest stages of MCI. This possibility is supported by the range of MoCA scores in this study, although these ranges were not significantly different between groups. Nonetheless, our findings may by biased by an unknown number of participants in either group that were in the earliest stages of MCI and thus evidencing early neurodegenerative tissue loss. The N-Back may also exhibit limitations inherent to the task regarding its use for the study of lateralized differences in structure-function relationships. As the functional foci of activation for N-Back changes with age and development, this tool may not be ideal for full identification of all frontal related working memory related neural correlates.

CONCLUSION

Normal physiological processes of aging are associated with neuronal circuitry changes, which may result in impaired cognition and behavior in some older individuals. Individuals that show poorer cognitive performance tend to show impairments of executive functions first (e.g., working memory, planning, and goal directed behavior), thus it has been postulated that neurons and circuits of the prefrontal cortex may be particularly vulnerable during normal aging in humans and non-human primates (Dickstein et al., 2013). During the aging process, there is evidence that neurons undergo morphological changes such as reduced complexity of dendritic arborization and dendritic length, as well as decreases in spine numbers. As spines are the major sites for excitatory synapses, changes in spine numbers could reflect a change in synaptic densities (Dickstein et al., 2007). These morphological changes may underlie surface area reductions, as neuron numbers remain relatively stable in older aged individuals lacking neurodegenerative diseases. As dendrites are pivotal in forming and maintaining neural networks, regulating synaptic plasticity, and integrating electrical inputs (Dickstein et al., 2007), it is perhaps not surprising that a potential marker of age-related change in dendritic morphology correlates with poorer performance on behavioral tasks.

There is great variability in cytoarchitectonic features of the cortex between individuals (Kringelbach and Rolls, 2004). The difficulty of deciphering the functional role of any brain region lies in the complexities of connections between and within brain structures, which may lend a single structure the ability to activate for a multitude of tasks. The N-Back task used in this study requires considerable vigilance and working memory processes to accurately detect target letters in the correct 2-Back pattern, a task that is quite challenging.

The right inferior frontal gyrus, an area implicated in cognitive inhibition and working memory, demonstrated significant reduction in surface area in older adults with lower working memory performance. The SFG, a crucial substrate of working memory processes, also exhibited a reduction in structural integrity. Finally, the MOF, a region shown to be necessary in coordination of working memory maintenance, manipulation, and monitoring processes also exhibited significantly reduced cortical surface area in low working memory groups. Taken together, these regions appear to play an important role in age-related working memory decline. The structural integrity of these three regions may also play an important role relative to compensatory processes previously found in functional MRI studies of N-Back performance. For example, deficits in these right frontal regions may interfere with compensatory engagement of left frontal structures found to activate in older adults able to maintain a high level of working memory performance. Future research investigating differences in both functional and structural connectivity between right and left frontal regions in high versus low working memory performers will be important for further evaluating this theory. In addition, these three frontal areas may prove to be important therapeutic targets

for brain stimulation or other methods capable of upregulating cerebral metabolism and function in brain regions showing decline.

ETHICS STATEMENT

The Institutional Review Board (IRB) at the University of Florida approved this study. Prior to any study procedures, all participants provided written informed consent. The study protocol was carried out in accordance with the Declaration of Helsinki, and the University of Florida Institutional Review Board approved all procedures in this study. All study participants were healthy older adults.

AUTHOR CONTRIBUTIONS

NN, AO, RC, EP, VB, and AW contributed text to the manuscript. AO, NN, and AW performed data analysis. All authors provided edits and approved the final version of the manuscript.

FUNDING

This work was supported in part by the McKnight Brain Research Foundation, Center for Cognitive Aging and Memory at the University of Florida, and the National Institute of Health (grant numbers KL2TR001429, K01AG050707-A1, R01AG054077 to AW).

REFERENCES

Aron, A. R., Robbins, T. W., and Poldrack, R. A. (2004). Inhibition and the right inferior frontal cortex. *Trends Cogn. Sci.* 8, 170–177. doi: 10.1016/j.tics.2004.02.010

Baddeley, A. (1992). Working memory. *Curr. Biol.* 255, 556–559. doi: 10.1016/j.cub.2009.12.014

Baddeley, A. (2000). The episodic buffer: a new component of working memory? *Trends Cogn. Sci.* 4, 417–423. doi: 10.1016/S1364-6613(00)01538-2

Barbey, A. K., Koenigs, M., and Grafman, J. (2011). Orbitofrontal contributions to human working memory. *Cereb. Cortex* 21, 789–795. doi: 10.1093/cercor/bhq153

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B.* 57, 289–300. doi: 10.2307/2346101

Boisgueheneuc, F., Du Levy, R., Volle, E., Seassau, M., Duffau, H., Kinkingnehun, S., et al. (2006). Functions of the left superior frontal gyrus in humans: a lesion study. *Brain* 129, 3315–3328. doi: 10.1093/brain/awl244

Cabeza, R. (2002). Hemispheric asymmetry reduction in older adults: the HAROLD model. *Psychol. Aging* 17, 85–100. doi: 10.1037/0882-7974.17.1.85

Cabeza, R., Anderson, N. D., Locantore, J. K., and McIntosh, A. R. (2002). Aging gracefully: compensatory brain activity in high-performing older adults. *NeuroImage* 17, 1394–1402. doi: 10.1006/nimg.2002.1280

Courtney, S. M., Petit, L., Haxby, J. V., and Ungerleider, L. G. (1998). The role of prefrontal cortex in working memory: examining the contents of consciousness. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 353, 1819–1828. doi: 10.1098/rstb.1998.0334

Curtis, C. E., and D'Esposito, M. (2003). Persistent activity in the prefrontal cortex during working memory. *Trends Cogn. Sci.* 7, 415–423. doi: 10.1016/S1364-6613(03)00197-9

Dale, A. M., Fischl, B., and Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 9, 179–194. doi: 10.1006/nimg.1998.0395

Dale, A. M., and Sereno, M. I. (1993). Improved localization of cortical activity by combining EEG and MEG with MRI cortical surface reconstruction: a linear approach. *J. Cogn. Neurosci.* 5, 162–176. doi: 10.1162/jocn.1993.5.2.162

Desikan, R. S., Segonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 31, 968–980. doi: 10.1016/j.neuroimage.2006.01.021

Dickstein, D. L., Kabaso, D., Rocher, A. B., Luebke, J. I., Wearne, S. L., and Hof, P. R. (2007). Changes in the structural complexity of the aged brain. *Aging Cell* 6, 275–284. doi: 10.1111/j.1474-9726.2007.00289.x

Dickstein, D. L., Weaver, C. M., Luebke, J. I., and Hof, P. R. (2013). Dendritic spine changes associated with normal aging. *Neuroscience* 251, 21–32. doi: 10.1016/j.neuroscience.2012.09.077

Dolcos, F., Rice, H. J., and Cabeza, R. (2002). Hemispheric asymmetry and aging: right hemisphere decline or asymmetry reduction. *Neurosci. Biobehav. Rev.* 26, 819–825. doi: 10.1016/S0149-7634(02)00068-4

Dotson, V. M., Szymkowicz, S. M., Sozda, C. N., Kirton, J. W., Green, M. L., O'Shea, A., et al. (2015). Age differences in prefrontal surface area and thickness in middle aged to older adults. *Front. Aging Neurosci.* 7:250. doi: 10.3389/fnagi.2015.00250

Falquez, R., Couto, B., Ibanez, A., Freitag, M. T., Berger, M., Arens, E. A., et al. (2014). Detaching from the negative by reappraisal: the role of right superior frontal gyrus (BA9/32). *Front. Behav. Neurosci.* 8:165. doi: 10.3389/fnbeh.2014.00165

Fischl, B., and Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U.S.A.* 97, 11050–11055. doi: 10.1073/pnas.200033797

Fischl, B., Liu, A., and Dale, A. M. (2001). Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans. Med. Imaging* 20, 70–80. doi: 10.1109/42.906426

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. doi: 10.1016/S0896-6273(02)00569-X

Fischl, B., Salat, D. H., van der Kouwe, A. J., Makris, N., Segonne, F., Quinn, B. T., et al. (2004a). Sequence-independent segmentation of magnetic resonance images. *Neuroimage* 23, S69–S84. doi: 10.1016/j.neuroimage.2004.07.016

Fischl, B., van der Kouwe, A., Destrieux, C., Halgren, E., Segonne, F., Salat, D. H., et al. (2004b). Automatically parcellating the human cerebral cortex. *Cereb. Cortex* 14, 11–22. doi: 10.1093/cercor/bhg087

Fischl, B., Sereno, M. I., and Dale, A. M. (1999a). Cortical surface-based analysis. II: inflation, flattening, and a surface-based coordinate system. *Neuroimage* 9, 195–207. doi: 10.1006/nimg.1998.0396

Fischl, B., Sereno, M. I., Tootell, R. B., and Dale, A. M. (1999b). High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum. Brain Mapp.* 8, 272–284. doi: 10.1002/(SICI)1097-019319998:4<272::AID-HBM10>3.0.CO;2-4

Freeman, S. H., Kandel, R., Cruz, L., Rozkalne, A., Newell, K., Frosch, M. P., et al. (2008). Preservation of neuronal number despite age-related cortical brain atrophy in elderly subjects without Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 67, 1205–1212. doi: 10.1097/NEN.0b013e31818fc72f

Fuster, J. M. (1988). "The prefrontal cortex," in *Comparative Neuroscience and Neurobiology*, ed. L. Irwin (Boston, MA: Birkhäuser), 107–109. doi: 10.1007/978-1-4899-6776-3_43

Gazzaley, A., Sheridan, M. A., Cooney, J. W., and D'Esposito, M. (2007). Age-related deficits in component processes of working memory. *Neuropsychology* 21, 532–539. doi: 10.1037/0894-4105.21.5.532

Glisky, E. L. (2007). *Changes in Cognitive Function in Human Aging*. Boca Raton, FL: CRC Press.

Goldman-Rakic, P. S. (1987). "Circuitry of primate prefrontal cortex and regulation of behavior by representational memory," in *Handbook of Physiology*, eds F. Plum and U. Mouncastle (Washington, DC: American Physiological Society), 373–417.

Goldman-Rakic, P. S. (1996). The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351, 1445–1453. doi: 10.1098/rstb.1996.0129

Han, X., Jovicich, J., Salat, D., van der Kouwe, A., Quinn, B., Czanner, S., et al. (2006). Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. *Neuroimage* 32, 180–194. doi: 10.1016/j.neuroimage.2006.02.051

Hentschel, S., and Kruggel, F. (2004). "Determination of the intracranial volume: a registration approach," in *Proceedings of the International Workshop on Medical Imaging and Virtual Reality*, (Berlin: Springer), 253–260.

Im, K., Lee, J. M., Lyttelton, O., Kim, S. H., Evans, A. C., and Kim, S. I. (2008). Brain size and cortical structure in the adult human brain. *Cereb. Cortex* 18, 2181–2191. doi: 10.1093/cercor/bhm244

Jovicich, J., Czanner, S., Greve, D., Haley, E., van der Kouwe, A., Gollub, R., et al. (2006). Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data. *Neuroimage* 30, 436–443. doi: 10.1016/j.neuroimage.2005.09.046

Kaup, A. R., Drummond, S. P. A., and Eyler, L. T. (2014). Brain functional correlates of working memory: reduced load-modulated activation and deactivation in aging without hyperactivation or functional reorganization. *J. Int. Neuropsychol. Soc.* 20, 945–950. doi: 10.1017/S1355617714000824

Kirchner, W. K. (1958). Age differences in short-term retention of rapidly changing information. *J. Exp. Psychol.* 55, 352–358. doi: 10.1037/h0043688

Klingberg, T. (2010). Training and plasticity of working memory. *Trends Cogn. Sci.* 14, 317–324. doi: 10.1016/j.tics.2010.05.002

Kringelbach, M. L., and Rolls, E. T. (2004). The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Prog. Neurobiol.* 72, 341–372. doi: 10.1016/j.pneurobio.2004.03.006

Kuperberg, G. R., Broome, M. R., McGuire, P. K., David, A. S., Eddy, M., Ozawa, F., et al. (2003). Regionally localized thinning of the cerebral cortex in schizophrenia. *Arch. Gen. Psychiatry* 60, 878–888. doi: 10.1001/archpsyc.60.9.878

Lemaitre, H., Goldman, A. L., Sambataro, F., Verchinski, B. A., Meyer-Lindenberg, A., Weinberger, D. R., et al. (2012). Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol. Aging* 33:617. doi: 10.1016/j.neurobiolaging.2010.07.013

Mograbi, D. C., Faria, C., de, A., Fichman, H. C., Paradela, E. M. P., and Lourenço, R. A. (2014). Relationship between activities of daily living and cognitive ability in a sample of older adults with heterogeneous educational level. *Ann. Indian Acad. Neurol.* 17, 71–76. doi: 10.4103/0972-2327.128558

Molnar-Szakacs, I., Iacoboni, M., Koski, L., and Mazziotta, J. C. (2005). Functional segregation within pars opercularis of the inferior frontal gyrus: evidence from fMRI studies of imitation and action observation. *Cereb. Cortex* 15, 986–994. doi: 10.1093/cercor/bhh199

Morrison, J. H., and Hof, P. R. (2002). Selective vulnerability of corticocortical and hippocampal circuits in aging and Alzheimer's disease. *Prog. Brain Res.* 136, 467–486. doi: 10.1016/S0079-6123(02)36039-4

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53, 695–699. doi: 10.1111/j.1532-5415.2005.53221.x

Nyberg, L., Cabeza, R., and Tulving, E. (1996). PET studies of encoding and retrieval: the HERA model. *Psychon. Bull. Rev.* 3, 135–148. doi: 10.3758/BF03212412

Owen, A. M., McMillan, K. M., Laird, A. R., and Bullmore, E. (2005). N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum. Brain Mapp.* 25, 46–59. doi: 10.1002/hbm.20131

Prabhakaran, V., Narayanan, K., Zhao, Z., and Gabrieli, J. D. E. (2000). Integration of diverse information in working memory within the frontal lobe. *Nat. Neurosci.* 3, 85–90. doi: 10.1038/71156

Reuter, M., Schmansky, N. J., Rosas, H. D., and Fischl, B. (2012). Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 61, 1402–1418. doi: 10.1016/j.neuroimage.2012.02.084

Rolls, E. T. (2004). The functions of the orbitofrontal cortex. *Brain Cogn.* 55, 11–29. doi: 10.1016/S0278-2626(03)00277-X

Rosas, H. D., Liu, A. K., Hersch, S., Glessner, M., Ferrante, R. J., Salat, D. H., et al. (2002). Regional and progressive thinning of the cortical ribbon in Huntington's disease. *Neurology* 58, 695–701. doi: 10.1212/WNL.58.5.695

Rowe, J. B., Toni, I., Josephs, O., Frackowiak, R. S., and Passingham, R. E. (2000). The prefrontal cortex: response selection or maintenance within working memory? *Science* 288, 1656–1660. doi: 10.1109/SSBI.2002.1233973

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S. R., Busa, E., et al. (2004). Thinning of the cerebral cortex in aging. *Cereb. Cortex* 14, 721–730. doi: 10.1093/cercor/bhh032

Salthouse, T. A., Mitchell, D. R., Skovronek, E., and Babcock, R. L. (1989). Effects of adult age and working memory on reasoning and spatial abilities. *J. Exp. Psychol. Learn. Mem. Cogn.* 15, 507–516. doi: 10.1037/0278-7393.15.3.507

Schnack, H. G., van Haren, N. E., Brouwer, R. M., Evans, A., Durston, S., Boomsma, D. I., et al. (2015). Changes in thickness and surface area of the human cortex and their relationship with intelligence. *Cereb. Cortex* 25, 1608–1617. doi: 10.1093/cercor/bht357

Scott, M., and Thacker, N. (2004). Cerebral cortical thickness measurements. *Tina Memo* 2004, 1–24.

Segonne, F., Dale, A. M., Busa, E., Glessner, M., Salat, D., Hahn, H. K., et al. (2004). A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 22, 1060–1075. doi: 10.1016/j.neuroimage.2004.03.032

Segonne, F., Pacheco, J., and Fischl, B. (2007). Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans. Med. Imaging* 26, 518–529. doi: 10.1109/TMI.2006.887364

Shefer, V. F. (1973). Absolute number of neurons and thickness of the cerebral cortex during aging, senile and vascular dementia, and Pick's and Alzheimer's diseases. *Neurosci. Behav. Physiol.* 6, 319–324. doi: 10.1007/BF01182672

Sled, J. G., Zijdenbos, A. P., and Evans, A. C. (1998). A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans. Med. Imaging* 17, 87–97. doi: 10.1109/42.668698

Wager, T. D., and Smith, E. E. (2003). Neuroimaging studies of working memory: a meta-analysis. *Cogn. Affect. Behav. Neurosci.* 3, 255–274. doi: 10.3758/CABN.3.4.255

Williams, K. N., and Kemper, S. (2010). Interventions to reduce cognitive decline in aging. *J. Psychosoc. Nurs. Ment. Health Serv.* 48, 42–51. doi: 10.3928/02793695-20100331-03

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 20 April 2017
doi: 10.3389/fnagi.2017.00102

Multiple Neuroimaging Measures for Examining Exercise-induced Neuroplasticity in Older Adults: A Quasi-experimental Study

*Lanxin Ji[1], Han Zhang[2,3], Guy G. Potter[4], Yu-Feng Zang[2,3], David C. Steffens[5], Hua Guo[1] and Lihong Wang[1,4,5]**

[1] Center for Biomedical Imaging Research, Department of Biomedical Engineering, Tsinghua University, Beijing, China, [2] Center for Cognition and Brain Disorders and the Affiliated Hospital, Hangzhou Normal University, Hangzhou, China, [3] Zhejiang Key Laboratory for Research in Assessment of Cognitive Impairments, Hangzhou, China, [4] Brain Imaging and Analysis Center, Duke University Medical Center, Durham, NC, USA, [5] Department of Psychiatry, University of Connecticut Health Center, Farmington, CT, USA

Physical exercise can improve physical and mental health. A number of imaging studies have examined the role of neuroplasticity in improving cognition with physical exercise; however, such neuroplasticity changes are not consistent across the reports partly due to small sample sizes in some studies. We thought to explore the concept that identifying consistent findings across multi-modality imaging measures would provide relatively reliable results. We designed a 6-week quasi-experiment with Wii-fitness exercise program in 24 healthy adults older than 60, and then examined the changes on neuroimaging measures including brain volume, the amplitude of low-frequency oscillation function (ALFF), regional homogeneity (ReHo), seed-based functional connectivity (FC), and the global efficiency of nodal connectivity during resting state. We focused on whether there were common regions showing changes after exercise across these measures and which measure was closely correlated with cognitive improvement. After the six-week exercise program, participants demonstrated a significant improvement in memory and executive function on neuropsychological tests, and in memory recall on an emotional memory task. The common brain regions that showed significant changes across different measures were the right striatum and the posterior cingulate (PCC). After exercise, the PCC showed decreased ReHo and increased volume, and the striatum did not show volume loss as the control group did and increased its FC with the cingulate, temporal, parietal, and occipital regions. Moreover, the connectivity change between the striatum and the thalamus was correlated with the improvement of executive function. This result implicates the striatum and the PCC associated network in physical exercise. Our work highlights the effectiveness of multi-modality neuroimaging measures in investigating neuroplasticity.

Keywords: physical exercise, neuroplasticity, aging, cognitive function, resting state functional connectivity, multiple imaging modality

OPEN ACCESS

Edited by:
Aurel Popa-Wagner, University of Rostock, Germany

Reviewed by:
David M. Schnyer, University of Texas at Austin, USA
Philipe De Souto Barreto, Toulouse University Hospital, France

****Correspondence:***
Lihong Wang
lwang@uchc.edu

Received: *02 March 2016*
Accepted: *30 March 2017*
Published: *20 April 2017*

Citation:
Ji L, Zhang H, Potter GG, Zang YF, Steffens DC, Guo H and Wang L (2017) Multiple Neuroimaging Measures for Examining Exercise-induced Neuroplasticity in Older Adults: A Quasi-experimental Study. Front. Aging Neurosci. 9:102. doi: 10.3389/fnagi.2017.00102

INTRODUCTION

Lack of physical exercise has been found to be one of the major contributors to development of Alzheimer disease (Barnes and Yaffe, 2011). Therefore, using physical exercise to improve cognitive function in older adults has gained increasing attention in the field of neuroscience research in recent years. There is abundant evidence in the literature showing that physical exercise not only can improve cardiovascular function, but also can improve cognitive function including speed, visual spatial, executive and cognitive control processing (Colcombe and Kramer, 2003; Lautenschlager et al., 2009; Barber et al., 2012). Neuroimaging research has been extensively conducted to investigate the training-induced neuroplasticity changes in the brain, however, thus far no consistent findings are found across reports partly due to the discrepancy of neuroimaging measures used among the studies in the literature (Colcombe et al., 2006; Boyke et al., 2008; Lustig et al., 2009). Studies measuring neuroplasticity change varied from brain volume, regional task-related activations, and perfusion change, to brain activity and functional connectivity (FC) during resting state, such as seed-based FC (Biswal et al., 1995), the amplitude of low-frequence fluctuation (ALFF, Zang et al., 2007), regional homogenity (ReHo, Zang et al., 2004), and graph theory analysis (Bullmore and Sporns, 2009). Functional connectivity and graph theory analysis have been widely used to measure correlations between spatially distinct brain areas. The ALFF of the resting-state fMRI signal has been suggested to reflect the intensity of regional spontaneous brain activity, while Reho is proposed to describe regional homogeneity of neural activities. While exercise-induced neuroplasticity changes in each of these neuroimaging measures have been reported previously, there are very few to integrate these methods together and investigate the common findings across the measures and compare which one is more closely correlated with changes in cognition.

Comparing the association between the changes of different neuroimaging measures with cognitive improvement would provide useful guidance for future imaging studies in exercise-induced neuroplasticity. Importantly, converging the findings from multiple neuroimaging measures would provide more reliable results than a single measure. Given the difficulty in executing exercise-intervention trial studies, some of the published exercise-related MRI studies in the literature have enrolled a small number of participants (Colcombe et al., 2006; Burdette et al., 2010; Holzschneider et al., 2012), which challenge the reliability of the reported results. We reason that identifying the common regions that show changes across different imaging modalities could be a reasonable solution for providing reliable results in studies with small sample sizes. Although each of these parameters reflects neuroplasticity with different neural mechanisms, those regions that repeatedly show changes in different types of measurements might have truly been shaped by physical exercises. To our best knowledge, there is only one cross-sectional study (Di et al., 2012) that has combined different imaging modalities which demonstrated that professional badminton training increased gray matter (GM) volume and the strength of neural oscillation (measured by ALFF) in the cerebellar regions. There are no longitudinal studies so far that have compared how consistent different neuroimaging measures are in reflecting neuroplasticity. Therefore, in this study, we aimed to investigate the consistent changes induced by physical exercises across different neuroimaging measures, and examine which measure is more closely correlated with cognitive improvement.

Previous studies have shown that participants who were in combined strength and aerobic training regimens improved cognitive function to a reliably greater degree than those who had aerobic training alone (Colcombe and Kramer, 2003). We posited that exercise programs in multiple domains that require the coordination of multiple neural systems to complete the exercise program might induce stronger neural plastic change than exercise in a single domain. Therefore, we designed a 6-week Wii-fitness exercise training program including aerobic, balance, weight lifting, and yoga. We expected this multi-domain exercise regimen would have a robust effect on cognitive function. The impact of exercise was evaluated on several imaging measurements including the brain GM volume, resting state low-frequency fluctuation activity, homogeneity, FC, as well as global connectivity efficiency of nodal networks in older adults. We hypothesized that motor and motor skill related regions such as striatum, motor cortex, and cerebellum, as well as attention and executive function related regions such as prefrontal cortex would reveal plasticity changes post exercise commonly in different imaging modalities. The plasticity changes in these regions may be associated with cognitive improvement after physical exercises.

MATERIALS AND METHODS

Participants

Twenty-four healthy subjects (70 $\pm$ 7.78 years; 12 females) were recruited through advertisements. Two subjects in the control group were left hand dominant, and the rest were right hand dominant. Exclusion criteria for the study were: (1) MRI contraindications and claustrophobia; (2) severe or unstable medical disorders, conditions, or drugs that may cause any condition that in the investigators' opinion might make the patients unsuitable for participating in the study (e.g., clinically significant cirrhosis, or heart disease); (3) any known current or past diagnosis of psychiatric disorders; (4) active suicidality or current suicidal risk as determined by the investigator; (5) significant handicaps that would interfere with neuropsychological testing or the inability to follow study procedures; (6) any known primary neurological disorders such as tumors, multiple sclerosis, or seizure disorder; (7) Mini-Mental State Examination (MMSE) <24; (8) any repetitive motion injury (e.g., tendinitis, bursitis, etc.); (9) extreme upper extremity arthritis; and (10) any other factor that in the investigators judgment may affect patient's safety or compliance. In addition, all subjects completed a demographic data form and a detailed questionnaire to screen out individuals who may be at-risk of injury during physical exercise. Subjects were also administered a neuropsychological testing battery

(see the Assessments on Cognitive and Psychological Function below). Subjects who had performance below 2 SD in any two tests of each cognitive domain (memory, executive function, or information processing speed) were removed from the study to ensure the cognitive status of all subjects being in normal range. The study received ethics committee approval by the Duke University School of Medicine Institutional Review Board. All subjects gave their written consents after being explained the purpose and procedures prior to the study.

Exercise Training and Experimental Procedure

The study was a quasi-experiment. The first four subjects were all controls to ensure the protocol work well. The rest of participants were randomly assigned by a research assistant either to participate in physical exercise training (Wii fit, Nintendo) ($n = 12$; five females; 67±6.40 years old) or to be on the no-training waiting list serving as a control group ($n = 12$; seven females; 73 ± 8 years old). Detailed demographic profiles of participants are summarized in **Table 1**. The exercise training program contained exercises in four domains, i.e., aerobic, balance, weight lifting, and yoga. A research assistant helped the participants to set up the Wii device and taught them how to do the Wii exercises. Participants were instructed to practice at home 30 min every day for 6 weeks. The Nintendo Wii system recorded exercise types, time, and duration automatically. A research assistant visited participants weekly to monitor the compliance and changes in mood. The mood state was evaluated by the Positive and Negative Affect Schedule (PANAS). One participant did not complete the PANAS form.

Assessments on Cognitive and Psychological Function

A neuropsychological testing battery was used to assess cognitive function. The battery included Immediate, Delayed, and Recognition Recall from Hopkins Verbal Learning Test-Revised (HVLT_Imm, HVLT_Delay and HVLT_Recog), Immediate and Delayed Story Recall from the Rivermead Behavioral Memory Test (RM_Imm and RM_Delay), WAIS-III Digit-Symbol Substitution Modality Test (DSST), WAIS-III Digit span, Trail Making Test (Trails A and Trails B), and Stroop Color and Word Test. All testing items were converted to z scores based on subjects' age, gender, race, and education level. We operationalized memory function using HVLT_Imm, HVLT_Delay, HVLT_Recog, RM_Imm and RM_Delay tests; executive function using Trails B and Stroop Color and Word Tests; working memory function using Digit Span; and information processing speed using the DSST and Trails A tests. The physical exercise training group completed the cognitive assessment both before and after the 6-week training. The control group only completed the initial tests on this battery, but not the retest after 6 weeks due to the failure of attempting to identify a second version of comparable neuropsychological testing battery. Since we did not examine the test–retest effect for the neuropsychological testing battery in this control group, we cannot rule out the possibility that significant improvement that we would find in cognitive function after physical exercise are due to a learning effect. Therefore, we further conducted a computerized memory task during the fMRI scan. The details of the task are introduced in the image acquisition section. There was one subject in the control group and two subjects in the physical exercise training group who didn't complete the memory

TABLE 1 | The demographic profile of participants and cognitive function at baseline.

	Control group ($N = 12$)	Training group ($N = 12$)	p-value
Age (years)	73.0 (8.00)	67.0 (6.40)	0.053
Sex (M/F)	5/7	7/5	0.684
Dominant hand (R/L)	10/2	12/0	0.481
Education (years)	16.3 (2.56)	17.2 (2.09)	0.35
Montgomery-Asberg Depression Scale	0.67 (1.30)	0.55 (1.29)	0.820
Memory Function	−0.03 (0.23)	0.17 (0.26)	0.58
RM_Imm (z)	0.35 (0.24)	0.22 (0.52)	0.81
RM_Delay (z)	0.34 (0.24)	0.22 (0.60)	0.85
HVLT_Imm (z)	−0.08 (0.25)	0.32 (0.26)	0.29
HVLT_Delay (z)	−0.28 (0.34)	0.20 (0.27)	0.30
HVLT_Recog (z)	−0.49 (0.34)	−0.01(0.26)	0.31
Executive Function	0.03 (0.23)	0.04 (0.28)	0.99
Trails B Making Test (z)	0.53 (0.32)	0.41 (0.43)	0.82
Stroop Color and Word Test (z)	−0.64 (0.21)	−0.34 (0.21)	0.32
Working Memory Function	−0.11 (0.24)	0.37 (0.32)	0.24
WAIS-III Digit Span (z)	−0.11 (0.24)	0.37 (0.32)	0.24
Information Processing Speed	0.74 (0.15)	0.78 (0.31)	0.89
DSST (z)	1.22 (0.19)	1.30 (0.36)	0.85
Trails A Making Test (z)	0.25 (0.23)	0.27 (0.39)	0.96

RM_Imm, RM_Delay: Immediate and Delayed Story Recall from the Rivermead Behavioral Memory Test. HVLT_Imm, HVLT_Delay and HVLT_Recog: Immediate, Delayed and Recognition Recall from Hopkins Verbal Learning Test-Revised DSST: WAIS-III Digit-Symbol Substitution Modality Test.

task. As mentioned earlier, the PNANS was used to assess mood state each week after exercise.

Imaging Acquisition

MRI scans were conducted at the Brain Imaging and Analysis Center on a 3.0 T GE EXCITE HD scanner (GE Medical Systems, Milwaukee, WI, USA). During each neuroimaging session, the following image acquisition protocols were used. First, sagittal T1-weighted spin-echo images were collected to identify landmarks for reference. Second, a T1-weighted 3D-SPGR sequence provided high-resolution anatomical images with 216 slices, slice thickness = 1 mm, acquisition matrix = 256 × 216 × 256, FOV (field of view) = 256 mm, TR (repetition time) = 7 ms, TE (echo time) = 3 ms, flip angle = 12°. Third, a 5-min resting-state fMRI scan was acquired using the following parameters: TR = 2000 ms, TE = 31 ms, FOV = 240 mm, flip angle = 90°, matrix = 64 × 64 × 34, slice thickness = 3.75 mm. Subjects were instructed to focus on a cross sign which was presented in the center of the screen. Fourth, a five-run emotional memory task-related fMRI scan was acquired. Three hours prior to the fMRI scan, subjects learned a set of happy, sad, and emotionally neutral pictures. They were asked to label the emotional category of the pictures and describe the details of the picture so that to remember these pictures. During the fMRI scan, the old pictures (learnt 3 h prior) as well as new pictures were presented randomly. In order to test immediate memory recall, half of the new pictures were also repeated (0–2 back). Subjects were asked to identify whether the pictures were old, new, or repeated pictures by pressing one of the three different buttons. The pictures used in the emotional memory task before (visit 1) and after (visit 2) 6 weeks were different to avoid learning effect. Given that we were only interested in comparing resting-state fMRI measures, we did not analyze the data during the emotional memory fMRI task in this report. Rather, the task was served as a behavior measure and only the results of memory recall performance are reported in this study.

MRI Structural Data Preprocessing and Analyses

The T1-weighted structural images were preprocessed using SPM8[1] running under the Matlab2012b environment. The images were initially preprocessed using VBM8 Toolbox[2] implemented in SPM8 and this involved segmentation, registration to the standard Montreal Neurological Institute (MNI) space and modulated normalization. A study-specific GM template was created from the preprocessed GM segments of all subjects using Template-O-Matic (TOM8) toolbox[3]. Then the spatially normalized GM segments were warped to the study-specific template and "modulated" by the Jacobian determinants of the deformations to account for local compression and expansion due to linear and non-linear transformation. Finally, the modulated GM volumes were smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM) (Draganski et al., 2011). One participant in the control group was excluded from the analysis due to the damage of images.

The students' two sample *t*-test was applied to examine significant differences between the two groups on the changes of the VBM map (visit 2 – visit 1) with age and sex being controlled. The threshold of significance was set at $p < 0.05$ using alphasim cluster correction [cluster size >389, connection criteria (rmm) = 5]. The alphasim correction was conducted on REST analysis toolkit (Song et al., 2011).

Resting State fMRI Data Preprocessing and Analyses

The resting state fMRI data was preprocessed using DPARSF[4] and SPM8 Toolbox[5]. The pre-processing steps included slice timing, realignment, spatial co-registration to each participant's own T1 image and then warping to the MNI space according to the deformation field information generated in structural T1 image processing, and resampled to 3 mm × 3 mm × 3 mm in voxel size. One participant in the physical exercise training group had head motion of more than 2.0 mm displacement or 2.0 degrees in maximum and was excluded. One subject in the control group was excluded because of damage to the T1 image.

Both the ALFF and ReHo analyses were conducted using DPARSF[2]. For ALFF (Zang et al., 2007), filtered time series (0.01–0.08 Hz) was transformed to a frequency domain with a fast Fourier transform (FFT) and the power spectrum was then obtained. The square root was thus calculated at each frequency of the power spectrum and the averaged square root was obtained across 0.01–0.08 Hz at each voxel. This averaged square root was taken as the ALFF.

The ReHo value was calculated to measure the similarity of the time series of a given voxel to its nearest 26 voxels (Zang et al., 2004). Through calculating the ReHo value of every voxel in the whole brain, individual ReHo maps were generated. Finally, the ALFF and ReHo maps were smoothed with a Gaussian kernel of 8 mm FWHM. To reduce the influence of individual variations, normalization of ReHo maps and ALFF was preformed through converting the maps to *z*-scores by subtracting the whole brain average ReHo/ALFF value and then dividing by the standard deviation of ReHo/ALFF values across all the voxels in the brain.

To examine the differences in the changes of ALFF and ReHo after 6 weeks between the physical exercise training group and control group, we first subtracted the ALFF and ReHo of visit 2 from visit 1 for each subject. Then the student's two-sample *t*-test was performed on the voxel-based difference maps of the two groups to calculate group difference in ALFF/ReHo changes. Age and gender effects were regressed out during the two-sample tests. Voxels with a *p*-value < 0.05 (corrected by AlphaSim, as implemented in the REST, with the following parameters: *p*-value at single voxel = 0.05, connection criteria (rmm) = 5, cluster size >389) were considered as having significant difference between the two groups.

[1] http://www.fil.ion.ucl.ac.uk/spm

[2] http://dbm.neuro.uni-jena.de/vbm8

[3] http://dbm.neuro.uni-jena.de/software/tom/

[4] http://rfmri.org/DPARSF

[5] http://www.fil.ion.ucl.ac.uk/spm/software/spm8/

To examine an effect of training on FC, pair-wise correlation analyses were conducted. For each participant, the Automated Anatomical Labeling template (AAL, Tzourio-Mazoyer et al., 2002) was used to divide the entire brain into 116 regions (including the cerebellum) as regions of interest. The mean time courses were extracted from each region and used to obtain a 116 × 116 correlation matrix of Pearson's correlation coefficients. This resulted in a 116×116 correlation matrix with 6670 [(116 × 115)/2] unique inter-regional correlation coefficients (r). Then we performed 6670 separate students' two sample t-tests on the difference of two visits (visit 2 – visit 1 within subjects). Multiple comparisons were corrected using a degree-based correction based on non-random data distribution patterns proposed by Chou et al. (2015) with 325069 ([61*73*61]) voxels. The regions which had at least 15 functional links (out of 116 × 116 matrix) connected to other regions with a significance of $p<0.05$ were considered significant.

We also evaluated the whole-brain FC changes at the network level for each node using the graph theory method. Based on the generated 116 × 116 correlation matrix as described above, we evaluated the following network properties (Burdette et al., 2010) of each subject using GRETNA toolbox[6]: (1) Nodal degree: A measure of connectivity to other regions of each node and (2) Global efficiency: A measure of the closeness of an individual node to all other nodes.

[6]http://www.nitrc.org/projects/gretna/

To confirm that there was no significant difference at baseline, we conducted two-sample t-tests on all neuroimaging measures.

Correlation between the Neuroimaging Measures and Cognitive Function

We have extracted those regions that have demonstrated a significant between-group difference for each measure as ROIs, and conducted ROI analyses to further examine whether the changes of our tested measures in those regions were correlated with any changes in cognitive function at each domain. Pearson's correlation analysis between neuroimaging measures with the four domains of cognitive measures was conducted separately.

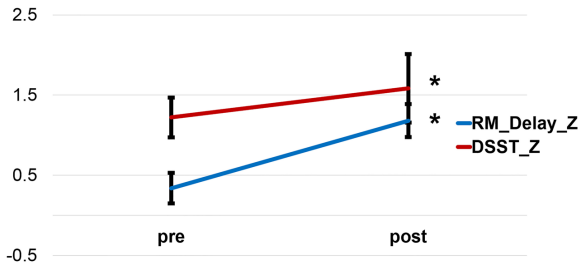


FIGURE 1 | Improvement of the Delayed Story Recall Test and the Digit-Symbol Substitution Test after a 6-week exercise training. RM_Delay, Delayed Story Recall Test. DSST, Digit-Symbol Substitution Test.

TABLE 2 | Descriptive statistics for the neuropsychological testing data and mood measurements before and after exercise.

	Pre training	Post training	p-value
	Mean (*SD*)	Mean (*SD*)	
Cognitive function			
Averaged z-score of all tests	0.12 (0.17)	0.32 (0.21)	0.147
Memory Function	−0.03 (0.23)	0.20 (0.35)	0.353
RM_Imm (z)	0.35 (0.24)	0.88 (0.41)	0.153
RM_Delay(z)	0.34 (0.24)	1.18 (0.43)	0.017*
HVLT_Imm (z)	−0.08 (0.25)	0.02 (0.28)	0.648
HVLT_Delay (z)	−0.28 (0.34)	−0.06 (0.42)	0.457
HVLT_Recog (z)	−0.49 (0.34)	−1.04 (0.38)	0.132
Executive Function	0.03 (0.23)	0.23 (0.21)	0.168
Trails B Making Test (z)	0.53 (0.32)	0.75 (0.24)	0.35
Stroop Color and Word Test (z)	−0.64 (0.21)	−0.52 (0.25)	0.464
Working Memory Function	−0.11 (0.24)	0.06 (0.20)	0.237
WAIS-III Digit Span (z)	−0.11 (0.24)	0.06 (0.21)	0.237
Information Processing Speed	0.74 (0.15)	0.93 (0.19)	0.092
DSST (z)	1.22 (0.19)	1.58 (0.21)	<0.001*
Trails A Making Test (z)	0.25 (0.23)	0.27 (0.27)	0.938
Memory task (Hit-False alarm rate)	−0.01 (< 0.01)	0.21 (0.03)	0.031*
Mood state			
PANAS_Positive	34.09 (1.85)	33.91 (1.80)	0.824
PANAS_Negative	11.27 (0.36)	11.09 (0.46)	0.774

Paired t-test, $p < 0.05$. RM_Imm, RM_Delay: Immediate and Delayed Story Recall from the Rivermead Behavioral Memory Test. HVLT_Imm, HVLT_Delay and HVLT_Recog: Immediate, Delayed and Recognition Recall from Hopkins Verbal Learning Test-Revised. DSST, WAIS-III Digit-Symbol Substitution Modality Test. PANAS, Positive and Negative Affect Schedule.

The threshold of significance was set at $p<0.01$ (0.05/4 cognition domains) to correct multiple comparisons.

RESULTS

Changes in Cognitive Function after the Exercise Training

Table 1 provides demographic and cognitive comparisons of the two groups at baseline. No significant differences were found between the two groups in any of the demographic profiles. There was no significant difference between the two groups in cognitive performance at baseline either (**Table 1**).

We examined the changes of neuropsychological tests before and after the physical exercise training in the exercise training group. All neuropsychological tests scores increased after exercise, except for HVLT_Recognition. This group demonstrated a significant improvement in memory during the Delayed Story Recall from the Rivermead Behavior Memory Test (RM_Delay) ($p = 0.017$) and in executive function during the WAIS-III Digit-Symbol Substitution Modality Test (DSST) ($p < 0.001$) after relative to before the 6-week exercise training (paired t-test) (**Figure 1**). However, after familywise error (FWE) correction for multiple comparisons, only DSST remained significant. **Table 2** provides the detailed descriptive statistics for the neuropsychological tests and mood measurements. Regarding participants' performance on the memory task during fMRI scan, the discrimination rate (i.e., Hit-False alarm rate) was used to assess the accuracy of recognition memory. We conducted two sample t-tests to

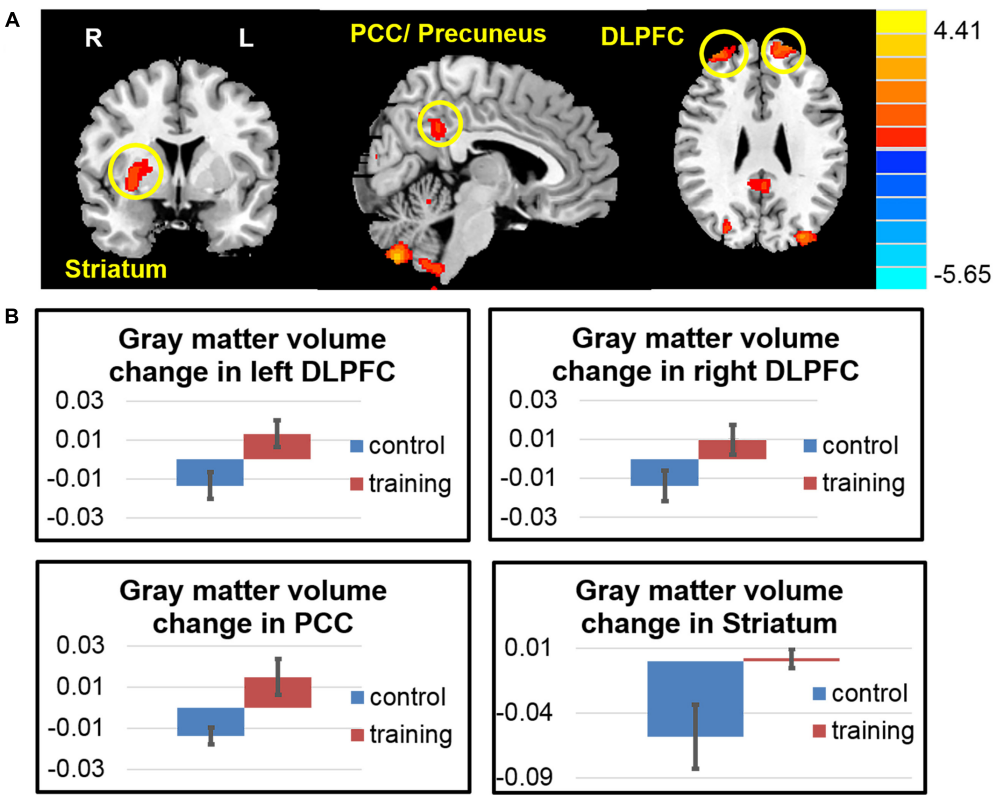


FIGURE 2 | Gray matter volume changes after exercise training. (A) The exercise training group had significantly increased gray matter volume in the dorsolateral prefrontal cortex (DLPFC), posterior cingulate cortex (PCC), striatum, hand motor area, occipital lobe and cerebellum. **(B)** Averaged gray matter intensity change in the regions of interest among two groups.

TABLE 3 | Regions increased gray matter volume after 6 weeks (visit 2 – visit 1) in the exercise group compared with the control group.

Region	Hemisphere	Cluster size	Peak MNI coordinates			Peak intensity (t)
			x	*y*	*z*	
Dorsolateral prefrontal cortex (DLPFC)	Left	904	−13.5	58.5	36	4.4129
Dorsolateral prefrontal cortex (DLFPC)	Right	1077	37.5	54	25.5	3.8444
Striatum (putamen)	Right	590	27	−1.5	−4.5	3.2625
Posterior cingulate / Precuneus cortex (PCC)	Right	904	6	−48	34.5	2.6374

compare the exercise training group with the control group in the improvement of the discrimination rate after vs. before 6 weeks (visit 2 − visit 1). The exercise training group showed significantly greater improvement in the discrimination rate than the control group ($p = 0.0313$). However, two subjects in the exercise training group had very low discrimination rate at their visit 1, and we speculated that they might have been somnolent during the scan. Given the low discrimination rate of the two subjects, the distribution was skewed. We further conducted the non-parameter Mann–Whitney test. The Mann–Whitney test confirmed that the discriminate rate of the exercise-training group increased significantly ($p = 0.035$) compared with the control group after versus before 6 weeks.

Gray Matter Volumetric Changes after the Exercise Training

The volumetric changes using VBM revealed that, compared with the control group, the exercise training group had significantly increased GM volume in the dorsolateral prefrontal cortex (DLPFC), posterior cingulate (PCC)/precuneus cortex, hand motor area, occipital lobe, and cerebellum (**Figure 2**). And the striatum did not show volume loss as the control group did. Detailed results are summarized in **Table 3**.

Functional Changes after the Exercise Training

The whole brain voxel-wise ALFF analysis revealed that the exercise training decreased ALFF in the PCC/precuneus cortex and increased ALFF in the left striatum extended to the anterior insula, right anterior insula, right caudate, right entorhinal cortex and left cerebellar cortex (**Figure 3** and **Table 4**) compared with the control group (two-sample t-test on the difference of two visits, $p < 0.05$, AlphaSim correction).

Consistent with the findings in the ALFF, the whole brain voxel-wise ReHo analysis also revealed an decrease in the PCC/precuneus area and increase ReHo in the right thalamus

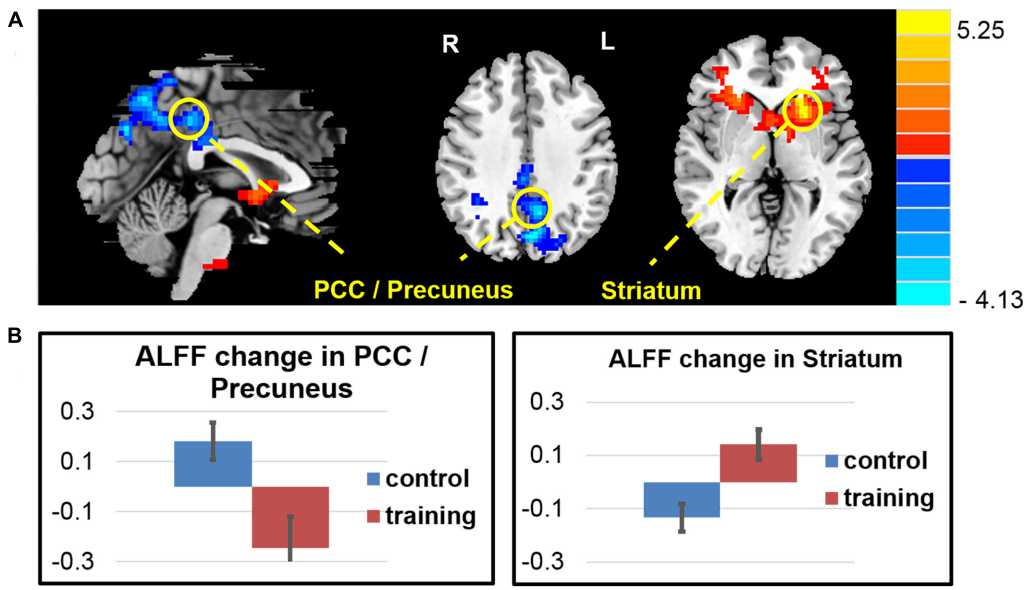


FIGURE 3 | Amplitude of low-frequency oscillation function (ALFF) changes after exercise training. (A) The exercise training-induced ALFF reduction in the PCC/precuneus area and ALFF increase in the left striatum extended to left anterior insula, right anterior insula, right caudate, right entorhinal cortex, and left cerebellar cortex. **(B)** Averaged ALFF z-score change of two groups in the regions of interest.

TABLE 4 | Regions changed ALFF after 6 weeks (visit 2 – visit 1) in the exercise group compared with the control group.

Region	Hemisphere	Cluster size	Peak intensity	Peak MNI coordinated		
				x	*y*	*z*
PCC / Precuneus	Both	824	−4.135	−3	−75	36
Striatum/Caudate /Insula	/	1149	5.2544	−24	15	0
Striatum	Left	/	5.2544	−24	15	0
Caudate	Right	/	3.6738	16	22	10
Insula	Left	/	4.2268	−33	19	−3
Insula	Right	/	3.4081	36	29	−1
Cerebellum	Left	716	4.7668	−12	−33	−39

and caudate, and the left middle frontal area (**Figure 4** and **Table 5**) in the exercise training group compared with the control group (two-sample *t*-test on the difference of two visits, $p < 0.05$, alphasim correction).

Among the 116 ROIs from the AAL template, the right striatum (including the putamen (AAL area 74) and the globus pallidus (AAL area 76)) were identified because their changes in FC (visit 2 – visit 1) with other brain regions were significantly different between the two groups, and the number of significantly changed connectivity paths was ≥15, corrected for multiple comparisons at $p < 0.05$. As shown in **Figure 5A**, the right putamen increased connectivity with the many regions in the brain including the superior frontal gyrus, median cingulate, thalamus, amygdala, temporal cortex, occipital cortex, and parietal cortex (including the default mode regions such as the PCC/precuneus and inferior parietal regions). Similarly, the right globus pallidus (**Figure 5B**) increased connectivity with the temporal and occipital regions, as well as the PCC/precuneus post vs. pre exercise.

The graph theory analysis did not find any statistically significant difference in the global efficiency of any nodes (visit 2 – visit 1) between the exercise training group and the control group.

Because of the small sample size, we used the simple *t*-tests on change scores to increase power. We also used a mixed-effect model to validate the result. As shown in Supplementary Figures 1–3 in Supplementary Materials, our main results were confirmed by the analysis with the mixed-effect model.

Correlations between the Neuroimaging Measures and Cognitive Function

After corrected for multiple comparisons, we did not find any significant correlation between the changes in any neuroimaging measurements with changes in any measures on cognitive function. However, the changes of FC between the right putamen and right thalamus showed marginally significant correlation with the change of the executive function ($r = 0.7071$, $p = 0.0150$, **Figure 6**).

DISCUSSION

After this short 6-week period of physical exercise, our participants showed significant improvement in their executive and memory function. Across different measures examined in our study, the commonly found brain regions that showed

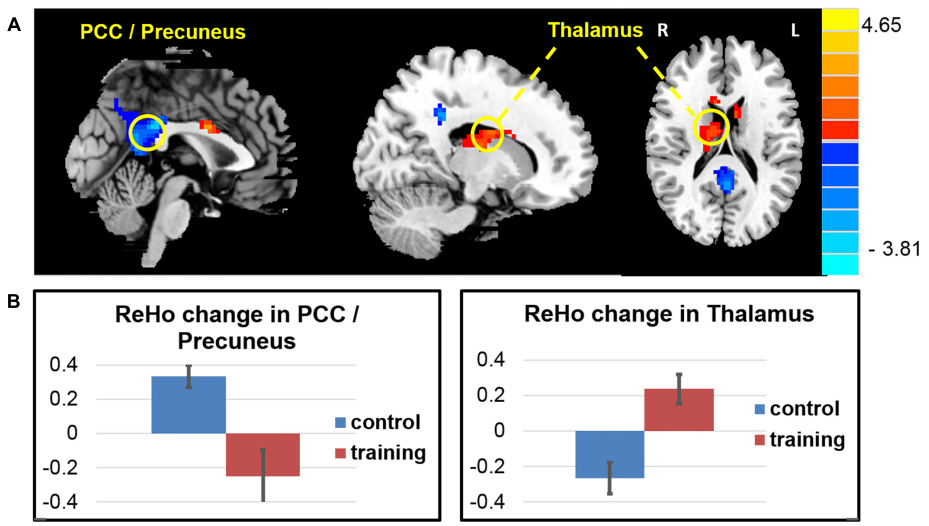
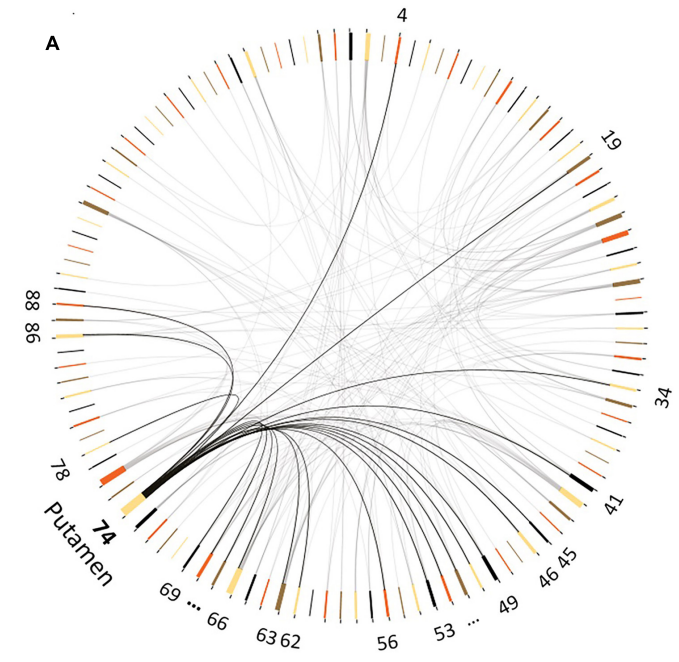


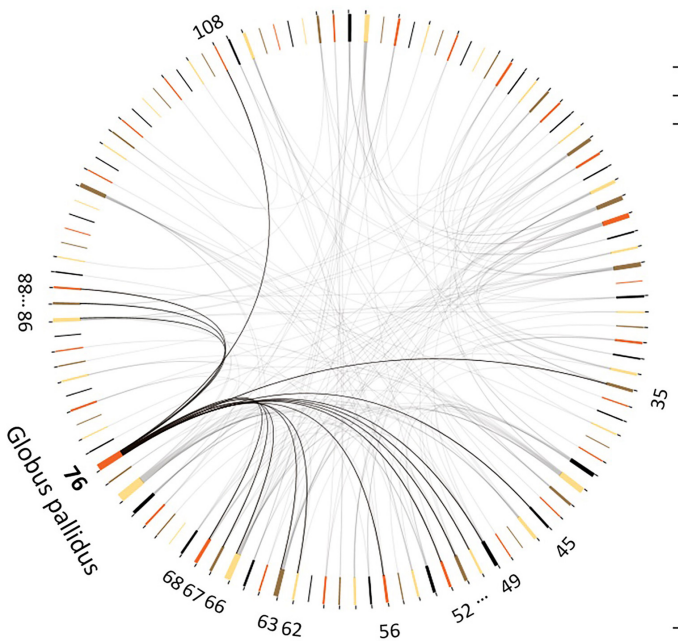
FIGURE 4 | ReHo changes after exercise training. (A) The exercise training causes ReHo reduction in the PCC/precuneus areas and ReHo increase in a cluster covering the right thalamus and caudate, and the left middle frontal area. **(B)** Averaged ReHo *z*-score change of two groups in the PCC/precuneus areas and caudate.

TABLE 5 | Regions changed ReHo after 6 weeks (visit 2 – visit 1) in the exercise group compared with the control group.

Region	Hemisphere	Cluster size	Peak intensity	Peak MNI coordinated		
				x	*y*	*z*
PCC / Precuneus	Both	434	−3.8160	−12	−69	36
Caudate / Thalamus	/	173	3.7863	3	0	27
Caudate	Right	/	3.1527	18	−6	24
Thalamus	Right	/	3.1375	15	−9	18
Middle Frontal area	Left	299	4.6513	−21	27	33



Corresponded regions of the labels	
Labels	Regions
4	Superior frontal gyrus, dorsolateral_R
19	Supplementary motor area_L
34	Median cingulate (and paracingulate gyri)_R
41	Amygdala_L
45	Cuneus_L
46	Cuneus_R
49	Superior occipital gyrus_L
50	Superior occipital gyrus_R
51	Middle occipital gyrus_L
52	Middle occipital gyrus_R
53	Inferior occipital gyrus_L
56	Fusiform_R
62	Inferior parietal gyrus_R
63	Supramarginal gyrus_L
66	Angular gyrus_R
67	Precuneus_L
68	Precuneus_R
69	Paracentral_Lobule_L
74	Lenticular nucleus, putamen_R
78	Thalamus_R
86	Middle temporal gyrus_R
88	Temporal pole (middle temporal gyrus)_R



Corresponded regions of the labels	
Labels	Regions
35	Posterior cingulate gyrus_L
45	Cuneus_L
49	Superior Occipital gyrus_L
50	Superior Occipital gyrus_R
51	Middle occipital gyrus_L
52	Middle occipital gyrus_R
56	Fusiform_R
62	Inferior parietal gyrus_R
63	Supramarginal gyrus_L
66	Angular_R
67	Precuneus_L
68	Precuneus_R
76	Lenticular nucleus, pallidum_R
86	Middle temporal gyrus_R
87	Temporal pole (middle temporal gyrus)_L
88	Temporal pole (middle temporal gyrus)_R
108	Cerebellum_R

FIGURE 5 | Functional connectivity changes after exercise training. (A) The right Putamen (AAL area 74) shows increased functional connectivity to other 21 regions. **(B)** The right Pallidum (AAL area 74) manifests increased functional connectivity to other 16 regions. Digit numbers represent the AAL labels.

significant changes after the 6-week exercise were the right striatum (including both the putamen and the globus pallidus) and the PCC/precuneus area. We noted a decrease in the striatum volume over the 6 weeks among the control non-exercise group. In comparison, the exercise training group showed no volume reduction in the right striatum, but increased functional connectivity between the right striatum and broad regions of the brain including the superior frontal gyrus, mid cingulate, amygdala, temporal, parietal (particularly the PCC/precuneus area), and the occipital cortices. This suggests an important

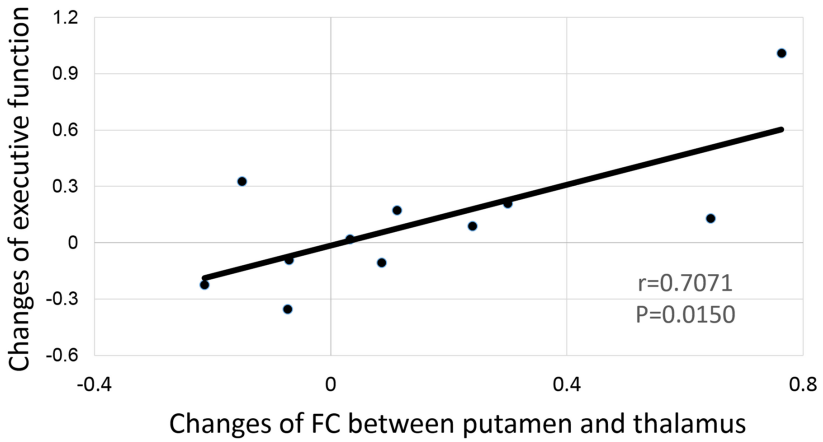


FIGURE 6 | Correlation between the functional connectivity change of the right putamen and the thalamus to the change of executive function.

role for exercise in preventing brain volume reduction, a phenomenon of brain aging. Importantly, the FC change between the right striatum and the right thalamus was marginally correlated with executive function. We integrated different measures to examine the training-induced neuroplasticity, and found that FC might be more sensitive among resting state functional activity/connectivity measures in evaluation of neuroplasticity related to cognitive improvement.

As a node of the cortico-striato-pallido-nigro-thalamo-cortical loop (DeLong et al., 1983), the striatum is well known for its role in motor planning, modulation of movement pathways (Alexander et al., 1986; Rolls, 1994), and a variety of cognitive processes including motivation and anticipation, procedural learning (Miyachi et al., 1997), as well as working memory (Voytek and Knight, 2010). As a supportive neural mechanism of the involvement of the striatum in exercise, increased metabolic capacity in the striatum has been found after 6-month exercise in rats (McCloskey et al., 2001). Increased brain-derived neurotropic factor (BDNF) levels in the striatum has also been reported after 6-week exercise in chronically stressed rats (Marais et al., 2009). FC analysis on the striatum by Di Martino et al. (2008) has also revealed that broad FC of the striatum with the rest of the neural systems is related to cognition, which is also consistent with our results. Therefore, it is not surprising that we found exercise could prevent volume loss and increase FC of the striatum to many other brain areas including the visual, temporal and default mode systems after exercise training, which could be related to the fact that all participants became skillful in the exercise programs they had practiced (based on the daily records of game performance level from the Wii console).

The other consistent change after exercise training occurred in the posterior cingulate cortex and precuneus area (PCC/precuneus). Although increased GM of the PCC/precuneus and increased FC between the PCC/precuneus and the striatum post exercise seems conflicting with the finding of reduced ALFF and ReHo in the PCC/precuneus, these results may not be hard to reconcile. Previous work has shown that the PCC is activated during mind wondering (Mason et al., 2007) and deactivated during meditation in mediators (Brewer et al., 2011). A recent real-time neurofeedback study has also shown that "undistracted awareness" such as "concentration" and "effortless doing" meditation has led to deactivation in the PCC (Garrison et al., 2013). It is possible that physical exercise can increase "effortless doing" ability with synchronized activity between the PCC and striatum and with low neural oscillation strength during resting state.

Although the current study focused on common findings among different imaging measures, we acknowledge that modality-specific results are also important because different measures reflect different neural mechanisms. For example, volumetric increase might be related to both neurogenesis and vasculogenesis/angiogenesis, whereas increased ALFF and ReHo during resting state should be related to increased amplitude or regional synchronization of automatic neural oscillations. We found significantly increased GM density in bilateral DLPFC, however, there was no significant changes in any of the functional measures in this region. It is possible that there was vasculogenesis/angiogenesis in the DLPFC post exercise without significantly enhancing neural function, or we can only find increased DLPFC activity when performing cognitive tasks but not during resting state. On the other hand, there was increased ALFF in the right entorhinal cortex without any volumetric changes. The entorhinal cortex is close to the hippocampus and critically involved in memory encoding (Eichenbaum and Lipton, 2008). However, the increased entorhinal ALFF did not correlate with cognitive performance, which could be related to the fact that our exercise training program involved in multiple tasks rather than memory specific. Future studies with exercise programs comparing how multiple task versus single learning task stimulate the hippocampal activation are necessary to further confirm the critical role of the hippocampus in physical exercise.

The significant increase in memory and executive function with 6-week exercise indicates that our mixed-domain exercise program is powerful and effective. Future studies in comparing of multi-domain versus single-domain exercise directly are

necessary. It would also be interesting to further investigate whether the plasticity change of the striatum can be universally related to executive function in any exercise program, or if it is only specific to certain types of exercise.

As discussed above, small sample size, no control on different types of physical exercises, and lack of different training duration as comparisons are major caveats of the current study. While these caveats limited our major conclusions, we do believe this pilot study has several strengths. To our knowledge, this is the first study that compared different neural imaging measurements to study the neural plasticity and demonstrated that common regions exist in showing changes across different neuroimaging measures. Therefore, examining changes in multiple neuroimaging measures is recommended in longitudinal studies with a small sample size. In addition, this is one of the few that has found a robust improvement in both behavioral and neural function in a short training period.

CONCLUSION

Our study demonstrated significant improvement in brain function as well as cognitive performance after 6 weeks of physical exercise training. Converging results indicate that our multi-domain exercise program could improve executive function through increasing function connectivity between the striatum and the thalamus. FC might be more sensitive among resting state functional activity/connectivity measures in evaluation of neuroplasticity related to cognitive improvement. In addition, our study suggests the effectiveness of multi-domain exercise training in improving cognitive function, and that identifying commonly changed regions across different imaging modalities could be an effective way to investigate neural plasticity.

AUTHOR CONTRIBUTIONS

LJ contributed in data analysis, interpretation of results, drafting the manuscript, approval for the final version for publication, and agreement to be accountable for the accuracy of all aspects of the work. HZ and Y-FZ contributed in data analysis, helped in interpretation of results and revising the manuscript, and approval for the final version for publication, and agreement to be accountable for the accuracy of all aspects of the work. GP and DS provided advice in study design and data collection, helped in interpretation of results and revising the manuscript, approval for the final version for publication, and agreement to be accountable for the accuracy of all aspects of the work. HG provided approval for the final version for publication. LW contributed in study design, data collection, helped in data analysis, interpretation of results, and revising the manuscript, approval for the final version for publication, and agreement to be accountable for the accuracy of all aspects of the work.

FUNDING

This research was supported by the Paul B. Beeson Career Developmental Award (K23-AG028982). LW, GP, and DS are supported by NIMH R01MH098301-01A1. GP is supported by NIMH Career Development Award (K23 MH087741). HZ is supported by National Natural Science Foundation of China (No. 81201156).

ACKNOWLEDGMENT

We sincerely thank Dr. Gary Glover at Stanford University for his advices in data analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2017.00102/full#supplementary-material

REFERENCES

Alexander, G. E., Delong, M. R., and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* 9, 357–381. doi: 10.1146/annurev.ne.09.030186.002041

Barber, S. E., Clegg, A. P., and Young, J. B. (2012). Is there a role for physical activity in preventing cognitive decline in people with mild cognitive impairment? *Age Ageing* 41, 5–8. doi: 10.1093/ageing/afr138

Barnes, D. E., and Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.* 10, 819–828. doi: 10.1016/S1474-4422(11)70072-2

Biswal, B., Zerrin Yetkin, F., Haughton, V. M., and Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar mri. *Magn. Reson. Med.* 34, 537–541. doi: 10.1002/mrm.1910340409

Boyke, J., Driemeyer, J., Gaser, C., Buchel, C., and May, A. (2008). Training-induced brain structure changes in the elderly. *J. Neurosci.* 28, 7031–7035. doi: 10.1523/JNEUROSCI.0742-08.2008

Brewer, J. A., Worhunsky, P. D., Gray, J. R., Tang, Y.-Y., Weber, J., and Kober, H. (2011). Meditation experience is associated with differences in default mode network activity and connectivity. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20254–20259. doi: 10.1073/pnas.1112029108

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–198. doi: 10.1038/nrn2575

Burdette, J. H., Laurienti, P. J., Espeland, M. A., Morgan, A., Telesford, Q., Vechlekar, C. D., et al. (2010). Using network science to evaluate exercise-associated brain changes in older adults. *Front. Aging Neurosci.* 2:23. doi: 10.3389/fnagi.2010.00023

Chou, Y. H., You, H., Wang, H., Zhao, Y. P., Hou, B., Chen, N. K., et al. (2015). Effect of repetitive transcranial magnetic stimulation on fMRI resting-state connectivity in multiple system atrophy. *Brain Connect.* 5, 451–459. doi: 10.1089/brain.2014.0325

Colcombe, S., and Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol. Sci.* 14, 125–130. doi: 10.1111/1467-9280.t01-101430

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., et al. (2006). Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. A* 61, 1166–1170. doi: 10.1093/gerona/61.11.1166

DeLong, M., Georgopoulos, A., and Crutcher, M. (1983). Cortico-basal ganglia relations and coding of motor performance. *Exp. Brain Res.* 7, 30–40. doi: 10.1007/978-3-642-68915-4_3

Di, X., Zhu, S., Jin, H., Wang, P., Ye, Z., Zhou, K., et al. (2012). Altered resting brain function and structure in professional badminton players. *Brain Connect.* 2, 225–233. doi: 10.1089/brain.2011.0050

Di Martino, A., Scheres, A., Margulies, D. S., Kelly, A., Uddin, L. Q., Shehzad, Z., et al. (2008). Functional connectivity of human striatum: a resting state FMRI study. *Cereb. Cortex* 18, 2735–2747. doi: 10.1093/cercor/bhn041

Draganski, B., Ashburner, J., Hutton, C., Kherif, F., Frackowiak, R. S. J., Helms, G., et al. (2011). Regional specificity of MRI contrast parameter changes in normal ageing revealed by voxel-based quantification (VBQ). *NeuroImage* 55, 1423–1434. doi: 10.1016/j.neuroimage.2011.01.052

Eichenbaum, H., and Lipton, P. A. (2008). Towards a functional organization of the medial temporal lobe memory system: role of the parahippocampal and medial entorhinal cortical areas. *Hippocampus* 18, 1314–1324. doi: 10.1002/hipo.20500

Garrison, K. A., Santoyo, J. F., Davis, J. H., Thornhill, T. A. I. V., Kerr, C. E., and Brewer, J. A. (2013). Effortless awareness: using real time neurofeedback to investigate correlates of posterior cingulate cortex activity in meditators' self-report. *Front. Hum. Neurosci.* 7:440. doi: 10.3389/fnhum.2013.00440

Holzschneider, K., Wolbers, T., Röder, B., and Hötting, K. (2012). Cardiovascular fitness modulates brain activation associated with spatial learning. *Neuroimage* 59, 3003–3014. doi: 10.1016/j.neuroimage.2011.10.021

Lautenschlager, N. T., Cox, K. L., Flicker, L., Foster, J. K., van Bockxmeer, F. M., Xiao, J. G., et al. (2009). Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial (vol 300, pg 1027, 2008). *JAMA* 301, 276–276. doi: 10.1001/jama.300.9.1027

Lustig, C., Shah, P., Seidler, R., and Reuter-Lorenz, P. (2009). Aging, training, and the brain: a review and future directions. *Neuropsychol. Rev.* 19, 504–522. doi: 10.1007/s11065-009-9119-9

Marais, L., Stein, D., and Daniels, W. U. (2009). Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. *Metab. Brain Dis.* 24, 587–597. doi: 10.1007/s11011-009-9157-2

Mason, M. F., Norton, M. I., Van Horn, J. D., Wegner, D. M., Grafton, S. T., and Macrae, C. N. (2007). Wandering minds: the default network and stimulus-independent thought. *Science* 315, 393–395. doi: 10.1126/science.1131295

McCloskey, D. P., Adamo, D. S., and Anderson, B. J. (2001). Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus. *Brain Res.* 891, 168–175. doi: 10.1016/S0006-8993(00)03200-5

Miyachi, S., Hikosaka, O., Miyashita, K., Kárádi, Z., and Rand, M. K. (1997). Differential roles of monkey striatum in learning of sequential hand movement. *Exp. Brain Res.* 115, 1–5. doi: 10.1007/PL00005669

Rolls, E. T. (1994). Neurophysiology and cognitive functions of the striatum. *Rev. Neurol.* 150, 648–660.

Song, X.-W., Dong, Z.-Y., Long, X.-Y., Li, S.-F., Zuo, X.-N., Zhu, C.-Z., et al. (2011). REST: a toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS ONE* 6:e25031. doi: 10.1371/journal.pone.0025031

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289. doi: 10.1006/nimg.2001.0978

Voytek, B., and Knight, R. T. (2010). Prefrontal cortex and basal ganglia contributions to visual working memory. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18167–18172. doi: 10.1073/pnas.1007277107

Zang, Y., Jiang, T., Lu, Y., He, Y., and Tian, L. (2004). Regional homogeneity approach to fMRI data analysis. *Neuroimage* 22, 394–400. doi: 10.1016/j.neuroimage.2003.12.030

Zang, Y. F., He, Y., Zhu, C. Z., Cao, Q. J., Sui, M. Q., Liang, M., et al. (2007). Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev.* 29, 83–91. doi: 10.1016/j.braindev.2006.07.002

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 24 April 2017
doi: 10.3389/fnagi.2017.00108

The Integrity of the Corpus Callosum Mitigates the Impact of Blood Pressure on the Ventral Attention Network and Information Processing Speed in Healthy Adults

Nichol M. L. Wong[1,2,3†], *Ernie Po-Wing Ma*[1,2,3†] *and Tatia M. C. Lee*[1,2,3,4*]

[1]*Laboratory of Neuropsychology, The University of Hong Kong, Hong Kong, Hong Kong,* [2]*Laboratory of Social Cognitive Affective Neuroscience, The University of Hong Kong, Hong Kong, Hong Kong,* [3]*Institute of Clinical Neuropsychology, The University of Hong Kong, Hong Kong, Hong Kong,* [4]*The State Key Laboratory of Brain and Cognitive Science, The University of Hong Kong, Hong Kong, Hong Kong*

Hypertension is a risk factor for cognitive impairment in older age. However, evidence of the neural basis of the relationship between the deterioration of cognitive function and elevated blood pressure is sparse. Based on previous research, we speculate that variations in brain connectivity are closely related to elevated blood pressure even before the onset of clinical conditions and apparent cognitive decline in individuals over 60 years of age. Forty cognitively healthy adults were recruited. Each received a blood pressure test before and after the cognitive assessment in various domains. Diffusion tensor imaging (DTI) and resting-state functional magnetic resonance imaging (rsfMRI) data were collected. Our findings confirm that elevated blood pressure is associated with brain connectivity variations in cognitively healthy individuals. The integrity of the splenium of the corpus callosum is closely related to individual differences in systolic blood pressure. In particular, elevated systolic blood pressure is related to resting-state ventral attention network (VAN) and information processing speed. Serial mediation analyses have further revealed that lower integrity of the splenium statistically predicts elevated systolic blood pressure, which in turn predicts weakened functional connectivity (FC) within the VAN and eventually poorer processing speed. The current study sheds light on how neural correlates are involved in the impact of elevated blood pressure on cognitive functioning.

Keywords: blood pressure, brain connectivity, cognitive function, aging, MRI

OPEN ACCESS

Edited by:
Clinton B. Wright, National Institute of Neurological Disorders and Stroke, USA

Reviewed by:
Ana M. Coto-Montes, Universidad de Oviedo Mieres, Spain
Lydia Yee, Otto-von-Guericke University Magdeburg, Germany

***Correspondence:**
Tatia M. C. Lee
tmclee@hku.hk

†These authors have contributed equally to this work.

Received: *27 January 2016*
Accepted: *04 April 2017*
Published: *24 April 2017*

Citation:
Wong NML, Ma EP-W and Lee TMC (2017) The Integrity of the Corpus Callosum Mitigates the Impact of Blood Pressure on the Ventral Attention Network and Information Processing Speed in Healthy Adults. Front. Aging Neurosci. 9:108. doi: 10.3389/fnagi.2017.00108

INTRODUCTION

Hypertension is highly prevalent in the aging population and the prevalence of it increases with age (Cheng et al., 2009). It is also a risk factor for other health problems, such as stroke (Strandgaard, 1996) and Alzheimer's disease (AD; Bermejo-Pareja et al., 2010). Furthermore, hypertension has been shown to be related to a broader deterioration of cognitive functions in individuals with AD (Bellew et al., 2004). Hypertension is also associated with poorer cognitive functioning in older adults without clinical conditions (e.g., dementia; Gifford et al., 2013). Hypertensive elderly individuals appear to demonstrate declines in measures of global cognition

(Goldstein et al., 2013) as well as in specific domains including working memory (Elias et al., 2010), attention (Hannesdottir et al., 2009) and executive functioning (Waldstein et al., 2005).

The mechanism underlying the relationship between elevated blood pressure and poorer cognitive functions remains unclear, and there is relatively little evidence on how this vascular risk factor provokes brain changes that precede late-life cognitive decline (Knopman et al., 2011; Carmichael, 2014). For instance, increased systolic blood pressure is associated with brain atrophy, reduced gray matter volume and white matter (WM) hyperintensities (Maillard et al., 2012), and increased diastolic blood pressure is associated with brain atrophy (Heijer et al., 2003). At the brain connectivity level, the functional frontoparietal connections are associated with the worsening of cognitive functions in hypertensive individuals, plausibly due to the deficits in WM integrity (Li et al., 2015), such that WM integrity deficits are noticeable in the splenium and are related to poorer global cognition (Gons et al., 2012). Presumably, brain injuries would increase the vulnerability to unhealthy aging, as exemplified by AD (Douaud et al., 2014).

Therefore, in the current study we investigate whether alterations in brain connectivity, accompanied by elevated blood pressure, could be identified before the onset of clinical conditions and apparent cognitive decline. The associations among blood pressure, brain connectivity and cognitive functions in individuals from the normotensive to moderate-severe hypertensive range are explored, using blood pressure measures as continuous metrics. Blood pressure is relatively modifiable (Mensah and Bakris, 2010), and a better understanding of its underlying neural mechanisms would help promote preventive efforts against cognitive decline and would highlight the importance of regulating blood pressure, which is of increasing interest to the general public. Thus, we investigate brain changes associated with elevated blood pressure at the topological and connectivity levels in cognitively healthy individuals aged 60–70 years using diffusion tensor imaging (DTI) and resting-state functional magnetic resonance imaging (rsfMRI). Adults would likely have a drastic increase in systolic and diastolic pressure in this critical period (Franklin et al., 1997).

DTI and rsfMRI provide information on the strength of structural and functional connectivity (FC), respectively. Compared to investigations of WM lesions at the macrostructural level (e.g., Maillard et al., 2012), fewer studies have examined the microstructural properties of WM using DTI. In addition, we also utilize independent components analysis (ICA; Beckmann and Smith, 2004), a whole-brain multivariate approach, to investigate the strength of FC within resting-state frontoparietal functional networks. We focus on the frontoparietal networks because they were found to be affected in hypertensive elderly individuals previously (Li et al., 2015). Different domains of cognitive function are investigated in our sample, including processing speed, working memory, selective and divided attention, visuospatial skills and executive functioning. Negative associations between blood pressure and WM integrity have been reported previously, including in individuals from the normotensive range (Kennedy and Raz, 2009; Leritz et al., 2010), and systolic blood pressure has been reported to be associated with reduced WM integrity in the corpus callosum (e.g., Delano-Wood et al., 2008; Maillard et al., 2012). Therefore, we hypothesize that elevated blood pressure, particularly systolic pressure, is related to reduced WM integrity in the splenium of the corpus callosum and weakened frontoparietal resting-state functional networks. We also hypothesize that frontoparietal functional networks largely explain the impact of elevated blood pressure on cognitive function.

MATERIALS AND METHODS

Participants

A sample of 40 healthy Chinese individuals was recruited from the community (**Table 1**). They were above 60 years of age and right-handed (Oldfield, 1971), scoring 24 or higher on the Mini Mental State Examination (MMSE; Folstein et al., 1975) and 8 or lower on the Geriatric Depression Scale-short form (GDS; Lesher and Berryhill, 1994). They did not have any history of vascular events (i.e., myocardial infarction, heart failure, stroke, or peripheral vascular disease), any history of cardiac, lung, liver, or renal failure, or any history of neurological or psychiatric disorders. Participants' blood pressure was measured before and after cognitive assessments. They underwent an MRI scanning session, in which structural T1-weighted, DTI and rsfMRI data were collected. All participants underwent all procedures either within the same day or across different days within the same month. All participants received a $100 supermarket coupon at the end of their participation.

This study was approved by the Faculty Ethics Panel of the Faculty of Social Sciences from The University of Hong Kong. Written informed consents were obtained from all participants.

Blood Pressure Measures

Each participant's systolic and diastolic brachial blood pressures were measured from their arms using a sphygmomanometer. Pulse pressure was obtained by calculating the difference between systolic and diastolic blood pressure. Every participant's blood pressure was measured twice (i.e., before and after cognitive assessment), and the average values were used.

Cognitive Measures

To assess the cognitive functions of our subjects, six domains were measured in the current study: processing speed, working memory, selective attention, divided attention, visuospatial skills and executive functioning.

Processing speed. A composite score of processing speed was obtained by adding the raw scores of the Digit Symbol Coding and Symbol Search according to the Wechsler Adult Intelligent Scale Third Edition—Taiwanese version (WAIS-III; Wechsler, 1997).

Working memory. A composite score of working memory was obtained by adding the raw scores of the Arithmetic and Digit Span according to the WAIS-III (Wechsler, 1997).

Selective attention. A validated Chinese translation of the Victoria version of the Stroop Color and Word Test (SCWT) was

TABLE 1 | Descriptors and cognitive functioning of the subjects.

Descriptors	Mean	S.D.	Minimum	Maximum
Systolic pressure	137.66	16.804	105.5	174.5
Diastolic pressure	81.01	10.676	64	101
Pulse pressure	56.64	12.953	35	87.5
Hypertensive treatment (Yes:No)	17.23	–	–	–
Age (years)	63.78	2.455	60	70
Gender (M:F)	14.26	–	–	–
Education (years)	10.45	3.544	4	20
MMSE	28.68	1.655	24	30
GDS	1.58	1.678	0	8
Composite score of processing speed	89.20	23.312	46	136
Composite score of working memory	30.05	6.164	18	44
Stroop interference index	1.3913	0.76341	0.1	3.6
CCT interference index	1.202	0.55767	0.2	2.45
JOL score	10.2	0.139	2	15
Total moves used in TOL	34.98	15.556	12	84
Total time spent in TOL	306.84	100.3233	145.9	590

CCT, Color Trails Test; GDS, Geriatric Depression Scale; JOL, Judgment of Line Orientation; MMSE, Mini Mental State Examination; S.D., standard deviation; TOL, Tower Of London Test.

used (Lee and Chan, 2000). An interference index was obtained by subtracting each participant's reaction time in the incongruent color-word condition (C) from that in the color-dots condition (D) and dividing the result by their reaction time in C.

Divided attention. The Color Trails Test (CTT) comprising Parts A and B was used (D'Elia et al., 1996). Part A required participants to join the digits in ascending order and Part B required participants to join the digits in ascending order with alternating colors. An interference index was obtained by subtracting each participant's reaction time in Part A from that in Part B and dividing the result by their reaction time in Part A.

Visuospatial skills. The 15-item short form of Judgment of Line Orientation (JLO) was used, comprising Form V items in the following order: 16, 9, 6, 2, 12, 30, 7, 17, 19, 28, 20, 21, 26, 24 and 22 (Qualls et al., 2000).

Executive functioning. The Tower of London test (TOL; Culbertson and Zillmer, 1999) was used with 10 problems of increasing complexity. Participants had a maximum of 2 min and 20 moves to solve each problem. Their total number of moves and the total time were recorded for analysis.

MRI Data Acquisition and Preprocessing

All participants' MRI data were acquired via a Philips 3T scanner with a standard 8-channel head coil. DTI data were acquired with TE = 65 ms, TR = 9426 ms, NEX = 2, flip angle = 90°, FOV = 225 × 225 mm, voxel size = 1.56 × 1.56 × 2 mm, one b = 0 reference and 32 b = 1000 diffusion directions and axial acquisition. rsfMRI data were acquired with TE = 30 ms, TR = 3000 ms, flip angle = 90°, FOV = 230 × 230 mm, voxel size = 2.88 × 2.88 × 4 mm, 160 volumes and axial acquisition.

The DTI data were first corrected for eddy current and motion distortions using FSL (FMRIB, Oxford, UK) and were then entered into the DSI studio[1] for tensor fitting and deterministic tractography using whole-brain seeding with fiber count = 100,000, turning angle = 60°, fractional anisotropy (FA) >0.13 threshold, step size = 0.78, minimum length = 10 and maximum length = 500. Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) was non-linearly registered to each subject's diffusion space and each subject's association matrix was then computed according to the 90 regions defined by the AAL atlas (i.e., cerebellum excluded). The number of streamlines between any pairs of regions was defined as strength of edges (**Figure 1A**). Using the Matlab-based package GAT (Hosseini et al., 2012) and the Small Worldness (SW; Humphries and Gurney, 2008), a measure of brain topology, was obtained for each matrix at a range of threshold densities between 0.24 and 0.33 with steps of 0.1 (**Figure 1B**). The minimum density was chosen so that none of the networks were fragmented, whereas the maximum density was chosen to avoid random networks (i.e., not random when SW >1), which are less likely to represent a biologically valid network (Kaiser and Hilgetag, 2006). The SW captures the trade-off between local clustering and path length and any changes in the index might indicate a shift in the balance of network segregation and integration (Rubinov and Sporns, 2010). The area under the curve (AUC; Ginestet et al., 2011) of the SW within the defined threshold density range of each association matrix was then calculated for topological analysis.

The DTI data that were corrected for eddy current and motion distortions were also entered into the FSL to obtain FA maps. Tract-Based Spatial Statistics (TBSS; Smith et al., 2006) implemented in the FSL were used. All of the FA maps were aligned to the default FMRIB58_FA template and were transformed to the Montreal Neurological Institute (MNI) standard space via a nonlinear registration. A mean FA image generated from the sample was produced and thinned to compute a mean FA skeleton with a threshold of FA >0.2. All subjects' FA maps were projected onto the mean FA skeleton for voxel-wise analyses of integrity within the main tracts (i.e., the skeleton).

The rsfMRI data were preprocessed with a whole-brain multivariate approach using MELODIC[2] implemented in the FSL. The first 10 volumes of each subject's data were first

[1]http://dsi-studio.labsolver.org/

[2]http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC

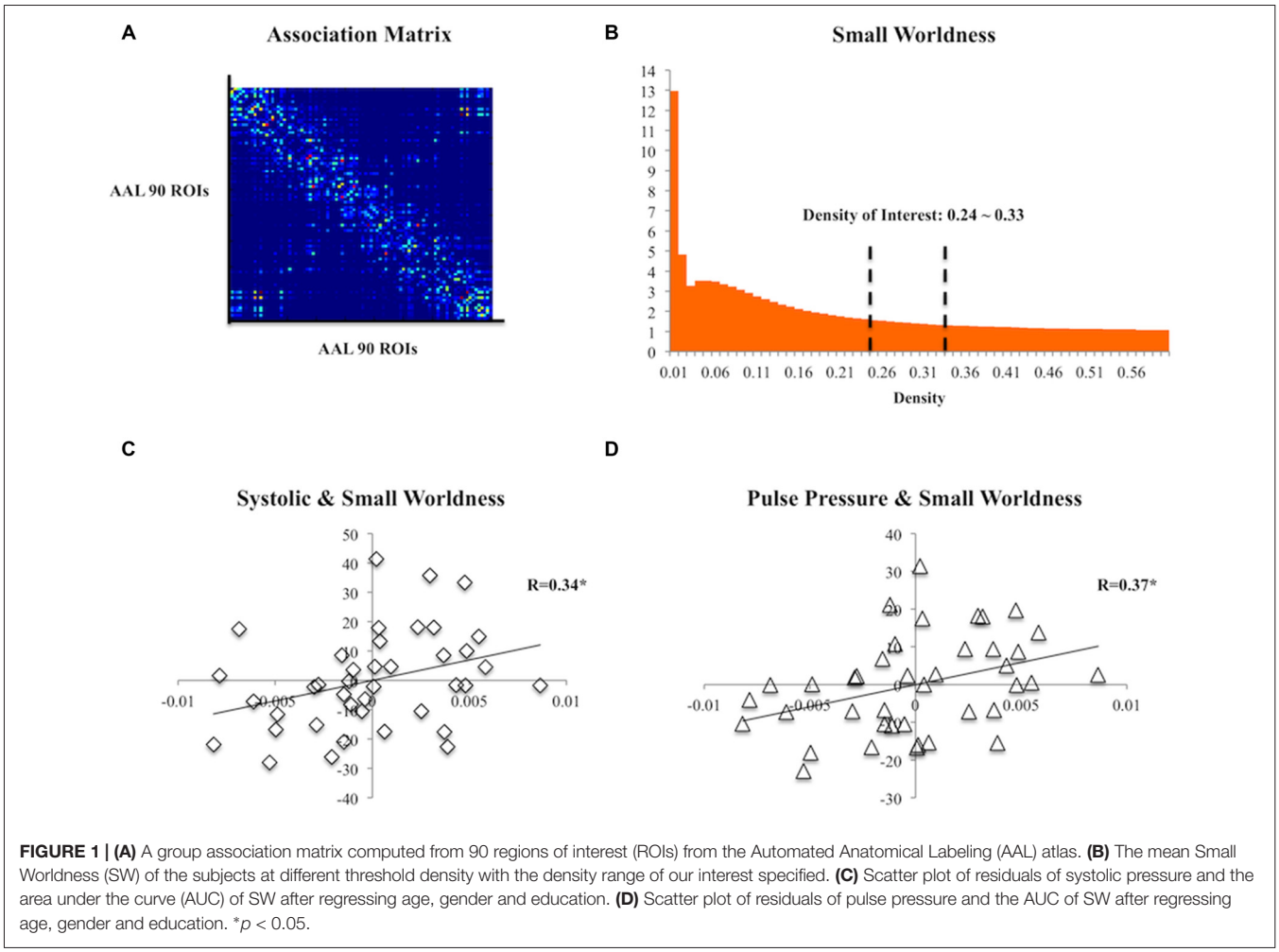


FIGURE 1 | (A) A group association matrix computed from 90 regions of interest (ROIs) from the Automated Anatomical Labeling (AAL) atlas. **(B)** The mean Small Worldness (SW) of the subjects at different threshold density with the density range of our interest specified. **(C)** Scatter plot of residuals of systolic pressure and the area under the curve (AUC) of SW after regressing age, gender and education. **(D)** Scatter plot of residuals of pulse pressure and the AUC of SW after regressing age, gender and education. *$p < 0.05$.

removed to account for signal stabilization. The data were then motion corrected, slice-timing corrected and skull-stripped. They were spatially normalized to the 4-mm MNI standard space with 12° of freedom via co-registering to their skull-stripped T1-weighted images with 6° of freedom. Finally, the data were smoothed with 5 mm FWHM and were high-pass filtered at 100 s. ICA was used to decompose the data into various spatiotemporal independent components (IC; i.e., 42 components were found). The spatial map of each IC was compared to three reference resting-state networks (Yeo et al., 2011) using cross-correlations to identify the IC of our sample that had significant spatial similarity (i.e., $R > 0.264$) with the three resting-state frontoparietal networks (i.e., frontoparietal control, dorsal attention and ventral attention; **Figure 2**). The significant threshold was determined using the table of critical values for Pearson's r with the corresponding degree of freedom. Significant ICs were then used to yield subject-specific FC maps through dual regression (Beckmann et al., 2009) in the FSL. This procedure involves regressing the component on each subject's data to obtain subject-specific time series first and then regressing the subject-specific time series on each subject's data again to obtain subject-specific connectivity map for each significant IC corresponding to the three resting-state networks. These final subject-specific connectivity maps were used for voxel-wise analysis of the FC between specific clusters of voxels and the resting-state network components.

Statistical Analysis

To rule out possible pre-post changes in blood pressure measures, repeated measures of analysis of covariance (ANCOVA) were performed on the blood pressure measures controlling for age, gender and education. Partial correlations between blood pressure measures and SW were investigated, controlling for age, gender and education. A general linear model (GLM) approach was used to identify how blood pressure measures could be related to brain connectivity at the voxel level. Specifically, systolic, diastolic, or pulse pressure was entered as a regressor, together with age, gender and education as covariate regressors on all subjects' FA maps masked by the mean FA skeleton, or resting-state FC data masked by the significant IC. Using the randomize function in FSL, permutation tests based on 5000 permutations were performed with a 0.05 significance level after correcting for family-wise error (FWE) using threshold-free cluster enhancement (TFCE). To further correct

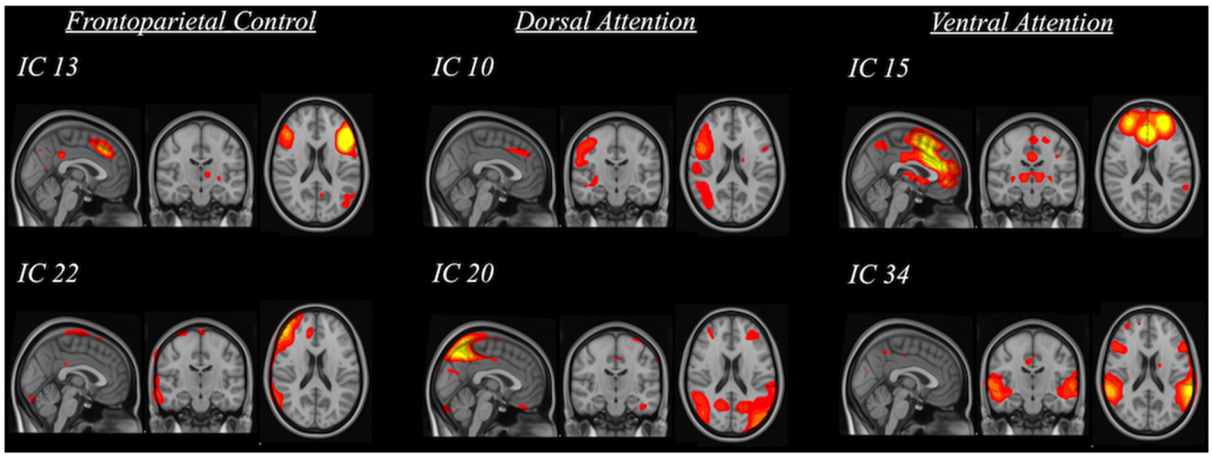


FIGURE 2 | Spatial maps of significant independent components (IC) that were grouped into three categories based on their relation to standard frontoparietal resting-state networks (i.e., frontoparietal control, dorsal attention and ventral attention).

for multiple testing of the components involved in the resting-state frontoparietal networks, a significance level of 0.005 was adopted for analyzing FC. The region of interest (ROI) approach was then applied and the mean values of regional FA and FC within significant clusters of the corresponding contrast were retrieved and partially correlated with the cognitive measures—including composite score of processing speed, composite score of working memory, interference index of SCWT, interference index of CTT, visuospatial performance on JLO and executive functioning captured by TOL—controlling for age, gender and education. Moderation analyses were performed to examine whether the associations between FA or FC and cognitive measures were dependent on the blood pressure measures. A parallel mediation model was then established to examine how brain variables might mediate the relationship between blood pressure measures and cognitive function in the subjects. Finally, serial mediation analyses were performed based on the findings from the moderation and parallel mediation analyses. Moderation and mediation analyses were performed using PROCESS macro[3] in SPSS v20.0 with 1000 bootstrap samples (Hayes, 2013). The significance of the mediation paths in the mediation model was based on the inferences from the 95% bias-corrected confidence interval (CI). The percent mediation was also reported as an effect size measure for the indirect effects in the mediation analyses.

RESULTS

Blood Pressure and Sample Characteristics

Blood pressure measures did not differ between pre- and post-assessment (systolic: $F_{(1,36)} = 0.042$, $p = 0.838$; diastolic: $F_{(1,36)} = 0.166$, $p = 0.686$; pulse pressure: $F_{(1,36)} = 0.000$, $p = 0.991$). The average value of each measure was used for the subsequent analyses.

Among all subjects, 17 of them were on drugs for hypertension treatment. The average duration was 124 months (minimum = 6, maximum = 360), and no significant age difference between those on drugs and others was detected ($F_{(1,36)} = 0.005$, $p = 0.943$).

None of the blood pressure measures were related to age, years of education, or scores of MMSE or GDS (all p values > 0.05). They did not correlate with scores of MMSE and GDS even when controlling for age, gender and years of education. Systolic pressure was related to diastolic and pulse pressure positively, controlling for age, gender and years of education (systolic and diastolic: $R_{(35)} = 0.642$, $p < 0.001$; systolic and pulse pressure: $R_{(35)} = 0.804$, $p < 0.001$; diastolic and pulse pressure: $R_{(35)} = 0.060$, $p = 0.724$).

Subjects' composite processing-speed scores were negatively correlated to their total time spent in TOL ($R_{(35)} = -0.606$, $p < 0.001$). Their composite scores of working memory were positively correlated with their performance in JLO ($R_{(35)} = 0.392$, $p = 0.016$). Their total moves and total time spent in TOL were correlated with each other ($R_{(35)} = 0.409$, $p = 0.012$).

Blood Pressure Relates to Cognitive Function

Both systolic ($R_{(35)} = -0.334$, $p = 0.044$) and pulse pressure ($R_{(35)} = -0.337$, $p = 0.041$) were negatively correlated with the composite score of the processing speed. Trending associations were observed in systolic pressure with the Stroop interference index ($R_{(35)} = -0.293$, $p = 0.079$) and with the total time spent in the TOL ($R_{(35)} = 0.315$, $p = 0.057$). Trending associations were also observed between pulse pressure and total moves ($R_{(35)} = 0.281$, $p = 0.093$) and with the total time spent in the TOL ($R_{(35)} = 0.311$, $p = 0.061$). Diastolic pressure did not correlate with any cognitive functions (all p values > 0.05), and none of the blood pressure measures were correlated with the composite

[3]http://www.processmacro.org/

score of working memory, the CTT interference index, or the visuospatial performance on JLO (all p values > 0.05).

Blood Pressure Relates to Brain Connectivity

At the topological level, both systolic ($R_{(35)} = 0.34$, $p = 0.04$) and pulse pressure ($R_{(35)} = 0.373$, $p = 0.023$) were positively correlated with SW (**Figures 1C,D**). Diastolic pressure did not correlate with SW ($R_{(35)} = 0.089$, $p > 0.05$).

Structurally, systolic pressure was negatively associated with FA largely in the splenium ($k = 1295$, MNI $x = -10$, $y = -39$, $z = 15$, $T_{peak} = 5.1$) and in the left posterior thalamic radiation (PTR; $k = 29$, MNI $x = -34, -60, 16$, $T_{peak} = 3.77$), whereas diastolic pressure was only negatively associated with the FA in the left PTR ($k = 272$, MNI $x = -31$, $y = -63$, $z = 15$, $T_{peak} = 4.66$). No significant clusters were associated with pulse pressure in the WM along the main tracts.

Functionally, systolic pressure was negatively associated to FC of the right superior temporal gyrus (RSTG) with the ventral attention network (VAN; $k = 24$, MNI $x = 70$, $y = -26$, $z = 8$, $Z_{peak} = 4.53$) and diastolic pressure was negatively associated to the FC of the left STG (LSTG) with the VAN ($k = 8$, MNI $x = -46$, $y = -34$, $z = 4$, $Z_{peak} = 5.71$; **Table 2**).

Brain Connectivity Relates to Cognitive Function

Structurally, the FA within the cluster of the left PTR (i.e., related to systolic pressure) was positively correlated with the composite processing-speed scores ($R_{(35)} = 0.345$, $p = 0.036$).

Functionally, the FC of the RSTG with the VAN was positively correlated with the Stroop interference index ($R_{(35)} = 0.398$, $p = 0.015$) and the composite score of processing speed ($R_{(35)} = 0.359$, $p = 0.029$) and negatively correlated with total time spent in the TOL ($R_{(35)} = -0.342$, $p = 0.038$).

Blood Pressure Moderates the Relationship of Brain Connectivity and Cognitive Function

Structurally, the association between FA within the cluster of the splenium of the corpus callosum and composite processing-speed scores was moderated by systolic (**Figure 3A**), diastolic and pulse pressure (systolic: $\Delta R^2 = 0.11$, $b = -14.64$, SE = 5.46, $p = 0.011$; diastolic: $\Delta R^2 = 0.03$, $b = -24.52$, SE = 10.73, $p = 0.029$; pulse pressure: $\Delta R^2 = 0.09$, $b = -21.82$, SE = 9.08, $p = 0.022$). The association between the FA within the cluster of the left PTR and the composite scores of working memory was moderated by pulse pressure ($\Delta R^2 = 0.09$, $b = -3.52$, SE = 1.55, $p = 0.030$). The association between the FA within the cluster of the splenium of the corpus callosum and the total moves used in TOL was moderated by systolic and pulse pressure (systolic pressure: $\Delta R^2 = 0.12$, $b = 10.48$, SE = 3.72, $p = 0.008$; pulse pressure: $\Delta R^2 = 0.15$, $b = 19.01$, SE = 5.95, $p = 0.003$). The association between the FA within the cluster of the left PTR and the total moves used in TOL was also moderated by pulse pressure ($\Delta R^2 = 0.08$, $b = 6.83$, SE = 3.13, $p = 0.036$). The association between the FA within the cluster of the splenium of the corpus callosum and the total time spent in TOL was moderated by systolic pressure ($\Delta R^2 = 0.11$, $b = 64.60$, SE = 29.03, $p = 0.033$). The association between the FA within the cluster of the splenium of the corpus callosum and the total time used in TOL was moderated by pulse pressure ($\Delta R^2 = 0.10$, $b = 101.55$, SE = 48.10, $p = 0.042$).

Functionally, the association between FC of RSTG with VAN and composite processing-speed scores was moderated by pulse pressure ($\Delta R^2 = 0.07$, $b = -0.06$, SE = 0.03, $p = 0.043$). The association between FC of LSTG with VAN and the Stroop interference index was moderated by pulse pressure ($\Delta R^2 = 0.09$, $b = -0.002$, SE = 0.001, $p = 0.045$). The association between FC of LSTG with VAN and the CTT interference index was moderated by systolic pressure ($\Delta R^2 = 0.12$, $b = -0.002$, SE = 0.001, $p = 0.028$).

Brain Connectivity Mediates the Relationship between Blood Pressure and Cognitive Function

Systolic pressure was negatively correlated with composite processing-speed scores. It was also significantly related to the FA of the left PTR and the FC of the RSTG with the VAN; and the FA of the left PTR and the FC of the RSTG with the VAN were also associated with composite processing-speed scores. Therefore, mediation analyses were performed to investigate whether the FA of the left PTR

TABLE 2 | Brain regions associated with blood pressure.

ROIs	Measures	Voxels	Peak[a]	MNI *x*	MNI *y*	MNI *z*
Systolic pressure-related						
Left SCC	FA	1295	5.1	−10	−39	14
Left FMA	FA	54	3.62	−29	−66	17
Right CP	FA	31	3.78	19	−15	−6
Left PTR	FA	29	3.77	−34	−60	16
Left PTR	FA	6	3.75	−31	−57	13
Right STG in VAN	FC	24	4.53	70	−26	8
Diastolic pressure-related						
Left PTR	FA	272	4.66	−31	−63	15
Left STG in VAN	FC	8	5.71	−46	−34	4

[a] *T_{peak} and Z_{peak} values are reported for the FA and the FC respectively. CP, cerebral peduncle; FA, fractional anisotropy; FC, functional connectivity; MNI, Montreal Neurological Institute; PTR, posterior thalamic radiation; ROI, region of interest; STG, superior temporal gyrus; SCC, splenium of corpus callosum.*

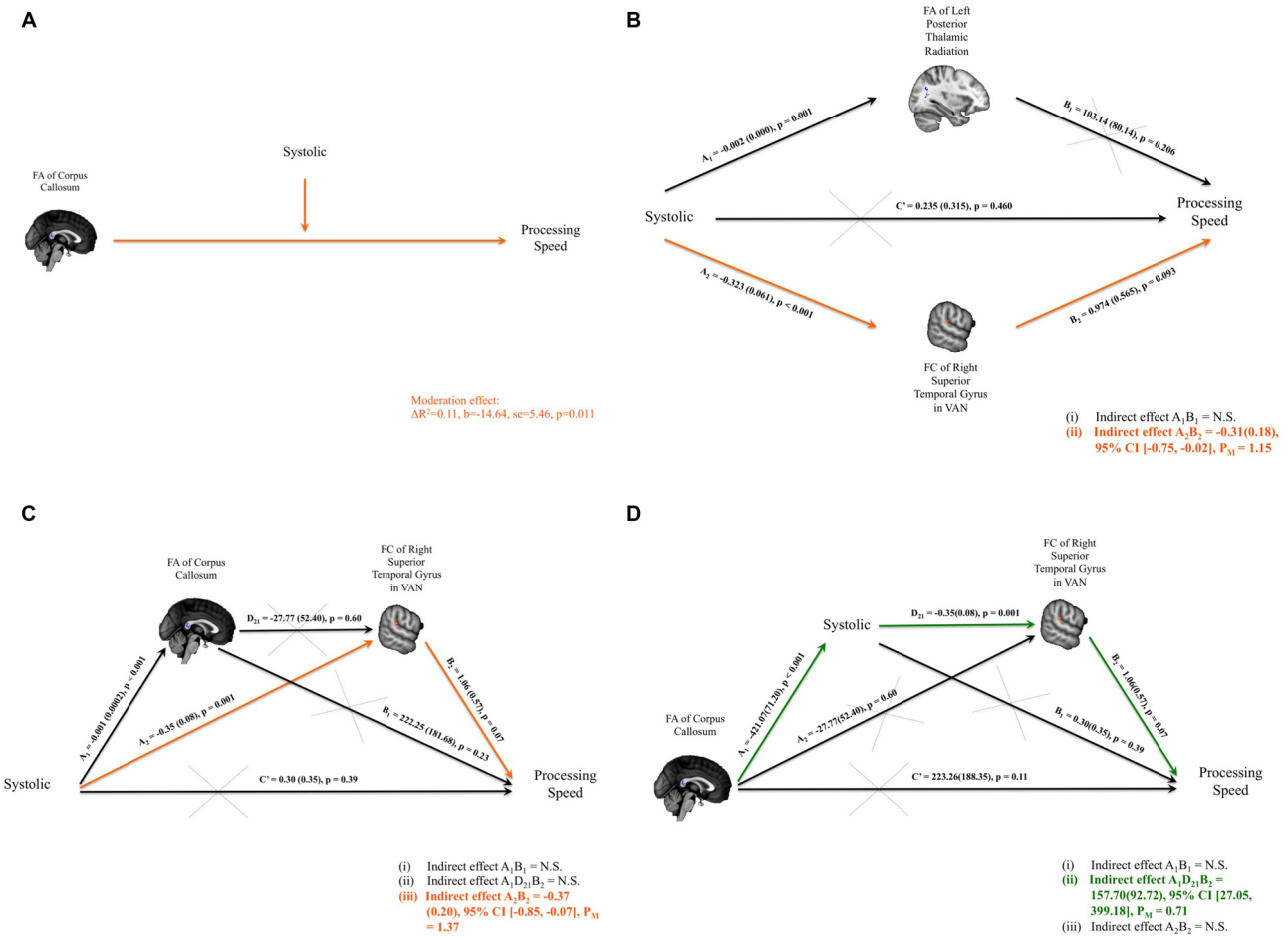


FIGURE 3 | (A) Significant interaction between the systolic blood pressure measure and the fractional anisotropy (FA) of corpus callosum on composite scores of processing speed was identified. **(B)** Parallel mediation models were set up to investigate whether FA of the left thalamic radiation and/or FC of right superior temporal gyrus (RSTG) with the ventral attention network (VAN) might mediate the relationship between systolic blood pressure measure and the composite scores of processing speed. Only the mediation path through FC of RSTG with VAN was significant. **(C)** Path model of the FA of the corpus callosum and the FC of RSTG with VAN that mediates the systolic pressure and processing speed. **(D)** Path model of systolic pressure and the FC of the RSTG with VAN that mediates the FA of the splenium of the corpus callosum and processing speed. Significant indirect paths are shaded in orange or green. Structural clusters are shaded in blue and functional clusters are shaded in red. N.S., not significant; P_M, Percent mediation.

and/or the FC of the RSTG with the VAN mediated the association between systolic pressure and composite processing-speed scores. Adopting the parallel mediation model, our results revealed that only FC of the RSTG with the VAN mediated the association between systolic pressure and processing speed (indirect effect = −0.31, SE = 0.18, 95% CI [−0.75, −0.02]; percent mediation = 1.15, SE = 24.55, 95% CI [−2.02, 24.28]; **Figure 3B**).

Serial Mediation Analysis

Moderation analysis established that the association between FA within the cluster of the splenium of the corpus callosum and composite processing-speed scores was moderated by systolic pressure. Through parallel mediation analysis, it was found that FC of the RSTG with the VAN mediated the association between systolic pressure and processing speed. Therefore, to investigate how the integrity of the corpus callosum might play a role in the impact of systolic pressure on VAN and processing speed, serial mediation analyses were performed. Two intriguing path models were proposed to investigate whether there are significant indirect paths that denote: (i) the impact of blood pressure (i.e., systolic pressure or pulse pressure) on the link between structural connectivity and FC and processing speed, or (ii) the impact of structural connectivity on the link between blood pressure and FC and processing speed. Importantly, our serial mediation analyses revealed that the lower FA of the splenium of the corpus callosum statistically predicted higher systolic pressure, which in turn allowed for the statistical prediction of weakened FC of the RSTG in the VAN and eventually poorer processing speeds in healthy subjects (indirect effect = 157.70, SE = 92.72, 95% CI [27.05, 399.18]; percent mediation = 0.71, SE = 37.56, 95% CI [0.01, 5.27]). Details of the models that were tested are presented in **Figures 3C,D**.

DISCUSSION

Our study provides preliminary evidence that elevated blood pressure is associated with detectable brain connectivity variations in adults without apparent cognitive impairment and that elevated blood pressure is related to slower information processing. To investigate the brain changes that are associated with elevated blood pressure at the topological and connectivity levels, we have investigated SW, WM integrity as captured by FA, and FC within three resting-state frontoparietal networks. In this study, we confirmed our hypothesis that brain changes at the topological (i.e., SW) level and connectivity (i.e., splenium of the corpus callosum and VAN) levels are related to elevated blood pressure. Importantly, the FC of the RSTG with the VAN of cognitively healthy subjects mediates the impact of elevated blood pressure on information-processing speed, with a preserved corpus callosum predicting more favorable blood pressure levels. Together, our results have suggested that the impact of elevated blood pressure on specific cognitive functions and the brain occurs earlier than the onset of clinical conditions.

Elevated Blood Pressure and Brain Differences

At the topological level, our study identified alterations in the structural topology of the brain in relation to elevated blood pressure. From our behavioral findings, individuals with higher blood pressure tended to have slower information-processing speeds with an upward shift of the SW property of their structural brain networking. SW captures the balance of network segregation and integration via clustering and short paths (Rubinov and Sporns, 2010), and a previous study has reported a similar upward alteration of SW in individuals with AD (He et al., 2008). In view of this, one plausible explanation for the systematic brain changes found in our study is that a disturbance in the networking function of the brain causes it to function at a less than optimal level (Strogatz, 2001). This may partly explain how elevated blood pressure is a high risk factor for developing AD: through its effect on neural mechanisms and brain changes.

Structurally, we observed lower WM integrity of the splenium of the corpus callosum in individuals with higher blood pressure. This parallels another study in the hypertensive elderly (Gons et al., 2012), and this association extends to hypertensive adults (Maillard et al., 2012). Although previous research has demonstrated how WM integrity in multiple fiber bundles could be related to cognitive functioning, our findings align with a previous study that reported that the relationship between variation of blood pressure and WM integrity across the brain might not be as direct and dispersed (Jacobs et al., 2013).

Functionally, connectivity within the frontoparietal networks (i.e., frontoparietal control network [FCN], dorsal attention network [DAN], VAN) was weakened in the elderly with higher blood pressure. The association between weakened frontoparietal network connectivity and higher blood pressure described in our study, parallels the results of a recent study reporting an alteration in the frontoparietal network of hypertensive individuals (Li et al., 2015). Importantly, we did not identify any disrupted structural frontoparietal connectivity relating to blood pressure as suggested in the previous study. This may be due to the fact that the previous study compared brain connectivity in hypertensive individuals to that in normotensive individuals, whereas we see blood pressure measures as a continuous metric and have examined the relationship between blood pressure and brain connectivity.

Corpus Callosum, Ventral Attention Network and Processing Speed

Based on the mediation analysis, the weakened connectivity of the RSTG in the VAN could largely explain the predictability of high systolic blood pressure on slower information processing speeds in cognitively healthy adults. The frontoparietal system—comprising three subsystems the FCN, the DAN, and the VAN (Yeo et al., 2011)—is important in maintaining mental well-being. The FCN plays a critical role in highly controlled adaptive processes (Cole et al., 2014). The DAN is involved in top-down goal-directed attention to external stimuli, and the VAN specializes in bottom-up stimulus-driven attention to behaviorally relevant, salient or unexpected stimuli (Corbetta and Shulman, 2002). The VAN is a right-lateralized ventral cortical network with the ventral frontal cortex (VFC) and temporoparietal regions as core components. The RSTG has been suggested to be a common site of damage that is related to cognitive problems, such as ventral attentional deficits (Marshall et al., 2002). Specifically, hypertensive individuals were often found to have changes in regional cerebral blood flow within superior temporal cortices (Dai et al., 2008) and over time (Beason-Held et al., 2007), suggesting its vulnerability to raised blood pressure. Variations of the FC of RSTG with the VAN due to elevated blood pressure are likely be related to variations of the bottom-up stimulus-driven attentional controls that would influence their information processing. The importance of good attentional control to maintain efficient processing in older adults is also demonstrated in another behavioral training study (Mackay-Brandt, 2011).

Additionally, the splenium of the corpus callosum is a major interhemispheric commissure with extensive connections linking the visual areas and the parietal and posterior cingulate regions (Knyazeva, 2013) and is closely related to the aging process (Voineskos et al., 2012). From our findings, variation of systolic blood pressure is related to the subject's processing speed. Moreover, we have found that the association between the WM integrity of the splenium of the corpus callosum and processing speed depends on systolic blood pressure levels. Our serial mediation analyses further identified that the WM integrity in the splenium of the corpus callosum could predict elevated systolic blood pressure and its impact on the VAN and processing speed, but elevated systolic blood pressure could not, in turn, predict the loss of integrity in the splenium of the corpus callosum. Literature might have suggested that higher blood pressure is a risk factor for WM lesions and hyperintensities (Maillard et al., 2012), and our results could

have revealed the WM integrity of the splenium of corpus callosum (SCC) as a good indicator of higher blood pressures and its impact. Loss of integrity of the corpus callosum is often related to the aging process (Voineskos et al., 2012), such that adults would likely have higher systolic pressure when they age (Franklin et al., 1997). Alternatively, the corpus callosum has been suggested to play an important role in resilience to stress. For instance, the WM microstructure in the corpus callosum in adults was associated with neuroticism, which could account for their resilience (Xu et al., 2012). The WM integrity of the corpus callosum was also suggested to be sensitive to early-life stress, confirming the implication of the corpus callosum in stress reactivity (Paul et al., 2008). On the other hand, biological response to stress was suggested to lead to raised blood pressure (e.g., Steptoe and Kivimäki, 2012). Drawing these findings together, this one-way statistical prediction might also be interpreted as an indication that the loss of integrity of the corpus callosum during aging (Voineskos et al., 2012) leads to raised blood pressure due to its implication in stress reactivity. It is plausible that other WM tracts (e.g., superior longitudinal fasciculus) are more related to the disruption of the frontoparietal resting-state connectivity due to hypertension (e.g., Li et al., 2015). Prospective studies will need to verify the findings and postulations.

Finally, trending associations were observed in systolic pressure with Stroop interference index and with the total time spent in the TOL. It is plausible that we did not have sufficient statistical power to detect significant correlations. Moreover, reaction time in perceptual and decision tasks is a basic indicator of information processing speed (Anstey et al., 2007). Therefore, as a measure of information processing speed, the composite processing-speed score could more readily be detected and has a more robust relationship with the systolic blood pressure measure. This result also parallels the finding in the study by Singh-Manoux and Marmot (2005).

Limitations

Our analyses have provided preliminary evidence concerning how blood pressure, structural and FC, and cognitive function might be related to each other on a relatively small sample of adults aged 60–70 years without apparent cognitive decline. We have applied multiple comparisons corrections for voxel-wise analyses to statistically control for errors from multiple comparisons, but the findings should be interpreted with caution and should be regarded as preliminary. Interpretations were made based on correlation, regression and mediation analyses. Owing to the nature of the cross-sectional design, we could not confirm the causality of the relationship without longitudinal data but could only make inferences from the statistical analyses. Therefore, a prospective longitudinal study on the relationship between the brain of cognitively healthy individuals and their blood pressure is needed. Lastly, the blood pressure measures were obtained from participants only before and after the cognitive assessment; the data could be improved by measuring blood pressure multiple times across a period to have a better approximation of participants' blood pressure levels.

Conclusions

This study has used a multi-modal neuroimaging approach to investigate how elevated blood pressure may be related to alterations in brain connectivity before the onset of clinical conditions and apparent cognitive decline. Our results have demonstrated the close relationships among elevated blood pressure, changes in brain topology and connectivity, and cognitive function. Although prospective studies are needed to validate our findings, our study has provided findings and shed light on how the functional brain is involved in the impact of elevated blood pressure on cognitive aging.

AUTHOR CONTRIBUTIONS

TMCL conceptualized the research idea and planned the study. EP-WM collected the data. NMLW and EP-WM analyzed and interpreted the data. NMLW wrote the manuscript. All authors read and approved the final manuscript. All authors had access to the data, and all authors agreed to submit the article for publication.

FUNDING

This study was supported by KKHo International Charitable Foundation and The University of Hong Kong May Endowed Professorship in Neuropsychology awarded to TMCL.

REFERENCES

Anstey, K. J., Mack, H. A., Christensen, H., Li, S. C., Reglade-Meslin, C., Maller, J., et al. (2007). Corpus callosum size, reaction time speed and variability in mild cognitive disorders and in a normative sample. *Neuropsychologia* 45, 1911–1920. doi: 10.1016/j.neuropsychologia. 2006.11.020

Beason-Held, L. L., Moghekar, A., Zonderman, A. B., Kraut, M. A., and Resnick, S. M. (2007). Longitudinal changes in cerebral blood flow in the older hypertensive brain. *Stroke* 38, 1766–1773. doi: 10.1161/STROKEAHA. 106.477109

Beckmann, C. F., Mackay, C. E., Filippini, N., and Smith, S. M. (2009). Group comparison of resting-state FMRI data using multi-subject ICA and dual regression. *Neuroimage* 47:S148. doi: 10.1016/S1053-8119(09) 71511-3

Beckmann, C. F., and Smith, S. M. (2004). Probabilistic independent component analysis for functional magnetic resonance imaging. *IEEE Trans. Med. Imaging* 23, 137–152. doi: 10.1109/tmi.2003.822821

Bellew, K. M., Pigeon, J. G., Stang, P. E., Fleischman, W., Gardner, R. M., and Baker, W. W. (2004). Hypertension and the rate of cognitive decline in patients with dementia of the alzheimer type. *Alzheimer Dis. Assoc. Disord.* 18, 208–213.

Bermejo-Pareja, F., Benito-León, J., Louis, E. D., Trincado, R., Carro, E., Villarejo, A., et al. (2010). Risk of incident dementia in drug-untreated arterial hypertension: a population-based study. *J. Alzheimers Dis.* 22, 949–958. doi: 10.3233/JAD-2010-101110

Carmichael, O. (2014). Preventing vascular effects on brain injury and cognition late in life: knowns and unknowns. *Neuropsychol. Rev.* 24, 371–387. doi: 10.1007/s11065-014-9264-7

Cheng, Y. L., Leung, C. B., Kwan, T. H., Chau, K. F., Tong, K. L., Li, C. S., et al. (2009). Screening for high blood pressure (BP): the hong kong experience. *Int. J. Cardiol.* 137:S126. doi: 10.1016/j.ijcard.2009.09.429

Cole, M. W., Repovš, G., and Anticevic, A. (2014). The frontoparietal control system: a central role in mental health. *Neuroscientist* 20, 652–664. doi: 10.1177/1073858414525995

Corbetta, M., and Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci.* 3, 201–215. doi: 10.1038/nrn755

Culbertson, W. C., and Zillmer, E. A. (1999). *The Tower of London, Drexel University, Research Version: Examiner's Manual.* New York, NY: Multi-Health Systems.

D'Elia, L. F., Satz, P., Uchiyama, C. L., and White, T. (1996). *Color Trails Test Professional Manual.* Odessa, FL: Psychological Assessment Resources, Inc.

Dai, W., Lopez, O. L., Carmichael, O. T., Becker, J. T., Kuller, L. H., and Gach, H. M. (2008). Abnormal regional cerebral blood flow in cognitively normal elderly subjects with hypertension. *Stroke* 39, 349–354. doi: 10.1161/STROKEAHA.107.495457

Delano-Wood, L., Bondi, M. W., Jak, A. J., Horne, N. R., Schweinsburg, B. C., Frank, L. R., et al. (2008). Stroke risk modifies regional white matter differences in mild cognitive impairment. *Neurobiol. Aging* 31, 1721–1731. doi: 10.1016/j.neurobiolaging.2008.09.013

Douaud, G., Groves, A. R., Tamnes, C. K., Westlye, L. T., Duff, E. P., Engvig, A., et al. (2014). A common brain network links development, aging, and vulnerability to disease. *Proc. Natl. Acad. Sci. U S A* 111, 17648–17653. doi: 10.1073/pnas.1410378111

Elias, M. F., Dore, G. A., Davey, A., Robbins, M. A., and Elias, P. K. (2010). From blood pressure to physical disability: the role of cognition. *Hypertension* 55, 1360–1365. doi: 10.1161/HYPERTENSIONAHA.110.149823

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Franklin, S. S., Gustin, I. V. W. IV, Wong, N. D., Larson, M. G., Weber, M. A., Kannel, W. B., et al. (1997). Hemodynamic patterns of age-related changes in blood pressure: the framingham heart study. *Circulation* 96, 308–315. doi: 10.1161/01.cir.96.1.308

Gifford, K. A., Badaracco, M., Liu, D., Tripodis, Y., Gentile, A., Lu, Z., et al. (2013). Blood pressure and cognition among older adults: a meta-analysis. *Arch. Clin. Neuropsychol.* 28, 649–664. doi: 10.1093/arclin/act046

Ginestet, C. E., Nichols, T. E., Bullmore, E. T., and Simmons, A. (2011). Brain network analysis: separating cost from topology using cost-integration. *PLoS One* 6:e21570. doi: 10.1371/journal.pone.0021570

Goldstein, F. C., Levey, A. I., and Steenland, N. K. (2013). High blood pressure and cognitive decline in mild cognitive impairment. *J. Am. Geriatr. Soc.* 61, 67–73. doi: 10.1111/jgs.12067

Gons, R. A., van Oudheusden, L. J. B., de Laat, K. F., van Norden, A. G. W., van Uden, I. W. M., Norris, D. G., et al. (2012). Hypertension is related to the microstructure of the corpus callosum: the RUN DMC study. *J. Alzheimers Dis.* 32, 623–631. doi: 10.3233/JAD-2012-121006

Hannesdottir, K., Nitkunan, A., Charlton, R. A., Barrick, T. R., MacGregor, G. A., and Markus, H. S. (2009). Cognitive impairment and white matter damage in hypertension: a pilot study. *Acta Neurol. Scand.* 119, 261–268. doi: 10.1111/j.1600-0404.2008.01098.x

Hayes, A. F. (2013). *Introduction to Mediation, Moderation, and Conditional Process Analysis.* New York, NY: Guilford.

He, Y., Chen, Z., and Evans, A. (2008). Structural insights into aberrant topological patterns of large-scale cortical networks in Alzheimer's disease. *J. Neurosci.* 28, 4756–4766. doi: 10.1523/JNEUROSCI.0141-08.2008

Heijer, T., Skoog, I., Oudkerk, M., de Leeuw, F. E., de Groot, J. C., Hofman, A., et al. (2003). Association between blood pressure levels over time and brain atrophy in the elderly. *Neurobiol. Aging* 24, 307–313. doi: 10.1016/s0197-4580(02)00088-x

Hosseini, S. M. H., Hoeft, F., and Kesler, S. R. (2012). GAT: a graph-theoretical analysis toolbox for analyzing between-group differences in large-scale structural and functional brain networks. *PLoS One* 7:e40709. doi: 10.1371/journal.pone.0040709

Humphries, M. D., and Gurney, K. (2008). Network 'small-world-ness': a quantitative method for determining canonical network equivalence. *PLoS One* 3:e0002051. doi: 10.1371/journal.pone.0002051

Jacobs, H. I. L., Leritz, E. C., Williams, V. J., Van Boxtel, M. P. J., van der Elst, W., Jolles, J., et al. (2013). Association between white matter microstructure, executive functions, and processing speed in older adults: the impact of vascular health. *Hum. Brain Mapp.* 34, 77–95. doi: 10.1002/hbm.21412

Kaiser, M., and Hilgetag, C. C. (2006). Nonoptimal component placement, but short processing paths, due to long-distance projections in neural systems. *PLoS Comput. Biol.* 2:e95. doi: 10.1371/journal.pcbi.0020095

Kennedy, K. M., and Raz, N. (2009). Pattern of normal age-related regional differences in white matter microstructure is modified by vascular risk. *Brain Res.* 1297, 41–56. doi: 10.1016/j.brainres.2009.08.058

Knopman, D. S., Penman, A. D., Catellier, D. J., Coker, L. H., Shibata, D. K., Sharrett, A. R., et al. (2011). Vascular risk factors and longitudinal changes on brain MRI: the ARIC study. *Neurology* 76, 1879–1885. doi: 10.1212/WNL.0b013e31821d753f

Knyazeva, M. G. (2013). Splenium of corpus callosum: patterns of interhemispheric interaction in children and adults. *Neural Plast.* 2013:639430. doi: 10.1155/2013/639430

Lee, T. M. C., and Chan, C. C. H. (2000). Stroop interference in chinese and english. *J. Clin. Exp. Neuropsychol.* 22, 465–471. doi: 10.1076/1380-3395(200008)22:4;1-0;FT465

Leritz, E. C., Salat, D. H., Milberg, W. P., Williams, V. J., Chapman, C. E., Grande, L. J., et al. (2010). Variation in blood pressure is associated with white matter microstructure but not cognition in african americans. *Neuropsychology* 24, 199–208. doi: 10.1037/a0018108

Lesher, E. L., and Berryhill, J. S. (1994). Validation of the geriatric depression scale—short form among inpatients. *J. Clin. Psychol.* 50, 256–260. doi: 10.1002/1097-4679(199403)50:2<256::AID-JCLP2270500218>3.0.CO;2-E

Li, X., Liang, Y., Chen, Y., Zhang, J., Wei, D., Chen, K., et al. (2015). Disrupted frontoparietal network mediates white matter structure dysfunction associated with cognitive decline in hypertension patients. *J. Neurosci.* 35, 10015–10024. doi: 10.1523/JNEUROSCI.5113-14.2015

Mackay-Brandt, A. (2011). Training attentional control in older adults. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 18, 432–451. doi: 10.1080/13825585.2011.568046

Maillard, P., Seshadri, S., Beiser, A., Himali, J. J., Au, R., Fletcher, E., et al. (2012). Effects of systolic blood pressure on white-matter integrity in young adults in the framingham heart study: a cross-sectional study. *Lancet Neurol.* 11, 1039–1047. doi: 10.1016/S1474-4422(12)70241-7

Marshall, J. C., Fink, G. R., Halligan, P. W., and Vallar, G. (2002). Spatial awareness: a function of the posterior parietal lobe? *Cortex* 38, 253–257; discussion 258–260. doi: 10.1016/s0010-9452(08)70654-3

Mensah, G. A., and Bakris, G. (2010). Treatment and control of high blood pressure in adults. *Cardiol. Clin.* 28, 609–622. doi: 10.1016/j.ccl.2010.08.002

Oldfield, R. C. (1971). The assessment and analysis of handedness: the edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Paul, R., Henry, L., Grieve, S., Guilmette, T., Niaura, R., Bryant, R., et al. (2008). The relationship between early life stress and microstructural integrity of the corpus callosum in a non-clinical population. *Neuropsychiatr. Dis. Treat.* 4, 193–201. doi: 10.2147/ndt.s1549

Qualls, C. E., Bliwise, N. G., and Stringer, A. Y. (2000). Short forms of the benton judgment of line orientation test: development and psychometric properties. *Arch. Clin. Neuropsychol.* 15, 159–163. doi: 10.1016/s0887-6177(98)00043-2

Rubinov, M., and Sporns, O. (2010). Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 52, 1059–1069. doi: 10.1016/j.neuroimage.2009.10.003

Singh-Manoux, A., and Marmot, M. (2005). High blood pressure was associated with cognitive function in middle-age in the Whitehall II study. *J. Clin. Epidemiol.* 58, 1308–1315. doi: 10.1016/j.jclinepi.2005.03.016

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., et al. (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* 31, 1487–1505. doi: 10.1016/j.neuroimage.2006.02.024

Steptoe, A., and Kivimäki, M. (2012). Stress and cardiovascular disease. *Nat. Rev. Cardiol.* 9, 360–370. doi: 10.1038/nrcardio.2012.45

Strandgaard, S. (1996). Hypertension and stroke. *J. Hypertens. Suppl.* 14, S23–S27. doi: 10.1097/00004872-199610003-00005

Strogatz, S. H. (2001). Exploring complex networks. *Nature* 410, 268–276. doi: 10.1038/35065725

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289. doi: 10.1006/nimg.2001.0978

Voineskos, A. N., Rajji, T. K., Lobaugh, N. J., Miranda, D., Shenton, M. E., Kennedy, J. L., et al. (2012). Age-related decline in white matter tract integrity and cognitive performance: a DTI tractography and structural equation modeling study. *Neurobiol. Aging* 33, 21–34. doi: 10.1016/j.neurobiolaging.2010.02.009

Waldstein, S. R., Brown, J. R. P., Maier, K. J., and Katzel, L. I. (2005). Diagnosis of hypertension and high blood pressure levels negatively affect cognitive function in older adults. *Ann. Behav. Med.* 29, 174–180. doi: 10.1207/s15324796abm2903_3

Wechsler, D. (1997). *WAIS-III Administration and Scoring Manual.* San Antonio, TX: The Psychological Corporation.

Xu, J., Kober, H., Carroll, K. M., Rounsaville, B. J., Pearlson, G. D., and Potenza, M. N. (2012). White matter integrity and behavioral activation in healthy subjects. *Hum. Brain Mapp.* 33, 994–1002. doi: 10.1002/hbm.21275

Yeo, B. T. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M., et al. (2011). The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J. Neurophysiol.* 106, 1125–1165. doi: 10.1152/jn.00338.2011

Conflict of Interest Statement: 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 Aging Neuroscience

ORIGINAL RESEARCH
published: 15 May 2017
doi: 10.3389/fnagi.2017.00132

Blood Pressure Control in Aging Predicts Cerebral Atrophy Related to Small-Vessel White Matter Lesions

Kyle C. Kern[1†], Clinton B. Wright[1†], Kaitlin L. Bergfield[2,3], Megan C. Fitzhugh[3], Kewei Chen[4,5,6], James R. Moeller[7], Nooshin Nabizadeh[1], Mitchell S. V. Elkind[8], Ralph L. Sacco[1], Yaakov Stern[7,8], Charles S. DeCarli[9] and Gene E. Alexander[2,3,6,10]*

[1] Department of Neurology, Evelyn F. McKnight Brain Institute, University of Miami Miller School of Medicine, Miami, FL, USA, [2] Neuroscience and Physiological Sciences Graduate Interdisciplinary Programs, University of Arizona, Tucson, AZ, USA, [3] Department of Psychology and Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, AZ, USA, [4] Computational Image Analysis Program, Banner Alzheimer Institute, Phoenix, AZ, USA, [5] School of Mathematics and Statistics, Arizona State University, Tempe, AZ, USA, [6] Arizona Alzheimers Consortium, Phoenix, AZ, USA, [7] Department of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, NY, USA, [8] Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, NY, USA, [9] Department of Neurology and Center for Neuroscience, University of California, Davis, Davis, CA, USA, [10] Department of Psychiatry and BIO5 Institute, University of Arizona, Tucson, AZ, USA

Cerebral small-vessel damage manifests as white matter hyperintensities and cerebral atrophy on brain MRI and is associated with aging, cognitive decline and dementia. We sought to examine the interrelationship of these imaging biomarkers and the influence of hypertension in older individuals. We used a multivariate spatial covariance neuroimaging technique to localize the effects of white matter lesion load on regional gray matter volume and assessed the role of blood pressure control, age and education on this relationship. Using a case-control design matching for age, gender, and educational attainment we selected 64 participants with normal blood pressure, controlled hypertension or uncontrolled hypertension from the Northern Manhattan Study cohort. We applied gray matter voxel-based morphometry with the scaled subprofile model to (1) identify regional covariance patterns of gray matter volume differences associated with white matter lesion load, (2) compare this relationship across blood pressure groups, and (3) relate it to cognitive performance. In this group of participants aged 60–86 years, we identified a pattern of reduced gray matter volume associated with white matter lesion load in bilateral temporal-parietal regions with relative preservation of volume in the basal forebrain, thalami and cingulate cortex. This pattern was expressed most in the uncontrolled hypertension group and least in the normotensives, but was also more evident in older and more educated individuals. Expression of this pattern was associated with worse performance in executive function and memory. In summary, white matter lesions from small-vessel disease are associated with a regional pattern of gray matter atrophy that is mitigated by blood pressure control, exacerbated by aging, and associated with cognitive performance.

Keywords: white matter hyperintensities, brain atrophy, hypertension, cerebrovascular disease, cognition, aging, scaled subprofile model, voxel-based morphometry

OPEN ACCESS

Edited by:
Pedro Rosa-Neto, McGill University, Canada

Reviewed by:
Richard Camicioli, University of Alberta, Canada
Gary A. Rosenberg, University of New Mexico, USA

***Correspondence:**
Clinton B. Wright
wright.clinton@gmail.com

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

Received: *25 October 2016*
Accepted: *19 April 2017*
Published: *15 May 2017*

Citation:
Kern KC, Wright CB, Bergfield KL, Fitzhugh MC, Chen K, Moeller JR, Nabizadeh N, Elkind MSV, Sacco RL, Stern Y, DeCarli CS and Alexander GE (2017) Blood Pressure Control in Aging Predicts Cerebral Atrophy Related to Small-Vessel White Matter Lesions. Front. Aging Neurosci. 9:132. doi: 10.3389/fnagi.2017.00132

INTRODUCTION

White matter hyperintensities (WMH) observed on T2 MRI are frequently discovered incidentally but are more prevalent with increased age, hypertension, and other cerebrovascular risk factors (Fazekas, 1989; DeCarli et al., 1995; de Leeuw et al., 2001). Although, the origins of WMHs are thought to be heterogeneous, ischemic small-vessel disease is one established etiology (DeCarli et al., 2005), and extensive lesions are associated with an increased risk of stroke (Fazekas et al., 1993), gait disturbance (Whitman et al., 2001), cognitive impairment and decline (de Groot et al., 2000), and dementia (Barber et al., 1999).

Hypertension (Salerno et al., 1992), subclinical elevated blood pressure (DeCarli et al., 1999), and WMH (Wen et al., 2006) are also associated with reduced gray matter volume (GMV). WMH and hypertension increase the risk of all-cause dementia (Barber et al., 1999; Launer et al., 2000), Alzheimer's disease (Hofman et al., 1997), and are independently linked to cognitive decline (van Swieten et al., 1991), with aspects of frontal lobe-mediated executive functions, memory, and processing speed preferentially affected (Junqué et al., 1990; Prins et al., 2005). Volumetric studies have found frontal and temporal lobe atrophy associated with WMH and aging (Raz et al., 2003; Raji et al., 2012), but the complex relationship between WMH, gray matter atrophy, cerebrovascular risk factors, and cognition is poorly understood. Since cognitive changes in aging may be mediated by gray matter loss (Raji et al., 2012), it is important to understand how WMH relate to gray matter volume, and which risk factors modify this relationship.

In this study we identify regional differences in GMV associated with WMH lesion load in a group of cognitively normal older adults and investigate the effects of blood pressure control, age, sex, education, and overall intelligence on this relationship, as well as examine the association between the WMH-associated GMV (WMH∼GMV) pattern and domain-specific cognitive performance.

METHODS

Participants

The Northern Manhattan Study (NOMAS) was designed to determine stroke incidence, risk factors, and prognosis in a race-ethnically diverse urban population. Study details have been published previously. (Sacco et al., 1997) Briefly, eligible participants were: (a) stroke-free; (b) greater than 40 years of age; and (c) residents of Northern Manhattan for at least 3 months in a household with a telephone and were enrolled between 1993 and 2001 ($N = 3{,}298$). Participants older than 50 years who remained stroke-free were invited to participate in a brain MRI substudy during annual telephone follow-up between 2003 and 2008. In addition, 199 household members of participants that met all NOMAS inclusion/exclusion criteria were enrolled to reach a total sample of 1,290. The study was approved by the Institutional Review Boards of Columbia University and the University of Miami and the protocol was designed and carried out in accordance with their recommendations. All participants provided informed written consent.

Sample Selection

For the current study, we identified 64 participants with normotension, controlled hypertension or uncontrolled hypertension. We obtained systolic and diastolic blood pressures measured at the brachial artery while seated with a mercury sphygmomanometer after a period of rest for 10 min. Two measurements were taken at least 1 h apart, and the values were averaged. Participants with a reported history of hypertension on antihypertensive medication were classified as having controlled hypertension if the mean blood pressure was less than 140 systolic and 90 diastolic. Participants were classified as uncontrolled hypertension if they had a previous diagnosis of hypertension and the mean blood pressure was greater than 140 systolic or 90 diastolic. Normotensive patients had no history of hypertension and were not taking antihypertensive medications. Groups were case-wise matched for age, sex, race, and educational attainment. Participants were classified as having finished 8th grade or less. The current study participants were similar to and reflective of the overall NOMAS population.

Cognitive Assessment

Participants in the NOMAS MRI substudy were administered a neuropsychological battery in either English or Spanish by trained research assistants on the day of MRI acquisition. As reported previously by Siedlecki et al. (2009) cognitive domains were created using an exploratory factor analysis to group tests with similar variance across a cohort of 796 participants without cognitive impairment. The cognitive domains in this study included memory, executive function, processing speed, language, and general intellectual ability (Gc) (Siedlecki et al., 2009). The neuropsychological tests used to represent each cognitive domain are in keeping with recommendations from the NINDS Canadian Stroke Network Vascular Cognitive Impairment Harmonization Standards. The memory domain was comprised of the components of a modified California Verbal Learning Test. Executive function was assessed with the Wechsler Adult Intelligence Scale letter-number sequencing test, the digit ordering task, the odd-man-out task, and a difference score from the Color Trails test (similar to Trailmaking Test form B). Processing speed was evaluated with the Grooved Pegboard task. Language (not previously reported) was assessed with the Controlled Oral Word Association Task, the Animal Naming test, and the Boston naming test. Finally, to estimate Gc (Cattell, 1987) as a surrogate for premorbid IQ (Siedlecki et al., 2009), we administered the Peabody Picture Vocabulary Test. Subtest scores were converted into Z-scores for each of the four domains based on the mean and standard deviation of all participants of the NOMAS brain MRI substudy. A self-reported depression scale, The Center for Epidemiologic Studies Depression Scale (CESD), was available in 58 of 64 participants. The CESD is a 20-question survey of depressive symptoms that was developed in 1977 and has been used in diverse populations for the epidemiologic study of depression (Radloff, 1977). ApoE genetic testing was available for a subgroup of 58 of the 64 participants from fasting blood samples drawn at the time of MRI. ApoE alleles were determined by *Hha*I digestion of PCR products amplified from genomic DNA as described previously.

Carriers of one or two alleles of ApoE-4 were collapsed into the same category and compared against carriers of only ApoE-3 or ApoE-2 (Hixson and Vernier, 1990; Willey et al., 2014).

Image Acquisition and Processing

Participants were scanned with a 1.5T scanner (Phillips, The Netherlands). A 3D T1 structural image (slice thickness 1.5 mm no gap, TE 2.1 ms, TR 20 ms, flip angle 20°) was acquired to assess gray matter volume (GMV). A T2 FLAIR sequence (FOV 250 mm, matrix 192 × 133 scaled to 256 × 256, slice thickness 3 mm no gap, TE 144 ms, TR 5,500 ms, TI 1900 ms, flip angle 90°) was used to identify WMH, total brain volumes (TBV), and total intracranial volumes (TIV), which were obtained using a validated, automatic segmentation algorithm as previously reported (DeCarli et al., 1992; Wright et al., 2005). GMV was converted into a fraction of TBV (GMF) for comparison. WMH volumes were converted into a percentage of TIV to correct for head size, and then log transformed (logWMH) to use in regression analyses, since raw volumes were not normally distributed.

T1 images were processed using SPM8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom; www.fil.ion.ucl.ac.uk/spm) Voxel Based Morphometry (VBM) (Ashburner and Friston, 2000) software to segment MRIs into gray matter, white matter and CSF after image intensity nonuniformity correction. The DARTEL (Diffeomorphic Anatomic Registration Through Exponentiated Lie algebra) toolbox was used to create a group-specific brain template aligned to a common MNI (Montreal Neurologic Institute) space by iteratively aligning tissue segmentations using a common tissue probability map. The gray matter maps were processed to preserve volume information with the spatial deformations. A 10 mm smoothing kernel was applied and statistics were performed on gray matter volume (GMV) maps across the sample at each voxel.

We used the Scaled Subprofile Model (SSM) (Moeller et al., 1987; Alexander and Moeller, 1994) to identify the regional pattern of VBM GMV differences associated with WMH load in the cohort. The SSM is a multivariate technique that tests for regional covariance patterns in neuroimaging scans without requiring conservative correction for multiple comparisons on a voxel-wise basis. This analytic method has been applied to numerous functional and structural imaging studies with PET and MRI (Eidelberg et al., 1995; Alexander et al., 1999; Smith et al., 2006). Applications to MRI VBM have demonstrated sensitivity in identifying regional patterns of gray matter changes related to aging and risk for Alzhiemer's disease (Alexander et al., 2006, 2008, 2012; Bergfield et al., 2010).

In our study, we used VBM and SSM to identify a pattern of GMV variability associated with logWMH volume and created a set of SSM subject scores that reflect the extent to which each individual expresses this WMH-related gray matter volume (WMH∼GMV) pattern. Individual subject scores were used in subsequent group analyses.

The SSM analysis was performed using MATLAB (Math Works, Natick, Massachusetts, USA). GMV images were smoothed and log transformed, and means across regions and participants were subtracted at each voxel. A Principal Component Analysis was performed to decompose the dataset into gray matter network components that explain the greatest variance in the neuroimaging data. To select the linear combination of gray matter network components that best model the variance in WMH across the cohort, we used the Akaike Information Criterion (AIC) (Burnham and Anderson, 2002) with multiple-regression. The first subset of components that together mapped the lowest AIC value in the regression model to identify the WMH∼GMV network was selected as the best set of component predictors. Total intracranial volume (TIV) was included as a covariate to account for differences in head size as previously described (Bergfield et al., 2010). To provide reliability estimates of the resulting WMH∼GMV pattern, we used bootstrap resampling with 500 iterations of the SSM multivariate regression, and used the means and standard deviations to calculate a Z-score at each voxel (Alexander et al., 2012). Thresholding at $Z \leq -2$ and $Z \geq 2$ created Z-maps identifying areas of GMV differences robustly associated with logWMH.

Statistics

Group differences were determined using ANOVA and chi-squared tests. Individual subject scores were calculated from the SSM multivariate regression that reflect the extent to which each participant expresses the WMH∼GMV pattern. These values were used to compare the strength of the WMH∼GMV association between uncontrolled hypertensive, controlled hypertensive, and normotensive participants using ANCOVA and ordinal linear regression while controlling for age and educational level. We tested the effects of age, sex, educational attainment and Gc on this relationship using multivariate linear regression. We also tested the association between expression of the WMH∼GMV pattern and cognitive performance using multivariate regression models that included the pattern subject scores and cognitive domain Z-scores for memory, executive function, processing speed, and language while controlling for age and level of education. A conservative bonferroni correction was applied to these 4 multivariate linear regression models for multiple comparisons and the corrected *p*-values reflect this. All tests were two-tailed and an alpha of 0.05 determined significance.

RESULTS

Participants included 64 adults ranging from 60 to 86 years of age (mean = 72 years). Twenty-two had uncontrolled hypertension, 21 had controlled hypertension, and 21 were normotensive. Groups were matched case-wise for age, sex, race and education. There were no differences between the study participants and the overall NOMAS population in age, ethnic distribution, smoking status, diabetes status, cognitive scores or CESD. There were no group differences in Gc or performance in each of the cognitive domains. Mean cognitive Z-scores were close to zero for each group and the mean of the four cognitive domains was greater than −1.5 for each participant, indicating a level of cognitive function representative of the NOMAS study without significant cognitive impairment. There were no group differences in GMF,

WMH volume, or total brain volume (TBV) after adjusting for head size with TIV. (**Table 1**)

Normotensive participants were not on antihypertensive medications. In the controlled hypertension group, all participants were on at least one antihypertensive medication and 5 participants were on 2 antihypertensives. In the uncontrolled hypertensive group, 9 participants were not on antihypertensive medication, 6 were on one antihypertensive, 6 were on 2 antihypertensives, and 1 was on 3 antihypertensives (Supplementary Table). The distribution of antihypertensive medication class was not different between the participants with controlled and uncontrolled hypertension.

More educated participants had better executive function and language scores ($p < 0.0001$) and tended to have non-significantly better memory ($p = 0.07$) and processing speed ($p = 0.10$) than those with less education. Participants with higher education had a trend for greater WMH load ($p = 0.07$) but no differences in adjusted TBV.

Gray matter VBM in combination with SSM and AIC identified the best linear combination of topographical GMV component patterns associated with WMH volumes in the sample. This model included components 1, 2, and 6 and accounted for 34.5% of the variance in logWMH ($R = 0.613$, adjusted $R^2 = 0.345$; **Figure 1**). The WMH∼GMV pattern included reduced GMV in the superior temporal gyri bilaterally, the left angular gyrus, and bilateral supramarginal and orbital gyri. GMV was relatively greater in the cingulate cortex bilaterally, the medial thalami and the basal forebrain (**Figure 2**).

In comparing blood pressure control groups, the uncontrolled hypertensive group had the greatest expression of the WMH∼GMV pattern (as indicated by the highest pattern subject scores), while the normotensive group had the lowest expression after adjusting for age, gender, education and Gc. The expression of the WMH∼GMV pattern was significantly greater in the uncontrolled hypertensive group compared to the normotensive group (ANCOVA: Main effect of group: F-statistic = 4.497, degrees of freedom (dof) = 2, $p = 0.015$), while the difference between uncontrolled hypertensive and controlled hypertensive groups, as well as the difference between controlled hypertensive and normotensive groups were not significant (**Figure 3A**). Using ordinal regression we tested the linear association between the WMH∼GMV pattern and blood pressure group while covarying for age and education and

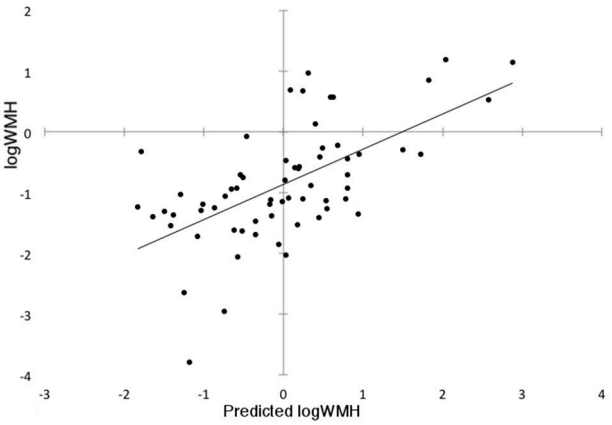


FIGURE 1 | Subject scores calculated from the Scaled Subprofile Model (SSM) gray matter volume covariance pattern predict log of white matter hyperintensity (WMH) volume across the cohort.

TABLE 1 | Clinical Characteristics.

	Total	Normotensives	Controlled hypertensives	Uncontrolled hypertensives	*P*-values*
N	64	21	22	21	
Age in years mean ± SD	72 ± 7	72 ± 7	71 ± 8	71 ± 7	0.80
Males:Female	21:43	7:14	7:15	7:14	0.99
never:former:current smoker	31:26:7	10:10:1	9:9:4	12:7:2	0.57
# with Diabetes	14	3	5	6	0.53
# with PVD	10	1	5	4	0.24
# with < 8th grade Education	35	12	12	11	0.95
IQ Z-score mean ± SD	0.08 ± 0.81	0.13 ± 0.97	0.06 ± 0.80	0.06 ± 0.68	0.95
Memory Z-score mean ± SD	0.07 ± 0.79	0.03 ± 0.88	0.15 ± 0.75	0.03 ± 0.73	0.85
Executive Function Z-score mean ± SD	–0.14 ± 0.81	–0.12 ± 0.84	–0.07 ± 0.80	–0.24 ± 0.81	0.80
Processing Speed Z-score mean ± SD	0.01 ± 0.35	–0.03 ± 0.30	0.07 ± 0.40	–0.02 ± 0.34	0.54
Language Z-score mean ± SD	0.01 ± 0.75	0.15 ± 0.89	–0.01 ± 0.69	–0.11 ± 0.67	0.54
Total Cerebral Volume cc ± SD	1138 ± 118	1137 ± 99	1130 ± 118	1146 ± 139	0.91
Gray Matter Fraction ± SD	0.56 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.75
WMH Volume cc mean ± SD	7.7 ± 9.1	5.6 ± 7.7	9.0 ± 8.3	8.4 ± 11.1	0.45
WMH Brain Fraction mean ± SD %†	0.66 ± 0.73 %	0.48 ± 0.61%	0.78 ± 0.70%	0.70 ± 0.85%	0.38

WMH, white matter hyperintensities; SD, standard deviation; PVD, peripheral vascular disease.

**P-values reflect Chi-squared test for gender, smoking, diabetes, PVD, and education, One-way ANOVA for all others.*

† Total WMH Volume/Total Intracranial Volume.

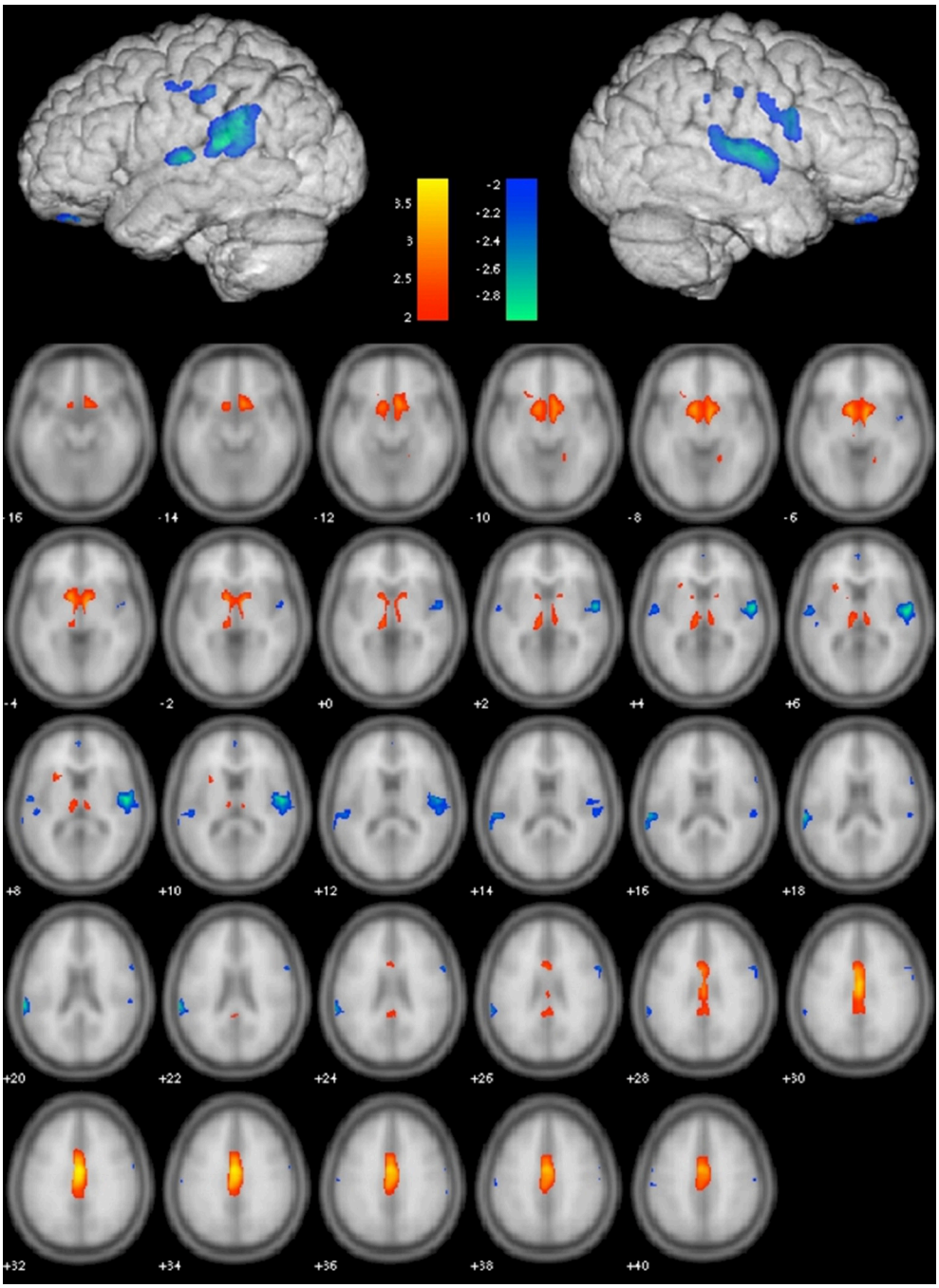


FIGURE 2 | Gray Matter Volume (GMV) Covariance Pattern predicting log of white matter hyperintensity (WMH) volume across the entire cohort. Color bars depict *Z*-scores. Blue shows areas of reduced GMV $Z \leq -2$ associated with greater WMH while red shows relative volume preservation with $Z \geq 2$.

confirmed that blood pressure group predicted expression of the WMH∼GMV pattern ($B = 0.281$, adjusted $R^2 = 0.064$, $p = 0.024$). Neither sex nor Gc showed a significant association with the WMH∼GMV pattern.

The WMH∼GMV pattern was expressed more in older participants (main effect F-statistic = 26.627, dof = 1, $p < 0.001$), but there was no age by blood pressure group interaction (**Figure 3B**). Participants with higher education also had greater expression of the WMH∼GMV pattern (main effect F-statistic = 7.138, dof = 1, $p = 0.01$), but there was no education by group interaction (**Figure 3C**).

Using multivariate linear regression, both increasing age ($B = 0.504$, adjusted $R^2 = 0.242$, $p < 0.001$) and greater educational attainment ($B = 0.289$, adjusted $R^2 = 0.069$, $p = 0.021$) were

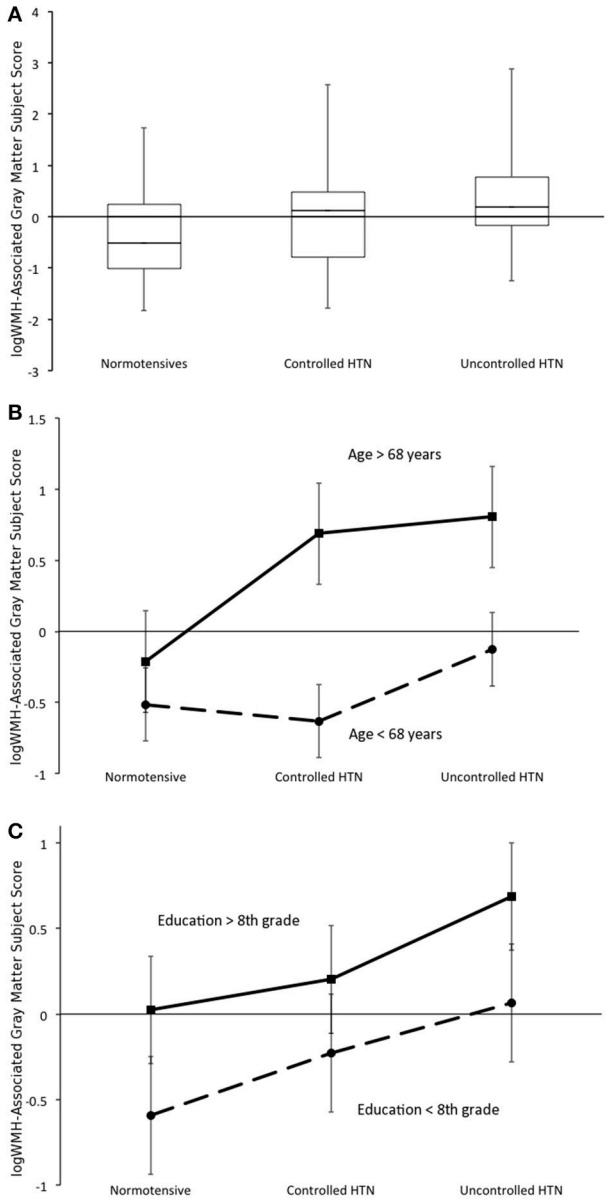


FIGURE 3 | (A) Expression of the white matter hyperintensity-associated gray matter volume (WMH∼GMV) pattern differs across blood pressure control groups while covarying for age and educational attainment. Boxplot depicts entire range (error bars), 1st quartile, median, and 3rd quartile. **(B)** Main effect of age on blood pressure group x WMH∼GMV pattern expression. **(C)** Main effect of education on blood pressure group x WMH∼GMV pattern expression interaction. Error bars show 95% confidence intervals **(B,C)**.

associated with greater expression of the WMH∼GMV pattern despite having preserved cognitive performance.

Greater expression of the WMH∼GMV pattern was also associated with worse memory ($B = -0.277$, $p = 0.012$, corrected $p = 0.048$) and executive function ($B = -0.315$, $p = 0.004$, corrected $p = 0.016$) while adjusting for age and education (**Figure 4**). However, WMH∼GMV pattern expression was not associated with processing speed, or language scores. Lower GMF was also independently associated with worse memory ($R = 0.42$, $p = 0.01$), but not executive function, language or processing speed. Cognitive scores were not associated with logWMH or adjusted TBV.

In the subgroup of 58 participants with CESD scores (20 normotensive, 19 controlled hypertensive, 19 uncontrolled hypertensive), depressive symptoms were not associated with WMH, TBV, expression of WMH∼GMV pattern, or any of the cognitive domains.

APOE genetic testing was available on 58 of the 64 participants. Twenty-eight percent have at least 1 APOE-4 allele. There were more APOE-4 alleles in the normotensive group (normotensive 7/17, controlled hypertensives 6/21, uncontrolled hypertensives 3/20). Using logistic regression, APOE-4 status was not significantly associated with brain volume, WMH volume, expression of the WMH∼GMV pattern, or any of the cognitive domains.

DISCUSSION

Using a multivariate neuroimaging approach incorporating both gray and white matter imaging techniques, we identified a pattern of GMV differences associated with WMH lesion load in a group of older adults without significant cognitive impairment. Areas of relatively smaller cortical GMV in the bilateral superior temporal and temporal-parietal junctions as well as the orbital cortex may reflect brain regions particularly sensitive to the effects of small-vessel disease. Our finding that this WMH∼GMV relationship was strongest in patients with uncontrolled hypertension, and weakest in normotensives supports this conclusion. Previous studies found that uncontrolled blood pressure predisposes to white matter disease, (van Swieten et al., 1991; de Leeuw et al., 2002) and that successful treatment reduces the risk of developing white matter lesions. (Liao et al., 1996) In our study, group differences in WMH volume or TBV were not significant nor predictive, but we identified a regional pattern of GMV differences associated with WMH that provides a potential mechanism for the link between poor blood pressure control, gray matter atrophy and age-related cognitive changes. (Nagai et al., 2008) Other studies have also demonstrated associations between WMH and GM atrophy (Appelman et al., 2009). Raji et al. (2012) found an association between WMH and GMV in a similar pattern involving the frontal and temporal-parietal regions independent of age. We find that poor blood pressure control augments the effect of WMH on GM atrophy, even without differences in WMH volume or TBV.

We also identified areas of relative brain volume preservation across the cingulate gyrus, the basal forebrain and the thalamus that reflect regions less affected by cerebral small-vessel disease, or perhaps indicate areas more strongly influenced by non-vascular mechanisms such as amyloid deposition. In a VBM study of mild cognitive impairment and Alzheimer's disease patients without cardiovascular disease or extensive cerebrovascular disease, Karas et al. demonstrated both thalamic and cingulate cortex atrophy compared to healthy controls

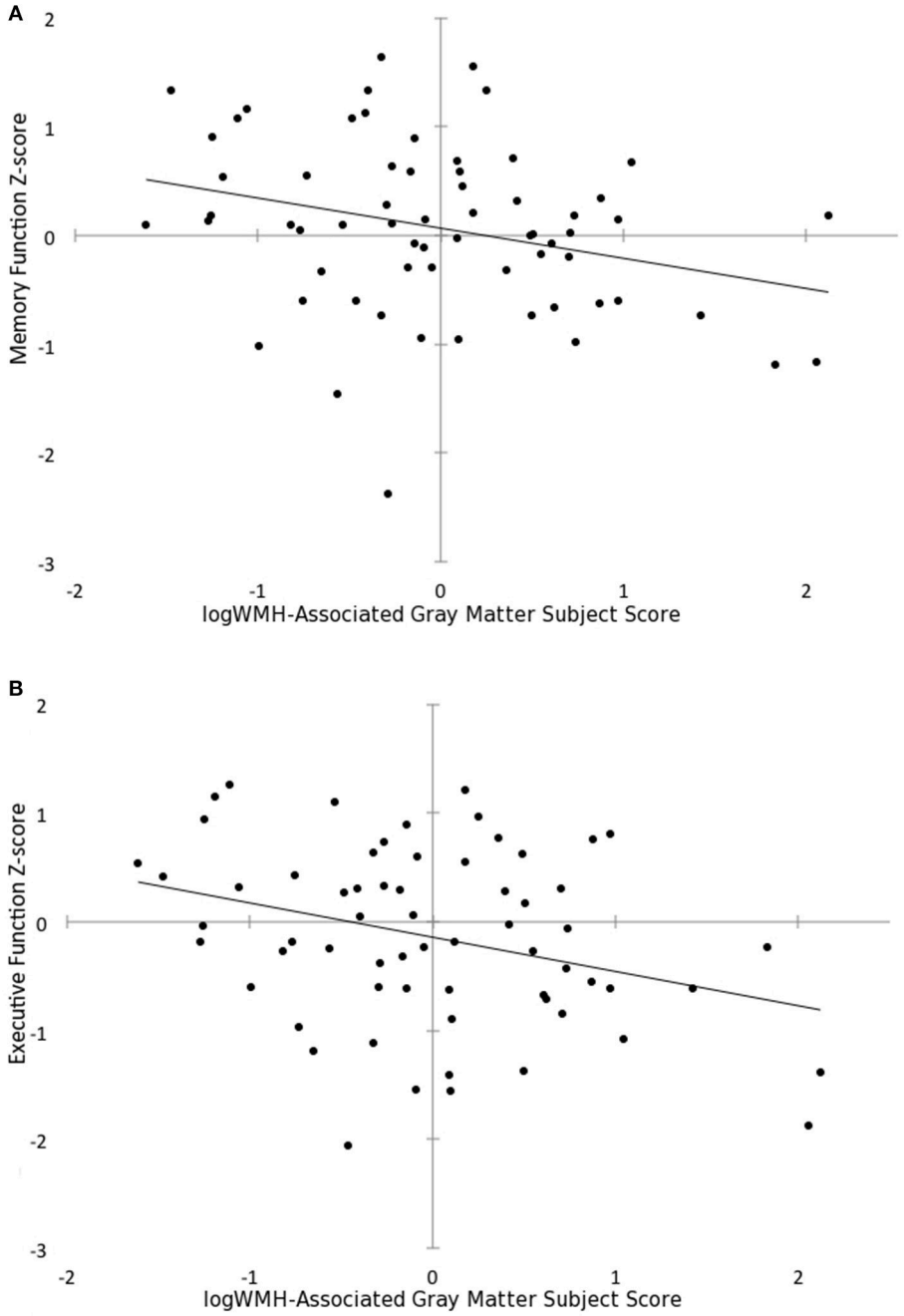


FIGURE 4 | Subject scores for the white matter hyperintensity-associated gray matter volume pattern predicts (A) memory function Z-score ($B = -0.277$, $p = 0.012$) and **(B)** executive function Z-score ($B = -0.315$, $p = 0.004$) after adjusting for age and educational attainment.

(Karas et al., 2004). However most studies identify extensive overlap between risk factors for Alzheimer's disease and WMH. Some theorize that amyloid deposition and vascular damage may act synergistically in the clinical manifestation of dementia (Erten-Lyons et al., 2013; Provenzano et al., 2013).

The effect of age on the WMH∼GMV relationship reveals that older individuals may be more susceptible to brain atrophy resulting from small-vessel disease. Indeed previous studies found differential effects of both hypertension and age (Appelman et al., 2009) as well as interactions between them (Strassburger et al., 1997; Raji et al., 2012). Arteriolar sclerosis that occurs with both high blood pressure and aging of the vascular system likely underlies endothelial dysfunction, reduced vasoreactivity, and subsequent ischemia. Chronic ischemia results in both demyelination and possibly Wallerian degeneration (Appelman et al., 2009). The deleterious

effects of these vascular changes on GM is less clear, in part because imaging modalities sensitive to WMH are not sensitive to GMV changes. While our findings are cross-sectional, these data raise the possibility that better blood pressure control could prevent cortical atrophy, especially in older individuals, and prospective studies are needed to directly test this hypothesis. The ongoing SPRINT-MIND trial (Systolic Pressure Intervention Trial) may help in this regard (ClinicalTrials.gov: NCT01206062). Meanwhile, evidence from the Systolic Hypertension in Europe (Syst-Eur) trial demonstrated that antihypertensive treatment reduced the incidence of dementia (Forette et al., 1998).

The expression of a WMH∼GMV pattern is clinically relevant in our study since it was associated with cognitive performance, while neither WMH volume nor adjusted TBV showed such a relationship. Greater expression of the WMH∼GMV pattern, after adjusting for age and education, predicted lower memory and executive function scores, despite our sample being cognitively intact overall. This finding is consistent with previous studies showing an association between memory function and both white (de Groot et al., 2000) and gray matter insult (DeCarli et al., 1995). Raji et al. (2012) concluded that while hypertension was associated with WMH, the cognitive effects of hypertension may be mediated by gray matter atrophy. The association in our study with executive function was even stronger after adjusting for age and education, which is also consistent with previous studies (DeCarli et al., 1995; Prins et al., 2005). Executive dysfunction has previously been associated with age, frontal lobe atrophy and frontal lobe WMH, and has been implicated as a marker for vascular cognitive impairment (Salthouse et al., 2003; Wright et al., 2008). By using a multivariate analysis of structural T1 and T2 differences, we identified a potential neuroimaging biomarker that may be more sensitive to early changes in domain-specific cognition in the elderly than conventional measures such as WMH or TBV alone. Longitudinal studies could evaluate this pattern as a biomarker for cognitive decline in aging. In contrast to previous studies (Ylikoski et al., 1993; Prins et al., 2005) we did not find WMH to be predictive of processing speed, but we were limited to only one processing speed test with low inter-subject variance.

In this community-based sample of older participants with preserved cognition, we found that those with more education had a trend for greater WMH load and significantly greater expression of WMH-associated cerebral atrophy, despite similar cognitive performance. These findings support the idea that education contributes to cognitive reserve, wherein cognitive function is preserved despite structural deterioration (Stern, 2002; Bennett et al., 2003). Cognitive reserve relates to lifestyle characteristics including higher education and occupational complexity (Stern et al., 1995; Bennett et al., 2003; Le Carret et al., 2003), and is thought to reflect greater efficiency in cognitive processes, permitting greater resistance to structural insult. Reserve may also reflect adaptive neural processes and recruitment of additional or more extensive brain areas to circumvent structural pathology (Stern, 2002). Alternatively, genetic predisposition responsible for cognitive resilience may instead permit higher educational attainment.

There are several limitations to this study. We used WMH volume, a summary measure for global white matter damage reflective of small-vessel disease. While easily acquired on commonly available clinical MRI scans, FLAIR WMH are associated with heterogeneous underlying histopathology: from mild perivascular tissue damage surrounding lipohyalinotic arterioles with minimal axonal loss, to more severe ischemic damage with extensive myelin and axonal loss (Gouw et al., 2008). We emphasized hypertensive vascular risk in this study, but WMH may also result from other factors including Alzheimer's disease, alcoholism or head trauma. Furthermore, regional WMH distribution may affect the sensitivity of detection on MRI, as well as alter the extent and pattern of cognitive impairment. Future studies using diffusion tensor imaging may elucidate the link between regional WM insult, gray matter atrophy, cerebrovascular risk factors, and cognition. Finally, we highlight the importance of blood pressure control on preventing brain structural changes associated with cognitive decline. However, our cross-sectional study cannot fully evaluate the effect of the duration of blood pressure control. Nor is it powered to assess the class effects of different antihypertensive medications. Larger longitudinal studies are needed to evaluate optimization of blood pressure treatment to limit structural and cognitive changes.

CONCLUSIONS

We evaluated a race-ethnically diverse, community-based population of older adults without cognitive impairment. In doing so we avoided the selection bias of hospital or referral based studies but controlled many of the known risk factors for WMH, gray matter atrophy, and cognitive decline by matching groups for age, sex, and education to focus primarily on the effects of blood pressure control on these outcomes. Rather than assess WM or gray matter pathology alone, we used an integrative, multivariate approach that evaluated modifiers of a structural relationship between GMV and WMH, as a marker of cerebral small-vessel disease. We identified a pattern of WMH∼GMV differences that was sensitive to the effects of blood pressure control and predictive of cognitive performance. We conclude that controlling blood pressure may limit the effects of small-vessel disease and aging on cerebral gray matter atrophy, potentially delaying or preventing the onset of cognitive sequelae.

AUTHOR CONTRIBUTIONS

KK contributed to data analyses, interpretation and manuscript preparation. CW contributed to study oversight, recruitment, data preparation, analyses, interpretation and manuscript preparation. KB, MF, KC, and NN contributed to data preparation, analyses and interpretation. JM, ME, RS, YS, and CD contributed to study oversight, recruitment, and data analyses and interpretation. GA contributed to study oversight, data analyses, interpretation, and manuscript preparation.

FUNDING

CW receives federal grant support (K02 NS 059729, R01 HL 108623, SPRINT MRI WFUHS 330214, R01 NS 29993) and private foundation support (American Heart Association Bugher Center project 14BFSC17690003). He receives royalties from UpToDate for 2 vascular dementia chapters. ME receives compensation for providing consultative services for BioTelemetry/Cardionet, BMS-Pfizer Partnership, Boehringer-Ingelheim, Daiichi-Sankyo, Janssen Pharmaceuticals, and Sanofi-Regeneron Partnership; receives research support from diaDexus Inc., Bristol- Myers Squibb/Sanofi Pharmaceuticals Partnership, and the NIH/NINDS; has given expert legal opinions on behalf of Organon (NuvaRing and stroke litigation) and Hi-Tech; and serves on the National, Founders Affiliate, and New York City chapter boards of the American Heart Association/American Stroke Association. He receives royalties from UpToDate for chapters related to stroke. RS receives federal grant support (R01 NS 29993), private foundation support (American Heart Association Bugher Center), and pharma research support (Boehringer Ingelheim). GA receives federal grant support from the National Institute on Aging (R01 AG049464, P30 AG019610) and funding from the State of Arizona and ADHS, the Advanced Research Institute for Biomedical Imaging, and the McKnight Brain Research Foundation. CD receives federal grant support. YS receives federal grant support. The remaining authors declare no conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

We thank Janet De Rosa MPH, NOMAS project coordinator. The authors appreciate support from the National Institutes of Neurological Disorders and Stroke, the National Institute on Aging, the state of Arizona and Arizona Department of Health Services, Arizona Advanced Research Institute for Biomedical Imaging, and the Evelyn F. McKnight Brain Research Foundation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2017.00132/full#supplementary-material

REFERENCES

Alexander, G. E., Bergfield, K. L., Chen, K., Reiman, E. M., Hanson, K. D., Lin, L., et al. (2012). Gray matter network associated with risk for Alzheimer's disease in young to middle-aged adults. *Neurobiol. Aging* 33, 2723–2732. doi: 10.1016/j.neurobiolaging.2012.01.014

Alexander, G. E., Chen, K., Aschenbrenner, M., Merkley, T. L., Santerre-Lemmon, L. E., Shamy, J. L., et al. (2008). Age-related regional network of magnetic resonance imaging gray matter in the rhesus macaque. *J. Neurosci.* 28, 2710–2718. doi: 10.1523/JNEUROSCI.1852-07.2008

Alexander, G. E., Chen, K., Merkley, T. L., Reiman, E. M., Caselli, R. J., Aschenbrenner, M., et al. (2006). Regional network of magnetic resonance imaging gray matter volume in healthy aging. *Neuroreport* 17, 951–956. doi: 10.1097/01.wnr.0000220135.16844.b6

Alexander, G. E., Mentis, M. J., Van Horn, J. D., Grady, C. L., Berman, K. F., Furey, M. L., et al. (1999). Individual differences in PET activation of object perception and attention systems predict face matching accuracy. *Neuroreport* 10, 1965–1971. doi: 10.1097/00001756-199906230-00032

Alexander, G. E., and Moeller, J. R. (1994). Application of the scaled subprofile model to functional imaging in neuropsychiatric disorders: a principal component approach to modeling brain function in disease. *Hum. Brain Mapp.* 2, 79–94. doi: 10.1002/hbm.460020108

Appelman, A. P., Exalto, L. G., van der Graaf, Y., Biessels, G. J., Mali, W. P., and Geerlings, M. I. (2009). White matter lesions and brain atrophy: more than shared risk factors? A systematic review. *Cerebrovasc. Dis.* 28, 227–242. doi: 10.1159/000226774

Ashburner, J., and Friston, K. J. (2000). Voxel-based morphometry–the methods. *Neuroimage* 11, 805–821. doi: 10.1006/nimg.2000.0582

Barber, R., Scheltens, P., Gholkar, A., Ballard, C., McKeith, I., Ince, P., et al. (1999). White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. *J. Neurol. Neurosurg. Psychiatry* 67, 66–72. doi: 10.1136/jnnp.67.1.66

Bennett, D. A., Wilson, R. S., Schneider, J. A., Evans, D. A., Mendes de Leon, C. F., Arnold, S. E., et al. (2003). Education modifies the relation of AD pathology to level of cognitive function in older persons. *Neurology* 60, 1909–1915. doi: 10.1212/01.WNL.0000069923.64550.9F

Bergfield, K. L., Hanson, K. D., Chen, K., Teipel, S. J., Hampel, H., Rapoport, S. I., et al. (2010). Age-related networks of regional covariance in MRI gray matter: reproducible multivariate patterns in healthy aging. *Neuroimage* 49, 1750–1759. doi: 10.1016/j.neuroimage.2009.09.051

Burnham, K. P., and Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Heidelberg: Springer-Verlag.

Cattell, R. B. (1987). *Intelligence: its Structure, Growth and Action*. Amsterdam: Elsevier.

DeCarli, C., Fletcher, E., Ramey, V., Harvey, D., and Jagust, W. J. (2005). Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke* 36, 50–55. doi: 10.1161/01.STR.0000150668.58689.f2

DeCarli, C., Maisog, J., Murphy, D. G., Teichberg, D., Rapoport, S. I., and Horwitz, B. (1992). Method for quantification of brain, ventricular, and subarachnoid CSF volumes from MR images. *J. Comput. Assist. Tomogr.* 16, 274–284. doi: 10.1097/00004728-199203000-00018

DeCarli, C., Miller, B. L., Swan, G. E., Reed, T., Wolf, P. A., Garner, J., et al. (1999). Predictors of brain morphology for the men of the NHLBI twin study. *Stroke* 30, 529–536. doi: 10.1161/01.STR.30.3.529

DeCarli, C., Murphy, D. G., Tranh, M., Grady, C. L., Haxby, J. V., Gillette, J. A., et al. (1995). The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults. *Neurology* 45, 2077–2084. doi: 10.1212/WNL.45.11.2077

de Groot, J. C., de Leeuw, F. E., Oudkerk, M., van Gijn, J., Hofman, A., Jolles, J., et al. (2000). Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann. Neurol.* 47, 145–151. doi: 10.1002/1531-8249(200002)47:2<145::AID-ANA3>3.0.CO;2-P

de Leeuw, F. E., de Groot, J. C., Achten, E., Oudkerk, M., Ramos, L. M., Heijboer, R., et al. (2001). Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J. Neurol. Neurosurg. Psychiatry* 70, 9–14. doi: 10.1136/jnnp.70.1.9

de Leeuw, F. E., de Groot, J. C., Oudkerk, M., Witteman, J. C., Hofman, A., van Gijn, J., et al. (2002). Hypertension and cerebral white matter lesions in a prospective cohort study. *Brain* 125, 765–772. doi: 10.1093/brain/awf077

Eidelberg, D., Moeller, J. R., Ishikawa, T., Dhawan, V., Spetsieris, P., Chaly, T., et al. (1995). Early differential diagnosis of Parkinson's disease with 18F-fluorodeoxyglucose and positron emission tomography. *Neurology* 45, 1995–2004. doi: 10.1212/WNL.45.11.1995

Erten-Lyons, D., Woltjer, R., Kaye, J., Mattek, N., Dodge, H. H., Green, S., et al. (2013). Neuropathologic basis of white matter hyperintensity accumulation with advanced age. *Neurology* 81, 977–983. doi: 10.1212/WNL.0b013e3182a43e45

Fazekas, F. (1989). Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. *Eur. Neurol.* 29, 164–168. doi: 10.1159/000116401

Fazekas, F., Kleinert, R., Offenbacher, H., Schmidt, R., Kleinert, G., Payer, F., et al. (1993). Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* 43, 1683–1683. doi: 10.1212/WNL.43.9.1683

Forette, F., Seux, M. L., Staessen, J. A., Thijs, L., Birkenhäger, W. H., Babarskiene, M. R., et al. (1998). Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. *Lancet* 352, 1347–1351. doi: 10.1016/S0140-6736(98)03086-4

Gouw, A. A., Seewann, A., Vrenken, H., Van Der Flier, W. M., Rozemuller, J. M., Barkhof, F., et al. (2008). Heterogeneity of white matter hyperintensities in Alzheimer's disease: post-mortem quantitative MRI and neuropathology. *Brain* 131, 3286–3298. doi: 10.1093/brain/awn265

Hixson, J. E., and Vernier, D. T. (1990). Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J. Lipid Res.* 31, 545–548.

Hofman, A., Ott, A., Breteler, M., Bots, M. L., Slooter, A. J., van Harskamp, F., et al. (1997). Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* 349, 151–154. doi: 10.1016/S0140-6736(96)09328-2

Junqué, C., Pujol, J., Vendrell, P., Bruna, O., Jódar, M., Ribas, J. C., et al. (1990). Leuko-araiosis on magnetic resonance imaging and speed of mental processing. *Arch. Neurol.* 47, 151–156. doi: 10.1001/archneur.1990.00530020047013

Karas, G. B., Scheltens, P., Rombouts, S. A., Visser, P. J., Van Schijndel, R. A., Fox, N. C., et al. (2004). Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. *Neuroimage* 23, 708–716. doi: 10.1016/j.neuroimage.2004.07.006

Launer, L. J., Ross, G. W., Petrovitch, H., Masaki, K., Foley, D., White, L. R., et al. (2000). Midlife blood pressure and dementia: the Honolulu-Asia aging study. *Neurobiol. Aging* 21, 49–55. doi: 10.1016/S0197-4580(00)00096-8

Le Carret, N., Lafont, S., Mayo, W., and Fabrigoule, C. (2003). The effect of education on cognitive performances and its implication for the constitution of the cognitive reserve. *Dev. Neuropsychol.* 23, 317–337. doi: 10.1207/S15326942DN2303_1

Liao, D., Cooper, L., Cai, J., Toole, J. F., Bryan, N. R., Hutchinson, R. G., et al. (1996). Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. *Stroke* 27, 2262–2270. doi: 10.1161/01.STR.27.12.2262

Moeller, J. R., Strother, S. C., Sidtis, J. J., and Rottenberg, D. A. (1987). Scaled subprofile model: a statistical approach to the analysis of functional patterns in positron emission tomographic data. *J. Cereb. Blood Flow Metab.* 7, 649–658. doi: 10.1038/jcbfm.1987.118

Nagai, M., Hoshide, S., Ishikawa, J., Shimada, K., and Kario, K. (2008). Ambulatory blood pressure as an independent determinant of brain atrophy and cognitive function in elderly hypertension. *J. Hypertens.* 26, 1636–1641. doi: 10.1097/HJH.0b013e3283018333

Prins, N. D., van Dijk, E. J., den Heijer, T., Vermeer, S. E., Jolles, J., Koudstaal, P. J., et al. (2005). Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain* 128, 2034–2041. doi: 10.1093/brain/awh553

Provenzano, F. A., Muraskin, J., Tosto, G., Narkhede, A., Wasserman, B. T., Griffith, E. Y., et al. (2013). White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease? *JAMA Neurol.* 70, 455–461. doi: 10.1001/jamaneurol.2013.1321

Radloff, L. S. (1977). The CES-D scale: a self-report depression scale for research in the general population. *Appl. Psychol. Meas.* 1, 385–401. doi: 10.1177/014662167700100306

Raji, C. A., Lopez, O. L., Kuller, L. H., Carmichael, O. T., Longstreth, W. T. Jr., Gach, H. M., et al. (2012). White matter lesions and brain gray matter volume in cognitively normal elders. *Neurobiol. Aging* 33, 834 e837–e816. doi: 10.1016/j.neurobiolaging.2011.08.010

Raz, N., Rodrigue, K. M., and Acker, J. D. (2003). Hypertension and the brain: vulnerability of the prefrontal regions and executive functions. *Behav. Neurosci.* 117, 1169–1180. doi: 10.1037/0735-7044.117.6.1169

Sacco, R. L., Roberts, J. K., Boden-Albala, B., Gu, Q., Lin, I. F., Kargman, D. E., et al. (1997). Race-ethnicity and determinants of carotid atherosclerosis in a multiethnic population. The Northern Manhattan Stroke Study. *Stroke* 28, 929–935. doi: 10.1161/01.STR.28.5.929

Salerno, J. A., Murphy, D. G., Horwitz, B., DeCarli, C., Haxby, J. V., Rapoport, S. I., et al. (1992). Brain atrophy in hypertension. A volumetric magnetic resonance imaging study. *Hypertension* 20, 340–348. doi: 10.1161/01.HYP.20.3.340

Salthouse, T. A., Atkinson, T. M., and Berish, D. E. (2003). Executive functioning as a potential mediator of age-related cognitive decline in normal adults. *J. Exp. Psychol. Gen.* 132, 566–594. doi: 10.1037/0096-3445.132.4.566

Siedlecki, K. L., Stern, Y., Reuben, A., Sacco, R. L., Elkind, M. S., and Wright, C. B. (2009). Construct validity of cognitive reserve in a multiethnic cohort: The Northern Manhattan Study. *J. Int. Neuropsychol. Soc.* 15, 558–569. doi: 10.1017/S1355617709090857

Smith, J. F., Chen, K., Johnson, S., Morrone-Strupinsky, J., Reiman, E. M., Nelson, A., et al. (2006). Network analysis of single-subject fMRI during a finger opposition task. *Neuroimage* 32, 325–332. doi: 10.1016/j.neuroimage.2005.12.010

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *J. Int. Neuropsychol. Soc.* 8, 448–460. doi: 10.1017/S1355617702813248

Stern, Y., Tang, M. X., Denaro, J., and Mayeux, R. (1995). Increased risk of mortality in Alzheimer's disease patients with more advanced educational and occupational attainment. *Ann. Neurol.* 37, 590–595. doi: 10.1002/ana.410370508

Strassburger, T. L., Lee, H. C., Daly, E. M., Szczepanik, J., Krasuski, J. S., Mentis, M. J., et al. (1997). Interactive effects of age and hypertension on volumes of brain structures. *Stroke* 28, 1410–1417. doi: 10.1161/01.STR.28.7.1410

van Swieten, J. C., Geyskes, G. G., Derix, M. M., Peeck, B. M., Ramos, L. M., van Latum, J. C., et al. (1991). Hypertension in the elderly is associated with white matter lesions and cognitive decline. *Ann. Neurol.* 30, 825–830. doi: 10.1002/ana.410300612

Wen, W., Sachdev, P. S., Chen, X., and Anstey, K. (2006). Gray matter reduction is correlated with white matter hyperintensity volume: a voxel-based morphometric study in a large epidemiological sample. *Neuroimage* 29, 1031–1039. doi: 10.1016/j.neuroimage.2005.08.057

Whitman, G. T., Tang, Y., Lin, A., and Baloh, R. W. (2001). A prospective study of cerebral white matter abnormalities in older people with gait dysfunction. *Neurology* 57, 990–994. doi: 10.1212/WNL.57.6.990

Willey, J. Z., Gardener, H., Moon, Y. P., Yoshita, M., DeCarli, C., Cheung, Y. K., et al. (2014). Lipid profile components and subclinical cerebrovascular disease in the northern manhattan study. *Cerebrovasc. Dis.* 37, 423–430. doi: 10.1159/000362920

Wright, C. B., Festa, J. R., Paik, M. C., Schmiedigen, A., Brown, T. R., Yoshita, M., et al. (2008). White matter hyperintensities and subclinical infarction: Associations with psychomotor speed and cognitive flexibility. *Stroke* 39, 800–805. doi: 10.1161/STROKEAHA.107.484147

Wright, C. B., Paik, M. C., Brown, T. R., Stabler, S. P., Allen, R. H., Sacco, R. L., et al. (2005). Total homocysteine is associated with white matter hyperintensity volume: the Northern Manhattan Study. *Stroke* 36, 1207–1211. doi: 10.1161/01.STR.0000165923.02318.22

Ylikoski, R., Ylikoski, A., Erkinjuntti, T., Sulkava, R., Raininko, R., and Tilvis, R. (1993). White matter changes in healthy elderly persons correlate with attention and speed of mental processing. *Arch. Neurol.* 50, 818–824. doi: 10.1001/archneur.1993.00540080029009

Conflict of Interest Statement: 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 Aging Neuroscience

REVIEW
published: 08 June 2018
doi: 10.3389/fnagi.2018.00177

Non-invasive Brain Stimulation: Probing Intracortical Circuits and Improving Cognition in the Aging Brain

Joyce Gomes-Osman[1,2,3], Aprinda Indahlastari[4†], Peter J. Fried[3†], Danylo L. F. Cabral[1], Jordyn Rice[1], Nicole R. Nissim[4], Serkan Aksu[4], Molly E. McLaren[4] and Adam J. Woods[4]**

[1] Department of Physical Therapy, University of Miami Miller School of Medicine, Miami, FL, United States, [2] Evelyn F. McKnight Brain Institute, University of Miami Miller School of Medicine, Miami, FL, United States, [3] Berenson-Allen Center for Noninvasive Brain Stimulation, Division of Cognitive Neurology, Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [4] Department of Clinical and Health Psychology, Department of Neuroscience, Center for Cognitive Aging and Memory, McKnight Brain Institute, University of Florida, Gainesville, FL, United States

OPEN ACCESS

Edited by:
Thomas Wisniewski,
School of Medicine, New York University, United States

Reviewed by:
Sean Kevin Meehan,
University of Michigan, United States
Kai-Hsiang Chuang,
The University of Queensland, Australia

***Correspondence:**
Joyce Gomes-Osman
j.gomes@miami.edu
Adam J. Woods
ajwoods@phhp.ufl.edu

[†] Co-second authors.

Received: 13 December 2017
Accepted: 22 May 2018
Published: 08 June 2018

Citation:
Gomes-Osman J, Indahlastari A, Fried PJ, Cabral DLF, Rice J, Nissim NR, Aksu S, McLaren ME and Woods AJ (2018) Non-invasive Brain Stimulation: Probing Intracortical Circuits and Improving Cognition in the Aging Brain. Front. Aging Neurosci. 10:177. doi: 10.3389/fnagi.2018.00177

The impact of cognitive aging on brain function and structure is complex, and the relationship between aging-related structural changes and cognitive function are not fully understood. Physiological and pathological changes to the aging brain are highly variable, making it difficult to estimate a cognitive trajectory with which to monitor the conversion to cognitive decline. Beyond the information on the structural and functional consequences of cognitive aging gained from brain imaging and neuropsychological studies, non-invasive brain stimulation techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) can enable stimulation of the human brain *in vivo*, offering useful insights into the functional integrity of intracortical circuits using electrophysiology and neuromodulation. TMS measurements can be used to identify and monitor changes in cortical reactivity, the integrity of inhibitory and excitatory intracortical circuits, the mechanisms of long-term potentiation (LTP)/depression-like plasticity and central cholinergic function. Repetitive TMS and tDCS can be used to modulate neuronal excitability and enhance cortical function, and thus offer a potential means to slow or reverse cognitive decline. This review will summarize and critically appraise relevant literature regarding the use of TMS and tDCS to probe cortical areas affected by the aging brain, and as potential therapeutic tools to improve cognitive function in the aging population. Challenges arising from intra-individual differences, limited reproducibility, and methodological differences will be discussed.

Keywords: cognitive aging, non-invasive brain stimulation, TMS, tDCS, cognition, neuromodulation

INTRODUCTION

Aging, Cognitive Aging, and Cognitive Impairment

Over the next 50 years, the number of adults aged 60 years or older will double, quickly reaching 1.4 billion people worldwide (United Nations, Department of Economic and Social Affairs, Population Division, 2015). The maintenance of cognitive health and concerns regarding memory loss are consistently cited as top concerns by this population (National Association of Area Agencies on Aging, 2015). Therefore, a greater understanding of the processes underlying the aging brain is relevant.

Cognitive aging is the decline in cognitive abilities resulting from physiologic change with age. The cognitive trajectory of cognitive aging is unique to each individual, resulting from inherent differences in the genetic make-up, life experiences, and level of education. The consequences of cognitive aging can range from subtle, even imperceptible, changes that do not impact quality of life, to more severe declines that antedate dementia (Harada et al., 2013). Even in the absence of disease, cognitive aging can result in selective impairment of certain cognitive domains. Episodic memory begins to decline during mid-life (Nyberg et al., 1996), while semantic memory decreases later, as individuals become elders (Nyberg, 2004; Rönnlund et al., 2005). Executive functioning, especially mental flexibility and response inhibition, also show age-dependent decreases (Wecker et al., 2005). Furthermore, psychomotor processing speed slows (Salthouse, 2010), and the ability to focus attention and/or multi-task becomes more difficult (Carlson et al., 1995; Darowski et al., 2008; Salthouse, 2010).

The neurobiological underpinnings of cognitive aging are multifactorial. Cognitive impairments may result from brain atrophy (cortical thinning or decreased gray matter volume), especially to the prefrontal cortex, temporal lobes, and hippocampus (Raz et al., 2004; DeCarli et al., 2005). The frontal lobe, which is the last to mature (around 25 years old) and the first to start thinning with age (Salat et al., 2004), plays an essential role in higher-order cognitive processes and a variety of critical executive functioning processes such as planning, decision-making, problem solving, and working memory (Nissim et al., 2017). Furthermore, demyelination or lesions to white matter tracts (Dong et al., 2015), imbalances in dopamine and serotonin (Mukherjee et al., 2002; Nyberg, 2004), decreased brain-derived neurotrophic factor (Mattson et al., 2004), increased monoamine oxidase, and deposition of amyloid beta (Aβ) are all linked to increased free radicals and dendritic spine loss (Hsieh et al., 2006).

The highly individual trajectories of cognitive aging pose a challenge clinically in that it is difficult to estimate one's cognitive trajectory and predict conversion to more severe clinical states, such as cognitive impairment or dementia. Annually, ∼15–20% of adults above 65 years of age will present with mild cognitive impairment (MCI) (Roberts and Knopman, 2013). In addition, more than 30% of individuals with MCI will develop Alzheimer's disease (AD) (Ward et al., 2013) or other types of dementia (Mitchell and Shiri-Feshki, 2009) within 5 years.

Great strides have been made in the study of the consequences of cognitive aging at the macroscopic level in humans. With the advent of neuroimaging techniques, preclinical pathophysiological changes have been identified as early as 10–15 year before individuals exhibit any cognitive changes (Sperling et al., 2011). In the currently accepted biomarker model of the preclinical stage of AD, evidence of Aβ accumulation and synaptic dysfunction are the first preclinical features observed through cerebrospinal fluid measurements, positron emission tomography and functional magnetic resonance imaging (fMRI) (Sperling et al., 2011).

Non-invasive brain stimulation (NIBS) modalities, which permit direct or indirect electrical stimulation of the human brain *in-vivo*, can be useful as adjunct to other neuroimaging tools in the study of cognitive aging and impairment. NIBS techniques, including transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), can be used to assess the functional integrity of intracortical circuits during synaptic dysfunction. Additionally, previous studies have shown the potential therapeutic roles of NIBS to reestablish or normalize activity and metabolism in brain areas affected by aging. The present review will focus on the available literature regarding the use of NIBS in the form of TMS and tDCS in the study of cognitive aging and cognitive decline.

TRANSCRANIAL MAGNETIC STIMULATION (TMS)

TMS is a means of indirect electrical stimulation via electromagnetic induction. Briefly, to generate a single TMS pulse, an electric current is passed through a copper coil generating a magnetic field that is perpendicular to the plane of the coil (Kobayashi and Pascual-Leone, 2003). The rapid rate of change of the magnetic field induces a secondary electric field in the underlying brain tissue (Kobayashi and Pascual-Leone, 2003) with the potential to depolarize neuronal membranes.

With the TMS coil placed on the subject's scalp overlying the motor cortex, by increasing the stimulus output, it is possible to alter the rate of change of the magnetic field so that the induced electrical current can depolarize layer-V pyramidal neurons directly, as well as indirectly through trans-synaptic activation of nearby interneurons (Kobayashi and Pascual-Leone, 2003).The depolarization of the pyramidal neuron propagates along the corticospinal pathway, and elicits a motor evoked potential (MEP) if the firing threshold is sufficient to trigger this process in a population of pyramidal neurons. The amplitude and latency of MEPs induced by the single pulse can be recorded with surface electrodes placed on the muscles of interest, such as the contralateral first dorsal interosseous (FDI) or abductor pollicis brevis (APB). TMS incorporates a variety of techniques. Single-pulse and paired-pulse TMS comprise paradigms that enable the probing of the cortical reactivity and functional integrity of excitatory and inhibitory intracortical circuits. Repetitive TMS protocols have traditionally been employed for therapeutic use, but also enable an assessment of mechanisms of plasticity.

Single-Pulse TMS

Single-pulse TMS is the building block of all TMS protocols, but on its own, can be used to probe the reactivity of the motor cortex (as well as non-motor regions if electroencephalography is used concurrently). The most ubiquitous measure in TMS is the motor threshold (MT). The MT is defined as the percentage of the maximum stimulator output required to elicit MEPs of a minimum amplitude in at least 50% of consecutive trials [typically 50 μV at rest (resting MT; rMT), or 200 μV during ∼20% of maximum contraction (active MT; aMT); (Rossi et al., 2009)]. The most current recommendations are that the MT be drawn from the number of MEPs acquired over 20 responses (i.e., the intensity that produces MEPs in 10/20 trials), to increase reliability (Rossini et al., 2015).

Locally, the MT assesses the reactivity of the motor pathway from the motor cortex, along the corticospinal tract, and peripheral muscle. Globally, the MT is influenced by whole-brain structural changes that alter the scalp-to-cortex distance, as they alter the amount of energy required to bring corticospinal neurons to threshold. Stokes et al. (2007) assessed the influence of increasing coil-to-cortex distance utilizing acrylic separators, and found that for every millimeter, there was an increase in ∼2.8% in the MT. Given that age-related atrophy of cortical tissue and increased ventricular volume can increase the coil-to-cortex distance, the MT is a relevant measure to explore in the aging brain. Another metric of overall corticomotor excitability obtained using TMS single pulses is the input-output (IO; also stimulus-response or recruitment) curve. The IO curve evaluates changes in MEP intensity in response to systematically varying TMS intensity and can be influenced by the patterns of recruitment and synchronization of neurons that contribute to the corticospinal volley (Devanne et al., 1997; Chen et al., 1998). This stimulus-response relationship is best determined with a full range of stimulus intensities from sub-threshold (null responses) to a saturation plateau (where further increases in intensity do not elicit larger MEPs). The plotted curve is typically sigmoidal in nature and can be successfully fitted using a Boltzmann function (Devanne et al., 1997).

The IO curve gives rise to a number of distinct parameters can be used to evaluate cortico-motor excitability. Among these the most common are: MEP_{MAX} (the largest MEP that can be elicited, corresponding to the plateau); S_{50} (the stimulation intensity that produces an MEP corresponding to 50% of the maximum); the maximal slope (corresponding to the steepest part of the curve), and the x-intercept (the stimulus intensity where the tangent of slope crosses) (Devanne et al., 1997; Ridding and Rothwell, 1997; Carroll et al., 2001). The advantage of using the input-output curves is that each parameter can be used as an outcome measure, providing complementary information about the excitability at a specific levels of excitability of the corticospinal pathway (Kukke et al., 2014). One challenge for collecting IO curves is being able to stimulate at high enough intensities to reach the plateau. This is especially pertinent for aging given the impact of cortical atrophy on coil-to-cortex distance discussed above. An alternative approach is to normalize MEP responses from TMS to the maximum M-wave that can be elicited by peripheral electrical stimulation.

Single-Pulse TMS in Cognitive Aging and Impairment

While there is evidence for increased rMT in older adults (McGinley et al., 2010; Young-Bernier et al., 2012), some studies also found rMT to be unchanged when compared to young adults (Peinemann et al., 2001; Oliviero et al., 2006; Opie and Semmler, 2014). This discrepancy may be explained by individual patterns of cortical atrophy that are difficult to account for in a cross-sectional analysis unless coil-to-cortex distance can be individually assessed with structural magnetic resonance imaging (MRI).

Another interpretation for the lack of agreement in the age-related differences in rMT is the possibility that the MT goes through different stages during the transition from young adulthood to cognitive aging. In support of this idea, Shibuya et al. (2016) assessed the rMT in 113 individuals with ages ranging from 20 to 83 years of age, making comparisons between subgroups in each decade (Shibuya et al., 2016). The pattern of age-related changes in rMT followed a quadratic curve, with significant differences in each decade. Beginning at 20 years, the rMT increased until age 50, slowly decreasing after that (Shibuya et al., 2016). Ultimately, longitudinal studies are needed to confirm this hypothesis.

Beyond rMT, there is evidence that the stimulus-response relationship may itself be altered in the aging brain. Pitcher et al. (2003) compared the input-output curves of young adults and older adults. The older cohort demonstrated a rightward shift in the x-intercept, wherein higher intensities were necessary to achieve the maximum MEP, while the slope and rMT were not different (Pitcher et al., 2003). The implication of these results is that common approaches for evaluating cortical excitability, such as measuring the intensity necessary to achieve an MEP of 1 mV or averaging the amplitude of MEPs at a specific intensity relative to the rMT, may be misleading because they would represent excitability at different points on the input-output curves for each subject. Thus, future studies should consider incorporating input-output curves into their neurophysiologic assessments in older adults.

In individuals with cognitive impairment due to AD or vascular dementia, the majority of studies have found evidence of cortical hyperexcitability, evidenced by a reduction of rMT when compared with their cognitively intact peers (Carvalho et al., 1997; Di Lazzaro et al., 2002, 2004; Pennisi et al., 2011). However, one study did not find differences in MT between older healthy adults, individuals with early-onset dementia and individuals with fronto-temporal dementia (Pierantozzi et al., 2004), attributing their results to having a homogeneous sample with strict age and cognition-matched controls, though failure to control for coil-to-cortex distance cannot be ruled out.

It may seem counterintuitive that individuals with cognitive impairment would have reduced rMT, since they are more likely

to have atrophy (and thus increased cortex-to-coil distance, which would *increase* rMT). This finding might be partly explained by metabolic changes in the neurotransmitter systems that regulate resting membrane potential. There is evidence for a disruption in the availability of neurotransmitters such as gamma-Aminobutyric acid (GABA), glutamate and acetylcholine (Ferreri et al., 2003; Di Lazzaro et al., 2004; Koliatsos et al., 2006) that are consistent with the increase in cortical excitability (and a reduction in the rMT) in AD. Furthermore, there is increasing evidence that hyperexcitability (as indexed by rMT) is related to the severity of cognitive dysfunction in AD (Khedr et al., 2011). Future studies should seek to fully address the relationship between changes in neurotransmitters and rMT in individuals with cognitive impairment.

Taken together, longitudinal measurements of the MT may be more useful to capture the electrophysiological correlates of the gray matter volume loss and degeneration of white matter associated with the aging brain than cross-sectional measurements. Furthermore, few studies report structural or cognitive performance data in the participants. Given the variability in trajectories in cognitive aging, it would be desirable to collect data on the baseline neuropsychological performance and structural integrity of cortical areas, to gain a better understanding of the electrophysiological implications of cognitive and volumetric changes in cognitive aging.

Despite the high variability in cross-sectional measurements of rMT, there is high test-retest reliability for rMT measurements in healthy older adults [intraclass correlation coefficient (ICC) of 94; (Christie et al., 2007)]. This finding has been replicated with the further finding that reproducibility (as measured by Cronbach's α) was higher with using monophasic TMS pulses ($\alpha = 0.94$) then biphasic TMS pulses ($\alpha = 0.83$) (Fried et al., 2017). In individuals with AD, reproducibility of the rMT is high and equivalent with both monophasic and biphasic TMS ($\alpha = 0.98$). The aMT is more variable ($\alpha = 0.77$ for healthy older adults and 0.85 for individuals with AD) (Fried et al., 2017). While there may be changes in the MT throughout the lifespan, the high reproducibility of this measure within an individual may be useful to demonstrate such transitions.

While the test-retest reliability for measurements of the input-output curve has not been performed in older adults, there is evidence on the test-retest reliability of MEPs along different points of the input-output curve in young adults (Brown et al., 2017). The ICC was highest at 130% rMT (0.70), and decreased at higher levels of intensity (0.68 at 150% rMT, and 0.64 at 175% rMT) (Brown et al., 2017). Future studies should investigate the reliability of input-output curves in older adults.

Paired-Pulse TMS Techniques

Paired-pulse TMS refers to the delivery of two TMS pulses in close succession, wherein the first, or conditioning pulse (CP) influences the second, suprathreshold test pulse (TP) (Kobayashi and Pascual-Leone, 2003). Depending on the intensity of the CP and inter-pulse interval, a conditioned TP will result in an MEP of higher or lower amplitude when compared with an unconditioned TP. Different paired-pulse protocols have been developed to probe excitatory and inhibitory intracortical circuits. Short-interval intracortical inhibition (SICI), intracortical facilitation (ICF) and long-interval intracortical inhibition (LICI) are paired-pulse paradigms that use cortical TMS as the CP, whereas short-afferent inhibition (SAI) uses peripheral electrical stimulation as the CP (Kujirai et al., 1993; Tokimura et al., 2000).

SICI, ICF, and LICI

Short-interval intracortical inhibition (SICI) is assessed when a subthreshold CP (typically delivered at 70–80% rMT) is delivered 1–6 ms prior to the TP (Kujirai et al., 1993; Sanger et al., 2001). There is evidence to support that SICI reflects a form of GABA-a receptor mediated inhibition (Kujirai et al., 1993). Using the same intensity of CP and TP and a slightly longer inter-stimulus interval (ISI) (8–30 ms), one is able to probe intracortical circuits that are associated with facilitation of MEP responses (intracortical facilitation [ICF]) (Kujirai et al., 1993; Sanger et al., 2001). Although the exact mechanisms responsible ICF are not fully understood, it is considered a net-facilitation that is associated with an increased N-methyl-D-aspartate (NMDA) receptor mediated facilitation, combined with a weaker GABA-a-receptor mediated inhibition (Hanajima et al., 1998). When both CP and TP are delivered at suprathreshold intensity (typically 110–130% rMT), and separated by 50–200 ms, a GABA-b receptor mediated inhibition can be observed, termed LICI (Kujirai et al., 1993; Werhahn et al., 1999; Sanger et al., 2001).

SAI

Short-afferent inhibition (SAI) is a different type of paired-pulse paradigm wherein a peripheral electrical stimulation serves as the CP with a TMS pulse as a TP. In SAI, short (200 μs) electrical currents are applied to the median nerve (above the perceptual sensory threshold) followed 18–50 ms by a suprathreshold TMS pulse over the homologous region of primary motor cortex (M1) (Tokimura et al., 2000). SAI has been attributed *at least partly* to a cholinergic mechanism (Di Lazzaro et al., 2002), and thus, has been utilized in studies assessing the functional integrity of cholinergic circuits in the aging brain. Future studies investigating the mechanisms underlying SAI are needed.

Paired-Pulse TMS Techniques in Cognitive Aging and Impairment

The evidence examining paired-pulse measures in cognitive aging and cognitive impairment has produced mixed results. SICI in older adults has been shown to be decreased (Peinemann et al., 2001; Marneweck et al., 2011), increased (McGinley et al., 2010), and unaltered (Oliviero et al., 2006; Opie and Semmler, 2014). SICI was shown to be non-significantly reduced in individuals with AD (Di Lazzaro et al., 2002), but decreased in individuals with early-onset AD (Pierantozzi et al., 2004) and individuals with FTD (Benussi et al., 2017). ICF was found to be both unaltered (Peinemann et al., 2001; Shibuya et al., 2016) and decreased (McGinley et al., 2010) in older adults, unaltered in individuals with AD and decreased in individuals with FTD (Benussi et al., 2017). LICI was found to be both increased (McGinley et al., 2010) and decreased (Opie and Semmler, 2014)

in older adults, decreased in individuals with FTD and both decreased (Brem et al., 2013) and unaltered in individuals with AD (Benussi et al., 2017).

Inconsistencies in the effects of paired-pulse protocols across studies can be attributed to several factors. One important factor may be the selection of ISI. Each paired-pulse protocol is associated with a relatively wide range of possible ISIs and the optimal ISI may be different across individuals or between normal and pathological states of cognitive aging. While the majority of TMS paired-pulse studies utilized various ISI's (Peinemann et al., 2001; Di Lazzaro et al., 2002; Pierantozzi et al., 2004; Oliviero et al., 2006; Shibuya et al., 2016; Benussi et al., 2017), others employed only a single ISI for all subjects (McGinley et al., 2010; Marneweck et al., 2011; Opie and Semmler, 2014). Since different individuals will likely show optimal responses at different ISIs, the use of paired-pulse curves (in which the impact of the CP is plotted against the ISI) may decrease the variability and improve the outcomes of future studies.

Another important factor contributing to the variability of paired-pulse protocols is the choice of stimulus intensity for the CP. For SICI and ICF the following CP's were used: 70% rMT (Marneweck et al., 2011; Shibuya et al., 2016); 75% rMT (Peinemann et al., 2001); 80% MT during an active contraction (Opie and Semmler, 2014); 95% rMT (Di Lazzaro et al., 2002; Oliviero et al., 2006; McGinley et al., 2010). In studies that assessed LICI, the conditioning stimulus was given at an intensity that would elicit MEPs in the magnitude of 0.5–1 mV (McGinley et al., 2010) and 120% RMT (Opie and Semmler, 2014). Future studies should standardize the methodology in paired-pulse studies, as variability in methodology introduces unnecessary bias in the comparison across studies.

In addition to the technical aspects mentioned above, some variability in paired-pulse effects may be due to heterogeneity in the neurocognitive status of the control group. Of all studies that assessed paired-pulse techniques, only half (Di Lazzaro et al., 2002; Pierantozzi et al., 2004; Silbert et al., 2006; Marneweck et al., 2011; Pennisi et al., 2011; Young-Bernier et al., 2012, 2014) employed screening of cognitive function for older healthy adults. In addition, two of these (Young-Bernier et al., 2012, 2014) included individuals outside the normative values on the Montreal Cognitive Assessment, but who were classified as healthy on the basis other clinical determinants. The remaining studies did not report if they screened older healthy adults for cognitive impairment (Carvalho et al., 1997; Peinemann et al., 2001; Di Lazzaro et al., 2004; Oliviero et al., 2006; McGinley et al., 2010; Opie and Semmler, 2014; Shibuya et al., 2016).

Unlike paired-pulse TMS, SAI has more consistently been shown to be altered in the aging brain. Two studies found decreased SAI in older adults (Young-Bernier et al., 2012, 2014), but one study found that SAI was unaltered in the comparison between older and young adults (Oliviero et al., 2006). Four studies (Di Lazzaro et al., 2002, 2004; Young-Bernier et al., 2014; Benussi et al., 2017) have reported that individuals with mild to moderate AD demonstrated impaired SAI. Further, one study found that SAI measures were associated with age, independent of the diagnosis of AD or age of onset (Di Lorenzo et al., 2016).

Part of the consistency exhibited in SAI when compared with the TMS paired-pulse paradigms could be explained by the fact that all but one study (Young-Bernier et al., 2014) used various ISI's to characterize SAI. In addition, four (Di Lazzaro et al., 2002, 2004; Di Lorenzo et al., 2016; Benussi et al., 2017) of these six studies normalized the CP to each individual by standardizing it to the N20 component latency of the somatosensory evoked potential of the median nerve. These procedures decrease the influence of inter-individual factors on the overall response. Another potential factor is that central cholinergic intracortical circuits show reliable decays with aging and are primarily implicated in the pathogenesis of AD (Bhandari et al., 2016; Kandimalla and Reddy, 2017). While glutamatergic circuits are also implicated (Kandimalla and Reddy, 2017), these circuits are only partially probed with ICF. Lastly, the precise roles of GABAergic circuits, probed by SICI and LICI in aging and cognitive impairment, are less clear (Bhandari et al., 2016; Kandimalla and Reddy, 2017).

Finally, it is worth noting that many of the studies using paired-pulse techniques may simply be underpowered to reliably detect significant difference between groups given the inter-individual variability in their effects. To this effect, the reproducibility of paired-pulse TMS measures was examined in older healthy adults and individuals with AD (Fried et al., 2017). In the healthy group, reproducibility was moderate for SICI (Cronbach's $\alpha = 0.68$), low to none for ICF ($\alpha = 0.11$) and high for LICI ($\alpha = 0.98$), likely due to floor effects from near complete inhibition. By comparison, all three measures were highly reproducible in the AD group ($\alpha \geq 0.81$) (Fried et al., 2017). The reproducibility of SAI in middle aged adults was moderate (ICC = 0.65–0.67; Brown et al., 2017), but has not been assessed in older adults or those with pathological cognitive aging. Future studies can use these cohort-specific measures of reliability to adjust effect and sample size calculations (Fried et al., 2017).

REPETITIVE TMS (rTMS)

Repetitive TMS (rTMS) involves the delivery "trains" of repeated TMS pulses at a set intensity and frequency to a given cortical area (Kobayashi and Pascual-Leone, 2003). rTMS differs from the approaches discussed above in that it offers the possibility of modulating the activity of the stimulated area for a period that outlasts the stimulation application (Kobayashi and Pascual-Leone, 2003). Many factors influence the aftereffects of rTMS, including the frequency, pattern, intensity, and duration of the stimulation. In addition, the basal or ongoing level of activity within the targeted area and associated networks (i.e., state-dependency) can influence the outcome of rTMS (Silvanto et al., 2007).

Conventional rTMS is characterized by the delivery of individual pulses at regular intervals (Kobayashi and Pascual-Leone, 2003). Knowledge of the neurophysiology of rTMS comes primarily from studying its effects in M1. In neurotypical individuals under normal conditions, on-off patterns of high-frequency rTMS (10–20 Hz) tend to increase cortico-motor (i.e.,

lower rMT and/or higher MEP amplitude), while continuous low frequency rTMS (∼1 Hz) tends to reduce excitability (Kobayashi and Pascual-Leone, 2003). In clinical settings (e.g., in the case of medication-resistant major depression) rTMS is used therapeutically with the aim of normalizing aberrant activity in the targeted site and associated networks.

More recently, researchers and clinicians have explored more complex patterned stimulation consisting of *bursts of pulses* at regular intervals (Oberman and Pascual-Leone, 2013). The most commonly used patterned rTMS protocol is Theta-burst stimulation (TBS) (Huang et al., 2005). Modeled after classical protocols for inducing plasticity in the cortex and hippocampal formation, TBS consists of the delivery of a low intensity 50 Hz burst triplet (three pulses with 20 ms inter-pulse interval) repeated at 5 Hz (Huang et al., 2005). TBS is typically applied in one of two patterns: continuous TBS (cTBS), in which the bursts are delivered continuously, has been shown to reduce MEP amplitudes, while intermittent TBS (iTBS), in which the bursts are delivered in 2-s trains with an 8-s delay, has been shown to increase MEP amplitude (Huang et al., 2005). When applied for 600 pulses, cTBS and iTBS have been shown to induce modulation of MEP amplitude for up to 45 min, creating "plasticity curves" that resemble long-term depression (LTD) and long-term potentiation (LTP), respectively in terms of its biochemistry and temporal profile of the effects (Huang et al., 2005). Thus there is a growing interest in the application of TBS to assess the mechanisms of cortical plasticity.

Evidence from human and rodent pharmacology, neuroimaging, and biochemistry studies have shown that the aftereffects of iTBS and cTBS are NMDA receptor-dependent (Huang et al., 2007) and reflect changes in the activity of GABAergic synapses on layer-V pyramidal cells (Stagg et al., 2009b; Funke and Benali, 2011). Huang et al. (2007) found that memantine, a NMDA receptor antagonist, abolished the aftereffects of both iTBS and cTBS, providing evidence that glutamatergic synapses are required for TBS aftereffects. Using magnetic resonance spectroscopy, cTBS was shown to increase GABA metabolism in the stimulated region. Using magnetic resonance spectroscopy, cTBS was shown to increase GABA metabolism in the stimulated region (Stagg et al., 2009b).

Studies of calcium-binding proteins in rodents have shown iTBS likely achieves facilitation by reducing parvalbumin-mediated inhibition of the pyramidal cell output, while cTBS increases inhibition of the pyramidal cell likely by reducing calbindin expression that affects dendritic integration (Funke and Benali, 2011). Complementary evidence from invasive epidural recordings in humans demonstrated iTBS modulates layer-V pyramidal cell activity via monosynaptic connections (Di Lazzaro et al., 2005), while cTBS activates a more complex, trans-synaptic network of interneurons acting on distal dendrites of layer-V pyramidal cells (Di Lazzaro et al., 2008).

The net-effect on the cortical excitability following each stimulation paradigm has been linked to differing patterns of calcium entry in the post-synaptic cell that resemble the patterns of LTP and LTD observed in classical studies of plasticity induction in the trisynaptic pathway of the hippocampus (Huang et al., 2011). In iTBS, interleaving two-second intervals of high-frequency stimulation with 8 s of rest is consistent with a model of stable *high rate* of intracellular calcium in the post-synaptic cell observed in traditional LTP experiments (Huang et al., 2011). By comparison, the uninterrupted high-frequency stimulation of cTBS is associated with an initial rise and eventual *decay in the rate* of calcium entry, leading to an increased overall level of calcium consistent with traditional LTD experiments (Huang et al., 2011). Given the similarities of iTBS and cTBS to LTP and LTD, respectively, the impact of these protocols on cortico-motor excitability is increasingly used to assess the efficacy of neuroplastic mechanisms in relation to aging, disease, and therapeutic interventions (Freitas et al., 2011; Fried et al., 2016, 2017; Gomes-Osman et al., 2017). In addition, given the fact that it leads to modulation of neural activity beyond the stimulation period, TBS has also been employed as a cognitive therapeutic tool.

TBS and rTMS to Assess Plasticity in Cognitive Aging and Impairment

Freitas et al. (2011) was the first study to use TBS to gain insights into the impact of normal aging on the mechanisms of plasticity. The authors applied cTBS to M1 in 36 individuals across a wide age range (18–81 y). The authors found negative correlations between age and both the magnitude and duration of cTBS aftereffects, suggesting that LTD-like plasticity is progressively reduced with increasing age. To confirm these findings and further investigate the impact of age, future studies should be performed with sufficient sample sizes to allow for comparisons between age ranges (i.e., per decade or quartile) and ultimately follow the same subjects as they transition from middle to old age.

There have been several additional studies that used TBS to investigate differences in the mechanisms of plasticity between normal and pathological aging, and their behavioral consequences. Fried et al. (2016) compared the response of iTBS to M1 in 24 cognitively intact older adults with type-2 diabetes mellitus (T2DM) to 16 demographically similar non-T2DM controls. Compared with controls, the T2DM group showed significantly less facilitation MEPs. Moreover, there was a positive correlation between iTBS-induced modulation of MEPs and verbal learning performance, suggesting a global decline in the efficacy of LTP-like plasticity in T2DM.

Trebbastoni et al. (2016) compared the impact of conventional 5 Hz rTMS to M1 on MEPs between 40 individuals with MCI and 20 older healthy adults. The participants received clinical, neurologic and neuropsychological assessments at baseline, and were followed for 4 years to monitor for conversion to AD. The authors reported a decrease in response to the rTMS protocol in individuals with MCI, which was correlated with time to conversion to AD. These results raise the possibility that rTMS could be a useful prognostic tool in the assessment of AD and related dementia.

The majority of cognitive aging-related studies using TBS and rTMS to probe the mechanisms of plasticity have focused on individuals with AD (Koch et al., 2012, 2014; Di Lorenzo

et al., 2016; Fried et al., 2017). In the first of a series of studies, Koch et al. (2012) compared the response of 14 individuals with moderate AD and 14 older healthy adults to three different TBS protocols targeting M1: iTBS, cTBS, and coTBS (a facilitatory variant of cTBS combining continuous delivery of 300 pulses with a 1-min contraction of the first and second digits). In comparison to controls, individuals with AD showed a decreased response to iTBS and coTBS, but not cTBS, suggesting impaired LTP and spared LTD in AD. Similar to the findings in T2DM, the reduced response to iTBS was correlated with decreased performance on the delayed recall of the Rey's figure copy visuospatial task.

A subsequent study by the same group (Koch et al., 2014) replicated the decreased M1 iTBS response in 20 individuals with moderate AD. Moreover, LTP-like plasticity was recovered after a 4-week treatment with the dopamine agonist rotigotine, and was accompanied by an improvement in global cognition and executive function. These data indicate implicate dopaminergic pathways in the reestablishment of LTP-like plasticity, thus providing a potential therapeutic target.

Di Lorenzo et al. (2016) also evaluated iTBS and cTBS to M1 in 54 individuals with AD and 24 demographically similar older adults, confirming the prior findings of abnormal iTBS response and normal cTBS response in AD (Koch et al., 2012, 2014). Further, the authors found that iTBS not only failed to facilitate MEPs in AD, rather it led to the suppression of MEP responses similar to cTBS. An 18-month follow-up assessment demonstrated that the extent of the impaired response to iTBS was associated with more severe cognitive decline (Di Lorenzo et al., 2016).

While the aforementioned studies above suggest a potential usefulness in using TBS and conventional rTMS to assess the mechanisms of plasticity in older adults with and without cognitive impairment, a number of studies in young and older adults have highlighted the challenge posed by considerable inter- and intra-individual variability in the response to these protocols (Maeda et al., 2000; Vernet et al., 2014; Vallence et al., 2015; Fried et al., 2017; Schilberg et al., 2017). For example, Hamada et al. (2013) observed that only 25% of their 56 participants demonstrated the "expected" response to iTBS and cTBS (i.e., facilitation and suppression of MEPs, respectively). This variability was not related to age, gender, testing time, or difference in MT or amplitude of baseline responses. Interestingly, by altering the coil orientation (and thus, manipulating the direction of the resultant induced current in the brain), they found that at least 50% of this variability could be explained by individual differences in the latency of MEPs resulting from direct vs. indirect activation of layer-V pyramidal cells (Maeda et al., 2000).

Furthermore, Cheeran et al. (2008) demonstrated variability in the response to iTBS and cTBS could be partly explained by a commonly observed polymorphism of the brain-derived neurotrophic (BDNF) gene. The presence of a met allele in the BDNF gene has also been associated with higher test-retest variability in response to iTBS in older adults, including those with T2DM or AD (Fried et al., 2017). In that same study, Fried et al. (2017) examined the reliability of iTBS to M1 in 36 adults, including 9 with probable mild-to-moderate AD, 15 with T2DM and 12 older healthy adults. iTBS was at best associated with low reproducibility (Cronbach's $\alpha \leq 0.50$) in the older healthy adults and moderate to high reproducibility in individuals with AD (Cronbach's α $0.53 \geq 0.81$). The authors hypothesized that greater reproducibility in the clinical population could be linked to a neurobiological "rigidity" in the mechanisms that support LTP-like plasticity induction. While the reproducibility of iTBS has been evaluated in older adults, such data is not available for cTBS and rTMS. Additional contributing factors implicated with the variability of iTBS outcomes in older adults were the between-session time, rMT and baseline MEP amplitudes (Fried et al., 2017). Responses were more variable if the sessions were conducted within 7 days, possibly as a result of subtle longer-lasting changes in the expression of GABAergic precursors (Trippe et al., 2009). Echoing a point first raised in section Single-Pulse TMS, setting the intensity of subsequent stimulation based on a set level percentage of rMT may introducing additional variance given age-related changes in the input-output curve of TMS (Pitcher et al., 2003) and the relationships between changes in RMT, baseline MEP amplitude, and the impact of iTBS (Fried et al., 2017).

In summary, the potential usefulness of TBS and conventional rTMS to measure the mechanisms of plasticity in older adults across the spectrum of cognitive aging must be considered in context of the current limitations of high inter- and intra-individual variability. However, at least for AD (which comprise the majority of the findings to date), consistent results across studies coupled with higher reproducibility suggest that these measures are more stable, and therefore, more useful in this population. Furthermore, the associations between modulation of MEPs and disease progression, symptom severity, and response to treatment with cognitive behavior point to their clinical relevance. The insights offered by studying variability and its causes in older clinical populations are critical to the widespread implementation of these protocols. Thus, future studies should aim to replicate those findings with larger samples and investigate the reproducibility of cTBS and rTMS in older adults, with and without cognitive impairment.

Utility of the Motor Cortex for TMS-Based Assessments of Neurophysiology and Neuroplasticity in Cognitive Aging and Impairment

The vast majority of TMS based studies in cognitive aging described in this paper, and indeed in the field overall, are neurophysiological assessments probing the function of the motor cortex. Much of this has until recently been out of necessity, since the output of the cortico-spinal pathway provides the only objective response to a suprathreshold TMS pulse (i.e., a MEP). While improved technology and methods of data cleaning and analysis have expanded the potential of concurrent use of TMS and electroencephalogram (TMS-EEG) for assessing physiology in non-motor brain regions (Pascual-Leone and Taylor, 2011), the motor cortex remains the most well-characterized region and MEPs represent the gold standard for measuring the neurophysiological response to TMS. There is no

doubt that restricting assessments to the motor cortex present a limitation to understanding the complex neurophysiology of the aging brain, especially as the motor cortex is rarely the target for disease-related pathologies, including those of AD.

However, there is an important benefit to conducting these assessments in a region, which is not directly affected by a particular disease. Take for example the case of AD, which is characterized by progressive spread of amyloid and tau depositions, cortical hypometabolism and atrophy. While the pathology of AD will eventually spread to involve the motor cortex, this is only at the latest stage of the disease (Braak and Braak, 1998), and the majority of studies to characterize or treat AD symptoms focus on patients at earlier stages of the disease, including preclinical populations where an intervention may be the most effective. Thus, while TMS based measurements in the motor cortex cannot inform on the function of brain regions directly affected by AD pathology, abnormal findings in motor cortex and be used to infer about the state of the brain overall rather than local pathology.

By comparison, if the measurements were performed in a region that was directly affected (such as association regions of frontal, parietal, or temporal lobes) it would be challenging to disentangle the contributions of local vs. global brain changes when making conclusions. Moreover, as TMS measurements are increasingly performed in non-motor brain regions, those findings, even in healthy cognitive aging, will still need to be explained and understood in the context of those same assessments performed in motor cortex (Gedankien et al., 2017).

Therapeutic Role of rTMS in Cognitive Aging and Impairment

The rationale for the use of rTMS therapeutically is based in part on studies in elderly individuals that have reported altered patterns of activation in the prefrontal cortex associated with deficits in episodic memory (Kim, 2011). Moreover, participants receiving daily high-frequency rTMS to the left DLPFC for depression performed better in working memory, executive function, objective memory, and fine motor speed after treatment (Martis et al., 2003). Given the impairments observed in the retrieval and encoding of episodic memory exhibited in individuals with cognitive impairment (Nyberg et al., 1996), researchers are increasingly directing rTMS to cortical regions that operate in the execution of these functions. Episodic memory relies on activation of several areas, such as the medial temporal lobe, medial prefrontal cortex, and posterior parietal cortex (Wang et al., 2006). Among these, only the posterior parietal cortex is easily accessible to TMS. In addition, neuroimaging studies have demonstrated altered patterns of functional activity in the right DLPFC and hippocampus are related to memory decline in individuals with MCI and AD (Maillet and Rajah, 2013).

Single-session studies have been useful in contributing to knowledge of the consequences of the altered neurophysiology in the production of cognitive output in the aging brain and highlighted potential targets for multi-session studies. In older healthy adults, the performance of a prospective memory task can be augmented with iTBS to the Frontopolar cortex (Vidal-Piñeiro et al., 2014; Debarnot et al., 2015). In individuals with MCI, stimulation of the prefrontal cortex with high-frequency rTMS can improve processing speed, visuomotor coordination, executive function, and associative memory (Rektorova et al., 2005; Solé-Padullés et al., 2006). Alternatively, low-frequency rTMS to the right prefrontal cortex can improve recognition memory in individuals with MCI, and verbal and non-verbal memory in older healthy adults (Turriziani et al., 2012). Finally, iTBS to the right dorsolateral prefrontal cortex (R-DLPFC) can improve action and object naming in individuals with AD (Cotelli et al., 2008). Future studies should evaluate the efficacy of these interventions in multi-session studies with larger and better-characterized cohorts.

However, multi-session studies are more appropriate for drawing insights regarding therapeutic effectiveness. The studies discussed in this section are grouped as pertaining to a therapeutic role because they propose multi-session interventions involving rTMS and TBS.

Across all studies, the most common stimulation target has been the DLPFC. In older adults and individuals with MCI, this was the only therapeutic target studied to date. Kim et al. (2012) randomized 16 individuals to receive real 10 rTMS (13 2-s trains, inter-train interval of 15 s, 780 pulses/session) or sham to the left DLPFC for 5 days, and found improved inhibitory control, only in the real condition. Drumond Marra et al. (2015) randomized a group of 31 individuals with MCI to either undergo 10 sessions of real 10 Hz rTMS over the left DLPFC (5 s, with an inter-train interval of 25 s, 2,000 pulses/session) or sham. The authors found significant improvements in episodic memory post-stimulation and executive function only in the real group, retained at a follow-up assessment performed 1 month later.

In individuals with AD, two studies proposed the use of rTMS targeting the DLPFC in isolation. In the study by Cotelli et al. (2011). Ten individuals with moderate AD underwent 20 sessions of 20 Hz rTMS (50 trains, 2-s trains, inter-train interval of 28 s, 2,000 pulses/session) over 4 weeks. The authors found significant improvements in sentence comprehension at weeks 2 and 4, and at a follow-up performed 12 weeks post-intervention. Ahmed et al. (2012) randomized 45 individuals with mild-to-severe AD to one of 3 paradigms of DLPFC stimulation, delivered over 5 days: 20 Hz (20 5-s trains, inter-train interval of 25 s, 2,000 pulses/session); 1 Hz (2,000 pulses/session); and sham. The authors found that only individuals with mild to moderate AD showed any significant improvements. Participants in the 20 Hz group improved in global cognition, those in both the 20 and 1 Hz groups improved in instrumental daily activities, but the 20 Hz groups retained their improvements during a follow-up at 1 and 3 months post-stimulation.

A number of studies have used more than more target in individuals with AD. Zhao et al. (2017) recruited 30 participants and randomized them to 10 Hz rTMS (20 10-s trains, inter-train interval of 20 s, 2,000 pulses/session) targeting the following areas: bilateral DLPFC, bilateral parietal cortex, Broca's and Wernicke's area (superior temporal gyrus), or sham for 30 sessions over 6 weeks. The authors reported no between-group differences in any of the outcomes reported.

Five studies examined the use of rTMS targeting multiples areas combined with cognitive training in individuals with mild-to-moderate AD (Bentwich et al., 2011; Rabey et al., 2013; Rabey and Dobronevsky, 2016; Gandelman-Marton et al., 2017; Nguyen et al., 2017). All studies utilized cognitive training paradigms designed to engage the stimulated areas, with grammar, comprehension, action and object naming, and spatial memory and attention tasks.

Most of the studies (Bentwich et al., 2011; Rabey et al., 2013; Rabey and Dobronevsky, 2016; Gandelman-Marton et al., 2017; Nguyen et al., 2017) employed similar stimulation paradigms, consisting of 10 Hz rTMS (20–25 2-s trains, 1,200 pulses/session), targeting the following areas: bilateral DLPFC, bilateral parietal somatosensory association cortex, Broca's and Wernicke's area (superior temporal gyrus). Bentwich et al. (2011) recruited 8 participants, Rabey et al. (2013) recruited 15 participants, and Gandelman-Marton et al. (2017) recruited 8 participants, who underwent 54 sessions over 18 weeks. Rabey and Dobronevsky (2016) recruited 30 participants, who underwent 30 sessions over 6 weeks. All four studies demonstrated significant improvements in global cognition and activities of daily living both at 1.5 and 4.5 months following participation in the study, but Rabey et al. (2013) additionally demonstrated an increase in the participants' subjective perception of clinical change.

Nguyen et al. (2017) targeted the prefrontal and parietal cortices bilaterally, and Broca's and Wernicke's areas using 10 Hz rTMS (20 2-s trains, 400 pulses/session). Ten participants were recruited and underwent 25 sessions over 5 weeks. The authors found improvement in the overall score, and apathy and dependence sections of the Alzheimer's Disease assessment scale cognitive sub score (ADAS-Cog). Furthermore, a separate analysis limited to the five best responders revealed that their improvements remained at 6 months follow-up, while the improvements attained by the rest of the study sample did not.

With the possible exception of Rabey and Dobronevsky (2016), the results of the studies above are severely limited due to the small sample sizes and large inter-individual variability, resulting in low statistical power. In this scenario, it has been stipulated that effect sizes may be equally or even more useful than the simple consideration of statistical differences at estimating the clinical meaningfulness of trials (Ottenbacher, 1995; Musselman, 2007). For this reason, we calculated the effect sizes associated with the comparisons between interventions and control/comparison group from the studies above and considered that clinically meaningful changes to be *significant differences that were associated with an effect of at least a moderate size*. Effect sizes were computed using the Cohen's d (Cohen, 1988), calculated for within-group measures by computing the average standard deviation of both repeated measures (Cumming, 2012), and for between-group comparisons by dividing the difference between the means by the pooled standard deviation (Cohen, 1988) (**Table 1**). Effect sizes were interpreted based on published values: trivial effect ($d < 0.2$), small effect ($d = 0.2$–0.5), moderate effect ($d = 0.5$–0.8), and large effect ($d > 0.8$) (Cohen, 1988).

Many significant within-group improvements were associated with at least moderate effect sizes. Regarding global cognition measured by the Mini Mental Status Exam (MMSE), multi-target 10 Hz rTMS combined with cognitive training (Bentwich et al., 2011; Rabey and Dobronevsky, 2016; Gandelman-Marton et al., 2017) was associated with moderate-to-high effect ($d = 1.01$–2.00, $d = 0.62$, and $d = 0.64$–1.24, respectively). In addition, 10 Hz rTMS in isolation (Zhao et al., 2017) was associated with a moderate effect ($d = 0.65$). Furthermore, multi-target 10 Hz rTMS combined with cognitive training (Bentwich et al., 2011; Gandelman-Marton et al., 2017; Nguyen et al., 2017) was also associated moderate-to-high effect for global cognition measured by ADAS-cog was also associated with a moderate to large effect ($d = 0.68$, $d = 0.68$, $d = 1.03$). A moderate-to-high effect was also associated with 10 Hz rTMS in isolation (Zhao et al., 2017; $d = 0.72$–1.07).

Additional clinically relevant effects were associated with 10 Hz rTMS in isolation were improved memory with 10 Hz rTMS (Drumond Marra et al., 2015; $d = 0.56$-1.16), verbal learning (Zhao et al., 2017; $d = 0.73$–1.01), and inhibitory control (Kim et al., 2012; $d = 0.51$). With 20 Hz rTMS in isolation, large effects were observed with improvements in sentence comprehension (Cotelli et al., 2011) ($d = 1.54$–1.57). All of the remaining within-group significant differences were associated with a lower than moderate effect.

Six studies (Bentwich et al., 2011; Cotelli et al., 2011; Kim et al., 2012; Drumond Marra et al., 2015; Rabey and Dobronevsky, 2016; Nguyen et al., 2017; Zhao et al., 2017) had a control/comparison group and presented their data with sufficient detail to allow for between-group Cohen's *d*. The improvements in the language domain associated with 20 Hz rTMS to the DLPFC was associated with a large effect ($d = 1.54$) (Cotelli et al., 2011). In addition, the improvement associated with global cognition associated with the multi-target 10 Hz rTMS combined with cognitive training was also large ($d = 1.53$) (Nguyen et al., 2017). All of the remaining significant between-group differences were associated with lower than moderate effect.

In summary, the above evidence suggests that after an intervention consisting of multi-target high-frequency rTMS combined with cognitive training as described above, individuals may present with clinically meaningful improvements in global cognition. This finding was consistent and replicated by four different studies. In addition, individuals participating in a high-frequency rTMS to the DLPFC can also achieve improvements in global cognition, but this finding was less consistent. Additionally, these individuals may exhibit clinically meaningful improvements in in memory, verbal learning, inhibitory control and sentence comprehension.

One important caveat is that the clinical meaningfulness of high-frequency rTMS, with or without cognitive training was limited when compared to a control condition. In this scenario, it may be possible to improve in global cognition when participating in multi-target rTMS with cognitive training and sentence comprehension when undergoing high-frequency rTMS in isolation. One limitation from the studies above is that none employed a comparison group; therefore, it is unclear if these results would persist in the comparison to an actual intervention. Future studies should employ active comparisons

TABLE 1 | Study characteristics from articles proposing rTMS as an intervention for cognitive improvement in individuals with cognitive aging and cognitive impairment.

Study	*n* Group status: age (mean ± *SD*)	Control/ Comparison	- Target area - Localization method	TMS parameters - Mode - Frequency - MT%	- Length of intervention - Sessions	TMS parameters - Trains - Duration - Interval - Total pulses	Cognitive domain	Neuropsychological test	Significant findings (treatment group)	Effect size (Cohen's *d*) between groups	Effect size (Cohen's *d*) within group
Kim et al., 2012	16 OHA Active (8): 63.50 (±4.57) Sham (8): 62.75 (±5.50)	Sham site	L-DLPFC	- OFF /HF /rTMS - 10 Hz - 30%	- 1 week - 5 sessions	- 39 - 2s - NR - 780	1—Inhibitory control (RT) 2—ATT control	1 and 2–Modified Stroop task	↑ performance RT (incongruent trials) ↑ ATT control	DATA NOT SUFICIENT (not significant)	- RT congruent: 0.16 - RT incongruent: 0.51**
Drumond Marra et al., 2015	34 [aMCI (31) and naMCI (3)] Active (15): 65.1 (± 3.5) Sham (19): 65.2 (± 4.1)	Sham coil	- L-DLPFC - 5 cm anterior method	- HF/rTMS - 10 Hz - 110%	- 2 weeks - 10 sessions	- NR - 5 s - 25 s - 2,000	1—everyday MEM 2—EF	1 - RBMT, Logical memory II (delayed), WAIS III; 2 – TMTB and VF (animal naming).	↑ everyday MEM at 2 and 4 weeks ↑ EF (at 4 weeks)	- RBMT T1: 0.27 - RBMT T2: 0.57 - Logical memory II T1: −0.15 - Logical memory II—T2: −0.21 - Letter-number T1: 0.03 - Letter-number T2: 0.59 - TMTB T1: −0.34 - TMTB T2: 0.29 - Verbal fluency/animal T1: 0.08 - Verbal fluency/animal T2: −0.03	**WITHIN GROUPS (T0 × T1)** - RBMT: 0.92** - Logical memory II: −0.03** - Letter-number: −0.20 - TMTB: −0.21** - Verbal fluency/animal: −0.09* **WITHIN GROUPS (T0 × T2)** - RBMT: 1.16** - Logical memory II: 0.56** - Letter-number: 0.3 - TMTB: 0.12 - Verbal fluency/animal: 0.06** **WITHIN GROUPS (T1 X T2)** - RBMT: 0.18 - Logical memory II: 0.57 - Letter-number: 0.52** - TMTB: 0.29** - Verbal fluency/ animal: 0.14
Cotelli et al., 2011	10 [moderate AD] Real-real (5): 71.2 (±6.1) Placebo-real (5): 74.4 (±3.8)	Placebo-real group: 2 weeks placebo + 2 week of real	- L-DLPFC - Template MRI guiding	- OFF/HF/rTMS - 20 Hz - 100%	- 4 weeks - 20 sessions	- 40 - 2 s - 28 s - 2,000	1—GC 2—MEM 3—EF 4—ADL and IADL; and 5—Language.	1—MMSE and Cognitive estimation test; 2 and 3 —picture-naming task 4—ADL and IADL task; 5—SC-BADA.	↑ Language (Persistent beneficial effects (along 2, 4, and 12 weeks) of rTMS in the real-real group on sentence comprehension in AD patients analyzed via SC-BADA)	**Variable: (2 weeks)/(4 weeks)/(12 weeks)** - MMSE: 0/−0.26/0.58 - ADL: −0.67/−0.67/−0.67 - IADL: −0.34/−0.34/−0.34 - Picture-naming task (objects): 0.55/0.71/1.05 - Picture-naming task (actions): 0.94/0.45/0.22 - Battery (Sentence comprehension): 1.54**/−0.25/0.35 - AAT (token test): 0.63/0.89/0.16 - AAT (Repetition): 0.13/0.8/0.54 - AAT (Writing): 0.03/0.02/0.03 - AAT (Naming): 0.11/0.28/0.73 - AAT (Comprehension): 0.2/0.12/0.82 - Serial curve position (primacy): −45/−0.66/−1.00 - Serial curve position (recency): 0.91*/0.86*/ 0.3* - Serial curve position (first item): −0.66/−0.87/−0.62 - Cognitive estimation test (errors): 0.18/0.53/0.65 - Cognitive estimation test (bizarreness): 0.29/0.69/0.93	**Variable: (BASELINE × 2 weeks)/(BASELINE × 4 weeks)/(BASELINE × 12 weeks)** - MMSE: −0.07/−0.26/0.07 - ADL: 0/0/0 - IADL: 0/0/0 - Picture-naming task (objects): −0.13/0.08/0.27 - Picture-naming task (actions): 0.82/0.56/0.62 - Battery (Sentence comprehension): 1.57**/0.92/1.54** - AAT (token test): 0.22/0.68/0 - AAT (Repetition): −0.23/0.24/−0.04 - AAT (Writing): 0.41/0.61/0.53 - AAT (Naming): 0.06/0.55/1.31AAT (Comprehension): 1.08*/0.89/1.94 - Serial curve position (primacy): 0.31*/0/−0.1 - Serial curve position (recency): 0.44/0.8*/0.73* - Serial curve position (first item): 0/−0.1/−0.9* - Cognitive estimation test (errors): 0.54/0.65/0.48 - Cognitive estimation test (bizarreness): −0.27/0/0.4

(Continued)

TABLE 1 | Continued

Study	*n* Group status: age (mean ± *SD*)	Control/ Comparison	Target area Localization method	TMS parameters - Mode - Frequency - MT%	Length of intervention Sessions	TMS parameters - Trains - Duration - Interval - Total pulses	Cognitive domain	Neuropsychological test	Significant findings (treatment group)	Effect size (Cohen's *d*) between groups	Effect size (Cohen's *d*) within group
Cotelli et al., 2011	10 [moderate AD] Real-real (5): 71.2 (±6.1) Placebo-real (5): 74.4 (±3.8)	Placebo-real group: 2 weeks placebo + 2 week of real	- L-DLPFC - Template MRI guiding	- OFF/HF/rTMS - 20 Hz - 100%	- 4 weeks - 20 sessions	- 40 - 2 s - 28 s - 2,000	1—GC 2—MEM 3—EF 4—ADL and IADL; and 5—Language.	1—MMSE and Cognitive estimation test; 2 and 3—picture-naming task 4—ADL and IADL task; 5—SC-BADA.	↑ Language (Persistent beneficial effects (along 2, 4, and 12 weeks) of rTMS in the real-real group on sentence comprehension in AD patients analyzed via SC-BADA)	**Variable: (2 weeks)/(4 weeks)/(12 weeks)** - MMSE: 0/−0.26/0.58 - ADL: −0.67/−0.67/−0.67 - IADL: −0.34/−0.34/−0.34 - Picture-naming task (objects): 0.55/0.71/1.05 - Picture-naming task (actions): 0.94/0.45/0.22 - Battery (Sentence comprehension): 1.54**/−0.25/0.35 - AAT (token test): 0.63/0.89/0.16 - AAT (Repetition): 0.13/0.8/0.54 - AAT (Writing): 0.03/0.02/0.03 - AAT (Naming): 0.11/0.28/0.73 - AAT (Comprehension): 0.2/0.12/0.82 - Serial curve position (primacy): −45/−0.66/−1.00 - Serial curve position (recency): 0.91*/0.86*/ 0.3* - Serial curve position (first item): −0.66/−0.87/−0.62 - Cognitive estimation test (errors): 0.18/0.53/0.65 - Cognitive estimation test (bizarreness): 0.29/0.69/0.93	**Variable: (BASELINE × 2 weeks)/(BASELINE × 4 weeks)/(BASELINE × 12 weeks)** - MMSE: −0.07/−0.26/0.07 - ADL: 0/0/0 - IADL: 0/0/0 - Picture-naming task (objects): −0.13/0.08/0.27 - Picture-naming task (actions): 0.82/0.56/0.62 - Battery (Sentence comprehension): 1.57**/0.92/1.54** - AAT (token test): 0.22/0.68/0 - AAT (Repetition): −0.23/0.24/−0.04 - AAT (Writing): 0.41/0.61/0.53 - AAT (Naming): 0.06/0.55/1.31AAT (Comprehension): 1.08*/0.89/1.94 - Serial curve position (primacy): 0.31*/0/−0.1 - Serial curve position (recency): 0.44/0.8*/0.73* - Serial curve position (first item): 0/−0.1/−0.9* - Cognitive estimation test (errors): 0.54/0.65/0.48 - Cognitive estimation test (bizarreness): −0.27/0/0.4

(Continued)

TABLE 1 | Continued

Study	*n* Group status: age (mean ± *SD*)	Control/ Comparison	- Target area - Localization method	TMS parameters - Mode - Frequency - MT%	- Length of intervention - Sessions	TMS parameters - Trains - Duration - Interval - Total pulses	Cognitive domain	Neuropsychological test	Significant findings (treatment group)	Effect size (Cohen's *d*) between groups	Effect size (Cohen's *d*) within group
Ahmed et al., 2012	Total: 45 [mild to moderate and severe AD] Age: 68.4	Sham DLPFC	- R-DLPFC and L-DLPFC - 5 cm anterior method	- HF and LF/rTMS - 1–20 Hz - 90–100%	- 5 consecutive days - 5 sessions	- 20 - 5 s - 25 s - 2,000	1—GC; and 2—IADL	1—MMSE; and 2—IADL.	↑ GC and IADL in mild to moderate AD, not in severe (HF showed better improvement than LF) along 1 and 3 months.	**Legend: Variable: (after last session/1 month/3 months) MILD to MODERATE (20HZ × 1 Hz)** - MMSE: 1.56/1.61/1.84 **SEVERE (20HZ × 1 Hz)** MMSE: 0.42/1.48/1.15 **MILD to MODERATE (20HZ × SHAM)** - MMSE: 2.00/3.00/3.22 **SEVERE (20HZ ×SHAM)** - MMSE: 1.3/1.67/2.03 **MILD to MODERATE (1HZ × SHAM)** - MMSE: 2.00/3.00/3.22 **SEVERE (1HZ × SHAM)** - MMSE: 1.3/1.67/2.03	**Legend: Variable: (BASELINE × AFTER)/(BASELINE × 1 MONTH)/(BASELINE × 3 MONTHS) 20Hz** - MILD to MODERATE MMSE: 1.01**/1.66**/2.00** - SEVERE MMSE: 0.33/0/0.44 **1Hz:** - MILD to MODERATE MMSE: 0.05/0.30/0.34 - SEVERE MMSE: 0/−1.00/-0.68 **SHAM:** - MILD to MODERATE MMSE: 0.07/−0.17/−0.33 - SEVERE MMSE: 0.15/0/0
Bentwich et al., 2011	8 [mild to moderate AD] Age: 75.4 (±4.4)	No control	- R-DLPFC and L-DLPFC; BR-WE; R-pSAC and L-pSAC - Subject MRI guiding	- HF/rTMS/ COG - 10 Hz - 90–110%	- 18 weeks - 54 sessions	- 2 - 5 s - 30 s - 2,000	1—GC; and 2—IADL	1—ADAS-cog, CGIC, and MMSE; 2—ADAS-ADL	↑ ADAS-cog at 6 and 18 weeks (great improvement) ↑ ADAS-ADL and MMSE at 18 weeks ↑ CGIC (no statistical power)	No control group	**BASELINE × 6 WEEKS** - ADAS-COG: 0.68** - CGIC: −2 - MMSE: 0.62** - ADAS-ADL: 0.33** **BASELINE × 4.5 months** - ADAS-COG: 0.85** - CGIC: −4.57 - MMSE: −0.42 - ADAS-ADL: 0.09
Gandelman-Marton et al., 2017	8 (mild AD) Age: 75.5 (4.3)	No control	- Frontal, temporal, and parieto-occipital regions - Template MRI guiding	- ON/HF/rTMS/ COG - 10 Hz - 70–90%	- 18 weeks - 54 sessions (5x/week and 2x/week)	- 20 - 2 s - 40 s - 1,200	1—GC	1—ADAS-cog, CGIC, and MMSE;	↑ MMSE 6 weeks ↑ ADAS-cog (6 and 4.5 months	No control group	**BASELINE × 6 WEEKS** - ADAS-COG: 0.68** - MMSE: 0.65** **BASELINE × 4.5 months** - ADAS-COG: 0.84** - MMSE: −0.37

(Continued)

TABLE 1 | Continued

Study	*n* Group status: age (mean ± *SD*)	Control/ Comparison	- Target area Localization method	TMS parameters - Mode - Frequency - MT%	- Length of intervention - Sessions	TMS parameters - Trains - Duration - Interval - Total pulses	Cognitive domain	Neuropsychological test	Significant findings (treatment group)	Effect size (Cohen's *d*) between groups	Effect size (Cohen's *d*) within group
Nguyen et al., 2017	10 (AD) Age: 73 (7.2)	No control	- 6 brain areas (R-DLPFC, L-DLPFC, R-PC, L-PC, BR-WE) - MRI guided	- HF/rTMS/ COG - 10 Hz - 100%	- 5 weeks - 25 sessions (5 days a week with a 6 months follow up)	- 20 - 2 s - 40 s - NR	- 1—GC - 2—MEM - 3—ATT and PS - 4—Language - 5—Visuospatial	- 1—MMSE, ADAS-cog; FAB - 2—Dubois score; - 3—Stroop color; - 4—Word recognition and word recall scores 5	↑ ADAS-cog at 5 weeks (scores returned after follow up, but not in best responders) ↑ Improvement of the performance on all task (test-retest effect and an effective learning) - After correction only task from parietal and languages were significant	- ADAS-cog (Best scores × Worst scores): 1.53**	**Variable: (Baseline × 6 weeks)/(Baseline × 6 months)** - ADAS-cog: 1.03**/0** - ADAS-cog recognition: 0.45/0.89 - ADAS-cog word recall: 0.2/0 - MMSE score: 0.28/−0.31 - MMSE language score: 0.57/0.5 - Dubois score: −0.8/−0.74 - FAB score: 0.15/−0.07 - Stroop color: −0.45/0.17 **Variable (Cognitive task): (Baseline × 6 weeks)** - R-DLPFC (action naming): 7.3 - R-DLPFC (subject naming): 6.4 - R- DLPFC (word recall): 7.3 - L-DLPFC (action naming): 8.06 - L-DLPFC (subject naming): 7.52 - L- DLPFC (word recall): 6.10 - R-PC (red rectangle recognition): 8.9** - R-PC (blue rectangle recognition): 8.99** - R-PC (letter recognition): 9.14** - Broca (sentence similarity): 7.19 - Broca (right/wrong words): 9.52 - Wernicke (words/pseudowords): 11.37** - Wernicke (categories): 15.21**

(Continued)

TABLE 1 | Continued

Study	*n* Group status: age (mean ± *SD*)	Control/ Comparison	- Target area - Localization method	TMS parameters - Mode - Frequency - MT%	- Length of intervention - Sessions	TMS parameters - Trains - Duration - Interval - Total pulses	Cognitive domain	Neuropsychological test	Significant findings (treatment group)	Effect size (Cohen's *d*) between groups	Effect size (Cohen's *d*) within group
Zhao et al., 2017	30 [AD mild group and moderate group)] Age: 68.4	Non rTMS sham group	Parietal P3/P4 and posterior temporal T5/T6	- HF/rTMS - 20 Hz - NR	- 6 weeks - 30 sessions (1 session /day and 5 days/week)	- 20 - 10 s - 20 s - 2,000	- 1—GC - 2—MEM	- 1—ADAS-COG, MMSE, MoCA - 2—WHO-UCLA AVLT	↑ ADAS-cog, MMSE and WHO-UCLA AVLT score after treatment and 6 weeks along	**ALL (6 weeks/12 weeks)** - ADAS-cog: 0.62/0.57 - MMSE: 0.28/0.29 - MoCA: 0.07/0.07 - WHO-UCLA-AVLT: 0/0.11 **MILD (6 weeks/12 weeks)** - ADAS-cog: 0.79/0.76 - MMSE: 0.23/0.39 - MoCA: 0.03/0.27 - WHO-UCLA-AVLT: 0.19/0.53 **MODERATE (6 weeks/12 weeks)** - ADAS-cog: 0.51/0.60 - MMSE: 0/0.06 - MoCA: −0.08/-0.05 - WHO-UCLA-AVLT: −0.09/−0.16	**ALL (Baseline x Post 6 weeks)/(Baseline x 12 weeks)** - ADAS-cog: 0.72**/0.90** - MMSE: 0.64*/0.89** - MoCA: 0.36/0.96 - WHO-UCLA-AVLT: 0.42/0.73** **MILD (Baseline x Post 6 weeks)/(Baseline x 12 weeks)** - ADAS-cog: 0.87**/1.07** - MMSE: 0.48/1.24** - MoCA: 0.53/0.86** - WHO-UCLA-AVLT: 0.38/1.01** **MODERATE (Baseline x Post 6 weeks)/(Baseline x 12 weeks)** - ADAS-cog: 0.53/0.74 - MMSE: 0.41/0.73 - MoCA: 0.22/0.44 - WHO-UCLA-AVLT: 0.61/0.73 **COGNITIVE DOMAIN 6 x 12 weeks: (All/Mild/Moderate)** - MEMORY: 0.19**/0.12**/0.31 - LANGUAGE: 0.14**/0.11**/0.13 - EXECUTIVE FUNCTION: −0.2/0**/−0.7
Rabey and Dobronevsky, 2016	Total: 30 [mild to moderate AD] Treat (7): 72.6 (± 8.9) Sham (8): 75.4 (± 9.07)	No control	L-IFG (Broca); L-STG (Wernicke); L-DLPFC; R-DLPFC; R-pSAC and L-pSAC	- ON/HF/rTMS/ COG - 10 Hz - 90–110%	- 6 weeks - 30 sessions (1-h daily sessions, 5 days per week)	- 20 > 5 - 2 s > 2 s - NR - 1,200 > 100 = 1,300	1—GC	1—ADAS-cog and MMSE	↑ ADAS-cog and MMSE along 6 weeks	No control group	- ADAS-cog: 0.32** - MMSE: 0.62**
Rabey et al., 2013	Total: 15 [mild to moderate AD] Treat (7): 72.6 (± 8.9) Sham (8): 75.4 (± 9.07)	Sham coil	R-DLPFC and L-DLPFC; BR-WE; R-pSAC and L-pSAC - Subject MRI guiding	- HF/rTMS/ COG - 10 Hz - 90–110%	–	- 20 - 2 s - NR - 1,200	1—GC	1—ADAS-cog and CGIC.	↑ ADAS-cog and CGIC along 6 and 18 weeks	Not enough information	Not enough information

*n, number of subjects; SD, standard deviation; TMS, Transcranial Magnetic Stimulation; rTMS, repetitive TMS; rtMS-COG, rTMS plus cognitive training; MT%, Motor Threshold percentage; OHA, Older Healthy Adults; AD, Alzheimer's Disease; MCI, Mild Cognitive Impairment; aMCI, amnestic MCI; naMCI – nonamnestic MC; T0, baseline; T1, first assessment after treatment; T2, second assessment after treatment; L-DLPFC, left dorsolateral prefrontal cortex; R-DLPFC, right dorsolateral prefrontal cortex; L-IPL, left inferior parietal lobe; PFC, Prefrontal cortex; R-IFG, right inferior frontal gyrus; R-STG, right superior temporal gyrus; BR-WE, Broca and Wernicke area; L-pSAC, left parietal somatosensory association cortex; R-pSAC, right parietal somatosensory association cortex; L-PC, left parietal cortex; R-PC, right parietal cortex; HF, High Frequency; LF, Low frequency; ON, online stimulation; OFF, offline stimulation; RT, reaction time; GC, global cognition EF, executive function; MEM, memory; ATT, attention; IADL, instrumental activities of daily living; VSP, visuospatial; ADL, activities of daily living; NAM, naming; PS – processing speed; MMSE, Mini-Mental State Examination; IADL, Instrumental Activities of Daily Living;; AAT, Aachener Aphasic Test; ADAS-cog, Alzheimer Disease Assessment Scale-Cognitive; ADAS-ADL, Alzheimer Disease Assessment Scale-ADL; CGIC, Clinical Global Impression of Change scale; NPI, Neuropsychiatric Inventory; TMT, Trail Making Test; ↑, improvement of; NA, Not applied; SC-BADA, Battery for Analysis of Aphasic Deficits; WHO-UCLA-AVLT, World Health Organization-University of California-Los Angeles Auditory Verbal Learning Test; MoCA, Montreal Cognitive Assessment; MIS, Memory Impairment Screen; FCRT, Free and Cued Recall Test; AVLT, Auditory Verbal Learning Test; RBMT, Rivermead Behavioral Memory Test; RT, reaction time; VFT, Verbal fluency test; STR, stroop test; RCFT-R, Rey Complex Figure Test and Recognition Trial; MRI, Magnetic Resonance Imaging; NR, not reported; **, significant finding ($p < 0.05$); *, trend to significance ($0.05 < p < 0.1$).*

in their study design to gain a better understanding of the efficacy of these techniques.

CHALLENGES POSED AND OPPORTUNITIES OFFERED BY THE VARIABILITY OF TMS

As discussed in many of the individual sections above, inter- and intra-individual variability remains a challenge to the widespread use of TMS as a neurophysiological probe and a neuromodulatory intervention. While this problem is not unique to the study of cognitive aging, there are specific considerations that must be addressed. For starters, much of our knowledge of how TMS interacts with the brain comes from the study of younger, relatively healthy individuals. Thus, it may be necessary to develop age-specific normative values if these TMS assessments are to be used to distinguishing normal from pathological cognitive aging. Furthermore, TMS measures that are already highly variable across subjects within a given group are likely to have poor diagnostic utility in older populations, especially given the highly individualized trajectories of cognitive aging and the large heterogeneity of age-related pathologies.

Similarly, the problem of intra-individual variability, or reproducibility, of certain TMS measures is a direct limitation to their use as prognostic tools to track individual decline or response to therapeutic interventions. Fortunately, these problems are increasingly recognized within the TMS scientific community leading to several recent reviews (Gonsalvez et al., 2017), meta-analyses (Wischnewski and Schutter, 2015; Chung et al., 2016), and direct investigations into the magnitude and sources of inter- and intra-individual variability (Cheeran et al., 2008; Chang et al., 2016; Schilberg et al., 2017), including recent analyses of older, clinical populations (Christie et al., 2007; Fried et al., 2017).

While variability is often thought of purely in terms of the limitations it confers, there may be opportunities to exploit inter-individual variability to gain a deeper understanding into the neurobiology of cognitive aging. One such example is in the study of T2DM, a major contributor to pathological cognitive aging in its own right as well as a risk factor for dementia (Arvanitakis et al., 2004). As mentioned in section TBS and rTMS to Assess Plasticity in Cognitive Aging and Impairment, Fried et al. (2016) compared older diabetics without cognitive complaints to non-T2DM controls. Not only did the T2DM show a decreased response to iTBS, there was a strong association in the T2DM group between the modulation of MEP and verbal learning performance. Thus, the variation in response to iTBS within the T2DM group was actually informative as to more general disruption in LTP-like plasticity that also impacted learning and memory. Similarly, while several studies have used motor thresholds and other TMS measures as evidence of hyperexcitability in AD (Ferreri et al., 2003; Di Lazzaro et al., 2004; Koliatsos et al., 2006), the variation in rMT may be itself predictive of cognitive dysfunction. Finally, in order to fully leverage the sources of variability to better inform on the neurobiology of cognitive aging, it may be necessary to look beyond straightforward analysis of variance or simple linear regression techniques when analyzing data.

TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS)

While TMS is a method that functions through direct generation of action potentials in cortical tissue using electromagnetic induction, tDCS is an alternate NIBS method that achieves neuromodulation of both cortical and subcortical tissue through sub-threshold alteration of resting membrane potentials using direct electrical stimulation. tDCS is safe to use and has shown potential for enhancing cognitive processes and intervening on disease states (e.g., depression, chronic pain, recovery after stroke) (Nitsche and Paulus, 2000; Fregni et al., 2006; Reis et al., 2009; Medeiros et al., 2012; Ahn et al., 2017). In tDCS, a weak electrical current (typically 1–2 mA) is delivered to underlying brain tissue through electrodes placed over the scalp (Nitsche and Paulus, 2000; Nitsche et al., 2008). Saline-soaked sponges, or carbon loaded rubber pads with conductive gel are used as electrodes for direct current to flow between the anode (positive polarity) and cathode (negative polarity) electrode. Current flows between the electrodes may alter the sub-threshold resting membrane potential of neurons, resulting in either neural excitation or inhibition of cortical and subcortical tissues (Bolzoni et al., 2013; Stagg et al., 2013). Unlike TMS, tDCS has the potential to impact underlying subcortical tissue either directly or indirectly. Computational modeling (Sadleir et al., 2010; Shahid et al., 2014; Indahlastari et al., 2016) and intracranial recording data (Opitz et al., 2016; Huang et al., 2017) have demonstrated that electrical current passing between tDCS electrodes was found in both intervening cortical and subcortical tissue and thus may directly stimulate these brain regions (i.e., direct effect). In addition, effects on subcortical tissue can also be achieved indirectly through complex connectivity of brain network within the stimulated and unstimulated cortical and subcortical brain regions (Weber et al., 2014).

The parameters used in tDCS are assumed to dictate whether the neural response to stimulation under and between the anode or cathode electrode is excitatory or inhibitory. These parameters are consisted of stimulation duration and frequency, current intensity, as well as electrode size and location (Prehn and Flöel, 2015). Studies have shown that stimulation intensity of 1 mA results in depolarization of neurons under the anode and hyperpolarization under the cathode electrode (Nitsche and Paulus, 2000). However, stimulation at 2 mA has previously been shown to elicit net excitatory response under both the anode and cathode electrodes (Batsikadze et al., 2013). In addition, the effects of tDCS can outlast the period of stimulation from minutes to hours post-stimulation depending on the duration of stimulation (Bindman et al., 1964; Monte-Silva et al., 2013). The size of stimulation can be achieved by using different types of electrodes. Conventional tDCS offers a broader stimulation area via two large (e.g., 35 cm^2) rectangular electrodes (one anode, one cathode). High-definition tDCS (HD-tDCS) can

provide more focal stimulation regions by using smaller disc electrodes (1 cm diameter) in a 4 × 1 ring configuration (e.g., anode in the center surrounded by four cathode electrodes) (Kuo et al., 2013). Therefore, the choice of conventional vs. HD-tDCS is based on the location of desired target brain regions. Electrode montages used in tDCS were selected such that the intended stimulation regions would receive a sufficient current dose. Studies of cognition in tDCS typically follow a functional targeting approach that specifically aims to stimulate underlying brain regions implicated in the cognitive abilities of interest for a given study. For example, the most common intended stimulation area for cognitive aging is the frontal lobe in an effort to impact executive functions such as working memory and error awareness (Nissim et al., 2017). In addition, decline in frontal structures and function are a hallmark of the cognitive aging process and represent one of the brain areas most impacted by advanced age (Lemaitre et al., 2012)—supporting the frontal lobes as a target for tDCS intervention. In AD population, the temporoparietal and temporal regions were chosen as the stimulation target areas since impairments in these regions have been correlated with impaired verbal and visual recognition observed in AD patients (Ferrucci et al., 2008).

Studies in both humans and animals have suggested that the acute effects (during stimulation) vs. the after-effects (post stimulation) are relying on different mechanisms of action (Liebetanz et al., 2002; McLaren et al., 2017). While the exact mechanisms of action are not well-understood, studies have shown that sodium and potassium channels are necessary for the acute effects of stimulation, whereas the after-effects of stimulation are NMDA receptor, GABA/Glutamate, and calcium channel dependent (Stagg et al., 2009a). The mechanisms of action that produce the after-effects of stimulation are particularly important when we consider cognitive aging and age-related cognitive decline. As previously discussed, certain aspects of cognition start to decline with aging (e.g., working memory, attention). The ability to modulate the excitatory response of brain tissues for longer periods of time can potentially enhance LTP, which is the basis of learning and memory. LTP indicates a persistent synaptic strengthening, providing the capacity of modifying neural connectivity that can support signal transmission between neurons (Bliss and Lomo, 1973; Barnes, 2003; Monte-Silva et al., 2013). By altering LTP and neuroplasticity in older adults, tDCS shows promise to remediate cognitive aging and age-related diseases (Nitsche and Paulus, 2000; Nitsche et al., 2008).

tDCS to Remediate Cognitive Aging in Healthy Older Adults

Prior research in healthy older adults shows promise for the application of tDCS to remediate age-related cognitive decline and enhance cognition. Stephens and Berryhill (2016) examined tDCS paired with working memory training in 90 healthy older adults. Participants were randomly assigned to receive sham, 1 mA, or 2 mA of stimulation for 15 min while completing five sessions of working memory training. The anode electrode was placed over the right DLPFC (F4), and the cathode was placed over the contralateral cheek. Assessments were given pre- and post-intervention and all participants showed improvements on the trained verbal and visual working memory tasks. The group receiving 2 mA of stimulation showed a significant increase in far transfer benefits (processing speed, cognitive flexibility, arithmetic) at 1 month after intervention. This observation suggested that cognitive training paired with 2 mA of tDCS might induce overall improvements to daily life activities in older adults.

Park et al. (2013) assessed duration of tDCS effects combined with cognitive training on working memory in healthy older adults. Forty older adults were randomly assigned to sham or active tDCS during 10 sessions (over 2-weeks, 5 days/per week) of computer-assisted cognitive training (30 min a day). tDCS current was delivered over the bilateral prefrontal cortex (F3/F4) at 2 mA for 30 min in the active group. Using two stimulators, two anodes were placed over F3 and F4 and two cathodes were placed on the non-dominant arm. The sham group had an identical montage of electrodes with the stimulation device powered off after 30 s. Neuropsychological assessments were performed at baseline and 1-month post intervention. Significant improvements in digit span forward tests were seen in active vs. sham groups. The verbal working memory accuracy improvement was maintained for 28 days post intervention. These results suggested that tDCS might enhance cognitive training outcomes on cognitive functions in healthy older adults that lasted beyond the stimulation period.

In a task-based fMRI study with tDCS, Meinzer et al. (2013) examined the effects of improved language function induced by tDCS in 20 healthy older adults (age 60–76) and included healthy younger adults (aged 19–31) as controls. Active tDCS was delivered during a resting state scan followed by a semantic word generation task (1 mA for 17 min). The anode was placed over the left inferior frontal gyrus (IFG) (10–20 EEG system corresponding to the intersection of T3-F3 and F7-C3 and the midpoint between F7-F3) and the cathode on the contralateral supraorbital region. Reduced performance on semantic word-generation tasks in healthy older adults associated with enhanced task-related activity in bilateral IFG activation was found during sham stimulation. The active stimulation in older adults produced significantly higher performance comparable to the younger controls, and significantly reduced task-related hyperactivity in bilateral prefrontal cortex (PFC). Increased connectivity was also observed between the left IFG and the language related cortical areas during the resting state in active stimulation compared to sham. These results suggested that enhanced functional connectivity might be the basis for increasing neural efficiency marked by reduced activation with improved performance in task related cortical areas. Therefore, these results showed potentials of tDCS at enhancing cognitive processes in older adults with direct impact on underlying neural response patterns.

Harty et al. (2014) investigated the effects of tDCS on error awareness in healthy older adults by stimulating the right and left DLPFC during a sham-controlled, single-blind crossover trial. Participants were separated into four groups (24 healthy older

adults per group). The study tested the influence of current polarity and electrode location (anode over F3 or F4, and cathode over Cz), on error monitoring. During tDCS application of 1 mA, participants performed a computerized test of error awareness (5 blocks, each 7.5 min and 1-min resting time within each block), a Go/No-go response inhibition task that required constant monitoring to detect errors. The group with anode placements over the right DLPFC (F4) was the only group to experience improved error detection during the task. This study suggested that the right DLPFC might have a larger causal role on error awareness compared to the left DLPFC.

Therapeutic Role of tDCS to Remediate Mild Cognitive Impairment and Dementia in Older Adults

Previous studies have investigated the effects of tDCS in MCI populations and have found improvements in a variety of areas (e.g., face-name association memory, non-verbal recognition memory, attention) (Birba et al., 2017). In a double-blind, sham-controlled, within-subject study, Meinzer et al. (2015), applied tDCS at 1 mA for 20 min over the left ventral IFG (Brodmann areas 44/45) with the anode electrode placed on the left vIFG and the cathode on the right supraorbital region (Meinzer et al., 2012) in MCI participants. This study aimed to assess the effects of tDCS on cognition using a semantic word generation task. Multi-modal neuroimaging was acquired in a 3 Tesla MRI comparing resting state connectivity and task-related brain activity in active vs. sham tDCS groups. The active group improved significantly in word retrieval performance up to the same level as found in healthy older adults. Overall, findings from this study suggested that tDCS might improve cognitive performances in older adults with MCI by decreasing bilateral hyperactivity in PFC (Meinzer et al., 2015).

Yun et al. (2016) investigated the effects of repeated tDCS application on glucose metabolism and cognitive performance in MCI participants ($N = 16$). tDCS intensity of 2 mA was applied for 30 min, three times per week for 3 weeks in the active condition with the anode over left DLPFC (F3) and cathode over the right DLPFC (F4) for bilateral frontal stimulation. Using Positron emission tomography (PET), they found a significant increase in cerebral metabolic activity in the medial prefrontal cortex, precuneus, midtemporal regions, and the anterior cingulate cortices in the active group over sham. Multifactorial Memory Questionnaire (MMQ) was performed to assess participant's subjective memory functioning. MMQ scores and glucose metabolism were significantly improved in the active group over sham. Therefore, active tDCS can potentially change the regional brain metabolism as well as transient memory function in MCI participants (Steffener et al., 2009).

Studies using tDCS in AD participants have demonstrated positive effects on cognitive function when tDCS was applied during task execution (Hsu et al., 2015). Ferrucci et al. (2008) assessed the effects of tDCS applied over the temporoparietal areas in probable AD participants ($N = 10$). In this within-subject study, all participants received two active (reversed polarity) and one sham tDCS session over the temporoparietal areas. tDCS was delivered bilaterally via two pairs of electrodes. Each pair consisted of an electrode placed on the scalp (P3-T5 left side; P6-T4 right side) and another on the right deltoid muscle. For each session, recognition memory and visual attention were assessed pre-stimulation and 30-min post-stimulation. Accuracy for word recognition increased significantly with the anode placed over the temporoparietal region and reduced with the cathode over the temporoparietal region for the active tDCS sessions. There was no change in accuracy observed during the sham session (Ferrucci et al., 2008). This study demonstrated that stimulation over the temporoparietal areas might affect recognition memory in participants with AD. Further studies are necessary using repeated sessions in conjunction with therapeutic interventions (e.g., cognitive training) for treatments of cognitive decline in AD participants.

Boggio et al. (2012) examined the effects of tDCS on visual memory in AD participants in this within-subject study ($N = 15$). Cognitive functions were evaluated before and after the stimulation. tDCS was delivered at 2 mA for 30 min per day for five consecutive days. Bilateral stimulation was achieved using two anode electrodes placed over the temporal lobes (T3 and T4) and the cathode over the right deltoid muscle. All participants received active and sham stimulation, with sessions randomized by order and separated by an average of 71.1 days to avoid possible carry over effects. The results showed a main effect of active tDCS on enhancing visual memory performance over sham at baseline. However, there was no difference in general cognitive performance measured between active and sham (Boggio et al., 2012). This study demonstrated the therapeutic benefit of tDCS on visual memory in AD participants. Future studies aimed at optimizing intervention protocols can be explored to evaluate if specific task enhancements can be transferred to other cognitive domains.

Vascular dementia is the second highest prevalent form of dementia after AD. Vascular lesions typically result in cognitive slowing on a global scale including frontal lobe executive dysfunction and attention deficits, and local deficits at the lesion site (Hachinski et al., 2006; André et al., 2016). André et al. (2016) examined the effects of tDCS over the left DLPFC on visual short-term memory, executive function and working memory in participants with mild vascular dementia in a parallel-group design ($N = 21$; 13 active 8 sham). At-home tDCS at 2 mA for 20 min was performed over four consecutive days, with the anode placed over the left DLPFC (F3) and cathode over the contralateral supraorbital area (Fp2). In this study, participants completed cognitive assessments on the first day of stimulation, final day of stimulation, and 2-weeks later. The assessments were picture-naming task to assess visual short-term memory, the N-Back task to assess verbal working memory, and the Go/No-go task to assess executive control. Improvements were observed in both the active and sham groups. While the observed outcomes might be due to test-retest effects from repeated testing, the tasks could be considered as cognitive training, which is a promising tool for rehabilitation. Visual recall and reaction times on the N-Back and Go/No-go task were improved significantly in the active group over sham. This study provided compelling evidence for

therapeutic potentials of tDCS combined with cognitive training or behavioral protocols aimed at preventing cognitive decline or rehabilitation of cognitive faculties.

SUMMARY AND LIMITATIONS OF tDCS STUDIES TARGETING COGNITIVE AGING AND IMPAIRMENT IN OLDER ADULTS

Collectively, clinical studies of tDCS in both healthy and impaired older adults have shown potential in improving cognition and functional independence in aging population. **Table 2** summarizes a variety of stimulation parameters (electrode placement, duration, intensity, electrode size) of tDCS studies cognitive aging and impairment as mentioned in the previous sections. Cohen's *d* effect sizes were included in the table to demonstrate tDCS effects at the group level. Moderate to high effect sizes indicated that tDCS showed positive results in remediating cognitive aging and a variety of dementia-related cognitive impairments. Overall, the studies reported in the previous sections suggested that applications of 1–2 mA tDCS for at least 15 min showed moderate effects (Cohen's $d > 0.5$) to improve targeted cognitive functions in older adults. The majority of these tDCS studies (Ferrucci et al., 2008; Boggio et al., 2012; Park et al., 2013; Meinzer et al., 2015; André et al., 2016; Stephens and Berryhill, 2016; Yun et al., 2016) utilized electrode placements over the frontal lobe with memory as the most studied domain. However, it is difficult to identify a single parameter set with the highest potential for success due to the lack of common outcome measures and variation in the cognitive domains targeted across studies.

As the study of tDCS as an intervention for cognitive aging and dementia is a nascent field, the collective data presented in this paper perhaps best indicate the promise of this approach for future investigation and dose-response refinement. In addition, these studies converge in demonstrating stimulation of frontal cortices as a desirable target for intervention approaches in cognitive aging and dementia. At present, the first Phase III tDCS trial targeting remediation of cognitive aging was recently initiated and is underway (Woods et al., 2018). Trials of this type as well as further research integrating multimodal neuroimaging with tDCS in the study of cognitive aging and dementia will greatly improve our overall understanding of treatment efficacy and potentially provide a window to strategies for treatment optimization.

Factors impacting individual response to tDCS deserves further consideration and study. The clinical studies to date were mainly focused on treatment effects at group-level compared to individual effects. Individual variability in brain anatomy plays a critical role in the distribution and intensity of current flow delivered to the brain during tDCS, and thus may alter individual treatment effects (Minhas et al., 2012; Kessler et al., 2013; Woods et al., 2016). Finite element studies on current flow modeling using realistic head models have been used to predict the effects of inter-subject variability (Miranda et al., 2003; Datta et al., 2009; Opitz et al., 2013; Laakso et al., 2015). At present, while current flow modeling is available, individual assessments of current dosage *in-vivo* resulting from tDCS are limited (Kasinadhuni et al., 2017). Future studies are needed to investigate the cognitive effects from tDCS at the individual level and factors that may alter efficacy.

TMS AND tDCS USE IN COGNITIVE AGING: MOVING FORWARD

NIBS such as TMS and tDCS are promising as methods for non-invasively probing cortical circuits *in-vivo*. Since its introduction in the 1980's, TMS has seen widespread use as a non-invasive means to assess cortico-motor neurophysiology and as a clinical intervention for certain chronic disorders, such as medical resistant major depression. Beyond these applications, evidence generated over the last years and summarized in the present review, highlights additional TMS application for aging such as the evaluation of the functional integrity of intracortical GABAergic, glutamatergic, and cholinergic circuits, the assessment of mechanisms of LTP and LTD-like plasticity, and novel potential therapeutic targets for stimulation. While tDCS literature spans less than 20 years, it is quickly becoming a common methodology for non-invasive brain stimulation in both research and clinical settings. As a safe and relatively painless method for modulating the excitability of brain tissue, tDCS has strong potential for application in a variety of aging-related disorders and conditions (Bikson et al., 2016; Szymkowicz et al., 2016; Ahn et al., 2017; Fazeli et al., 2017). Therefore, researchers and clinicians are exploring the use of these techniques to provide mechanistic insight into the pathophysiology of cognitive aging and cognitive impairment and as a potential means to characterize, or even slow or reverse cognitive decline.

The results of TMS and tDCS studies presented herein have been mixed, largely owing to the inherent challenge of studying a large heterogeneous population (older adults with and without cognitive impairment) without a consistent agreed-upon set of parameters for stimulation, or measurable and sensitive outcomes of cognition. These are necessary factors to move the field forward and improve both the characterization of individuals at baseline, and the responsiveness to therapeutic interventions. While several technique-specific sources of variability were identified and discussed in the previous sections (individual, genetic, methodological), collectively, group-level data from interventional applications of TMS and tDCS demonstrated clinically meaningful, positive behavior results, with the most promising and reliable parameters presented in **Tables 1**, **2**. High-frequency rTMS was associated with improvements in global cognition, memory, verbal learning, inhibitory control and sentence comprehension. tDCS was associated with improvements in memory, working memory, language production, error awareness. However, one area of consistency across both rTMS and tDCS is the prevalence of studies targeting the frontal lobe in an attempt to enhance cognitive functions. This is perhaps not surprising, as the frontal lobe is one of the brain structures that changes most significantly due to advanced age, in the absence of neurodegeneration, and

TABLE 2 | Study characteristics from articles proposing tDCS as an intervention for cognitive improvement in individuals with cognitive aging and cognitive impairment.

Study	Mean Age (year)/ sample size	Population	Intended stimulation region	Anode	Cathode	Electrode size (cm2)	Current (mA)	Duration (min)	Outcome measure	Cognitive domain	Effect size
Stephens and Berryhill, 2016	68.2/*N* = 90	OHA	Frontal	F4	Contralateral cheek	35	2	15	Weekly Calendar Planning Activity; Road Law & Road Craft Test	Working Memory	0.56
Park et al., 2013	69.7/*N* = 40	OHA	Frontal	F3; F4	non-dominant arm	25	2	30	Digit span; 2-Back verbal working memory task	Working Memory	0.63
Meinzer et al., 2013	68.2/*N* = 20	OHA	Frontal	left vlFG	right supraorbital	35	1	17	Semantic word generation	Language Production	1.21
Harty et al., 2014	72.1/*N* = 48	OHA	Frontal	F4	Cz	35	1	37.5	Go/No-go response inhibition task	Error Awareness	0.49
Meinzer et al., 2015	67.4/*N* = 36	MCI	Frontal	left vlFG	right supraorbital	35	1	20	Semantic Word Retrieval	Memory - Non-verbal Recognition	1
Yun et al., 2016	74.8/*N* = 16	MCI	Frontal	F3	F4	25	2	30	MMQ-C (contentment)	Subjective Memory Complaints	1.11
Ferrucci et al., 2008	75.2/*N* = 10	AD	Temporoparietal	P6; T4	right deltoid muscle	32	1.5	25	Word recognition task accuracy	Memory—Verbal Recognition	2.8
Boggio et al., 2012	78.9/*N* = 15	AD	Temporal	T3; T4	right deltoid muscle	35	2	30	Visual recognition task performance	Memory—Visual Recognition	0.35
André et al., 2016	63–94*/*N* = 21	MVD	Frontal	F3	Fp2	35	2	20	Reaction time on 2-Back task	Working Memory	0.73

*N, number of subjects; tDCS, transcranial direct current stimulation; OHA, Older Healthy Adults; AD, Alzheimer's Disease; MCI, Mild Cognitive Impairment; MVD, Coronary Microvascular Disease; cm^2, square centimeter; mA, miliampere; F3, left DLPFC; F4, right DLPFC; vlFG, Brodmann areas 44/45 (inferior frontal gyrus); P6, right side temporoparietal; T3, inferior gyri of temporal lobe; T4, fusiform gyrus of temporal lobe; Cz, vertex; Fp2, contralateral supraorbital area; MMQ, Multifactorial Memory Questionnaire; *, age mean not reported.*

is associated with a myriad of cognitive abilities that decline with age.

Future studies aimed at addressing the limitations highlighted in the present study have the potential to further improve the efficacy of treatment effects at a group level. However, not all identified sources of variability can be constrained (for example, sources related with intra-individual differences in health status, anatomy or genetic make-up). For this reason, future studies should incorporate the characterization of these sources of variability in their samples and investigate the cognitive effects from TMS and tDCS further at individual level, rather than relegate this important point to the discussion section as a means to explain muddled or unexpected results. Further, the stimulation parameters included in this study are for using TMS and tDCS separately. Therefore, further consideration for parameters may be needed when applying multiple NIBS techniques simultaneously (Hamada et al., 2012). Although outside the scope of the current paper, other NIBS approaches using transcranial alternating current stimulation or transcranial random noise stimulation methods may also hold promise for cognitive aging and dementia. While the body of literature for these NIBS techniques is limited, these methods deserve additional study in this domain.

Furthermore, age-related changes in brain structure and resulting consequences for tDCS current flow and TMS-induced intracranial current are important factors that influence outcomes and require significant further study. In addition, understanding how age-related change in functional brain response impacts TMS and tDCS outcomes may provide important information for optimizing treatment gains in these studies. As we find better methods for understanding how the electrical current impacts non-motor tissue, we will have more ability to customize stimulation parameters such as current dosage and electrode placements, potentially creating paradigms that can be individualized.

In this context, neuroimaging (PET, MRI, EEG, etc.) can help. While TMS and tDCS can be applied in the absence of imaging, the integration of NIBS with multimodal human neuroimaging allows a more thorough investigation of structural and functional brain differences that are relevant for improving overall efficacy of TMS and tDCS outcomes. For instance, the use of fMRI to provide functional targeting for tDCS montage design (Woods et al., 2014) and rTMS targets (Drysdale et al., 2017) for intervention applications will serve to refine intervention protocols. Half of the interventional rTMS studies included in this review (Bentwich et al., 2011; Cotelli et al., 2011; Rabey et al., 2013; Gandelman-Marton et al., 2017; Nguyen et al., 2017) employed MRI-guided rTMS, providing support for the increased dissemination of the utility of integrating neuroimaging with NIBS (**Table 1**).

Furthermore, structural and functional imaging can be done prior to the stimulation to predict the responsiveness of individuals to NIBS interventions based on predicted or *in vivo* mapping of current flow, or individually select cortical targets based on task or resting-state activation, respectively (Weigand et al., 2017; Boes et al., 2018). With improved understanding of how current flow and intensity within specific brain regions impact behavioral outcomes, it may be possible to move toward an individualized approach for NIBS and parameter design for optimization of outcomes and patient selection. In addition, the increased use of neuronavigated systems to deliver TMS enables greater precision during stimulation.

With strong promise for a wide variety of applications in older adult populations, both TMS and tDCS represent NIBS techniques that may serve to address growing public health concerns for a rapidly growing elder population. Refining the application of these methods by taking the steps above will be important to pushing these methods further into clinical translational application in cognitive aging populations.

AUTHOR CONTRIBUTIONS

JG-O, AW, AI participated in the study concept, manuscript preparation and revision. JR, DC, NN, SA, MM, AI participated in the data extraction and manuscript preparation. PF participated in the study concept and manuscript preparation.

FUNDING

JG-O was supported by a grant from the Evelyn F. McKnight Brain Institute at University of Miami Miller School of Medicine. AW, AI, and NN were supported by the National Institute on Aging of the National Institutes of Health under Award Number R01AG054077 and K01AG050707. MM was supported by the National Institute on Aging of the National Institutes of Health under Award Number T32 AG020499. NN was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under University of Florida Clinical and Translational Science Awards TL1TR001428 and UL1TR001427. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. AW, AI, NN, and MM, were also supported by the Center for Cognitive Aging and Memory at the University of Florida and the McKnight Brain Research Foundation.

REFERENCES

Ahmed, M. A., Darwish, E. S., Khedr, M., Yasser, M. E. S., and Anwer, M. A. (2012). Effects of low versus high frequencies of repetitive transcranial magnetic stimulation in cognitive function and cortical excitability in Alzheimer's dementia. *J. Neurol.* 259, 83–92. doi: 10.1007/s00415-011-6128-4.

Ahn, H., Woods, A. J., Kunik, M. E., Bhattacharjee, A., Chen, Z., Choi, A., et al. (2017). Efficacy of transcranial direct current stimulation over primary motor cortex (anode) and contralateral supraorbital area (cathode) in clinical pain severity and mobility performance in persons with knee osteoarthritis: an experimenter- and participant-Bl. *Brain Stimul.* 10, 902–909. doi: 10.1016/j.brs.2017.05.007

André, S., Heinrich, S., Kayser, F., Menzler, K., Kesselring, J., Khader, P. H., et al. (2016). At-home tDCS of the left dorsolateral prefrontal cortex improves visual short-term memory in mild vascular dementia. *J. Neurol. Sci.* 369, 185–190. doi: 10.1016/j.jns.2016.07.065

Arvanitakis, Z., Wilson, R. S., Bienias, J. L., Evans, D. A., and Bennett, D. A. (2004). Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. *Arch. Neurol.* 61, 661–666. doi: 10.1001/archneur.61.5.661

Barnes, C. A. (2003). Long-term potentiation and the ageing brain. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 358, 765–772. doi: 10.1098/rstb.2002.1244

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F., and Nitsche, A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.* 591, 1987–2000. doi: 10.1113/jphysiol.2012.249730

Bentwich, J., Dobronevsky, A., Aichenbaum, S., Shorer, R., Peretz, R., Michael Khaigrekht, M., et al. (2011). Beneficial effect of repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease: a proof of concept study. *J. Neural Transm.* 118, 463–471. doi: 10.1007/s00702-010-0578-1

Benussi, A., Di Lorenzo, F., Dell'Era, V., Cosseddu, M., Alberici, A., Caratozzolo, S., et al. (2017). Transcranial magnetic stimulation distinguishes Alzheimer Disease from frontotemporal dementia. *Neurology* 89, 665–672. doi: 10.1212/WNL.0000000000004232

Bhandari, A., Radhu, N., Farzan, F., Mulsant, B. H., Rajji, T. K., Daskalakis, Z. J., et al. (2016). A meta-analysis of the effects of aging on motor cortex neurophysiology assessed by transcranial magnetic stimulation. *Clin. Neurophysiol.* 127, 2834–2845. doi: 10.1016/j.clinph.2016.05.363

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, L., Adnan, T., et al. (2016). Safety of transcranial direct current stimulation: evidence based update 2016. *Brain Stimul.* 9, 641–661. doi: 10.1016/j.brs.2016.06.004

Bindman, L. J., Lippold, O. C., and Redfearn, J. W. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* 172, 369–382. doi: 10.1113/jphysiol.1964.sp007425

Birba, A., Ibáñez, A., Sedeño, L., Ferrari, J., García, A. M., and Zimerman, M. (2017). Non-invasive brain stimulation: a new strategy in mild cognitive impairment? *Front. Aging Neurosci.* 9:16. doi: 10.3389/fnagi.2017.00016

Bliss, T. V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232, 331–356. doi: 10.1113/jphysiol.1973.sp010273

Boes, A. D., Uitermarkt, B. D., Albazron, F. M., Lan, M. J., Liston, C., Pascual-Leone, A., et al. (2018). Rostral anterior cingulate cortex is a structural correlate of repetitive TMS treatment response in depression. *Brain Stimul.* 11, 575–581. doi: 10.1016/j.brs.2018.01.029

Boggio, P. S., Ferrucci, R., Mameli, F., Martins, D., Martins, O., Vergari, M., et al. (2012). Prolonged visual memory enhancement after direct current stimulation in Alzheimer's disease. *Brain Stimul.* 5, 223–230. doi: 10.1016/j.brs.2011.06.006.

Bolzoni, F., Pettersson, L. G., and Jankowska, E. (2013). Evidence for long-lasting subcortical facilitation by transcranial direct current stimulation in the Cat. *J. Physiol.* 591, 3381–3399. doi: 10.1113/jphysiol.2012.244764

Braak, H., and Braak, E. (1998). Evolution of neuronal changes in the course of Alzheimer's disease. *J. Neural Transm. Suppl.* 53, 127–140.

Brem, A. K., Atkinson, N. J., Seligson, E. E., Pascual-Leone, A., Hansen, N., and Ferrer, I. (2013). Differential pharmacological effects on brain reactivity and plasticity in Alzheimer's disease. *Front. Psychiatry* 4:124. doi: 10.3389/fpsyt.2013.00124

Brown, K. E., Lohse, K. R., Mayer, I. M. S., Strigaro, G., Desikan, M., Casula, E. P., et al. (2017). The reliability of commonly used electrophysiology measures. *Brain Stimul.* 10, 1102–1111. doi: 10.1016/j.brs.2017.07.011

Carlson, M. C., Hasher, L., Zacks, R. T., and Connelly, S. L. (1995). aging, distraction, and the benefits of predictable location. *Psychol. Aging* 10, 427–436. doi: 10.1037/0882-7974.10.3.427

Carroll, T. J., Riek, S., and Carson, R. G. (2001). Reliability of the input–output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *J. Neurosci. Methods.* 112, 193–202. doi: 10.1016/S0165-0270(01)00468-X

Carvalho, M., de Mendonça, A., de Miranda, P. C., Garcia, C., and Luís, M. L. S. (1997). Magnetic stimulation in Alzheimer's Disease. *J. Neurol.* 244, 304–307.

Chang, W. H., Fried, P. J., Saxena, S., Jannati, A., Gomes-Osman, J., Kim, Y. H., et al. (2016). Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. *Clin. Neurophysiol.* 127, 2892–2897. doi: 10.1016/j.clinph.2016.04.001

Cheeran, B., Talelli, P., Mori, F., Koch, G., Suppa, A., Edwards, M., et al. (2008). A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. *J. Physiol.* 58623, 5717–5725. doi: 10.1113/jphysiol.2008.159905

Chen, R., Tam, A., Bütefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., et al. (1998). Intracortical inhibition and facilitation in different representations of the human motor cortex. *J. Neurophysiol.* 80, 2870–2881.

Christie, A., Fling, B., Crews, R. T., Mulwitz, L. A., and Kamen, G. (2007). Reliability of motor-evoked potentials in the ADM muscle of older adults. *J. Neurosci. Methods* 164, 320–324. doi: 10.1016/j.jneumeth.2007.05.011

Chung, S. W., Hill, A. T., Rogasch, N. C., Hoy, K. E., and Fitzgerald, P. B. (2016). Use of theta-burst stimulation in changing excitability of motor cortex: a systematic review and meta-analysis. *Neurosci. Biobehav. Rev.* 63, 43–64. doi: 10.1016/j.neubiorev.2016.01.008

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences, 2nd Edn*. Hillsdale, NJ: Erlbaum.

Cotelli, M., Calabria, M., Manenti, R., Rosini, S., Zanetti, O., Cappa, S. F., et al.. (2011). Improved language performance in Alzheimer disease following brain stimulation. *J. Neurol. Neurosurg. Psychiatr.* 82, 794–797. doi: 10.1136/jnnp.2009.197848

Cotelli, M., Manenti, R., Cappa, S. F., Zanetti, O., and Miniussi, C. (2008). Transcranial magnetic stimulation improves naming in Alzheimer disease patients at different stages of cognitive decline. *Eur. J. Neurol.*. 15, 1286–1292. doi: 10.1111/j.1468-1331.2008.02202.x

Cumming, G. (2012). *Understanding the New Statistics: Effect Sizes, Confidence Intervals, and Meta-Analysis*. Melbourne, VIC: La Trobe University.

Darowski, E. S., Helder, E., Zacks, R. T., Hasher, L., and Hambrick, D. Z. (2008). Age-related differences in cognition: the role of distraction control. *Neuropsychology* 22, 638–644. doi: 10.1037/0894-4105.22.5.638

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., and Bikson, M. (2009). Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul.* 2, 201–207. doi: 10.1016/j.brs.2009.03.005

Debarnot, U., Crépon, B., Orriols, E., Abram, M., Charron, S., Lion, S., et al. (2015). Intermittent theta burst stimulation over left BA10 Enhances virtual reality-based prospective memory in healthy aged subjects. *Neurobiol. Aging* 36, 2360–2369. doi: 10.1016/j.neurobiolaging.2015.05.001

DeCarli, C., Massaro, J., Harvey, D., Hald, J., Tullberg, M., Au, R., Beiser, A., et al. (2005). Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. *Neurobiol. Aging* 26, 491–510. doi: 10.1016/j.neurobiolaging.2004.05.004

Devanne, H., Lavoie, B. A., and Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Exp. Brain Res.* 114, 329–338. doi: 10.1007/PL00005641

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C., et al. (2004). Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatr.* 75, 555–559. doi: 10.1136/jnnp.2003.018127

Di Lazzaro, V., Oliviero, A., Tonali, P. A., Marra, A., Daniele, A., Profice, P., et al. (2002). Noninvasive *in vivo* assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology* 59, 392–397. doi: 10.1212/WNL.59.3.392

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Oliviero, A., Mazzone, P., et al. (2008). The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *J. Physiol.* 58616, 3871–3879. doi: 10.1113/jphysiol.2008.152736

Di Lazzaro, V., Pilato, F., Saturno, E., Oliviero, A., Dileone, M., Mazzone, P., et al. (2005). Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J. Physiol.* 5653, 945–950. doi: 10.1113/jphysiol.2005.087288

Di Lorenzo, F., Ponzo, V., Bonnì, S., Motta, C., Serra, P. C., Bozzali, M., et al. (2016). Long-term potentiation–like cortical plasticity is disrupted in Alzheimer's disease patients independently from age of onset. *Ann. Neurol.* 80, 202–210. doi: 10.1002/ana.24695.

Dong, C., Nabizadeh, N., Caunca, M., Cheung, Y. K., Rundek, T., Elkind, M. S., et al. (2015). Cognitive correlates of white matter lesion load and brain atrophy: the northern Manhattan study. *Neurology* 85, 441–449. doi: 10.1212/WNL.0000000000001716

Drumond Marra, H. L., Livia, H., Myczkowski, M. L., Maia Memória, C., Arnault, D., Ribeiro, L. P., et al. (2015). Transcranial magnetic stimulation to address mild cognitive impairment in the elderly: a randomized controlled study, transcranial magnetic stimulation to address mild cognitive impairment in the elderly: a randomized controlled study. *Behav. Neurol.* 2015:287843. doi: 10.1155/2015/287843

Drysdale, A. T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., et al. (2017). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nat. Med*. 23, 28–38. doi: 10.1038/nm.4246.

Fazeli, P. L., Woods, A. J., Pope, C. N., Vance, D. E., and Ball, K. K. (2017). Effect of transcranial direct current stimulation combined with cognitive training on cognitive functioning in older adults with HIV: a pilot study. *Appl. Neuropsychol. Adult* 11, 1–12. doi: 10.1080/23279095.2017.1357037

Ferreri, F., Pauri, F., Pasqualetti, P., Fini, R., Forno, D. D., and Rossini, P. M. (2003). Motor cortex excitability in Alzheimer's Disease: a transcranial magnetic stimulation study. *Ann. Neurol.* 53, 102–108. doi: 10.1002/ana.10416

Ferrucci, R., Mameli, F., Guidi, I., Mrakic-Sposta, S., Vergari, M., Marceglia, S., et al. (2008). Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology* 71, 493–498. doi: 10.1212/01.wnl.0000317060.43722.a3

Fregni, F., Boggio, P. S., Nitsche, M. A., Marcolin, M. A., Rigonatti, A. P., and Pascual-Leone, A. (2006). Treatment of major depression with transcranial direct current stimulation. *Bipolar Disord.* 8, 203–204. doi: 10.1111/j.1399-5618.2006.00291.x

Freitas, C., Perez, J., Knobel, M., Tormos, J. M., Oberman, L., Eldaief, M., et al. (2011). Changes in cortical plasticity across the lifespan. *Front. Aging Neurosci.* 3:5. doi: 10.3389/fnagi.2011.00005

Fried, P. J., Jannati, A., Davila-Pérez, P., and Pascual-Leone, A. (2017). Reproducibility of single-pulse, paired-pulse, and intermittent theta-burst TMS measures in healthy aging, type-2 diabetes, and Alzheimer's disease. *Front. Aging Neurosci.* 9:263. doi: 10.3389/fnagi.2017.00263

Fried, P. J., Schilberg, L., Brem, A. K., Saxena, S., Wong, B., Cypess, A. M., et al. (2016). Humans with type-2 diabetes show abnormal long-term potentiation-like cortical plasticity associated with verbal learning deficits. *J. Alzheimers Dis.* 55, 89–100. doi: 10.3233/JAD-160505

Funke, K., and Benali, A. (2011). Modulation of cortical inhibition by rTMS - findings obtained from animal models. *J. Physiol.* 589, 4423–4435. doi: 10.1113/jphysiol.2011.206573

Gandelman-Marton, R., Aichenbaum, S., Dobronevsky, E., Khaigrekht, M., and Rabey, J. M. (2017). Quantitative EEG after brain stimulation and cognitive training in Alzheimer disease. *J. Clin. Neurophysiol*. 34, 49–54. doi: 10.1097/WNP.0000000000000301

Gedankien, T., Fried, P. J., Pascual-Leone, A., and Shafi, M. M. (2017). Intermittent theta-burst stimulation induces correlated changes in cortical and corticospinal excitability in healthy older subjects. *Clin. Neurophysiol*. 128, 2419–2427. doi: 10.1016/j.clinph.2017.08.034

Gomes-Osman, J., Cabral, D. F., Hinchman, C., Jannati, A., Morris, T. P., and Pascual-Leone, A. (2017). The effects of exercise on cognitive function and brain plasticity – a feasibility trial. *Restor. Neurol. Neurosci*. 35, 547–556. doi: 10.3233/RNN-170758

Gonsalvez, I., Baror, R., Fried, P., Santarnecchi, E., and Pascual-Leone, A. (2017). Therapeutic noninvasive brain stimulation in Alzheimer's disease. *Curr. Alzheimer Res*. 14, 362–376. doi: 10.2174/1567205013666160930113907.

Hachinski, V., Iadecola, C., Petersen, R. C., Breteler, M. M., Nyenhuis, D. L., Black, S. E., et al. (2006). National institute of neurological disorders and stroke-Canadian stroke network vascular cognitive impairment harmonization standards. *Stroke* 37, 2220–2241. doi: 10.1161/01.STR.0000237236.88823.47

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., and Rothwell, J. C. (2013). The role of interneuron networks in driving human motor cortical plasticity. *Cereb. Cortex* 23, 1593–1605. doi: 10.1093/cercor/bhs147

Hamada, M., Strigaro, G., Murase, N., Sadnicka, A., Galea, J. M., Edwards, M. J., et al. (2012). Cerebellar modulation of human associative plasticity. *J. Physiol.* 590, 2365–2374. doi: 10.1113/jphysiol.2012.230540

Hanajima, R., Ugawa, Y., Terao, Y., Sakai, K.,Furubayashi, T., Machii, K., et al. (1998). Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. *J. Physiol.* 509, 607–618.

Harada, C. N., Marissa, C., Love, M. C. N., and Triebel, K. (2013). Normal cognitive aging. *Clin. Geriatr. Med*. 29, 737–752. doi: 10.1016/j.cger.2013.07.002

Harty, S., Robertson, I. H., Miniussi, C., Sheehy, O. C., Devine, C. A., McCreery, S., et al. (2014). Transcranial direct current stimulation over right dorsolateral prefrontal cortex enhances error awareness in older age. *J. Neurosci.* 34, 3646–3652. doi: 10.1523/JNEUROSCI.5308-13.2014

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., et al. (2006). AMPAR removal underlies abeta-induced synaptic depression and dendritic spine loss. *Neuron* 52, 831–843. doi: 10.1016/j.neuron.2006.10.035

Hsu, W. Y., Ku, Y., Zanto, T. P., and Gazzaley, A. (2015). Effects of noninvasive brain stimulation on cognitive function in healthy aging and Alzheimer's disease: a systematic review and meta-analysis. *Neurobiol. Aging* 36, 2348–2359. doi: 10.1016/j.neurobiolaging.2015.04.016.

Huang, Y. Z., Chen, R. S., Rothwell, J. C., and Wen, H. Y. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin. Neurophysiol.* 118, 1028–1032. doi: 10.1016/j.clinph.2007.01.021

Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., and Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron* 45, 201–206. doi: 10.1016/j.neuron.2004.12.033

Huang, Y. Z., Liu, A. A., Lafon, B., Friedman, D., Dayan, M., Wang, X., et al. (2017). Measurements and models of electric fields in the *in vivo* human brain during transcranial electric stimulation. *Elife* 6:e18834. doi: 10.7554/eLife.18834

Huang, Y. Z., Rothwell, J. C., Chen, R. S., Lu, C. S., and Chuang, W.-L. (2011). The theoretical model of theta burst form of repetitive transcranial magnetic stimulation. *Clin. Neurophysiol.* 122, 1011–1018. doi: 10.1016/j.clinph.2010.08.016

Indahlastari, A., Chauhan, M., Schwartz, B., and Sadleir, R. J. (2016). Changing head model extent affects finite element predictions of transcranial direct current stimulation distributions. *J. Neural Eng*. 13:066006. doi: 10.1088/1741-2560/13/6/066006

Kandimalla, R., and Reddy, P. H. (2017). Therapeutics of neurotransmitters in Alzheimer's disease. *J. Alzheimers Dis.* 57, 1049–1069. doi: 10.3233/JAD-161118

Kasinadhuni, A. K., Indahlastari, A., Chauhan, M., Schär, M., Mareci, T. H., and Sadleir, R. J. (2017). Imaging of current flow in the human head during transcranial electrical therapy. *Brain Stimul.* 10, 764–772. doi: 10.1016/j.brs.2017.04.125

Kessler, S. K., Minhas, P., Woods, A. J., Rosen, A., Gorman, C., and Bikson, M. (2013). Dosage considerations for transcranial direct current stimulation in children: a computational modeling study. *PLoS ONE* 8:e76112. doi: 10.1371/journal.pone.0076112

Khedr, E. M., Ahmed, M. A., Darwish, E. S., and Ali, A. M. (2011). The relationship between motor cortex excitability and severity of Alzheimer's disease: a transcranial magnetic stimulation study. *Neurophysiol. Clin*. 41, 107–113. doi: 10.1016/j.neucli.2011.03.002

Kim, H. (2011). Neural activity that predicts subsequent memory and forgetting: a meta-analysis of 74 fMRI studies. *Neuroimage* 54, 2446–2461. doi: 10.1016/j.neuroimage.2010.09.045

Kim, S. H., Han, H. J., Ahn, H. M., Kim, S. A., and Kim, S. E. (2012). Effects of five daily high-frequency rTMS on stroop task performance in aging individuals. *Neurosci. Res*. 74, 256–260. doi: 10.1016/j.neures.2012.08.008

Kobayashi, M., and Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol.* 2, 145–156. doi: 10.1016/S1474-4422(03)00321-1

Koch, G., Di Lorenzo, F., Bonnì, S., Giacobbe, V., Bozzali, M., Caltagirone, C., et al. (2014). Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. *Neuropsychopharmacology* 39, 2654–2661. doi: 10.1038/npp.2014.119

Koch, G., Di Lorenzo, F., Bonnì, S., Ponzo, V., Caltagirone, C., and Martorana, A. (2012). Impaired LTP-but not LTD-like cortical plasticity in Alzheimer's disease patients. *J. Alzheimers Dis.* 31, 593–599. doi: 10.3233/JAD-2012-120532

Koliatsos, V. E., Kecojevic, A., Troncoso, J. C., Gastard, M. C., Bennett, C. A., and Schneider, J. A. (2006). Early involvement of small inhibitory cortical interneurons in Alzheimer's Disease. *Acta Neuropathol.* 112, 147–162. doi: 10.1007/s00401-006-0068-6

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., et al. (1993). Cotrical inhibition in human motor cortex. *J. Physiol.* 471, 501–519. doi: 10.1113/jphysiol.1993.sp019912

Kukke, S. N., Paine, R. W., Chao, C. C., de Campos, A. C., and Hallett, M. (2014). Efficient and reliable characterization of the corticospinal system using transcranial magnetic stimulation. *J. Clin. Neurophysiol.* 31, 246–252. doi: 10.1097/WNP.0000000000000057

Kuo, H. I., Bikson, M., Datta, A., Minhas, P., Paulus, W., Kuo, M.-F., et al. (2013). Comparing cortical plasticity induced by conventional and high-definition 4 × 1 ring tDCS: a neurophysiological study. *Brain Stimul.* 6, 644–648. doi: 10.1016/j.brs.2012.09.010

Laakso, I., Tanaka, S., Koyama, S., De Santis, V., and Hirata, A. (2015). Inter-subject variability in electric fields of motor cortical tDCS. *Brain Stimul.* 8, 906–913. doi: 10.1016/j.brs.2015.05.002.

Lemaitre, H., Goldman, A. L., Sambataro, F., Verchinski, B. A., Meyer-Lindenberg, A., Weinberger, D. R., et al. (2012). Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol. Aging* 33:617. doi: 10.1016/j.neurobiolaging.2010.07.013

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125, 2238–2247. doi: 10.1093/brain/awf238

Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H., and Pascual-Leone, A. (2000). Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. *Exp. Brain Res.* 133, 425–430. doi: 10.1007/s002210000432

Maillet, D., and Rajah, M. N. (2013). Association between prefrontal activity and volume change in prefrontal and medial temporal lobes in aging and dementia: a review. *Ageing Res. Rev.* 12, 479–489. doi: 10.1016/j.arr.2012.11.001

Marneweck, M., Loftus, A., and Hammond, G. (2011). Short-interval intracortical inhibition and manual dexterity in healthy aging. *Neurosci. Res.* 70, 408–414. doi: 10.1016/j.neures.2011.04.004.

Martis, B., Alam, D., Dowd, S. M., Hill, S. K., Sharma, R. P., Rosen, C., et al. (2003). Neurocognitive effects of repetitive transcranial magnetic stimulation in severe major depression. *Clin. Neurophysiol.* 114, 1125–1132. doi: 10.1016/S1388-2457(03)00046-4

Mattson, M. P., Maudsley, S., and Martin, B. (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 27, 589–594. doi: 10.1016/j.tins.2004.08.001

McGinley, M., Hoffman, R. L., Russ, D. W., Thomas, J. S., and Clark, B. C. (2010). Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. *Exp. Gerontol.* 45, 671–678. doi: 10.1016/j.exger.2010.04.005

McLaren, M. E., Nissim, N. R., and Woods, A. J. (2017). The effects of medication use in transcranial direct current stimulation: a brief review. *Brain Stimul.* 11, 52–58. doi: 10.1016/j.brs.2017.10.006.

Medeiros, L. F., de Souza, I. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., et al. (2012). Neurobiological effects of transcranial direct current stimulation: a review. *Front. Psychiatry* 3:110. doi: 10.3389/fpsyt.2012.00110

Meinzer, M., Antonenko, D., Lindenberg, R., Hetzer, S., Ulm, L., Avirame, K., et al. (2012). Electrical brain stimulation improves cognitive performance by modulating functional connectivity and task-specific activation. *J. Neurosci.* 32, 1859–1866. doi: 10.1523/JNEUROSCI.4812-11.2012

Meinzer, M., Lindenberg, R., Phan, M. T., Ulm, L., Volk, C., and Flöel, A. (2015). Transcranial direct current stimulation in mild cognitive impairment: behavioral effects and neural mechanisms. *Alzheimers Dement.* 11, 1032–1040. doi: 10.1016/j.jalz.2014.07.159

Meinzer, R. L., Antonenko, D., Flaisch, T., and Flöel, A. (2013). Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. *J. Neurosci.* 33, 12470–11278. doi: 10.1523/JNEUROSCI.5743-12.2013

Minhas, P., Bikson, M., Woods, A. J., Rosen, A. R., and Kessler, S. K. (2012). Transcranial direct current stimulation in pediatric brain: a computational modeling study. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2012, 859–862. doi: 10.1109/EMBC.2012.6346067

Miranda, P. C., Hallett, M., and Basser, P. J. (2003). The electric field induced in the brain by magnetic stimulation: A 3-D finite-element analysis of the effect of tissue heterogeneity and anisotropy. *IEEE Trans. Biomed. Eng.* 50, 1074–1085. doi: 10.1109/TBME.2003.816079

Mitchell, A. J., and Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia–meta-analysis of 41 robust inception cohort studies. *Acta Psychiatr. Scand.* 119, 252–265. doi: 10.1111/j.1600-0447.2008.01326.x

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., et al. (2013). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimul.* 6, 424–432. doi: 10.1016/j.brs.2012.04.011

Mukherjee, J., Christian, B. T., Dunigan, K. D., Shi, B., Narayanan, T. K., Satter, M., et al. (2002). Brain imaging of18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse* 46, 170–188. doi: 10.1002/syn.10128

Musselman, K. E. (2007). Clinical significance testing in rehabilitation research: what, why, and how? *Phys. Ther. Rev.* 12, 287–296. doi: 10.1179/108331907X223128

Nguyen, J. P., Suarez, A., Kemoun, G., Meignier, M., Le Saout, E., Damier, P., et al. (2017). Repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease. *Neurophysiol. Clin.* 47, 47–53. doi: 10.1016/j.neucli.2017.01.001

Nissim, N. R., O'Shea, A. M., Bryant, V., Porges, E. C., Cohen, R., and Woods, A. J. (2017). Frontal structural neural correlates of working memory performance in older adults. *Front. Aging Neurosci.* 8:328. doi: 10.3389/fnagi.2016.00328

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 1, 206–23. doi: 10.1016/j.brs.2008.06.004

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nyberg, L. (2004). Intact frontal memory effect in older age and dementia. *Neuron* 42, 701–702. doi: 10.1016/j.neuron.2004.05.016

Nyberg, L., Backman, L., Erngrund, K., Olofsson, U., and Nilsson, L. G. (1996). Age differences in episodic memory, semantic memory, and priming: relationships to demographic, intellectual, and biological factors. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 51, 234–240. doi: 10.1093/geronb/51B.4.P234

Oberman, L., and Pascual-Leone, A. (2013). Changes in plasticity across the lifespan: cause of disease and target for intervention. *Prog. Brain Res.* 207, 91–120. doi: 10.1016/B978-0-444-63327-9.00016-3

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., and M., Dileone, M., et al. (2006). Effects of aging on motor cortex excitability. *Neurosci. Res.* 55, 74–77. doi: 10.1016/j.neures.2006.02.002

Opie, G. M., and Semmler, J. G. (2014). Age-related differences in short- and long-interval intracortical inhibition in a human hand muscle. *Brain Stimul.* 7, 665–672. doi: 10.1016/j.brs.2014.06.014

Opitz, A., Falchier, A., Yan, C. G., Yeagle, E. M., Linn, G. S., Megevand, P., et al. (2016). Spatiotemporal structure of intracranial electric fields induced by transcranial electric stimulation in humans and nonhuman primates. *Sci. Rep.* 6:31236. doi: 10.1038/srep31236

Opitz, A., Legon, W., Rowlands, A., Bickel, W. K., Paulus, W., and Tyler, W. J. (2013). Physiological observations validate finite element models for estimating subject-specific electric field distributions induced by transcranial magnetic stimulation of the human motor cortex. *Neuroimage* 81, 253–364. doi: 10.1016/j.neuroimage.2013.04.067

Ottenbacher, K. J. (1995). Why rehabilitation research does not work (as well as we think it should). *Arch. Phys. Med. Rehabil.* 76, 123–129.

Park, S. H., Seo, J. H., Kim, Y. H., and Ko, M. H. (2013). Long-term effects of transcranial direct current stimulation combined with computer-assisted cognitive training in healthy older adults. *Neuroreport* 25, 122–126. doi: 10.1097/WNR.0000000000000080

Pascual-Leone, A., and Taylor, M. J. (2011). A developmental framework of Brain and cognition from infancy to old age. *Brain Topogr.* 24, 183–186. doi: 10.1007/s10548-011-0197-7

Peinemann, A., Lehner, C., Conrad, B., Siebner, H. R., Roman, H., and Siebner, H. R. (2001). Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex. *Neurosci. Lett.* 313, 33–36. doi: 10.1016/S0304-3940(01)02239-X

Pennisi, G., Ferri, R., Alagona, G., Pennisi, M., Malaguarnera, G., Motta, M., et al. (2011). Motor cortex hyperexcitability in subcortical ischemic vascular dementia. *Arch. Gerontol. Geriatr.* 53, 111–113. doi: 10.1016/j.archger.2010.07.004

Pierantozzi, M., Panella, M., Palmieri, M. G., Koch, G., Giordano, A., Marciani, M. G., et al. (2004). Different TMS patterns of intracortical inhibition in early onset Alzheimer dementia and frontotemporal dementia. *Clin. Neurophysiol.* 115, 2410–2418. doi: 10.1016/j.clinph.2004.04.022

Pitcher, J. B., Ogston, K. M., and Miles, T. S. (2003). Age and sex differences in human motor cortex input-output characteristics. *J. Physiol.* 546, 605–613. doi: 10.1113/jphysiol.2002.029454

Prehn, K., and Flöel, A. (2015). Potentials and limits to enhance cognitive functions in healthy and pathological aging by tDCS. *Front. Cell. Neurosci.* 9:355. doi: 10.3389/fncel.2015.00355

Rabey, J. M., and Dobronevsky, E. (2016). Repetitive transcranial magnetic stimulation (rTMS) combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: clinical experience. *J. Neural Transm.* 123, 1449–1455. doi: 10.1007/s00702-016-1606-6

Rabey, J. M., Dobronevsky, E., Aichenbaum, S., Gonen, O., Marton, R. G., and Khaigrekht, M. (2013). Repetitive transcranial magnetic stimulation combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: a randomized, double-blind study. *J. Neural Transm.* 120, 813–819. doi: 10.1007/s00702-012-0902-z

Raz, N., Gunning-Dixon, F., Head, D., Rodrigue, K. M., Williamson, A., and Acker, J. D. (2004). Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. *Neurobiol. Aging* 25, 377–396. doi: 10.1016/S0197-4580(03)00118-0

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., et al. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1590–1595. doi: 10.1073/pnas.0805413106.

Rektorova, I., Megova, S., Bares, M., and Rektor, I. (2005). Cognitive functioning after repetitive transcranial magnetic stimulation in patients with cerebrovascular disease without dementia: a pilot study of seven patients. *J. Neurol. Sci.* 229–230, 157–161. doi: 10.1016/j.jns.2004.11.021

Ridding, M. C., and Rothwell, J. C. (1997). Stimulus/response curves as a method of measuring motor cortical excitability in man. *Electroencephalogr. Clin. Neurophysiol.* 105, 340–344.

Roberts, R., and Knopman, S. K. (2013). Classification and epidemiology of MCI. *Clin. Geriatr. Med.* 29, 753–772. doi: 10.1016/j.cger.2013.07.003

Rönnlund, M., Nyberg, L., Bäckman, L., and Nilsson, L.-G. (2005). Stability, growth, and decline in adult life span development of declarative memory: cross-sectional and longitudinal data from a population-based study. *Psychol. Aging* 20, 3–18. doi: 10.1037/0882-7974.20.1.3

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Rossini, P., Burke, D., Chen, R., Cohen, L., Daskalakis, Z., Di Iorio, R., et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. *Clin. Neurophysiol.* 126, 1071–1107. doi: 10.1016/j.clinph.2015.02.001

Sadleir, R. J., Vannorsdall, T. D., Schretlen, D. J., and Gordon, B. (2010). Transcranial direct current stimulation (tDCS) in a realistic head model. *Neuroimage* 51, 1310–1318. doi: 10.1016/j.neuroimage.2010.03.052

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S. R., Evelina Busa, E., et al. (2004). Thinning of the cerebral cortex in aging. *Cereb. Cortex* 14, 721–730. doi: 10.1093/cercor/bhh032

Salthouse, T. A. (2010). Influence of age on practice effects in longitudinal neurocognitive change. *Neuropsychology* 24, 563–572. doi: 10.1037/a0019026

Sanger, T. D., Garg, R. R., and Chen, R. (2001). Interactions between two different inhibitory systems in the human motor cortex. *J. Physiol.* 530, 307–317. doi: 10.1111/j.1469-7793.2001.0307l.x

Schilberg, L., Schuhmann, T., and Sack, A. T. (2017). Interindividual variability and intraindividual reliability of intermittent theta burst stimulation-induced neuroplasticity mechanisms in the healthy brain. *J. Cogn. Neurosci.* 29, 1022–1032. doi: 10.1162/jocn_a_01100

Shahid, S., Wen, P., and Ahfock, T. (2014). Assessment of electric field distribution in anisotropic cortical and subcortical regions under the influence of tDCS. *Bioelectromagnetics* 35, 41–57. doi: 10.1002/bem.21814

Shibuya, K., Park, S. B., Geevasinga, N., Huynh, W., Simon, N. G., Menon, P., et al. (2016). Threshold tracking transcranial magnetic stimulation: effects of age and gender on motor cortical function. *Clin. Neurophysiol.* 127, 2355–2361. doi: 10.1016/j.clinph.2016.03.009

Silbert, L. C., Nelson, C., Holman, S., Eaton, R., Oken, B. S., Lou, J. S., et al. (2006). Cortical excitability and age-related volumetric MRI changes. *Clin. Neurophysiol.* 117, 1029–1036. doi: 10.1016/j.clinph.2006.02.003

Silvanto, J., Muggleton, N. G., Cowey, A., and Walsh, V. (2007). Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. *Eur. J. Neurosci.* 25, 1874–1881. doi: 10.1111/j.1460-9568.2007.05440.x

Solé-Padullés, C., Bartrés-Faz, D., Junqué, C., Clemente, I. C., Molinuevo, J. L., Bargalló, N., et al. (2006). Repetitive transcranial magnetic stimulation effects on brain function and cognition among elders with memory dysfunction. a randomized sham-controlled study. *Cereb. Cortex* 16, 1487–1493. doi: 10.1093/cercor/bhj083

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., et al. (2011). Toward defining the preclinical stages of Alzheimer's disease: recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 280–292. doi: 10.1016/j.jalz.2011.03.003

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., et al. (2009a). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J. Neurosci.* 29, 5202–5206. doi: 10.1523/JNEUROSCI.4432-08.2009

Stagg, C. J., Lin, R. L., Mezue, M., Segerdahl, A., Kong, Y., Xie, J., et al. (2013). Widespread modulation of cerebral perfusion induced during and after transcranial direct current stimulation applied to the left dorsolateral prefrontal cortex. *J. Neurosci.* 33, 11425–11131. doi: 10.1523/JNEUROSCI.3887-12.2013

Stagg, C. J., Wylezinska, M., Matthews, P. M., Johansen-Berg, H., Jezzard, P., Rothwell, J. C., et al. (2009b). Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. *J. Neurophysiol.* 101, 2872–2877. doi: 10.1152/jn.91060.2008

Steffener, J., Brickman, A., Rakitin, B. C., Gazes, Y., and Stern, Y. (2009). The impact of age-related changes on working memory functional activity. *Brain Imaging Behav.* 3, 142–153. doi: 10.1007/s11682-008-9056-x

Stephens, J. A., and Berryhill, M. E. (2016). Older adults improve on everyday tasks after working memory training and neurostimulation. *Brain Stimul.* 9, 553–559. doi: 10.1016/j.brs.2016.04.001

Stokes, M. G., Chambers, C. D., Gould, I. C., English, T., McNaught, E., McDonald, O., et al. (2007). Distance-adjusted motor threshold for transcranial magnetic stimulation. *Clin. Neurophysiol.* 118, 1617–1625. doi: 10.1016/j.clinph.2007.04.004

Szymkowicz, S. M., McLaren, M. E., Suryadevara, U., and Woods, A. J. (2016). Transcranial direct current stimulation use in the treatment of neuropsychiatric disorders: a brief review. *Psychiatr. Ann.* 46, 642–646. doi: 10.3928/00485713-20161006-01

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., Insola, A., et al. (2000). Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J. Physiol.* 523, 503–513. doi: 10.1111/j.1469-7793.2000.t01-1-00503.x

Trebbastoni, A., Pichiorri, F., D'Antonio, F., Campanelli, A., Onesti, E., Ceccanti, M., et al. (2016). Altered cortical synaptic plasticity in response to 5-Hz repetitive transcranial magnetic stimulation as a new electrophysiological finding in amnestic mild cognitive impairment converting to Alzheimer's disease: results from a 4-Year prospective cohort study. *Front. Aging Neurosci.* 7:253. doi: 10.3389/fnagi.2015.00253

Trippe, J., Mix, A., Aydin-Abidin, S., Funke, K., and Benali, A. (2009). θ burst and conventional low-frequency rTMS differentially affect GABAergic neurotransmission in the rat cortex. *Exp. Brain Res.* 199, 411–421. doi: 10.1007/s00221-009-1961-8

Turriziani, P., Smirni, D., Zappalà, G., Mangano, G. R., Oliveri, M., and Cipolotti, L. (2012). Enhancing memory performance with rTMS in healthy subjects and

individuals with mild cognitive impairment: the role of the right dorsolateral prefrontal cortex. *Front. Hum. Neurosci.* 6:62. doi: 10.3389/fnhum.2012.00062

National Association of Area Agencies on Aging, National Council on Aging and United Healthcare (2015). *The 2015 United States of Aging Survey*.

United Nations, Department of Economic and Social Affairs, Population Division (2015). *World Population Ageing*.

Vallence, A.-M., Goldsworthy, M. R., Hodyl, N. A., Semmler, J. G., Pitcher, J. B., and Ridding, M. C. (2015). Inter- and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. *Neuroscience* 304, 266–278. doi: 10.1016/j.neuroscience.2015.07.043

Vernet, M., Bashir, S., Yoo, W. K., Oberman, L., Mizrahi, I., Ifert-Miller, F., et al. (2014). Reproducibility of the effects of theta burst stimulation on motor cortical plasticity in healthy participants. *Clin. Neurophysiol.* 125, 320–326. doi: 10.1016/j.clinph.2013.07.004

Vidal-Piñeiro, D., Martin-Trias, P., Arenaza-Urquijo, E. M., Sala-Llonch, R., Clemente, I. C., Mena-Sánchez, I., et al. (2014). Task-dependent activity and connectivity predict episodic memory network-based responses to brain stimulation in healthy aging. *Brain Stimul.* 7, 287–296. doi: 10.1016/j.brs.2013.12.016

Wang, L., Zang, Y., He, Y., Liang, M., Zhang, X., Tian, L., et al. (2006). Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. *Neuroimage* 31, 496–504. doi: 10.1016/j.neuroimage.2005.12.033

Ward, A., Tardiff, S., Dye, C., and Arrighi, H. M. (2013). Rate of conversion from prodromal Alzheimer's disease to Alzheimer's dementia: a systematic review of the literature. *Dement. Geriatr. Cogn. Dis. Extra* 3, 320–332. doi: 10.1159/000354370

Weber, M. J., Messing, S. B., Rao, H., Detre, J. A., and Thompson-Schill, S. L. (2014). Prefrontal transcranial direct current stimulation alters activation and connectivity in cortical and subcortical reward systems: a tDCS-fMRI study. *Hum. Brain Mapp.* 35, 3673–3686. doi: 10.1002/hbm.22429

Wecker, N. S., Kramer, J. H., Hallam, B. J., and Delis, D. C. (2005). Mental flexibility: age effects on switching. *Neuropsychology* 19, 345–352. doi: 10.1037/0894-4105.19.3.345

Weigand, A., Horn, A., Caballero, R., Cooke, D., Stern, A. P., Taylor, S. F., et al. (2017). Prospective validation that subgenual connectivity predicts antidepressant efficacy of transcranial magnetic stimulation sites. *Biol. Psychiatry*. doi: 10.1016/j.biopsych.2017.10.028. [Epub ahead of print].

Werhahn, K. J., Kunesch, E., Noachtar, S., Benecke, R., and Classen, J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J. Physiol.* 517, 591–597.

Wischnewski, M., and Schutter, D. J. (2015). Efficacy and time course of theta burst stimulation in healthy humans. *Brain Stimul.* 8, 685–692. doi: 10.1016/j.brs.2015.03.004

Woods, A. J., Antal, A., Bikson, M., Boggio, P. S., Brunoni, A. R., Celnik, P., et al. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin. Neurophysiol.* 127, 1031–1048. doi: 10.1016/j.clinph.2015.11.012

Woods, A. J., Cohen, R., Marsiske, M., Alexander, G. E., Czaja, S. J., and Wu, S. (2018). Augmenting cognitive training in older adults (The ACT Study): design and methods of a Phase III tDCS and cognitive training trial. *Contemp. Clin. Trials* 65, 19–32. doi: 10.1016/j.cct.2017.11.017

Woods, A. J., Hamilton, R. H., Kranjec, A., Minhaus, P., Bikson, M., Yu, J., et al. (2014). Space, time, and causality in the human brain. *Neuroimage* 92, 285–297. doi: 10.1016/j.neuroimage.2014.02.015

Young-Bernier, M., Davidson, P. S., and Tremblay, F. (2012). Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. *Neurobiol. Aging* 33, 835.e1–835.e11. doi: 10.1016/j.neurobiolaging.2011.08.012

Young-Bernier, M., Tanguay, A. N., Davidson, P. S., and Tremblay, F. (2014). Short-latency afferent inhibition is a poor predictor of individual susceptibility to rTMS-induced plasticity in the motor cortex of young and older adults. *Front. Aging Neurosci.* 6:182. doi: 10.3389/fnagi.2014.00182

Yun, K., Song, I. U., and Chung, Y. A. (2016). Changes in cerebral glucose metabolism after 3 weeks of noninvasive electrical stimulation of mild cognitive impairment patients. *Alzheimers Res. Ther.* 8:49. doi: 10.1186/s13195-016-0218-6

Zhao, J., Li, Z., Cong, Y., Zhang, J., Tan, M., Zhang, H., et al. (2017). Repetitive transcranial magnetic stimulation improves cognitive function of Alzheimer's disease patients. *Oncotarget* 8, 33864–33871. doi: 10.18632/oncotarget.13060

Conflict of Interest Statement: 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 Aging Neuroscience

REVIEW
published: 27 June 2019
doi: 10.3389/fnagi.2019.00145

Neuroimaging of Cerebral Small Vessel Disease and Age-Related Cognitive Changes

Michelle R. Caunca[1,2†], Andres De Leon-Benedetti[2†], Lawrence Latour[3], Richard Leigh[3] and Clinton B. Wright[3]*

[1]Division of Epidemiology and Population Health Sciences, Department of Public Health Sciences, Leonard M. Miller School of Medicine, Evelyn F. McKnight Brain Institute, University of Miami, Miami, FL, United States, [2]Department of Neurology, Leonard M. Miller School of Medicine, University of Miami, Miami, FL, United States, [3]National Institute of Neurological Diseases and Stroke (NINDS), National Institutes of Health, Bethesda, MD, United States

Subclinical cerebrovascular disease is frequently identified in neuroimaging studies and is thought to play a role in the pathogenesis of cognitive disorders. Identifying the etiologies of different types of lesions may help investigators differentiate between age-related and pathological cerebrovascular damage in cognitive aging. In this review article, we aim to describe the epidemiology and etiology of various brain magnetic resonance imaging (MRI) measures of vascular damage in cognitively normal, older adult populations. We focus here on population-based prospective cohort studies of cognitively unimpaired older adults, as well as discuss the heterogeneity of MRI findings and their relationships with cognition. This review article emphasizes the need for a better understanding of subclinical cerebrovascular disease in cognitively normal populations, in order to more effectively identify and prevent cognitive decline in our rapidly aging population.

Keywords: aging, neuroimaging, cognitive aging, brain MRI, cerebrovascular disease

OPEN ACCESS

Edited by:
P. Hemachandra Reddy, Texas Tech University Health Sciences Center, United States

Reviewed by:
Richard Camicioli, University of Alberta, Canada
Enrico Mossello, University of Florence, Italy

***Correspondence:**
Clinton B. Wright
clinton.wright@nih.gov

†Shared first authorship

Received: *25 January 2019*
Accepted: *31 May 2019*
Published: *27 June 2019*

Citation:
Caunca MR, De Leon-Benedetti A, Latour L, Leigh R and Wright CB (2019) Neuroimaging of Cerebral Small Vessel Disease and Age-Related Cognitive Changes. Front. Aging Neurosci. 11:145. doi: 10.3389/fnagi.2019.00145

INTRODUCTION

Recent advancements in medicine, global health, and biotechnology have led to prolonged life expectancy, and thus, a rapidly aging population (Ortman et al., 2014). As a result, the prevalence of age-related diseases has increased, including cardiometabolic disease that impacts cerebrovascular health. Subclinical cerebral small vessel disease (CSVD) has garnered attention in recent years and is strongly related to modifiable risk factors such as hypertension and diabetes, especially in midlife (Debette et al., 2011; Power et al., 2017). Managing cardiometabolic factors has become a priority in maintaining optimal brain health (Gorelick et al., 2017). Many studies examining the association between CSVD and cognitive aging have used cognitive impairment or dementia as outcomes. We can improve our understanding of cognitive aging by distinguishing between changes in magnetic resonance imaging (MRI) that are associated with normal vs. abnormal cognitive performance. Studying those who have remained cognitively intact in old age can provide insight into the prevention of significant cognitive decline and dementia.

Neuroimaging features of CSVD include small subcortical infarcts and lacunes, white matter hyperintensities (WMH) on MRI (hypodensities on computed tomography), prominent perivascular spaces, cerebral microbleeds (CMBs), disruption of the blood-brain barrier (BBB),

and diffusion-based markers of microstructural integrity (Alexander et al., 2007; Farrall and Wardlaw, 2009; Pantoni, 2010; Wardlaw et al., 2013b). These pathological entities form part of the American Heart Association (AHA)/American Stroke Association (ASA) definition of vascular contributions to cognitive impairment and dementia (VCID; Gorelick et al., 2011) and have been proposed as additions to recently published criteria for Alzheimer's disease (Sweeney et al., 2019). Though several markers of CSVD have been related to an increased risk of cognitive impairment (Vermeer et al., 2007; Taheri et al., 2011a; Debette et al., 2019), their natural course across the lifespan and impact on age-related, non-pathological cognitive decline, remains to be elucidated. Furthermore, these CSVD markers have heterogeneous etiologies.

Data from neuroepidemiologic studies can help us understand and quantify the public health burden of CSVD and provide results that are more generalizable and reproducible than those from small, non-representative, or clinical samples (Paus, 2010; Falk et al., 2013; Ganguli et al., 2018). The characterization of CSVD markers from brain imaging in large epidemiologic studies has increased in recent years due to the availability of standardized, valid, and reliable post-processing software. This review article summarizes evidence from epidemiologic studies that investigate the relationship of these CSVD markers to cognition in older adults. In order to better characterize the role of CSVD in age-related cognitive changes, we restricted our review to studies of non-demented older adults when discussing cognitive performance or decline as the outcome. When describing the general mechanisms or epidemiology of the CSVD marker, we included studies with cognitively mixed samples, especially for CSVD markers for which there is a dearth of literature. Terminology of most CSVD markers discussed is derived from the STRIVE panel (Wardlaw et al., 2013b), which aimed to achieve a consensus on image interpretation, acquisition, and reporting on markers of CSVD based on neuroimaging and pathological characteristics. For each CSVD marker, we review the definition, etiology, and epidemiology, as well as summarize the evidence of its relation to cognitive function in non-demented samples. The goal of this review article is to inform future research in age-related cognitive decline, better differentiate between normal and pathological brain aging, and generate hypotheses for how CSVD may contribute to normal and pathological cognitive aging.

WHITE MATTER HYPERINTENSITIES OF PRESUMED VASCULAR ORIGIN

Definition and Measurement

Historically, white matter lesions (WML) were first observed on CT and were named "leukoaraiosis" (Hachinski et al., 1987). Once the use of MRI became widespread, these lesions were better known as WMH, due to their hyperintense appearance on T2-weighted sequences (Wardlaw et al., 2013b). The addendum "of presumed vascular origin" is used to distinguish them from white matter (WM) abnormalities secondary to other causes, such as multiple sclerosis or the leukodystrophies (Wardlaw et al., 2013b).

In the research setting, lesion severity is generally characterized either semi-quantitatively by visual grading or quantitatively by volume (e.g., WMH volume or WMHV). Volumetric quantification of WMHs is more reliable and sensitive than visual rating scales of WM lesions (van den Heuvel et al., 2006b) and has become more common in recent years due to the increased availability of various automated and semi-automated software. These systems quantify WMHV based on the pixel intensity of a determined area on T2-weighted images in either an automated or semi-automated fashion (DeCarli et al., 1999; Jenkinson et al., 2012). The transverse component of the MR magnetization decays as a result of spin-spin interactions and is characterized by relaxation of the time-constant, T2. A number of pathological processes, including edema, gliosis, and demyelination, leads to an increase in T2 and a relative increase of the signal intensity in a T2-weighted image; the pathology appears "hyperintense" when compared to homologous healthy tissue. Because cerebral spinal fluid (CSF) also appears bright on a T2-weighted image, confounding the identification of lesions (such as at the boundaries of the ventricles), fluid attenuated inversion recovery (FLAIR) prepared imaging is often used to null the signal from CSF, leaving predominantly parenchyma.

Often, lesions are further divided into periventricular WMH (PWMH), located adjacent to the lateral ventricular wall, or deep WMH (DWMH), located in the immediate subcortical region (Fazekas et al., 1987). Some studies have shown high correlations between the two types suggesting this division is arbitrary (DeCarli et al., 2005). However, other studies have found differences in pathological correlates when comparing PWMH with DWMH (Shim et al., 2015), supporting the use of this categorization. In statistical analyses, WMHV is usually modeled as a proportion of total intracranial volume to account for individual differences in head size. Alternatively, intracranial volume can be treated as an additional covariate in multivariable analyses. To model WMH burden, categories of WMHV (e.g., quartiles) are often used to classify participants with extensive or large WMH burden, but to date these categories have not been translated into clinically meaningful amounts and radiologists generally read scans qualitatively when examining these changes.

Etiology and Pathological Correlates

WML are often ischemic in origin, developing as a consequence of arteriolosclerosis of the small arteries and arterioles that supply the WM, and damage to capillary beds and venous collagenosis has also been found (Moody et al., 1997; Pantoni, 2010; Wardlaw et al., 2015). Chronic ischemia results in vascular endothelial activation leading to the release of hypoxia-inducible factors, metalloproteinases (MMPs), and immunological mediators that cause hyaline wall thickening, smooth muscle cell loss, and narrowing of the vessel lumen (Fernando et al., 2006). These changes interfere with the auto-regulatory adaptations that would normally occur in response to attenuation of cerebral blood flow. Thus, focal

infarction (lacunes) or areas of demyelination result and are then observed as WMH on MRI. Pathological studies suggest that WMH are comprised of discontinuous ependyma and gliosis of WM fibers with demyelination and axonal damage (Fazekas et al., 1993).

There are several contributors to the age-related cerebral ischemia that underlie WMH. Vascular pathologies, such as venous collagenosis and arteriolar tortuosity due to hypertension, develop with age and may reduce blood flow to downstream small vessels, inducing a hypoxic environment (Brown et al., 2002). More directly, hypertension has been robustly associated with greater WML load (van Dijk et al., 2008; Gottesman et al., 2010; Debette et al., 2011; Godin et al., 2011; Hajjar et al., 2011; Marcus et al., 2011; Verhaaren et al., 2013), presumably through the induction of small vessel hypertensive vasculopathy and subsequent vascular remodeling (Laurent and Boutouyrie, 2015), especially in the periventricular region. Hypertension may also cause vascular injury that results in BBB dysfunction (see below), increasing vascular permeability and causing cerebral edema and activation of astrocytes in the WM (Wardlaw et al., 2003). Relevant to cognitive aging, Alzheimer's disease-related risk factors may also contribute to cerebral hypoperfusion. For example, epidemiologic evidence suggests that ApoE ε4 allele carriership and low serum amyloid levels are related to greater WML load (de Leeuw et al., 2004; Kaffashian et al., 2014). Low serum amyloid levels may reflect amyloid deposition in the cerebral vessel walls characteristic of cerebral amyloid angiopathy (CAA), which in turn reduces lumen diameter and diminishes blood flow (Bouras et al., 2006). Chronic ischemia alters mechanisms of perivascular lymphatic drainage of interstitial fluid, leading to the accumulation of amyloid and further exacerbating the hypoxic environment (Huang et al., 2010). More recent evidence from a smaller cognitively mixed study suggests that greater CSF $A\beta_{1-42}$ is associated with lower WMH burden (Al-Janabi et al., 2018). Similar trends have been observed in amyloid PET imaging studies (Goodheart et al., 2015), though this may differ by APOE ε4 allele status (Noh et al., 2014). In another smaller study of β-amyloid positive individuals (as detected by PET imaging), APOE ε2 allele carriers exhibited greater WM lesion load compared to APOE ε4 allele carriers (Groot et al., 2018). The exact relationship between neurodegenerative and cerebrovascular pathology is still unknown, but most recent studies among predemented individuals suggest that CSF $A\beta_{1-42}$ and WMH are associated with brain atrophy in an additive fashion (Bos et al., 2017).

In people with WMH, both BBB permeability and mean diffusivity are increased more than what would be expected by aging alone, even in areas of normal-appearing WM. The term WMH "penumbra" has been suggested to describe this, referring to decreased fractional anisotropy (FA) and higher mean diffusivity (MD) in apparently healthy tissues neighboring WMH (Maillard et al., 2011). Both FA and MD have been described as strong predictors of future stroke in healthy populations (Evans et al., 2016). This supports the notion that WMH are the most visible marker of a more diffuse process.

Epidemiology

The association between WMHs of presumed vascular origin and greater age has been well-documented (Brickman et al., 2008; Ikram et al., 2008; Morris et al., 2009; Chowdhury et al., 2011). Other demographic factors have also been examined with age adjusted in multivariable models, suggesting that factors such as race/ethnicity and sex explain variability in WMHs above and beyond age. For example, WMH volume or grade was reported to be greater in racial/ethnic minorities when compared with non-Hispanic Whites in diverse population-based studies (Brickman et al., 2008; Knopman et al., 2011). In contrast, the multi-ethnic Omni cohort of the Framingham Heart Study demonstrated decreased WMH volume compared with the mostly Caucasian FHS Offspring cohort, but this may be due to the younger mean age of the Omni cohort (Stavitsky et al., 2010). Some studies also found greater WM disease in women compared to men (Yue et al., 1997; de Leeuw et al., 2001; van Dijk et al., 2008), though the exact mechanism of this sex difference is unknown.

Cognition

Greater WML load has been related to worse general and domain-specific cognitive performance in community-based samples and several epidemiologic studies have specifically examined participants without cognitive impairment. Cross-sectional studies using data from non-demented participants and after adjusting for age have shown that greater WML load is associated with worse cognition (Koga et al., 2002; Au et al., 2006; Zhou et al., 2008; Godin et al., 2010; Vemuri et al., 2015), especially executive function (Lampe et al., 2019), processing speed (Au et al., 2006; van den Heuvel et al., 2006a; van Dijk et al., 2008; Raji et al., 2012; Knopman et al., 2015) and, to a lesser extent, episodic memory (Au et al., 2006). The associations with frontal lobe processes reflect damaged WM tracts relaying information to other parts of the brain.

Longitudinal analyses in non-demented people have also shown that those with greater WML load have significantly faster cognitive decline, similar in strength to the association of amyloid load to cognitive decline (Vemuri et al., 2015). Other longitudinal analyses have exhibited similar patterns, such that greater WML load is associated with greater cognitive decline, even after adjustment for age (Arvanitakis et al., 2016; Boyle et al., 2016).

Similar findings have also been demonstrated in cognitively normal participants who exhibit mild Parkinsonian signs (Camarda et al., 2018) and functional impairments (Murray et al., 2010; Wakefield et al., 2010; Dhamoon et al., 2018; Willey et al., 2018), further emphasizing the need to examine cognitively normal individuals who have other neurological deficits. Finally, region-specific WML load has also been examined in cognitively normal samples, and in particular, frontal lobe (Lampe et al., 2019) and periventricular (van den Heuvel et al., 2006a) WMLs are associated with worse cognitive performance. Generally, these associations are examined in multivariable models adjusted for age, suggesting that these associations are independent of aging.

Though these studies illustrate direct effects of WML load on cognitive performance, there are reports of indirect effects of WML load on cognition through markers of gray matter volume (Knopman et al., 2015; Rizvi et al., 2018). These data imply that cerebrovascular damage may cause degeneration of the surrounding brain parenchyma, potentially leading to cognitive impairment. However, the relationships between CSVD and neurodegeneration are complex and no consensus exists on whether the association is additive or synergistic.

LACUNES OF PRESUMED VASCULAR ORIGIN

Definition and Measurement

Lacunar infarcts are small subcortical infarcts that arise from occlusion of a single perforating cerebral artery (Fisher, 1982). Lacunar infarcts of presumed vascular origin have been further defined as round or ovoid cavities that are subcortical, fluid-filled, and measuring between 3 mm and about 15 mm in diameter (Wardlaw et al., 2013b). A peripheral hyperintense rim can often be observed on FLAIR sequences, although it is nonspecific and can also surround perivascular spaces (Wardlaw et al., 2013b). The distinction between lacunar infarcts and perivascular spaces is important; most studies utilize the minimal diameter of 3 mm to exclude small perivascular spaces. Studies also use an upper size limit of 15 mm, to differentiate lacunar infarcts from larger subcortical infarcts that reflect the involvement of more than one penetrating vessel (or cortical branch occlusions). This is due to tissue loss in older infarcts, and inflammation in the acute stage in the newer infarcts (Wardlaw et al., 2013b). Furthermore, many studies use the term "subclinical brain infarcts" or "silent brain infarcts," where lacunar infarcts are grouped together with cortical infarcts, ignoring their distinct etiologies. Typically, the associations with presence or absence of infarcts are studied, although the number and location are also considered.

In this review article, we consider studies that group together all brain infarcts and emphasize results related to lacunar infarcts when defined by the study. Though many studies group all brain infarcts together, most also report that the majority of these silent infarcts are lacunar in nature.

Etiology and Pathological Correlates

Pathologically, lacunar infarcts manifest as a result of lypohyalinosis and/or microatheroma of penetrating arteries, usually due to systemic hypertension or diabetes, and thus have a similar etiology to WML (Fisher, 1982; Prabhakaran et al., 2008; Knopman et al., 2011; Wardlaw et al., 2013b). Other mechanisms of disease include small emboli, atherosclerosis, and loss of endothelial integrity (Fisher, 1982; Wardlaw et al., 2003, 2009; Romero et al., 2009). Measures of subclinical carotid atherosclerosis have been associated with a higher incidence of lacunes (Brisset et al., 2013) and greater presence of subclinical brain infarcts (not excluding cortical infarcts; Romero et al., 2009; Caughey et al., 2018). Also, wider carotid lumen diameter has been related to a higher incidence of lacunes (Brisset et al., 2013), which may represent a compensatory response of the vessel to wall stiffness due to excessive extracellular matrix turnover, a lack of vascular smooth muscle cell proliferation, or apoptosis.

Epidemiology

The prevalence of lacunar infarcts varies between studies, but are generally associated with increasing age across all studies. The Cardiovascular Health Study (CHS) reported a 78.2% prevalence of infarct-like lesions in deep nuclear location, and a 10.1% prevalence in the posterior fossa (Bryan et al., 1997). The CHS has also reported incidence data on lacunar infarcts, finding that 59.8% of those with lacunes had a single lacune only, while 15.8% of those with infarcts had multiple lacunes only (Longstreth et al., 2002). The Northern Manhattan Study, consisting mostly of Hispanic/Latino participants, reported a prevalence of silent brain infarcts of 16%, and 82.9% were classified as subcortical (Prabhakaran et al., 2008; Wright et al., 2017). In the Rotterdam Study, the prevalence of silent brain infarcts (including cortical) was 7.2% (Vernooij et al., 2007), and of the 217 participants with silent brain infarcts, 202 had lacunar infarcts specifically (Vermeer et al., 2003). In aggregate, most subclinical brain infarcts are lacunar in nature.

Cognition

Lacunar infarcts have been associated with reduced cognitive performance in population-based cohort studies of elderly people, but few studies examined non-demented samples. Most studies examine these associations in multivariable models adjusting for age, so associations observed are independent of aging. A Japanese-based study found that multiple lacunar infarcts were associated with frontal lobe dysfunction specifically (Koga et al., 2009), which is consistent with a US-based cohort study (Knopman et al., 2015). The Rotterdam Study found that silent thalamic infarcts were specifically associated with steeper declines in memory performance, while nonthalamic infarcts were related to steeper declines in psychomotor speed (Vermeer et al., 2003). However, these studies are limited in terms of racial and ethnic diversity, which decreases their generalizability to the U.S. Overall, lacunar infarcts appear to be an important contributor to vascular cognitive impairment in population-based elderly cohorts, but more work is warranted to examine cognitive performance in non-demented elderly, especially in racial/ethnic minorities who are at higher risk for developing dementia and vascular disease compared to non-Hispanic whites (Mayeda et al., 2016; Benjamin E. J. et al., 2018).

PERIVASCULAR SPACES

Definition and Measurement

Also called Virchow-Robin spaces, perivascular spaces are extensions of the extracerebral fluid that surround small arteries and arterioles as they perforate the brain surface (Groeschel et al., 2006; Wardlaw et al., 2013b). They are not seen on conventional neuroimaging when small. However, the greater the resolution MRI is acquired, the more evident and more numerous they appear to be. Larger spaces located at the base

of the brain become increasingly apparent with aging (Groeschel et al., 2006; Wardlaw et al., 2013b). The STRIVE panel defines dilated perivascular spaces as fluid-filled spaces that follow the typical course of a vessel as it travels through the gray or WM (Wardlaw et al., 2013b). They can be confused for lacunar infarcts, and therefore, their diameter should be less than 3 mm when imaged perpendicular to the course of the vessel (Wardlaw et al., 2013b). Perivascular spaces tend to be more prominent in the inferior basal ganglia area, where they can reach up to 20 mm, even causing some mass effect (Wardlaw et al., 2013b). A recognized lesion known as the infraputaminal lacune is an enlarged perivascular space in the subinsular region and can be mistaken for lacunar infarction, but pathological data have shown it to be of non-vascular origin (Pullicino et al., 1995). Measurement of perivascular spaces also heavily depends on MRI resolution, and therefore, estimates may widely vary depending on the strength of MRI used (De Guio et al., 2016). The relevance of perivascular spaces as an MRI marker of cognitive aging remains understudied. Researchers recently attempted to establish uniform criteria for identifying perivascular spaces through population-based studies (Adams et al., 2015). As with other CVSD markers, the presence, as well as the location of these spaces, are important classifiers for research, and it is hoped this biomarker will be utilized in clinical practice. Data on perivascular spaces in large population-based studies is extremely limited, especially in non-demented samples.

Etiology and Pathological Correlates

Dilated perivascular spaces are thought to be caused by increased fluid exudation due to greater vascular permeability, obstruction of the lymphatic drainage system, or parenchymal atrophy (Groeschel et al., 2006; Adams et al., 2015; Ramirez et al., 2016). They have been pathologically correlated with pro-oxidative enzyme activation and complement activation in CAA (van Veluw et al., 2016), a vascular entity highly related to Alzheimer's dementia.

The cause of dilated perivascular spaces is still under debate. They have been associated with other vascular risk factors for CSVD, such as elevated blood pressure (Klarenbeek et al., 2013; Yakushiji et al., 2014; Yao et al., 2014; Gutierrez et al., 2015) and large-vessel abnormalities (Gutierrez et al., 2013; Riba-Llena et al., 2018), however these results are not consistent in the literature (Bouvy et al., 2016). Thus although perivascular spaces seem to share similar risk factors as other measures of CSVD, more research is warranted.

Epidemiology

Few population-based, epidemiological cohort studies have explored the prevalence or incidence of perivascular spaces, and moreover, these studies differ in terms of detection methods and data reported. However, across all studies, the load of perivascular spaces is significantly associated with greater age. The 3C-Dijon study reported that 28.5% of their non-demented and stroke-free cohort had 1–2 hippocampal dilated perivascular spaces, while 16% had >2 hippocampal dilated perivascular spaces (Yao et al., 2014). The Northern Manhattan Study, a mostly Hispanic/Latino elderly cohort, reported that 48% of their stroke-free sample had a perivascular spaces score of >4, which represents greater brain involvement (Gutierrez et al., 2013). A Japanese study of a neurologically healthy cohort reported prevalence rates for dilated perivascular spaces located in the basal ganglia and the centrum semiovale. It found that in the basal ganglia about 80% of their participants had mild, 11% had moderate, and about 3% had frequent or severe perivascular spaces. In the centrum semiovale, about 26% had mild, 51% had moderate, and 23% had frequent or severe perivascular spaces (Yakushiji et al., 2014). Overall, the reported prevalence varies tremendously in the literature, which is due in part to the lack of large, epidemiologic cohorts measuring this vascular entity in diverse populations.

Cognition

The association between perivascular spaces and cognitive performance in the elderly has yet to be completely explored, and data in non-demented samples is especially limited. The results from studies of both population- and clinic-based samples are mixed. In the Age, Gene/Environment, Susceptibility-Reykjavik Study, dilated perivascular spaces were related to faster cognitive decline, particularly in processing speed (Ding et al., 2017a). In the community-based 3C-Dijon MRI Study of dementia- and stroke-free participants, dilated perivascular spaces were not related to cognitive performance (Yao et al., 2014). These findings are consistent with some smaller studies of clinical populations with cognitively mixed participants (Benjamin P. et al., 2018), but inconsistent with others (Huijts et al., 2014). Though not an epidemiologic sample, a small study of healthy older men found that dilated perivascular spaces were associated with worse cognitive function (Maclullich et al., 2004). Further research in larger cohorts is needed to understand the etiology of perivascular spaces and if they contribute uniquely to vascular cognitive impairment.

CEREBRAL MICROBLEEDS

Definition and Measurement

MRI techniques that detect magnetic susceptibility allowed the recognition of CMBs, and this has improved as the technology has advanced (Greenberg et al., 2009). CMBs are defined as small round foci of signal void on T2*-weighted imaging measuring generally 2–5 mm in diameter, but sometimes up to 10 mm (Wardlaw et al., 2013b). Differences in the bulk magnetic susceptibility of tissue give rise to inhomogeneities in the magnetic field produced by the MR system. This results in an apparent decay of transverse magnetization (T2*) at a rate faster than that of spin-spin interactions (T2). Oxyhemoglobin is slightly diamagnetic and very similar to the neuropil, whereas deoxyhemoglobin, methemoglobin, and hemosiderin are paramagnetic owing to unpaired electrons. Red blood cells containing hemoglobin extravasate and undergo degradation in the perivascular space and parenchyma. The hemoglobin shifts the magnetic field over distances much greater than the underlying microscopic pathology, and in a sense, amplifies the effect to allow

visualization in an MR image sensitized to T2*. Sequences such as gradient recalled echo (GRE), susceptibility weighted imaging (SWI), or practically any echo-planar weighted image such as diffusion weighted imaging (DWI) or dynamic susceptibility contrast (DSC) perfusion weighted imaging suffer from signal drop out in regions containing the blood degradation products.

Although initially associated with the presence of a concomitant lobar hemorrhage, they have also been detected in community-based samples. They are related to bleeding-prone microangiopathy of different origins, with the imaging findings corresponding to hemosiderin-laden macrophages (Fazekas et al., 1999; Shoamanesh et al., 2011). Hemosiderin is a blood breakdown product that causes magnetic susceptibility-induced dephasing, or loss of orientation of the nuclei due to relaxation of the signal, leading to T2* signal loss (Viswanathan and Greenberg, 2011).

The location of CMBs differ by etiology, with a cortical (or lobar) location suggesting amyloid angiopathy, and deep locations (i.e., in the basal ganglia or thalamus) suggesting hypertensive vasculopathy (Knudsen et al., 2001; Greenberg et al., 2009). CAA is diagnosed and subtyped using the Boston criteria, which considers clinical and pathological data for diagnosis and includes the identification of microbleeds (Knudsen et al., 2001). Additionally, many of these studies also examine the number of CMBs present, though estimates for these analyses become less precise as the number of participants with >1 CMB is usually low.

Differences in MRI techniques and strength continue to be an issue in detecting CMB prevalence and incidence. The recent introduction of SWI into clinical practice has further improved the quantification of CMBs, but make it difficult to compare studies that use different techniques. SWI uses additional spin phase data from the MRI acquisition to enhance the appearance of CMBs and remove venous artifacts (Haacke et al., 2009). This can lead to an increase in the burden of CMBs detected when compared with traditional GRE imaging. **Figure 1** shows how when a GRE image is adapted into an SWI, hemosiderin deposition becomes more evident and the number of CMBs appear to increase. Further, definitions of CMBs have varied over time and standardization of the imaging definition will be important for future studies.

Etiology and Pathological Correlates

Many processes that weaken cerebrovascular integrity, and therefore increase the risk of bleeding, have been associated with CMBs. Common with other CSVD entities, elevated blood pressure is associated with CMBs, especially in deep locations, since deep CMBs are usually caused by hypertensive vasculopathy (Roob et al., 1999; Vernooij et al., 2008; Poels et al., 2010, 2011; Romero et al., 2014; Wiegman et al., 2014; Akoudad et al., 2015; Del Brutto et al., 2015). Further, systemic vasculopathies related to hypertension, including carotid stenosis, carotid intima-media thickness, and arterial stiffness, have also been associated with CMB presence (Poels et al., 2012b; Romero et al., 2016).

The other important etiology of CMBs is CAA, especially when CMBs are exclusively lobar or cortical. The APOE ε4 allele, a factor strongly related to amyloid angiopathy (Caselli et al., 2010; Tai et al., 2016), has been related to the increased prevalence and incidence of lobar location of CMB in several studies (Vernooij et al., 2008; Poels et al., 2010, 2011; Romero et al., 2014; Graff-Radford et al., 2017). Similar to CAA hemorrhages, lobar CMB tends to occur more frequently in the temporal lobe (Mesker et al., 2011). Additionally, APOE alleles ε4 and ε2 are more strongly related to clustering of lobar CMB when compared to carriers of the ε3 allele (Loehrer et al., 2014). Despite evidence for two separate etiologies, mixed pathology has also been described in several of these studies, which is not surprising given the older age of these participants. CMBs do not yet have diagnostic value with regard to CAA or hypertensive vasculopathy (Martinez-Ramirez et al., 2015).

Finally, systemic inflammation has also been suggested as a mechanism for CMB due to the microglial mediated clearance of blood products post-bleed that results in a detectable hypointense lesion on MRI. Romero et al. (2012) found that in subjects with at least 1 APOE ε2 or ε4 allele, high LpPLA2 levels, a known marker of vascular inflammation, was associated with deep CMB. In a subsequent report of the same cohort, CMB presence was associated with elevated levels of circulating tumor necrosis factor receptor 2 (TNFR2) and myeloperoxidase (Shoamanesh et al., 2015).

Epidemiology

Several epidemiologic studies have estimated the prevalence of CMBs in the general population, as well as its relation to age and vascular risk factors. Generally, greater age is related to greater odds of having a CMB. Prevalence rates differ widely across reports and range between 4.7% and 24.4% (Roob et al., 1999; Jeerakathil et al., 2004; Vernooij et al., 2008; Poels et al., 2010, 2011; Wiegman et al., 2014; Akoudad et al., 2015; Del Brutto et al., 2015; Caunca et al., 2016; Ding et al., 2017b; Graff-Radford et al., 2017; van Leijsen et al., 2017) due to the heterogeneity in the ages of participants, MRI techniques, and CMB definitions. For example, the Framingham Study initially found a prevalence of CMB of 4.7% (Jeerakathil et al., 2004). In a subsequent report, the prevalence for CMB was 8.8%, almost double their previous report, despite the mean age being similar (Romero et al., 2014). The difference in prevalence may be explained by a difference in MR strength (1.0T vs. 1.5T) since increased magnetic field strength enhances CMB detection (Scheid et al., 2007; Stehling et al., 2008). In a more diverse sample, the Northern Manhattan Study found a prevalence rate of 5% for CMBs (Caunca et al., 2016). The Washington Heights/Inwood Columbia Aging Project, also based in Northern Manhattan, found a prevalence of 27%; however, their study used stratified sampling based on Medicare enrollees (aged 65 and older), while the Northern Manhattan Study randomly sampled a younger population (aged 50 and older; Wiegman et al., 2014). Further, the former study had a smaller sample size than the latter, and CMB detection methods differed as well. In contrast, the Rotterdam Study has reported a prevalence of CMBs ranging from 15.3% to 28%

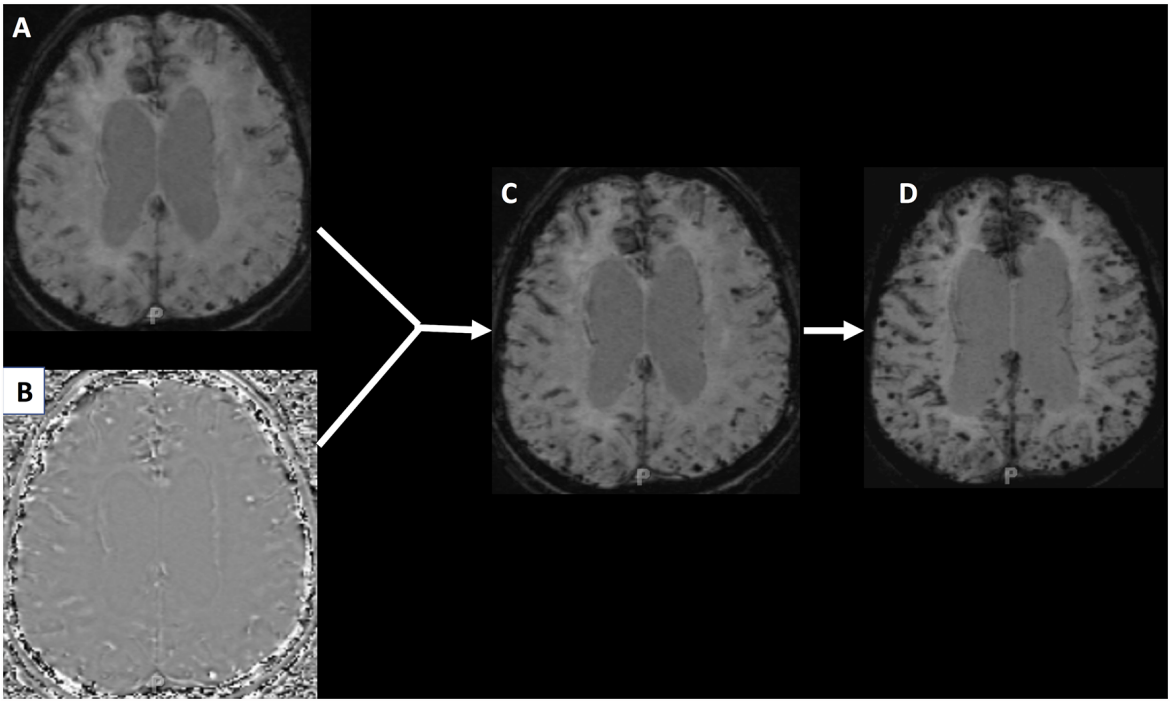


FIGURE 1 | Panel **(A)** shows a traditional gradient recalled echo (GRE) image of a patient with hemosiderin deposition. Panel **(B)** is a phase image that when manipulated and integrated with the GRE produces the susceptibility weighted image (SWI) seen in panel **(C)**. Panel **(D)** is a minimum intensity projection (MIP) image that makes the cerebral microbleeds (CMBs) even more conspicuous.

across several years (Vernooij et al., 2008; Poels et al., 2010, 2011). Similarly, the Atherosclerosis Risk in Communities Study found an overall prevalence of 24% in their biracial cohort using a 3T-strength MR machine (Graff-Radford et al., 2017). Generally, CMBs are more commonly located in lobar vs. deep locations (Jeerakathil et al., 2004; Caunca et al., 2016; Ding et al., 2017b; Graff-Radford et al., 2017), especially in the temporal lobe (Mesker et al., 2011). The incidence of CMBs also varies, but prospective studies in community-based populations are limited. The Rotterdam Study found a 3-year incidence rate of 10% for new CMBs (Poels et al., 2011), while the RUN DMC cohort exhibited an annual 2.2% incidence rate for CMBs over a 9-year period (van Leijsen et al., 2017). In an Ecuadorian, rural sample, the prevalence of CMBs was 11% (Del Brutto et al., 2015). In the Age, Gene/Environment Susceptibility-Reykjavik Study, the prevalence of CMBs was 16.8% (Ding et al., 2017b). Chinese population-based studies have found 10.1% (Han et al., 2018) and 32.3% (Hilal et al., 2014) of CMBs, respectively.

Cognition

The presence of one or more CMBs has been associated with decreased global cognitive performance, independent of age, but there is relatively limited evidence in non-demented, epidemiologic samples (Poels et al., 2012a; Ding et al., 2017b). Domain-specific cognitive performance has also been related to CMBs in non-demented samples (Poels et al., 2012a; Meier et al., 2014; Akoudad et al., 2016; Paradise et al., 2019), but results are mixed in terms of which CMB location is related to which cognitive domain. Overall, CMBs, especially lobar CMBs, appear to affect mostly executive function processes, which is consistent with the clinical presentation of vascular cognitive impairment.

BLOOD-BRAIN BARRIER DISRUPTION

Definition and Measurement

The BBB is a dynamic interface between the cerebral circulation and the central nervous system that limits and regulates the movement of cells and molecules between these two spaces *via* an interdependent network of cells and cell structures (Sandoval and Witt, 2008). An intact BBB generally acts to protect the brain; however, facilitated permeability of the BBB also plays an important role in healthy brain physiology, such as during sleep that controls the opening of the BBB (Pan and Kastin, 2017).

Gadolinium-enhanced MRI is the modality most commonly used to assess BBB integrity. Gadolinium based contrast agents (GBCAs) administered intravenously do not normally cross an intact BBB. When present, GBCAs dramatically shorten the T1 and T2 relaxation time-constants providing a mechanism to "enhance" the signal in regions where the BBB is compromised. Clearance of GBCAs is through the kidneys and most agents have a half-life in the vasculature of a couple of hours.

The typical clinical assessment of the BBB involves T1-weighted imaging before and after administration of

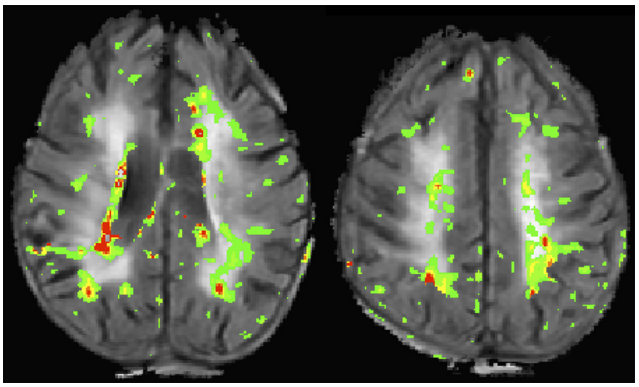


FIGURE 2 | This image shows two slices from a flair magnetic resonance imaging (MRI) of a patient with confluent white matter hyperintensities (WMH). A blood-brain barrier (BBB) permeability heatmap derived from a dynamic susceptibility contrast (DSC) sequence has been superimposed in color. Increasing BBB permeability follows the color sequence green, yellow, orange, red.

GBCAs. This approach is able to detect overt disruption of the BBB such as with brain tumors or multiple sclerosis, however, it is insensitive to more subtle gadolinium leakage. The most commonly used research tool for measuring BBB permeability is dynamic contrast enhanced (DCE) MRI which uses serial T1-weighted imaging to measure the transfer constant (Ktrans) during steady state. A more pragmatic approach uses DSC imaging, which is a modality commonly acquired for perfusion imaging in the clinical setting. Both DCE and DSC have been used to measure BBB disruption associated with CSVD (Taheri et al., 2011b; Arba et al., 2017). **Figure 2** shows an example of a BBB permeability heatmap derived from a DSC sequence superimposed on a FLAIR sequence for a patient with confluent WMH.

Etiology and Pathological Correlates

Dysfunction or disruption of the BBB is known to be associated with many diseases of the central nervous system (Weiss et al., 2009). BBB permeability also increases with normal aging but is accelerated in patients with WMH (Farrall and Wardlaw, 2009). White matter BBB disruption has been identified in patients suffering from lacunar stroke (Wardlaw et al., 2009), and vascular cognitive impairment (Taheri et al., 2011a). In patients with a history of lacunar stroke, BBB disruption is known to occur not only within the WMH but also in the normal appearing white matter (NAWM; Topakian et al., 2010). The pattern of WMH-associated BBB disruption tends to be located around the edges of WMH, stretching into the NAWM (Rosenberg et al., 2014; Huisa et al., 2015). It has been hypothesized, but not yet proven, that BBB disruption in the NAWM precedes the development of WMH (Wardlaw et al., 2013a).

BBB disruption has been suggested as an early stage in the cascade of events that leads to the development of WML (Rosenberg et al., 2016). Areas of the brain susceptible to intermittent hypoxia with reduced cerebrovascular autoregulation experience activation of the MMPs resulting in degradation of the endothelial basil lamina and tight junction proteins, opening the BBB. This hypoxic pro-inflammatory environment leads to the accumulation of free radicals and proteases resulting in myelin degradation through a non-immune mediated inflammatory demyelination (Rosenberg et al., 2016).

Epidemiology

Most studies looking at the relationship between BBB disruption and CSVD have been performed in patients who were identified due to the occurrence of an acute ischemic event. An analysis of an imaging data registry of stroke patients found that an increasing burden of traditional biomarkers for CSVD (WML, CMB, etc.) was associated with an increase in measures of BBB disruption both in the region of the ischemic event as well as in areas remote from the infarction (Arba et al., 2017). Another analysis of stroke patients with WML found a dissociation between the traditional CSVD risk factor of hypertension and the occurrence of BBB disruption (Gupta et al., 2018).

The incidence and prevalence of BBB disruption in the general population of patients with subclinical CSVD have not been examined in large population-based studies to date. BBB disruption detected at the time of a stroke can persist for months after the initial event, suggesting that it is a chronic process rather than merely a manifestation of the acute event. In a small study of patients with known vascular dementia, BBB disruption was present on serial imaging and appeared to migrate over time (Huisa et al., 2015). Recent data from clinical samples also seems to suggest that MRI measures of BBB disruption are not related to age (Nation et al., 2019), but further studies in larger, population-based samples are needed to explore age-related associations.

Cognition

Much of the research looking at BBB disruption has been focused on its relationship to the development of WML. It is possible that BBB disruption precedes the conversion of NAWM to WML. Since progressive WMLs are associated with cognitive decline, it is hypothesized that BBB disruption may be the earliest biomarker for identifying at-risk patients. This hypothesis is supported by one study that found BBB disruption preceded the development of post-stroke cognitive decline (Wardlaw et al., 2017). More recently, a smaller clinically-based study was conducted showing that BBB disruption was related to cognitive dysfunction, independent of Alzheimer's Disease imaging biomarkers as well as after adjustment for age (Nation et al., 2019). Participants in this study were selected from Alzheimer's Disease Research Centers, and included participants with early cognitive dysfunction. Thus, more work needs to be done in larger, representative samples to improve both internal and external validity.

DIFFUSION TENSOR IMAGING

Definition and Measurement

Diffusion tensor imaging (DTI) allows for 3-dimensional measurement of the displacement of water molecules within

tissues as a result of Brownian motion caused by heat (Basser et al., 1994; Basser and Pierpaoli, 1996; Alexander et al., 2007). Water diffusing in tissue interacts with cell membranes and other microstructural features that infer with, and decrease, the net displacement in comparison to that of bulk fluid. Some of these features, such fiber tracts formed by axons, have coherent structural orientation which in turn is reflected in the net displacement measured by DTI. The magnitude and the directional dependency can be decomposed and used from the DTI data to infer the structural integrity of the tissue.

Commonly, two measures of WM microstructural integrity are used: the MD and FA. The MD reflects the overall diffusion within a voxel or region of interest, with greater MD indicating more microstructural integrity loss (Soares et al., 2013). On the other hand, the FA reflects the degree of anisotropy (ranges from 0 to 1) or restriction of the water diffusion in a single direction. Values of FA approaching 1 indicate that the water diffusion is occurring in a single direction, which indicates better microstructural integrity (Soares et al., 2013).

Etiology and Pathological Correlates

Mechanisms of microstructural WM integrity loss are still being elucidated, and the etiology of WM microstructural integrity loss is multifactorial. Generally, vascular risk factors are strongly related. In a middle-aged, biracial sample, hypertension and greater time spent in sedentary activities were related to lower FA (Launer et al., 2015). Similarly, greater systolic blood pressure was related to decreased FA and increased MD, especially in the anterior corpus callosum and inferior fronto-occipital fasciculus in a mostly White sample (Maillard et al., 2012). More recently, studies have shown that both midlife and late-life vascular risk factors were related to worse microstructural integrity among older adults (Wang et al., 2015; Power et al., 2017). Relatedly, behavioral risk factors, such as smoking and diet, are also related to WM microstructural integrity (Gons et al., 2011; Launer et al., 2015; Gu et al., 2016). Finally, serum measures of systemic inflammation have also been associated with lower FA and greater MD, suggesting a potential inflammatory mechanism for microstructural integrity loss (Walker et al., 2017, 2018).

Studies have shown that non-demented APOE ε4 allele carriers exhibit increased MD, suggesting that the APOE ε4 allele may affect cerebral lipid metabolism and subsequently the integrity of the myelin of these WM tracts (Kljajevic et al., 2014; Operto et al., 2018). In a smaller study of cognitively normal participants from AD Research Centers, a greater phosphorylated tau-Aβ42 ratio was related to higher MD (Racine et al., 2019). These data are also consistent with the idea that microstructural integrity loss might be an earlier marker of pathological brain aging than other markers of CSVD.

Microstructural WM integrity has been studied in the context of WM lesions as seen on FLAIR images. Generally, in cognitively normal samples, greater WMHV is related to lower FA (Seiler et al., 2018). Importantly, WM microstructural integrity loss has been observed outside WMHs in aging populations, suggesting that WMHs represent the most severe damage to the WM, but WM injury continues outside these lesions (Maillard et al., 2011, 2014).

Epidemiology

In a sample of participants with ages ranging across the life-course, non-linear associations observed between WM microstructural integrity and age were observed, highlighting the need to consider changes in WM integrity in the context of different age spans (Slater et al., 2019). Similarly, in a sample of aging adults (age range 46–100 years), non-linear associations of FA and MD were observed with increasing age (Vinke et al., 2018). Prospective cohort studies have observed decreasing FA and increasing MD over 2-year follow-up period among older adults with an average age of about 70 years (de Groot et al., 2016). Among non-demented oldest-old (i.e., aged 90 years or older), age-related changes in WM microstructural integrity (as measured by MD and FA) has been observed in regions important to dementia risk (Bennett et al., 2017). In younger samples, greater age was also related to lower FA and greater MD (Maillard et al., 2012; Launer et al., 2015).

Cognition

Generally, studies have observed that greater MD and lower FA were related to faster decline in global cognition among non-demented older adults (Tuladhar et al., 2015; Wang et al., 2015), especially among APOE ε4 allele carriers (Wang et al., 2015). Domain-specific associations have also been observed in a prospective cohort studies of non-demented and stroke-free older adults, where both global and tract-specific FA and greater MD was related to worse processing speed, executive function, and motor speed (Vernooij et al., 2009; Cremers et al., 2016; Seiler et al., 2018). Others have found similar associations and suggest that the effect of upstream factors, such as diet, on cognitive decline may be mediated by WM microstructural integrity (Gu et al., 2016). However, more work needs to be done, especially in epidemiologic studies.

CONCLUSION

In summary, most CSVD markers, especially WM lesion load, subclinical infarcts, and diffusion measures, are related to cognitive decline independent of age. More research needs to be done to examine age-related cognitive changes in relation to BBB disruption and changes in diffusion-based markers of microstructural integrity. Understanding the relationship between normal aging and cognitive decline is a challenging endeavor. However, MRI markers of CSVD may offer a critical clue to understanding this relationship. Large, epidemiological neuroimaging studies have the potential to provide valuable information to guide research. The use of population-based cohorts ensures generalizability and affords the opportunity to study a variety of risk factors and outcomes related to neuroimaging markers. Despite the significant work that has been done with established MRI markers, further epidemiological research is warranted in understudied markers, such as perivascular spaces and BBB disruption. While work in aging populations can inform researchers and clinicians of the prevalence and incidence of these markers in age-related cognitive changes, future studies should

focus carefully on excluding cognitively abnormal participants particularly in studies that use cognitive performance as an outcome. This will facilitate the study of how these MRI markers relate to cognitive impairment in the context of normal aging.

AUTHOR CONTRIBUTIONS

CW contributed to the conception of this review article. MC and AL-B conducted the literature review and wrote the first draft of the manuscript. LL and RL wrote sections of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

FUNDING

This work was funded by the National Institute of Neurological Disease and Stroke (NINDS, F30NS103462), the Evelyn F. McKnight Brain Institute, and the Intramural Research Program at NINDS.

REFERENCES

Adams, H. H., Hilal, S., Schwingenschuh, P., Wittfeld, K., van der Lee, S. J., DeCarli, C., et al. (2015). *A priori* collaboration in population imaging: the uniform neuro-imaging of virchow-robin spaces enlargement consortium. *Alzheimers Dement.* 1, 513–520. doi: 10.1016/j.dadm.2015.10.004

Akoudad, S., Portegies, M. L., Koudstaal, P. J., Hofman, A., van der Lugt, A., Ikram, M. A., et al. (2015). Cerebral microbleeds are associated with an increased risk of stroke: the rotterdam study. *Circulation* 132, 509–516. doi: 10.1161/circulationaha.115.016261

Akoudad, S., Wolters, F. J., Viswanathan, A., de Bruijn, R. F., van der Lugt, A., Hofman, A., et al. (2016). Association of cerebral microbleeds with cognitive decline and dementia. *JAMA Neurol.* 73, 934–943. doi: 10.1001/jamaneurol.2016.1017

Alexander, A. L., Lee, J. E., Lazar, M., and Field, A. S. (2007). Diffusion tensor imaging of the brain. *Neurotherapeutics* 4, 316–329. doi: 10.1016/j.nurt.2007.05.011

Al-Janabi, O. M., Brown, C. A., Bahrani, A. A., Abner, E. L., Barber, J. M., Gold, B. T., et al. (2018). Distinct white matter changes associated with cerebrospinal fluid amyloid-β1–42 and hypertension. *J. Alzheimers Dis.* 66, 1095–1104. doi: 10.3233/jad-180663

Arba, F., Leigh, R., Inzitari, D., Warach, S., Luby, M., and Lees, K. R. (2017). Blood brain barrier leakage increases with small vessel disease in acute ischemic stroke. *Neurology* 89, 2143–2150. doi: 10.1212/wnl.0000000000004677

Arvanitakis, Z., Fleischman, D. A., Arfanakis, K., Leurgans, S. E., Barnes, L. L., and Bennett, D. A. (2016). Association of white matter hyperintensities and gray matter volume with cognition in older individuals without cognitive impairment. *Brain Struct. Funct.* 221, 2135–2146. doi: 10.1007/s00429-015-1034-7

Au, R., Massaro, J. M., Wolf, P. A., Young, M. E., Beiser, A., Seshadri, S., et al. (2006). Association of white matter hyperintensity volume with decreased cognitive functioning: the Framingham Heart Study. *Arch. Neurol.* 63, 246–250. doi: 10.1001/archneur.63.2.246

Basser, P. J., and Pierpaoli, C. (1996). Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J. Magn. Reson. B* 111, 209–219. doi: 10.1006/jmrb.1996.0086

Basser, P. J., Mattiello, J., and LeBihan, D. (1994). MR diffusion tensor spectroscopy and imaging. *Biophys. J.* 66, 259–267. doi: 10.1016/S0006-3495(94)80775-1

Benjamin, P., Trippier, S., Lawrence, A. J., Lambert, C., Zeestraten, E., Williams, O. A., et al. (2018). Lacunar Infarcts, but not perivascular spaces, are predictors of cognitive decline in cerebral small-vessel disease. *Stroke* 49, 586–593. doi: 10.1161/STROKEAHA.117.017526

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., et al. (2018). Heart disease and stroke statistics-2018 update: a report from the american heart association. *Circulation* 137, e67–e492. doi: 10.1161/CIR.0000000000000558

Bennett, I. J., Greenia, D. E., Maillard, P., Sajjadi, S. A., DeCarli, C., Corrada, M. M., et al. (2017). Age-related white matter integrity differences in oldest-old without dementia. *Neurobiol. Aging* 56, 108–114. doi: 10.1016/j.neurobiolaging.2017.04.013

Bos, I., Verhey, F. R., Ramakers, I., Jacobs, H. I. L., Soininen, H., Freund-Levi, Y., et al. (2017). Cerebrovascular and amyloid pathology in predementia stages: the relationship with neurodegeneration and cognitive decline. *Alzheimers Res. Ther.* 9:101. doi: 10.1186/s13195-017-0328-9

Bouras, C., Kövari, E., Herrmann, F. R., Rivara, C. B., Bailey, T. L., von Gunten, A., et al. (2006). Stereologic analysis of microvascular morphology in the elderly: Alzheimer disease pathology and cognitive status. *J. Neuropathol. Exp. Neurol.* 65, 235–244. doi: 10.1097/01.jnen.0000203077.53080.2c

Bouvy, W. H., Zwanenburg, J. J., Reinink, R., Wisse, L. E., Luijten, P. R., Kappelle, L. J., et al. (2016). Perivascular spaces on 7 Tesla brain MRI are related to markers of small vessel disease but not to age or cardiovascular risk factors. *J. Cereb. Blood Flow Metab.* 36, 1708–1717. doi: 10.1177/0271678x16648970

Boyle, P. A., Yu, L., Fleischman, D. A., Leurgans, S., Yang, J., Wilson, R. S., et al. (2016). White matter hyperintensities, incident mild cognitive impairment and cognitive decline in old age. *Ann. Clin. Transl. Neurol.* 3, 791–800. doi: 10.1002/acn3.343

Brickman, A. M., Schupf, N., Manly, J. J., Luchsinger, J. A., Andrews, H., Tang, M. X., et al. (2008). Brain morphology in older African Americans, Caribbean Hispanics and whites from northern Manhattan. *Arch. Neurol.* 65, 1053–1061. doi: 10.1001/archneur.65.8.1053

Brisset, M., Boutouyrie, P., Pico, F., Zhu, Y., Zureik, M., Schilling, S., et al. (2013). Large-vessel correlates of cerebral small-vessel disease. *Neurology* 80, 662–669. doi: 10.1212/WNL.0b013e318281ccc2

Brown, W. R., Moody, D. M., Challa, V. R., Thore, C. R., and Anstrom, J. A. (2002). Venous collagenosis and arteriolar tortuosity in leukoaraiosis. *J. Neurol. Sci.* 203–204, 159–163. doi: 10.1016/s0022-510x(02)00283-6

Bryan, R. N., Wells, S. W., Miller, T. J., Elster, A. D., Jungreis, C. A., Poirier, V. C., et al. (1997). Infarctlike lesions in the brain: prevalence and anatomic characteristics at MR imaging of the elderly—data from the Cardiovascular Health Study. *Radiology* 202, 47–54. doi: 10.1148/radiology.202.1.8988191

Camarda, C., Torelli, P., Pipia, C., Battaglini, I., Azzarello, D., Rosano, R., et al. (2018). Association between atrophy of the caudate nuclei, global brain atrophy, cerebral small vessel disease and mild parkinsonian signs in neurologically and cognitively healthy subjects aged 45–84 years: A crosssectional study. *Curr. Alzheimer Res.* 15, 1013–1026. doi: 10.2174/1567205015666180702111110

Caselli, R. J., Walker, D., Sue, L., Sabbagh, M., and Beach, T. (2010). Amyloid load in nondemented brains correlates with APOE ε4. *Neurosci. Lett.* 473, 168–171. doi: 10.1016/j.neulet.2010.02.016

Caughey, M. C., Qiao, Y., Windham, B. G., Gottesman, R. F., Mosley, T. H. and Wasserman, B. A.. (2018). Carotid Intima-media thickness and silent brain infarctions in a biracial cohort: the atherosclerosis risk in communities (ARIC) study. *Am. J. Hypertens.* 31, 869–875. doi: 10.1093/ajh/hpy022

Caunca, M. R., Del Brutto, V., Gardener, H., Shah, N., Dequatre-Ponchelle, N., Cheung, Y. K., et al. (2016). Cerebral microbleeds, vascular risk factors, and magnetic resonance imaging markers: the Northern Manhattan Study. *J. Am. Heart Assoc.* 5:e003477. doi: 10.1161/jaha.116.003477

Chowdhury, M. H., Nagai, A., Bokura, H., Nakamura, E., Kobayashi, S., and Yamaguchi, S. (2011). Age-related changes in white matter lesions, hippocampal atrophy, and cerebral microbleeds in healthy subjects without

major cerebrovascular risk factors. *J. Stroke Cerebrovasc. Dis.* 20, 302–309. doi: 10.1016/j.jstrokecerebrovasdis.2009.12.010

Cremers, L. G., de Groot, M., Hofman, A., Krestin, G. P., van der Lugt, A., Niessen, W. J., et al. (2016). Altered tract-specific white matter microstructure is related to poorer cognitive performance: the Rotterdam Study. *Neurobiol. Aging* 39, 108–117. doi: 10.1016/j.neurobiolaging.2015.11.021

de Groot, M., Cremers, L. G., Ikram, M. A., Hofman, A., Krestin, G. P., van der Lugt, A., et al. (2016). White matter degeneration with aging: longitudinal diffusion MR imaging analysis. *Radiology* 279, 532–541. doi: 10.1148/radiol.2015150103

De Guio, F., Jouvent, E., Biessels, G. J., Black, S. E., Brayne, C., Chen, C., et al. (2016). Reproducibility and variability of quantitative magnetic resonance imaging markers in cerebral small vessel disease. *J. Cereb. Blood Flow Metab.* 36, 1319–1337. doi: 10.1177/0271678X16647396

de Leeuw, F. E., de Groot, J. C., Achten, E., Oudkerk, M., Ramos, L. M., Heijboer, R., et al. (2001). Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J. Neurol. Neurosurg. Psychiatry* 70, 9–14. doi: 10.1136/jnnp.70.1.9

de Leeuw, F. E., Richard, F., de Groot, J. C., van Duijn, C. M., Hofman, A., Van Gijn, J., et al. (2004). Interaction between hypertension, APOE and cerebral white matter lesions. *Stroke* 35, 1057–1060. doi: 10.1161/01.STR.0000125859.71051.83

Debette, S., Schilling, S., Duperron, M. G., Larsson, S. C., and Markus, H. S. (2019). Clinical significance of magnetic resonance imaging markers of vascular brain injury: a systematic review and meta-analysis. *JAMA Neurol.* 76, 81–94. doi: 10.1001/jamaneurol.2018.3122

Debette, S., Seshadri, S., Beiser, A., Au, R., Himali, J. J., Palumbo, C., et al. (2011). Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology* 77, 461–468. doi: 10.1212/WNL.0b013e318227b227

DeCarli, C., Fletcher, E., Ramey, V., Harvey, D., and Jagust, W. J. (2005). Anatomical mapping of white matter hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke* 36, 50–55. doi: 10.1161/01.str.0000150668.58689.f2

DeCarli, C., Miller, B. L., Swan, G. E., Reed, T., Wolf, P. A., Garner, J., et al. (1999). Predictors of brain morphology for the men of the NHLBI twin study. *Stroke* 30, 529–536. doi: 10.1161/01.str.30.3.529

Del Brutto, V. J., Zambrano, M., Mera, R. M., and Del Brutto, O. H. (2015). Population-based study of cerebral microbleeds in stroke-free older adults living in rural ecuador: the atahualpa project. *Stroke* 46, 1984–1986. doi: 10.1161/strokeaha.115.009594

Dhamoon, M. S., Cheung, Y. K., Bagci, A., Alperin, N., Sacco, R. L., Elkind, M. S. V., et al. (2018). Periventricular white matter hyperintensities and functional decline. *J. Am. Geriatr. Soc.* 66, 113–119. doi: 10.1111/jgs.15149

Ding, J., Sigurethsson, S., Jonsson, P. V., Eiriksdottir, G., Charidimou, A., Lopez, O. L., et al. (2017a). Large perivascular spaces visible on magnetic resonance imaging, cerebral small vessel disease progression, and risk of dementia: the age, gene/environment susceptibility-reykjavik study. *JAMA Neurol.* 74, 1105–1112. doi: 10.1001/jamaneurol.2017.1397

Ding, J., Sigurethsson, S., Jonsson, P. V., Eiriksdottir, G., Meirelles, O., Kjartansson, O., et al. (2017b). Space and location of cerebral microbleeds, cognitive decline, and dementia in the community. *Neurology* 88, 2089–2097. doi: 10.1212/WNL.0000000000003983

Evans, T. E., O'Sullivan, M. J., de Groot, M., Niessen, W. J., Hofman, A., Krestin, G. P., et al. (2016). White matter microstructure improves stroke risk prediction in the general population. *Stroke* 47, 2756–2762. doi: 10.1161/strokeaha.116.014651

Falk, E. B., Hyde, L. W., Mitchell, C., Faul, J., Gonzalez, R., Heitzeg, M. M., et al. (2013). What is a representative brain? Neuroscience meets population science. *Proc. Natl. Acad. Sci. U S A* 110, 17615–17622. doi: 10.1073/pnas.1310134110

Farrall, A. J., and Wardlaw, J. M. (2009). Blood-brain barrier: ageing and microvascular disease—systematic review and meta-analysis. *Neurobiol. Aging* 30, 337–352. doi: 10.1016/j.neurobiolaging.2007.07.015

Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., and Zimmerman, R. A. (1987). MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *Am. J. Roentgenol.* 149, 351–356. doi: 10.2214/ajr.149.2.351

Fazekas, F., Kleinert, R., Offenbacher, H., Schmidt, R., Kleinert, G., Payer, F., et al. (1993). Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* 43, 1683–1689. doi: 10.1212/wnl.43.9.1683

Fazekas, F., Kleinert, R., Roob, G., Kleinert, G., Kapeller, P., Schmidt, R., et al. (1999). Histopathologic analysis of foci of signal loss on gradient-echo T2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. *Am. J. Neuroradiol.* 20, 637–642.

Fernando, M. S., Simpson, J. E., Matthews, F., Brayne, C., Lewis, C. E., Barber, R., et al. (2006). White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. *Stroke* 37, 1391–1398. doi: 10.1161/01.str.0000221308.94473.14

Fisher, C. M. (1982). Lacunar strokes and infarcts: a review. *Neurology* 32, 871–876. doi: 10.1212/wnl.32.8.871

Ganguli, M., Albanese, E., Seshadri, S., Bennett, D. A., Lyketsos, C., Kukull, W. A., et al. (2018). Population neuroscience: dementia epidemiology serving precision medicine and population health. *Alzheimer Dis. Assoc. Disord.* 32, 1–9. doi: 10.1097/wad.0000000000000237

Godin, O., Tzourio, C., Maillard, P., Mazoyer, B., and Dufouil, C. (2011). Antihypertensive treatment and change in blood pressure are associated with the progression of white matter lesion volumes: the Three-City (3C)-Dijon Magnetic Resonance Imaging Study. *Circulation* 123, 266–273. doi: 10.1161/circulationaha.110.961052

Godin, O., Tzourio, C., Rouaud, O., Zhu, Y., Maillard, P., Pasquier, F., et al. (2010). Joint effect of white matter lesions and hippocampal volumes on severity of cognitive decline: the 3C-Dijon MRI study. *J. Alzheimers Dis.* 20, 453–463. doi: 10.3233/jad-2010-1389

Gons, R. A., van Norden, A. G., de Laat, K. F., van Oudheusden, L. J., van Uden, I. W., Zwiers, M. P., et al. (2011). Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter. *Brain* 134, 2116–2124. doi: 10.1093/brain/awr145

Goodheart, A. E., Tamburo, E., Minhas, D., Aizenstein, H. J., McDade, E., Snitz, B. E., et al. (2015). Reduced binding of Pittsburgh Compound-B in areas of white matter hyperintensities. *Neuroimage Clin.* 9, 479–483. doi: 10.1016/j.nicl.2015.09.009

Gorelick, P. B., Furie, K. L., Iadecola, C., Smith, E. E., Waddy, S. P., Lloyd-Jones, D. M., et al. (2017). Defining optimal brain health in adults: a presidential advisory from the american Heart Association/American Stroke Association. *Stroke* 48, e284–e303. doi: 10.1161/str.0000000000000148

Gorelick, P. B., Scuteri, A., Black, S. E., Decarli, C., Greenberg, S. M., Iadecola, C., et al. (2011). Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 42, 2672–2713. doi: 10.1161/STR.0b013e3182299496

Gottesman, R. F., Coresh, J., Catellier, D. J., Sharrett, A. R., Rose, K. M., Coker, L. H., et al. (2010). Blood pressure and white-matter disease progression in a biethnic cohort: atherosclerosis risk in communities (ARIC) study. *Stroke* 41, 3–8. doi: 10.1161/strokeaha.109.566992

Graff-Radford, J., Simino, J., Kantarci, K., Mosley, T. H. Jr., Griswold, M. E., Windham, B. G., et al. (2017). Neuroimaging correlates of cerebral microbleeds: the ARIC study (Atherosclerosis Risk in Communities). *Stroke* 48, 2964–2972. doi: 10.1161/strokeaha.117.018336

Greenberg, S. M., Vernooij, M. W., Cordonnier, C., Viswanathan, A., Al-Shahi Salman, R., Warach, S., et al. (2009). Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol.* 8, 165–174. doi: 10.1016/S1474-4422(09)70013-4

Groeschel, S., Chong, W. K., Surtees, R., and Hanefeld, F. (2006). Virchow-Robin spaces on magnetic resonance images: normative data, their dilatation and a review of the literature. *Neuroradiology* 48, 745–754. doi: 10.1007/s00234-006-0112-1

Groot, C., Sudre, C. H., Barkhof, F., Teunissen, C. E., van Berckel, B. N. M., Seo, S. W., et al. (2018). Clinical phenotype, atrophy and small vessel disease in APOEε2 carriers with Alzheimer disease. *Neurology* 91, e1851–e1859. doi: 10.1212/wnl.0000000000006503

Gu, Y., Vorburger, R. S., Gazes, Y., Habeck, C. G., Stern, Y., Luchsinger, J. A., et al. (2016). White matter integrity as a mediator in the relationship between dietary nutrients and cognition in the elderly. *Ann. Neurol.* 79, 1014–1025. doi: 10.1002/ana.24674

Gupta, N., Simpkins, A. N., Hitomi, E., Dias, C., Leigh, R., and NIH Natural History of Stroke Investigators. (2018). White matter hyperintensity-associated blood-brain barrier disruption and vascular risk factors. *J. Stroke Cerebrovasc. Dis.* 27, 466–471. doi: 10.1016/j.jstrokecerebrovasdis.2017.09.026

Gutierrez, J., Elkind, M. S., Cheung, K., Rundek, T., Sacco, R. L., and Wright, C. B. (2015). Pulsatile and steady components of blood pressure and subclinical cerebrovascular disease: the Northern Manhattan Study. *J. Hypertens.* 33, 2115–2122. doi: 10.1097/hjh.0000000000000686

Gutierrez, J., Rundek, T., Ekind, M. S., Sacco, R. L., and Wright, C. B. (2013). Perivascular spaces are associated with atherosclerosis: an insight from the Northern Manhattan Study. *Am. J. Neuroradiol.* 34, 1711–1716. doi: 10.3174/ajnr.a3498

Haacke, E. M., Mittal, S., Wu, Z., Neelavalli, J., and Cheng, Y. C. (2009). Susceptibility-weighted imaging: technical aspects and clinical applications, part 1. *Am. J. Neuroradiol.* 30, 19–30. doi: 10.3174/ajnr.A1400

Hachinski, V. C., Potter, P., and Merskey, H. (1987). Leuko-araiosis. *Arch. Neurol.* 44, 21–23. doi: 10.1001/archneur.1987.00520130013009

Hajjar, I., Quach, L., Yang, F., Chaves, P. H., Newman, A. B., Mukamal, K., et al. (2011). Hypertension, white matter hyperintensities and concurrent impairments in mobility, cognition, and mood: the Cardiovascular Health Study. *Circulation* 123, 858–865. doi: 10.1161/circulationaha.110.978114

Han, F., Zhai, F. F., Wang, Q., Zhou, L. X., Ni, J., Yao, M., et al. (2018). Prevalence and risk factors of cerebral small vessel disease in a chinese population-based sample. *J. Stroke* 20, 239–246. doi: 10.5853/jos.2017.02110

Hilal, S., Saini, M., Tan, C. S., Catindig, J. A., Koay, W. I., Niessen, W. J., et al. (2014). Cerebral microbleeds and cognition: the epidemiology of dementia in Singapore study. *Alzheimer Dis. Assoc. Disord.* 28, 106–112. doi: 10.1097/WAD.0000000000000015

Huang, Y. H., Zhang, W. W., Lin, L., Feng, J., Zhao, X. X., Guo, W. H., et al. (2010). Could changes in arterioles impede the perivascular drainage of interstitial fluid from the cerebral white matter in leukoaraiosis? *Neuropathol. Appl. Neurobiol.* 36, 237–247. doi: 10.1111/j.1365-2990.2009.01049.x

Huijts, M., Duits, A., Staals, J., Kroon, A. A., de Leeuw, P. W., and van Oostenbrugge, R. J. (2014). Basal ganglia enlarged perivascular spaces are linked to cognitive function in patients with cerebral small vessel disease. *Curr. Neurovasc. Res.* 11, 136–141. doi: 10.2174/1567202611666140310102248

Huisa, B. N., Caprihan, A., Thompson, J., Prestopnik, J., Qualls, C. R., and Rosenberg, G. A. (2015). Long-term blood-brain barrier permeability changes in Binswanger disease. *Stroke* 46, 2413–2418. doi: 10.1161/strokeaha.115.009589

Ikram, M. A., Vrooman, H. A., Vernooij, M. W., van der Lijn, F., Hofman, A., van der Lugt, A., et al. (2008). Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. *Neurobiol. Aging* 29, 882–890. doi: 10.1016/j.neurobiolaging.2006.12.012

Jeerakathil, T., Wolf, P. A., Beiser, A., Hald, J. K., Au, R., Kase, C. S., et al. (2004). Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. *Stroke* 35, 1831–1835. doi: 10.1161/01.str.0000131809.35202.1b

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., and Smith, S. M. (2012). FSL. *Neuroimage* 62, 782–790. doi: 10.1016/j.neuroimage.2011.09.015

Kaffashian, S., Tzourio, C., Soumare, A., Dufouil, C., Zhu, Y., Crivello, F., et al. (2014). Plasma β-amyloid and MRI markers of cerebral small vessel disease: three-city dijon study. *Neurology* 83, 2038–2045. doi: 10.1212/wnl.0000000000001038

Klarenbeek, P., van Oostenbrugge, R. J., Lodder, J., Rouhl, R. P., Knottnerus, I. L., and Staals, J. (2013). Higher ambulatory blood pressure relates to enlarged Virchow-Robin spaces in first-ever lacunar stroke patients. *J. Neurol.* 260, 115–121. doi: 10.1007/s00415-012-6598-z

Kljajevic, V., Meyer, P., Holzmann, C., Dyrba, M., Kasper, E., Bokde, A. L., et al. (2014). The ε4 genotype of apolipoprotein E and white matter integrity in Alzheimer's disease. *Alzheimers Dement.* 10, 401–404. doi: 10.1016/j.jalz.2013.02.008

Knopman, D. S., Griswold, M. E., Lirette, S. T., Gottesman, R. F., Kantarci, K., Sharrett, A. R., et al. (2015). Vascular imaging abnormalities and cognition: mediation by cortical volume in nondemented individuals: atherosclerosis risk in communities-neurocognitive study. *Stroke* 46, 433–440. doi: 10.1161/strokeaha.114.007847

Knopman, D. S., Penman, A. D., Catellier, D. J., Coker, L. H., Shibata, D. K., Sharrett, A. R., et al. (2011). Vascular risk factors and longitudinal changes on brain MRI: the ARIC study. *Neurology* 76, 1879–1885. doi: 10.1212/WNL.0b013e31821d753f

Knudsen, K. A., Rosand, J., Karluk, D., and Greenberg, S. M. (2001). Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. *Neurology* 56, 537–539. doi: 10.1212/wnl.56.4.537

Koga, H., Takashima, Y., Murakawa, R., Uchino, A., Yuzuriha, T., and Yao, H. (2009). Cognitive consequences of multiple lacunes and leukoaraiosis as vascular cognitive impairment in community-dwelling elderly individuals. *J. Stroke Cerebrovasc. Dis.* 18, 32–37. doi: 10.1016/j.jstrokecerebrovasdis.2008.07.010

Koga, H., Yuzuriha, T., Yao, H., Endo, K., Hiejima, S., Takashima, Y., et al. (2002). Quantitative MRI findings and cognitive impairment among community dwelling elderly subjects. *J. Neurol. Neurosurg. Psychiatry* 72, 737–741. doi: 10.1136/jnnp.72.6.737

Lampe, L., Kharabian-Masouleh, S., Kynast, J., Arelin, K., Steele, C. J., Loffler, M., et al. (2019). Lesion location matters: the relationships between white matter hyperintensities on cognition in the healthy elderly. *J. Cereb. Blood Flow Metab.* 39, 36–43. doi: 10.1177/0271678X17740501

Launer, L. J., Lewis, C. E., Schreiner, P. J., Sidney, S., Battapady, H., Jacobs, D. R., et al. (2015). Vascular factors and multiple measures of early brain health: CARDIA brain MRI study. *PLoS One* 10:e0122138. doi: 10.1371/journal.pone.0122138

Laurent, S., and Boutouyrie, P. (2015). The structural factor of hypertension: large and small artery alterations. *Circ. Res.* 116, 1007–1021. doi: 10.1161/circresaha.116.303596

Loehrer, E., Ikram, M. A., Akoudad, S., Vrooman, H. A., van der Lugt, A., Niessen, W. J., et al. (2014). Apolipoprotein E genotype influences spatial distribution of cerebral microbleeds. *Neurobiol. Aging* 35, 899–905. doi: 10.1016/j.neurobiolaging.2013.09.012

Longstreth, W. T. Jr., Dulberg, C., Manolio, T. A., Lewis, M. R., Beauchamp, N. J. Jr., O'Leary, D., et al. (2002). Incidence, manifestations and predictors of brain infarcts defined by serial cranial magnetic resonance imaging in the elderly: the Cardiovascular Health Study. *Stroke* 33, 2376–2382. doi: 10.1161/01.str.0000032241.58727.49

Maclullich, A. M., Wardlaw, J. M., Ferguson, K. J., Starr, J. M., Seckl, J. R., and Deary, I. J. (2004). Enlarged perivascular spaces are associated with cognitive function in healthy elderly men. *J. Neurol. Neurosurg. Psychiatry* 75, 1519–1523. doi: 10.1136/jnnp.2003.030858

Maillard, P., Fletcher, E., Harvey, D., Carmichael, O., Reed, B., Mungas, D., et al. (2011). White matter hyperintensity penumbra. *Stroke* 42, 1917–1922. doi: 10.1161/STROKEAHA.110.609768

Maillard, P., Fletcher, E., Lockhart, S. N., Roach, A. E., Reed, B., Mungas, D., et al. (2014). White matter hyperintensities and their penumbra lie along a continuum of injury in the aging brain. *Stroke* 45, 1721–1726. doi: 10.1161/strokeaha.113.004084

Maillard, P., Seshadri, S., Beiser, A., Himali, J. J., Au, R., Fletcher, E., et al. (2012). Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. *Lancet Neurol.* 11, 1039–1047. doi: 10.1016/s1474-4422(12)70241-7

Marcus, J., Gardener, H., Rundek, T., Elkind, M. S., Sacco, R. L., Decarli, C., et al. (2011). Baseline and longitudinal increases in diastolic blood pressure are associated with greater white matter hyperintensity volume: the northern Manhattan study. *Stroke* 42, 2639–2641. doi: 10.1161/STROKEAHA.111.617571

Martinez-Ramirez, S., Romero, J. R., Shoamanesh, A., McKee, A. C., Van Etten, E., Pontes-Neto, O., et al. (2015). Diagnostic value of lobar microbleeds in individuals without intracerebral hemorrhage. *Alzheimers Dement.* 11, 1480–1488. doi: 10.1016/j.jalz.2015.04.009

Mayeda, E. R., Glymour, M. M., Quesenberry, C. P., and Whitmer, R. A. (2016). Inequalities in dementia incidence between six racial and ethnic groups over 14 years. *Alzheimers Dement.* 12, 216–224. doi: 10.1016/j.jalz.2015.12.007

Meier, I. B., Gu, Y., Guzaman, V. A., Wiegman, A. F., Schupf, N., Manly, J. J., et al. (2014). Lobar microbleeds are associated with a decline in executive functioning in older adults. *Cerebrovasc. Dis.* 38, 377–383. doi: 10.1159/000368998

Mesker, D. J., Poels, M. M., Ikram, M. A., Vernooij, M. W., Hofman, A., Vrooman, H. A., et al. (2011). Lobar distribution of cerebral microbleeds: the Rotterdam Scan Study. *Arch. Neurol.* 68, 656–659. doi: 10.1001/archneurol.2011.93

Moody, D. M., Brown, W. R., Challa, V. R., Ghazi-Birry, H. S., and Reboussin, D. M. (1997). Cerebral microvascular alterations in aging, leukoaraiosis and Alzheimer's disease. *Ann. N Y Acad. Sci.* 826, 103–116. doi: 10.1111/j.1749-6632.1997.tb48464.x

Morris, Z., Whiteley, W. N., Longstreth, W. T. Jr., Weber, F., Lee, Y. C., Tsushima, Y., et al. (2009). Incidental findings on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ* 339:b3016. doi: 10.1136/bmj.b3016

Murray, M. E., Senjem, M. L., Petersen, R. C., Hollman, J. H., Preboske, G. M., Weigand, S. D., et al. (2010). Functional impact of white matter hyperintensities in cognitively normal elderly subjects. *Arch. Neurol.* 67, 1379–1385. doi: 10.1001/archneurol.2010.280

Nation, D. A., Sweeney, M. D., Montagne, A., Sagare, A. P., D'Orazio, L. M., Pachicano, M., et al. (2019). Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat. Med.* 25, 270-276. doi: 10.3410/f.734853562.793555497

Noh, Y., Seo, S. W., Jeon, S., Lee, J. M., Kim, J. H., Kim, G. H., et al. (2014). White matter hyperintensities are associated with amyloid burden in APOE4 non-carriers. *J. Alzheimers Dis.* 40, 877–886. doi: 10.3233/JAD-130461

Operto, G., Cacciaglia, R., Grau-Rivera, O., Falcon, C., Brugulat-Serrat, A., Ródenas, P., et al. (2018). White matter microstructure is altered in cognitively normal middle-aged APOE-ε4 homozygotes. *Alzheimers Res. Ther.* 10:48. doi: 10.1186/s13195-018-0375-x

Ortman, J. M., Velkoff, V. A., and Hogan, H. (2014). "An aging nation: the older population in the United States," in *Current Population Reports* (Washington, DC: U.S. Census Bureau), 25–1140.

Pan, W., and Kastin, A. J. (2017). The blood-brain barrier: regulatory roles in wakefulness and sleep. *Neuroscientist* 23, 124–136. doi: 10.1177/1073858416639005

Pantoni, L. (2010). Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol.* 9, 689–701. doi: 10.1016/s1474-4422(10)70104-6

Paradise, M., Seruga, A., Crawford, J. D., Chaganti, J., Thalamuthu, A., Kochan, N. A., et al. (2019). The relationship of cerebral microbleeds to cognition and incident dementia in non-demented older individuals. *Brain Imaging Behav.* 13, 750–761. doi: 10.1007/s11682-018-9883-3

Paus, T. (2010). Population neuroscience: why and how. *Hum. Brain Mapp.* 31, 891–903. doi: 10.1002/hbm.21069

Poels, M. M., Ikram, M. A., van der Lugt, A., Hofman, A., Krestin, G. P., Breteler, M. M., et al. (2011). Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. *Stroke* 42, 656–661. doi: 10.1161/STROKEAHA.110.607184

Poels, M. M., Ikram, M. A., van der Lugt, A., Hofman, A., Niessen, W. J., Krestin, G. P., et al. (2012a). Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology* 78, 326–333. doi: 10.1212/WNL.0b013e3182452928

Poels, M. M., Zaccai, K., Verwoert, G. C., Vernooij, M. W., Hofman, A., van der Lugt, A., et al. (2012b). Arterial stiffness and cerebral small vessel disease: the Rotterdam Scan Study. *Stroke* 43, 2637–2642. doi: 10.1161/STROKEAHA.111.642264

Poels, M. M., Vernooij, M. W., Ikram, M. A., Hofman, A., Krestin, G. P., van der Lugt, A., et al. (2010). Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. *Stroke* 41, S103–S106. doi: 10.1161/strokeaha.110.595181

Power, M. C., Tingle, J. V., Reid, R. I., Huang, J., Sharrett, A. R., Coresh, J., et al. (2017). Midlife and late-life vascular risk factors and white matter microstructural integrity: the atherosclerosis risk in communities neurocognitive study. *J. Am. Heart Assoc.* 6:e005608. doi: 10.1161/jaha.117.005608

Prabhakaran, S., Wright, C. B., Yoshita, M., Delapaz, R., Brown, T., DeCarli, C., et al. (2008). Prevalence and determinants of subclinical brain infarction: the Northern Manhattan Study. *Neurology* 70, 425–430. doi: 10.1212/01.wnl.0000277521.66947.e5

Pullicino, P. M., Miller, L. L., Alexandrov, A. V., and Ostrow, P. T. (1995). Infraputaminal 'lacunes': clinical and pathological correlations. *Stroke* 26, 1598–1602. doi: 10.1161/01.str.26.9.1598

Racine, A. M., Merluzzi, A. P., Adluru, N., Norton, D., Koscik, R. L., Clark, L. R., et al. (2019). Association of longitudinal white matter degeneration and cerebrospinal fluid biomarkers of neurodegeneration, inflammation and Alzheimer's disease in late-middle-aged adults. *Brain Imaging Behav.* 13, 41–52. doi: 10.1007/s11682-017-9732-9

Raji, C. A., Lopez, O. L., Kuller, L. H., Carmichael, O. T., Longstreth, W. T. Jr., Gach, H. M., et al. (2012). White matter lesions and brain gray matter volume in cognitively normal elders. *Neurobiol. Aging* 33, 834.e7–834.e16. doi: 10.1016/j.neurobiolaging.2011.08.010

Ramirez, J., Berezuk, C., McNeely, A. A., Gao, F., McLaurin, J., and Black, S. E. (2016). Imaging the perivascular space as a potential biomarker of neurovascular and neurodegenerative diseases. *Cell. Mol. Neurobiol.* 36, 289–299. doi: 10.1007/s10571-016-0343-6

Riba-Llena, I., Jiménez-Balado, J., Castañé, X., Girona, A., López-Rueda, A., Mundet, X., et al. (2018). Arterial stiffness is associated with basal ganglia enlarged perivascular spaces and cerebral small vessel disease load. *Stroke* 49, 1279–1281. doi: 10.1161/strokeaha.118.020163

Rizvi, B., Narkhede, A., Last, B. S., Budge, M., Tosto, G., Manly, J. J., et al. (2018). The effect of white matter hyperintensities on cognition is mediated by cortical atrophy. *Neurobiol. Aging* 64, 25–32. doi: 10.1016/j.neurobiolaging.2017.12.006

Romero, J. R., Beiser, A., Seshadri, S., Benjamin, E. J., Polak, J. F., Vasan, R. S., et al. (2009). Carotid artery atherosclerosis, MRI indices of brain ischemia, aging, and cognitive impairment: the Framingham study. *Stroke* 40, 1590–1596. doi: 10.1161/STROKEAHA.108.535245

Romero, J. R., Preis, S. R., Beiser, A., DeCarli, C., D'Agostino, R. B., Wolf, P. A., et al. (2016). Carotid atherosclerosis and cerebral microbleeds: the framingham heart study. *J. Am. Heart Assoc.* 4:e002377. doi: 10.1161/jaha.115.002377

Romero, J. R., Preis, S. R., Beiser, A. S., DeCarli, C., Lee, D. Y., Viswanathan, A., et al. (2012). Lipoprotein phospholipase A2 and cerebral microbleeds in the Framingham Heart Study. *Stroke* 43, 3091–3094. doi: 10.1161/strokeaha.112.656744

Romero, J. R., Preis, S. R., Beiser, A., DeCarli, C., Viswanathan, A., Martinez-Ramirez, S., et al. (2014). Risk factors, stroke prevention treatments, and prevalence of cerebral microbleeds in the Framingham Heart Study. *Stroke* 45, 1492–1494. doi: 10.1161/strokeaha.114.004130

Roob, G., Schmidt, R., Kapeller, P., Lechner, A., Hartung, H. P., and Fazekas, F. (1999). MRI evidence of past cerebral microbleeds in a healthy elderly population. *Neurology* 52, 991–994. doi: 10.1212/wnl.52.5.991

Rosenberg, G. A., Bjerke, M., and Wallin, A. (2014). Multimodal markers of inflammation in the subcortical ischemic vascular disease type of vascular cognitive impairment. *Stroke* 45, 1531–1538. doi: 10.1161/strokeaha.113.004534

Rosenberg, G. A., Wallin, A., Wardlaw, J. M., Markus, H. S., Montaner, J., Wolfson, L., et al. (2016). Consensus statement for diagnosis of subcortical small vessel disease. *J. Cereb. Blood Flow Metab.* 36, 6–25. doi: 10.1038/jcbfm.2015.172

Sandoval, K. E., and Witt, K. A. (2008). Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol. Dis.* 32, 200–219. doi: 10.1016/j.nbd.2008.08.005

Scheid, R., Ott, D. V., Roth, H., Schroeter, M. L., and von Cramon, D. Y. (2007). Comparative magnetic resonance imaging at 1.5 and 3 Tesla for the evaluation of traumatic microbleeds. *J. Neurotrauma* 24, 1811–1816. doi: 10.1089/neu.2007.0382

Seiler, S., Fletcher, E., Hassan-Ali, K., Weinstein, M., Beiser, A., Himali, J. J., et al. (2018). Cerebral tract integrity relates to white matter hyperintensities, cortex volume and cognition. *Neurobiol. Aging* 72, 14–22. doi: 10.1016/j.neurobiolaging.2018.08.005

Shim, Y. S., Yang, D. W., Roe, C. M., Coats, M. A., Benzinger, T. L., Xiong, C., et al. (2015). Pathological correlates of white matter hyperintensities on magnetic resonance imaging. *Dement. Geriatr. Cogn. Disord.* 39, 92–104. doi: 10.1159/000366411

Shoamanesh, A., Kwok, C. S., and Benavente, O. (2011). Cerebral microbleeds: histopathological correlation of neuroimaging. *Cerebrovasc. Dis.* 32, 528–534. doi: 10.1159/000331466

Shoamanesh, A., Preis, S. R., Beiser, A. S., Vasan, R. S., Benjamin, E. J., Kase, C. S., et al. (2015). Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: framingham heart study. *Neurology* 84, 825–832. doi: 10.1212/wnl.0000000000001279

Slater, D. A., Melie-Garcia, L., Preisig, M., Kherif, F., Lutti, A., and Draganski, B. (2019). Evolution of white matter tract microstructure across the life span. *Hum. Brain Mapp.* 40, 2252–2268. doi: 10.1002/hbm.24522

Soares, J. M., Marques, P., Alves, V., and Sousa, N. (2013). A hitchhiker's guide to diffusion tensor imaging. *Front. Neurosci.* 7:31. doi: 10.3389/fnins.2013.00031

Stavitsky, K., Du, Y., Seichepine, D., Laudate, T. M., Beiser, A., Seshadri, S., et al. (2010). White matter hyperintensity and cognitive functioning in the racial and ethnic minority cohort of the Framingham Heart Study. *Neuroepidemiology* 35, 117–122. doi: 10.1159/000313443

Stehling, C., Wersching, H., Kloska, S. P., Kirchhof, P., Ring, J., Nassenstein, I., et al. (2008). Detection of asymptomatic cerebral microbleeds: a comparative study at 1.5 and 3.0 T. *Acad Radiol.* 15, 895–900. doi: 10.1016/j.acra.2008.01.013

Sweeney, M. D., Montagne, A., Sagare, A. P., Nation, D. A., Schneider, L. S., Chui, H. C., et al. (2019). Vascular dysfunction-The disregarded partner of Alzheimer's disease. *Alzheimers Dement.* 15, 158–167. doi: 10.1016/j.jalz.2018.07.222

Taheri, S., Gasparovic, C., Huisa, B. N., Adair, J. C., Edmonds, E., Prestopnik, J., et al. (2011a). Blood-brain barrier permeability abnormalities in vascular cognitive impairment. *Stroke* 42, 2158–2163. doi: 10.1161/STROKEAHA.110.611731

Taheri, S., Gasparovic, C., Shah, N. J., and Rosenberg, G. A. (2011b). Quantitative measurement of blood-brain barrier permeability in human using dynamic contrast-enhanced MRI with fast T1 mapping. *Magn. Reson. Med.* 65, 1036–1042. doi: 10.1002/mrm.22686

Tai, L. M., Thomas, R., Marottoli, F. M., Koster, K. P., Kanekiyo, T., Morris, A. W., et al. (2016). The role of APOE in cerebrovascular dysfunction. *Acta Neuropathol.* 131, 709–723. doi: 10.1007/s00401-016-1547-z

Topakian, R., Barrick, T. R., Howe, F. A., and Markus, H. S. (2010). Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. *J. Neurol. Neurosurg. Psychiatry* 81, 192–197. doi: 10.1136/jnnp.2009.172072

Tuladhar, A. M., van Norden, A. G., de Laat, K. F., Zwiers, M. P., van Dijk, E. J., Norris, D. G., et al. (2015). White matter integrity in small vessel disease is related to cognition. *Neuroimage Clin.* 7, 518–524. doi: 10.1016/j.nicl.2015.02.003

van den Heuvel, D. M., ten Dam, V. H., de Craen, A. J., Admiraal-Behloul, F., Olofsen, H., Bollen, E. L., et al. (2006a). Increase in periventricular white matter hyperintensities parallels decline in mental processing speed in a non-demented elderly population. *J. Neurol. Neurosurg. Psychiatry* 77, 149–153. doi: 10.1136/jnnp.2005.070193

van den Heuvel, D. M., ten Dam, V. H., de Craen, A. J., Admiraal-Behloul, F., van Es, A. C., Palm, W. M., et al. (2006b). Measuring longitudinal white matter changes: comparison of a visual rating scale with a volumetric measurement. *Am. J. Neuroradiol.* 27, 875–878.

van Dijk, E. J., Prins, N. D., Vrooman, H. A., Hofman, A., Koudstaal, P. J., and Breteler, M. M. (2008). Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke* 39, 2712–2719. doi: 10.1161/strokeaha.107.513176

van Leijsen, E. M. C., van Uden, I. W. M., Ghafoorian, M., Bergkamp, M. I., Lohner, V., Kooijmans, E. C. M., et al. (2017). Nonlinear temporal dynamics of cerebral small vessel disease: the RUN DMC study. *Neurology* 89, 1569–1577. doi: 10.1212/wnl.0000000000004490

van Veluw, S. J., Biessels, G. J., Bouvy, W. H., Spliet, W. G., Zwanenburg, J. J., Luijten, P. R., et al. (2016). Cerebral amyloid angiopathy severity is linked to dilation of juxtacortical perivascular spaces. *J. Cereb. Blood Flow Metab.* 36, 576–580. doi: 10.1177/0271678X15620434

Vemuri, P., Lesnick, T. G., Przybelski, S. A., Knopman, D. S., Preboske, G. M., Kantarci, K., et al. (2015). Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. *Brain* 138, 761–771. doi: 10.1093/brain/awu393

Verhaaren, B. F., Vernooij, M. W., de Boer, R., Hofman, A., Niessen, W. J., van der Lugt, A., et al. (2013). High blood pressure and cerebral white matter lesion progression in the general population. *Hypertension* 61, 1354–1359. doi: 10.1161/hypertensionaha.111.00430

Vermeer, S. E., Longstreth, W. T. Jr., and Koudstaal, P. J. (2007). Silent brain infarcts: a systematic review. *Lancet Neurol.* 6, 611–619. doi: 10.1016/s1474-4422(07)70170-9

Vermeer, S. E., Prins, N. D., den Heijer, T., Hofman, A., Koudstaal, P. J., and Breteler, M. M. (2003). Silent brain infarcts and the risk of dementia and cognitive decline. *N. Engl. J. Med.* 348, 1215–1222. doi: 10.1056/nejmoa022066

Vernooij, M. W., Ikram, M. A., Tanghe, H. L., Vincent, A. J., Hofman, A., Krestin, G. P., et al. (2007). Incidental findings on brain MRI in the general population. *N. Engl. J. Med.* 357, 1821–1828. doi: 10.1056/NEJMoa070972

Vernooij, M. W., Ikram, M. A., Vrooman, H. A., Wielopolski, P. A., Krestin, G. P., Hofman, A., et al. (2009). White matter microstructural integrity and cognitive function in a general elderly population. *Arch. Gen. Psychiatry* 66, 545–553. doi: 10.1001/archgenpsychiatry.2009.5

Vernooij, M. W., van der Lugt, A., Ikram, M. A., Wielopolski, P. A., Niessen, W. J., Hofman, A., et al. (2008). Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. *Neurology* 70, 1208–1214. doi: 10.1212/01.wnl.0000307750.41970.d9

Vinke, E. J., de Groot, M., Venkatraghavan, V., Klein, S., Niessen, W. J., Ikram, M. A., et al. (2018). Trajectories of imaging markers in brain aging: the Rotterdam Study. *Neurobiol. Aging* 71, 32–40. doi: 10.1016/j.neurobiolaging.2018.07.001

Viswanathan, A., and Greenberg, S. M. (2011). Cerebral amyloid angiopathy in the elderly. *Ann. Neurol.* 70, 871–880. doi: 10.1002/ana.22516

Wakefield, D. B., Moscufo, N., Guttmann, C. R., Kuchel, G. A., Kaplan, R. F., Pearlson, G., et al. (2010). White matter hyperintensities predict functional decline in voiding, mobility and cognition in older adults. *J. Am. Geriatr. Soc.* 58, 275–281. doi: 10.1111/j.1532-5415.2009.02699.x

Walker, K. A., Power, M. C., Hoogeveen, R. C., Folsom, A. R., Ballantyne, C. M., Knopman, D. S., et al. (2017). Midlife systemic inflammation, late-life white matter integrity and cerebral small vessel disease: the atherosclerosis risk in communities study. *Stroke* 48, 3196–3202. doi: 10.1161/strokeaha.117.018675

Walker, K. A., Windham, B. G., Power, M. C., Hoogeveen, R. C., Folsom, A. R., Ballantyne, C. M., et al. (2018). The association of mid-to late-life systemic inflammation with white matter structure in older adults: the Atherosclerosis Risk in Communities Study. *Neurobiol. Aging* 68, 26–33. doi: 10.1016/j.neurobiolaging.2018.03.031

Wang, R., Fratiglioni, L., Laukka, E. J., Lövdén, M., Kalpouzos, G., Keller, L., et al. (2015). Effects of vascular risk factors and APOE ε4 on white matter integrity and cognitive decline. *Neurology* 84, 1128–1135. doi: 10.1212/wnl.0000000000001379

Wardlaw, J. M., Doubal, F., Armitage, P., Chappell, F., Carpenter, T., Muñoz Maniega, S., et al. (2009). Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann. Neurol.* 65, 194–202. doi: 10.1002/ana.21549

Wardlaw, J. M., Doubal, F. N., Valdes-Hernandez, M., Wang, X., Chappell, F. M., Shuler, K., et al. (2013a). Blood-brain barrier permeability and long-term clinical and imaging outcomes in cerebral small vessel disease. *Stroke* 44, 525–527. doi: 10.1161/strokeaha.112.669994

Wardlaw, J. M., Smith, E. E., Biessels, G. J., Cordonnier, C., Fazekas, F., Frayne, R., et al. (2013b). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* 12, 822–838. doi: 10.1016/S1474-4422(13)70124-8

Wardlaw, J. M., Makin, S. J., Hernandez, M. C. V., Armitage, P. A., Heye, A. K., Chappell, F. M., et al. (2017). Blood-brain barrier failure as a core mechanism in cerebral small vessel disease and dementia: evidence from a cohort study. *Alzheimers Dement.* 13, 634–643. doi: 10.1016/j.jalz.2016.09.006

Wardlaw, J. M., Sandercock, P. A., Dennis, M. S., and Starr, J. (2003). Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? *Stroke* 34, 806–812. doi: 10.1161/01.str.0000058480.77236.b3

Wardlaw, J. M., Valdés Hernández, M. C., and Muñoz-Maniega, S. (2015). What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. *J. Am. Heart Assoc.* 4:001140. doi: 10.1161/jaha.114.001140

Weiss, N., Miller, F., Cazaubon, S., and Couraud, P. O. (2009). The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim. Biophys. Acta* 1788, 842–857. doi: 10.1016/j.bbamem.2008.10.022

Wiegman, A. F., Meier, I. B., Schupf, N., Manly, J. J., Guzman, V. A., Narkhede, A., et al. (2014). Cerebral microbleeds in a multiethnic elderly community: demographic and clinical correlates. *J. Neurol. Sci.* 345, 125–130. doi: 10.1016/j.jns.2014.07.024

Willey, J. Z., Moon, Y. P., Dhamoon, M. S., Kulick, E. R., Bagci, A., Alperin, N., et al. (2018). Regional subclinical cerebrovascular disease is associated with balance in an elderly multi-ethnic population. *Neuroepidemiology* 51, 57–63. doi: 10.1159/000490351

Wright, C. B., Dong, C., Perez, E. J., De Rosa, J., Yoshita, M., Rundek, T., et al. (2017). Subclinical cerebrovascular disease increases the risk of incident stroke and mortality: the northern manhattan study. *J. Am. Heart Assoc.* 6:e004069. doi: 10.1161/jaha.116.004069

Yakushiji, Y., Charidimou, A., Hara, M., Noguchi, T., Nishihara, M., Eriguchi, M., et al. (2014). Topography and associations of perivascular spaces in healthy adults: the Kashima scan study. *Neurology* 83, 2116–2123. doi: 10.1212/wnl.0000000000001054

Yao, M., Zhu, Y. C., Soumaré, A., Dufouil, C., Mazoyer, B., Tzourio, C., et al. (2014). Hippocampal perivascular spaces are related to aging and blood pressure but not to cognition. *Neurobiol. Aging* 35, 2118–2125. doi: 10.1016/j.neurobiolaging.2014.03.021

Yue, N. C., Arnold, A. M., Longstreth, W. T. Jr., Elster, A. D., Jungreis, C. A., O'Leary, D. H., et al. (1997). Sulcal, ventricular, and white matter changes at MR imaging in the aging brain: data from the cardiovascular health study. *Radiology* 202, 33–39. doi: 10.1148/radiology.202.1.8988189

Zhou, G., Ren, S., Chen, N., Duan, L., Zhang, Z., Fang, S., et al. (2008). Cerebral white matter lesions and cognitive function in a non-demented Chinese veteran cohort. *J. Int. Med. Res.* 36, 115–122. doi: 10.1177/147323000803600115

Conflict of Interest Statement: CW receives royalties for 2 chapters on Vascular Dementia from UpToDate.

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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership