PRECLINICAL BIOMARKERS AND FUNCTIONAL COMPENSATION IN BRAIN AGING

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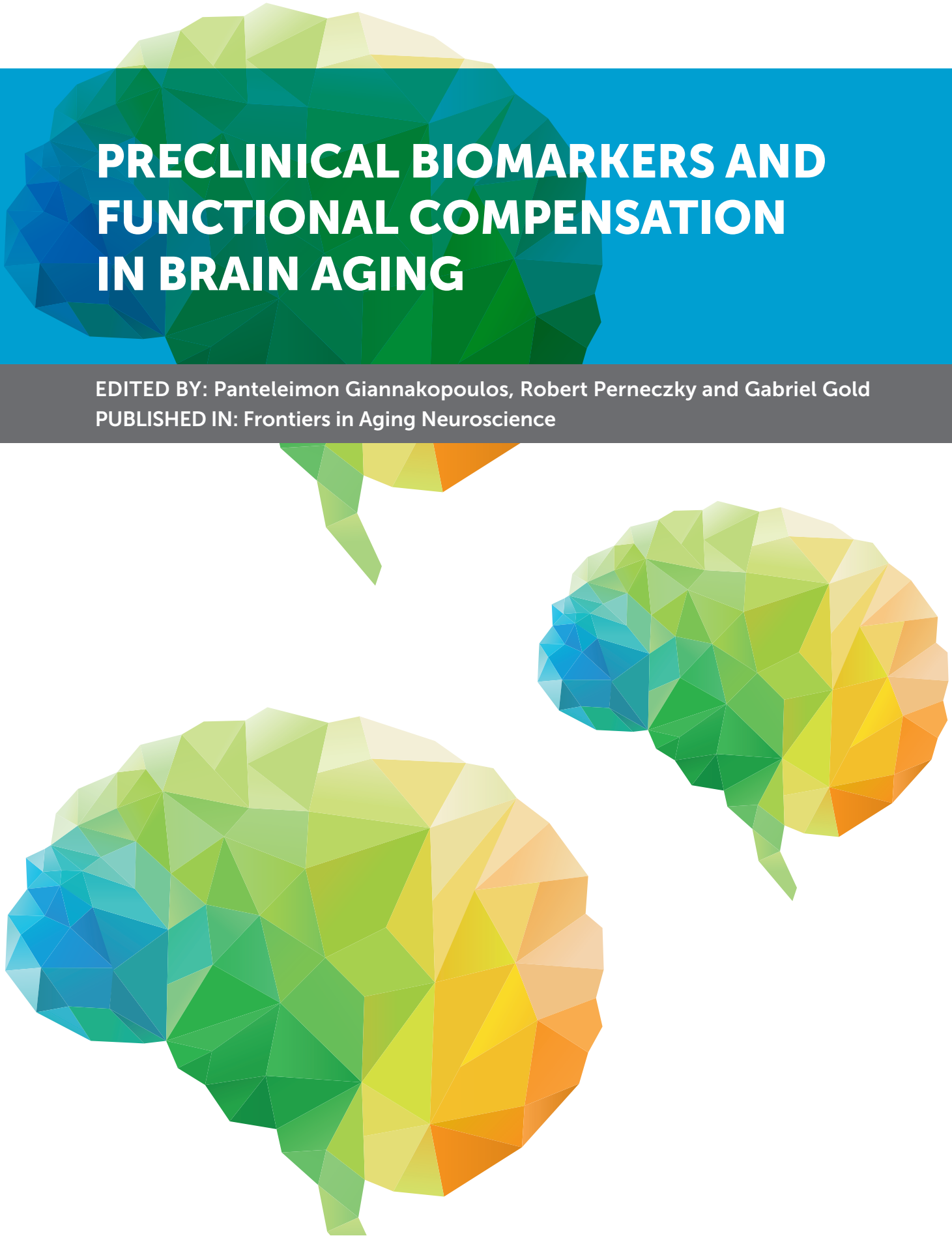

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PRECLINICAL BIOMARKERS AND FUNCTIONAL COMPENSATION IN BRAIN AGING

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Gender Differences in Elderly With Subjective Cognitive Decline

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Objective: Subjective cognitive decline (SCD), also known as significant memory concern (SMC), has been suggested as a manifestation of Alzheimer's Disease (AD) preceding mild cognitive impairment (MCI). This study assessed the impact of gender on cognition, amyloid accumulation, the volumes of hippocampus, entorhinal cortex (EC), fusiform and medial temporal lobe (MTA) and cerebrospinal fluid (CSF) pathology biomarkers in patients reporting SMC.

Methods: Twenty-nine males (mean age ± SD: 72.3 ± 5.7 years) and 40 females (mean age ± SD: 71.0 ± 5.1 years) with SMC from the AD Neuroimaging Initiative (ADNI) were included in the study. We explored the gender discrepancies in cognition, [^{18}F] AV45 amyloid positivity, volumes of hippocampus, EC, fusiform and MTA and CSF biomarkers.

Results: Compared with females, males showed significantly worse performance in Assessment Scale-cognitive subscale 13 (ADAS-13; $P = 0.004$) and lower amyloid deposition ($P < 0.001$). However, females showed greater advantage on the task of Rey Auditory Verbal Learning Test-5 (RAVLT-5) sum ($P = 0.021$), RAVLT-immediate recall ($P = 0.010$) and reduced volumes of the hippocampus, EC, fusiform and MTA ($P = 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.007$) than males. No gender differences were found in CSF Aβ42, CSF Tau and CSF P-tau ($P = 0.264$, $P = 0.454$, $P = 0.353$).

Conclusions: These findings highlight that gender discrepancies should be considered in the interpretation of cognitive measures when evaluating SMC.

Keywords: gender, significant memory concern, cognitive function, hippocampus, entorhinal cortex, medial temporal lobe

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[†]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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INTRODUCTION

Significant memory concern (SMC; also known as subjective cognitive decline (SCD) or subjective memory impairment), is defined as a self-reported cognitive complaints in the absence of objective cognitive deficits, which is common in older adults (Jessen et al., 2014; Jenkins et al., 2015). Recent mounting evidences indicated that SMC is a risk factor for future accelerated cognitive decline and progression to preclinical or clinical state of Alzheimer's disease (AD), with AD-type changes in amyloid deposition, neuroimaging and cerebrospinal fluid (CSF) biomarkers (Petersen, 2000; Visser et al., 2009; Reisberg et al., 2010; Perrotin et al., 2012; Scheef et al., 2012; Wang et al., 2012; Mitchell et al., 2014). Taken together, these results suggested that SMC might be an initial symptomatic indicator of preclinical AD (Jessen et al., 2014).

Gender-specific discrepancies in mild cognitive impairment (MCI) and AD have been observed (Roberts et al., 2012; Lin et al., 2015). Thus, sex-specific research in SMC is crucial to ensure early correct detection and pre-clinical intervention. However, to date, few studies have focused on the role of gender in SMC across a comprehensive profile of the cognitive assessment, neuroimaging and CSF AD biomarkers.

Therefore, the purpose of the current study was to go further to analyze whether the gender discrepancies are related to neuropsychological performance, CSF and positron emission tomography (PET) and magnetic resonance imaging (MRI) biomarkers of AD pathology in older adults reporting SMC.

MATERIALS AND METHODS

ADNI Study Design

Data used in the preparation of this article were obtained from the AD Neuroimaging Initiative (ADNI) database[1] during January 2018. The data collectors were blind to participant information during the experiments. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California-San Francisco. ADNI is a global research effort that actively supports the investigation and development of treatments that slow or stop the progression of AD and subjects have been recruited from over 50 sites across the US and Canada. The overall goal of ADNI is to determine biomarkers for use in AD clinical treatment trials. To date, it has three phases: ADNI1, ADNI GO and ADNI2, consisting of cognitively normal (CN) individuals, early MCI (EMCI), to late MCI (LMCI), and dementia or AD. For more information, see www.adni-info.org. This study was carried out in accordance with the recommendations of each ADNI site. The protocol was approved by the ADNI. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Subjects

The current study sample consisted of 69 ADNI-2 participants, including 29 males and 40 females. Participants were selected if they were diagnosed as having SMC. Diagnosis was made using the standard criteria described in the ADNI-2 procedures manual[2]. Briefly, SMC participants had subjective memory concerns as evaluated using the Cognitive Change Index (CCI; total score from first 12 items >16), which was based on selected items from a larger compilation of measures analyzed in an independent sample (Saykin et al., 2006), but no informant-reported memory complaints, and normal cognitive performance on the Wechsler Logical Memory Delayed Recall (LM-Delayed) and the Mini-Mental State Exam (MMSE).

[1]adni.loni.usc.edu

[2]http://www.adni-info.org

Neuropsychological Assessment

All participants underwent a standardized cognitive evaluation including the following items: (1) Global cognitive function: MMSE (Folstein et al., 1975), Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005), AD Assessment Scale-cognitive subscale 13 (ADAS-13; Mohs et al., 1997), Global Clinical Dementia Rating Scale (CDR-SB; Morris, 1993); (2) Memory: the Rey Auditory Verbal Learning Test (RAVLT), including trials 1–5 total recall (RAVLT-5 sum), 5-min delayed recall (RAVLT-immediate recall), 30-min delayed recall (RAVLT-delayed recall), yes-no recognition (RAVLT-recognition; Schmidt, 1996); (3) Attention/executive function: the Trail Making Test-A and B (TMT-A/B; Reitan, 1955); (4) Language: animal fluency, 30-item Boston Naming Task (BNT-30; Domoto-Reilly et al., 2012); (5) Visuospatial: clock Drawing Test (CDT; Brodaty and Moore, 1997); (6) 15-item Geriatric Depression Scale (GDS-15; Yesavage et al., 1982), Functional Assessment Questionnaire (FAQ; Pfeffer et al., 1982), Neuropsychiatric Inventory (NPI; Cummings et al., 1994).

Apolipoprotein E Genotyping

Apolipoprotein E (APOE; gene map locus 19q13.2) genotypes of the study subjects were obtained from the ADNI database[1]. All subjects were classified as APOE ε4 carriers with phenotypes ε2/ε4, ε3/ε4, and ε4/ε4, APOE ε4 non-carriers group with ε2/ε2, ε2/ε3 and ε3/ε3 genotypes.

Detection of CSF Aβ42, Tau and P-tau

The CSF Aβ42, Tau and P-tau immunoassays were used following a Roche Study Protocol at the University of Pennsylvania/ADNI Biomarker Laboratory, according to the preliminary kit manufacturer's instructions, as described in previous studies (Bittner et al., 2016). Values are given in pg/mL for both tau and Aβ42.

[^{18}F] AV 45 (Florbetapir) PET Scans

[^{18}F] AV 45 (Florbetapir) PET data were processed and acquired as described previously (Landau et al., 2012, 2013b). Mean florbetapir standard uptake value ratios (SUVRs) were measured within four regions (frontal, anterior cingulate, precuneus, and parietal cortex) and normalized to the whole cerebellum reference region. Participants were classified as amyloid positivity when the SUVRs were >1.11, and amyloid negativity when the SUVRs were $\leq$1.11 based on a previously established threshold (Landau et al., 2013a). For more detailed information about PET protocols and data, see the ADNI website[3].

Quantification of Volumes of Hippocampus, Entorhinal Cortex, Fusiform and Medial Temporal Lobe

The ADNI neuroimaging standardized procedure has been described in great detail elsewhere (Jack et al., 2008). ADNI-2 MRI data were acquired on a 3 Tesla MRI scanners using T1-weighted sagittal 3D magnetization-prepared rapid

[3]http://adni.loni.usc.edu/methods/

gradient-echo (MPRAGE) sequences. Cortical reconstruction and volumetric segmentation were obtained using FreeSurfer version 5.1 image analysis suite in ADNI 2[4] (McDonald et al., 2009), as described in previous reports (Fischl et al., 2001, 2002; Fleisher et al., 2005; Han et al., 2006). In this study, hippocampus, EC, fusiform and medial temporal lobe (MTA) volumes were evaluated. Further details on ADNI imaging protocols can be found at http://adni.loni.usc.edu/methods/documents/mri-protocols/.

Statistical Analysis

Demographic and clinical variables were compared between genders in the overall sample using Student's *t*-test by mean ± standard deviation (SD) according to the distribution, Mann–Whitney test for skewed distribution variables by median (M) and interquartile range (IQR), Chi-square test for categorical variables. All statistics were performed using SPSS software (version 23.0; IBM SPSS). All calculated tests were two-sided and statistical significance was set at $P < 0.05$. Figures were produced using GraphPad Prism 6.

[4]http://surfer.nmr.mgh.harvard.edu/

RESULTS

Demographic Characteristics by Gender

The overall sample was comprised of 69 participants including 29 males and 40 females were downloaded from the ADNI website. Socio-demographics and clinical characteristics of the study sample are presented in **Table 1**. In brief, females were less educated compared to males ($P = 0.036$) and males were reported with more alcohol drinking ($P = 0.038$). No gender differences were found in other variables (all $P > 0.05$).

Cognitive Profiles by Gender

Gender differences in neuropsychological performances in the study sample are demonstrated in **Table 2** and **Figure 1**. Men performed worse on ADAS-13 ($P = 0.004$), while women had statistically significant better cognitive function on RAVLT-5 sum ($P = 0.021$) and RAVLT-immediate recall ($P = 0.010$).

TABLE 1 | Demographic characteristics of subjects included in the study.

Characteristics	Total	Males (*n* = 29)	Females (*n* = 40)	*P* value
Age, years	71.6 ± 5.3	72.3 ± 5.7	71.0 ± 5.1	0.318
Education, years	16 (16–18)	18 (16–20)	16 (15–18)	0.036
Race, *n* (% White)	64 (92.8)	26 (40.6)	38 (59.4)	0.708
Ethnicity, *n* (% Not Hisp/Latino)	66 (95.7)	29 (43.9)	37 (56.1)	0.363
Marital status, *n* (% Married)	50 (72.5)	23 (46)	27 (54)	0.278
Right handedness, *n* (%)	58 (84.1)	26 (44.8)	32 (55.2)	0.280
APOE ε4 carriers, *n* (%)	25 (36.2)	8 (32)	17 (68)	0.203
Smoking, *n* (%)	30 (43.5)	15 (50)	15 (50)	0.239
Alcohol abuse, *n* (%)	3 (4.3)	3 (100)	0 (0)	0.038
Hypertension, *n* (%)	35 (50.7)	15 (42.9)	20 (57.1)	0.888
SBP (mmHg)	135.4 ± 17.5	132.1 ± 17.6	137.7 ± 17.2	0.191
DBP (mmHg)	73.3 ± 8.7	72.9 ± 9.4	73.5 ± 8.3	0.792

Abbreviations: APOE, apolipoprotein E; DBP, diastolic blood pressure; SBP, systolic blood pressure. Data are described as mean ± SD, median (M) and the interquartile range (IQR) unless otherwise specified. P values tested by Student's t-test, Mann-Whitney test and Chi-square test.

TABLE 2 | Clinical assessments by gender.

Variables	Total	Males (*n* = 29)	Females (*n* = 40)	*P* value
MMSE	29 (29–30)	29 (28–30)	29 (29–30)	0.917
MoCA	25.9 ± 2.4	25.4 ± 2.5	26.4 ± 2.3	0.098
ADAS-13	8.3 ± 3.7	9.8 ± 3.9	7.3 ± 3.3	0.004
CDR-SB	0 (0–0)	0 (0–0)	0 (0–0)	0.408
RAVLT-5 sum	46.6 ± 9.8	43.4 ± 10.9	48.9 ± 8.3	0.021
RAVLT-immediate recall	9.3 ± 3.3	8.1 ± 3.6	10.2 ± 2.7	0.010
RAVLT-delayed recall	7.4 ± 4.0	6.2 ± 4.4	8.2 ± 3.4	0.053
RAVLT-recognition	14 (12–14.5)	13 (10.5–14)	14 (12.25–15)	0.064
TMT-A	32.3 ± 10.6	34.1 ± 12.6	31.0 ± 8.9	0.229
TMT-B	82.2 ± 39.3	81.4 ± 32.0	82.8 ± 44.3	0.888
Animals fluency	20.9 ± 5.0	21.2 ± 4.9	20.7 ± 5.1	0.685
BNT-30	29 (28–30)	29 (28–29.5)	29 (28–30)	0.920
CDT	5 (4–5)	5 (4–5)	5 (5–5)	0.350
GDS-15	1 (0–2)	1 (0.5–1.5)	1 (0–2)	0.554
FAQ	0 (0–0)	0 (0–0.5)	0 (0–0)	0.843
NPI	0 (0–2.5)	0 (0–3)	0 (0–1.75)	0.632

Abbreviations: ADAS-13, Alzheimer's Disease Assessment Scale-cognitive subscale 13; BNT-30, Boston Naming Task; CDR-SB, Global Clinical Dementia Rating Scale; CDT, Clock Drawing Test; FAQ, Functional Assessment Questionnaire; GDS-15, Geriatric Depression Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NPI, Neuropsychiatric Inventory; RAVLT, Rey Auditory Verbal Learning Test; TMT, The Trail Making Test. Data are presented as mean ± SD, median (M) and the interquartile range (IQR). P values tested by Student's t-test, Mann-Whitney test.

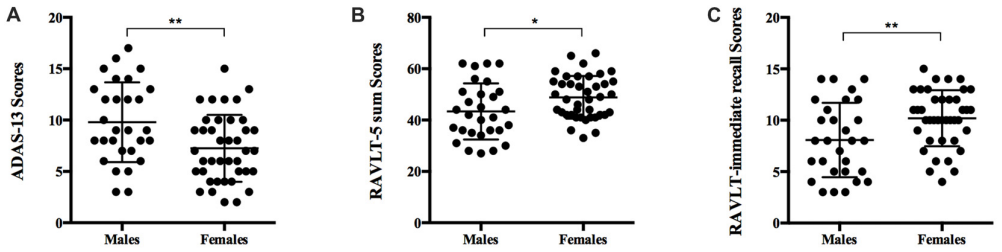

FIGURE 1 | Comparison of neuropsychological measures in men and women with significant memory concern (SMC). Scatter plots displaying cognitive function in males and females. **(A)** Men had significantly worse cognitive function as measured by ADAS-13 ($P = 0.004$). **(B)** Women showed greater advantages on RAVLT-5 sum ($P = 0.021$). **(C)** Women excelled at RAVLT-immediate recall ($P = 0.010$). ADAS-13, Alzheimer's Disease Assessment Scale-cognitive subscale 13; RAVLT, Rey Auditory Verbal Learning Test. *P* values tested by Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$.

However, there were no gender differences in performance on MMSE, MoCA, CDR-SB, RAVLT-delayed recall, RAVLT-recognition, TMT-A, TMT-B, Animals fluency, BNT-30, CDT, GDS-15, FAQ, NPI ($P = 0.917$, $P = 0.098$, $P = 0.408$, $P = 0.053$, $P = 0.064$, $P = 0.229$, $P = 0.888$, $P = 0.685$, $P = 0.920$, $P = 0.350$, $P = 0.554$, $P = 0.843$, $P = 0.632$).

CSF Biomarkers

There were no significant differences in CSF Aβ42, Tau and P-tau levels between males and females ($P = 0.264$, $P = 0.454$, $P = 0.353$; see **Table 3**).

Aβ Deposition in Different Groups

Significant decreases in [^{18}F] AV45 SUVRs were found in males compared with females ($P < 0.001$). Aβ positivity was detected in 17.4% of males, 82.6% of females respectively. The percentage of Aβ positive subjects was significantly higher in females vs. males ($P = 0.003$; **Table 3**).

Volumes of Hippocampus, Entorhinal Cortex, Fusiform and Medial Temporal Lobe by Gender

The volumes of hippocampus, EC, fusiform, medial temporal lobe (MTA) are illustrated in **Table 3**. There were significantly larger volumes of hippocampus, EC, fusiform and MTA in men compared to women ($P = 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.007$).

DISCUSSION

In the present study, we explored a statistically significant gender discrepancy in cognition function, Aβ deposition and brain volume in older adults with SMC. The results confirmed that women with SMC outperformed men with SMC on the tasks of RAVLT-5 sum and RAVLT-immediate recall, while the advantage was eliminated on the task of RAVLT-delayed recall and a floor effect might limit interpretation. However, we found that men with SMC were associated with worse performance on ADAS-13. No significant differences were observed between males and females on other cognitive domains. Our results are in concordance with previous studies showing that the female advantage in verbal memory task was more apparent than men (Herlitz et al., 1997; Sundermann et al., 2016a,b). These observations suggest that gender discrepancies among SMC subjects might be appropriate to a specific cognitive domain. If so, then implementing sex-adjusted norms in clinical memory tests might ameliorate the diagnostic accuracy in women.

Females with SMC participants showed increased Aβ deposition relative to males. Elevated risk of AD was in women compared to men, although the underlying mechanism remains elusive as previously reported (Seshadri et al., 2006). However, we did not discover differences in CSF Aβ42, Tau and P-tau between males and females with subjective cognitive impairment. It is noteworthy that we observed significant reduction in volumetric measurements of hippocampus, EC, fusiform gyrus and MTA in females with SMC subjects.

TABLE 3 | Measurements of cerebrospinal fluid (CSF) biomarkers, positron emission tomography (PET) and magnetic resonance imaging (MRI) in the subjects.

Variables	Total	Males (*n* = 29)	Females (*n* = 40)	*P* value
CSF Aβ42 (pg/mL)	1370.6 ± 614.2	1468.2 ± 587.2	1299.9 ± 630.9	0.264
CSF Tau (pg/mL)	243.3 ± 94.3	233.3 ± 90.6	250.6 ± 97.4	0.454
CSF P-tau (pg/mL)	22.4 ± 10.1	21.0 ± 9.1	23.3 ± 10.8	0.353
[^{18}F] AV45 SUVRs	1.06 (1.01–1.21)	1.01 (0.98–1.09)	1.11 (1.03–1.36)	<0.001
Aβ positivity, *n* (%)	23 (33.3)	4 (17.4)	19 (82.6)	0.003
Hippocampus (mm^3)	7678.7 ± 911.7	8095.7 ± 950.5	7376.4 ± 760.1	0.001
Entorhinal (mm^3)	3905.1 ± 568.2	4215.7 ± 532.8	3679.9 ± 485.1	<0.001
Fusiform (mm^3)	18853.0 ± 2209.3	20071.6 ± 2185.2	17969.5 ± 1782.7	<0.001
MTA (mm^3)	20823.3 ± 2776.2	21863.6 ± 3072.6	20069.1 ± 2296.1	0.007

Abbreviations: MTA, medial temporal atrophy; SUVR, standardized uptake values ratio. P values tested by Student's t-test, Mann-Whitney test and Chi-square test.

Consistent with the cognitive reserve theory (Klonoff and Landrine, 1992; Stern et al., 1994, 2003; Stern, 2002), better premorbid performance of women on verbal episodic memory tests might confer an advantage in the ability to maintain normal verbal memory performance despite reduced volumes of hippocampal, EC, fusiform gyrus, MTA and accumulating AD pathology (Sundermann et al., 2016a,b, 2017). However, women may have more accelerated decline once neuropathology reached a threshold level (Klonoff and Landrine, 1992; Stern et al., 1994).

Several limitations should be mentioned in the current study. First, a simple cross-sectional design used in the study does not definitively permit the theory that female advantage in verbal memory may act as a specific form of cognitive reserve, further longitudinal researches would be more necessary to closely confirm the conclusions. Second, The ADNI cohort included a potential selection bias based on the fact that ADNI participants had a high education level (Petersen et al., 2010; Grill et al., 2013), which may affect the generalizability to a greater population sections. Third, the relatively small group size could limit the interpretation of our results, larger sample of numbers need to be collected in future studies.

CONCLUSION

In summary, the present results highlighted the urgent need to consider the sex differences in cognition evaluation, which contributes to clinical diagnosis even in preclinical stages, such as SMC.

AUTHOR CONTRIBUTIONS

LW: conceived and designed the studies; wrote the article; is the corresponding author. LW and TT: performed the research; analyzed the data. All authors approved the final version of the manuscript for publication.

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[5]www.fnih.org

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Gene Expression Analysis Reveals Novel Gene Signatures Between Young and Old Adults in Human Prefrontal Cortex

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Human neurons function over an entire lifetime, yet the molecular mechanisms which perform their functions and protecting against neurodegenerative disease during aging are still elusive. Here, we conducted a systematic study on the human brain aging by using the weighted gene correlation network analysis (WGCNA) method to identify meaningful modules or representative biomarkers for human brain aging. Significantly, 19 distinct gene modules were detected based on the dataset GSE53890; among them, six modules related to the feature of brain aging were highly preserved in diverse independent datasets. Interestingly, network feature analysis confirmed that the blue modules demonstrated a remarkably correlation with human brain aging progress. Besides, the top hub genes including PPP3CB, CAMSAP1, ACTR3B, and GNG3 were identified and characterized by high connectivity, module membership, or gene significance in the blue module. Furthermore, these genes were validated in mice of different ages. Mechanically, the potential regulators of blue module were investigated. These findings highlight an important role of the blue module and its affiliated genes in the control of normal brain aging, which may lead to potential therapeutic interventions for brain aging by targeting the hub genes.

Keywords: normal brain aging, prefrontal cortical regions, transcriptomic, weighted gene correlation network analysis (WGCNA), hub gene

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INTRODUCTION

Brain aging is characterized by a progressive loss of physiological integrity including loss of gray and white matter volume, a general loss of dendritic spines, loss of synaptic plasticity, increased axonal bouton turnover rates, and elevated inflammation, leading to impaired function and increased vulnerability to neurodegenerative disease (Salthouse, 2009; Dorszewska, 2013; Grillo et al., 2013; Lopez-Otin et al., 2013). However, the systematic cellular mechanisms behind the normal brain aging phenotypic changes in the absence of neurodegenerative disease of healthy older adults are only barely understood. Inspiringly, precision medicine has emerged as a new approach to

health care base on the individual's molecular drivers of disease (Montine and Montine, 2015). Therefore, applying this tailored and molecular mechanism-based approach to understand and reduce the negative impacts of brain aging are very promising. Even though recent reports have suggested the distinct changes in the expression of genes at the single neuron level (Kadakkuzha et al., 2013), the systematic cellular mechanisms behind the normal brain aging phenotypic changes in the healthy older adults are only barely understood. One method to study molecular mechanisms of aging is the high-throughput technology. However, the biased process in large changes analysis of differential gene expression, as well as lacking the consideration of the relationship between changing genes as a whole are inevitable drawbacks for this method (Furlong, 2013; Lou et al., 2017).

In order to explore the dynamic changes for understanding the system-level properties of normal brain aging progress in an unbiased manner, one network approach, named weighted gene correlation network analysis (WGCNA) is proposed. It can group functionally correlated genes into modules (Langfelder and Horvath, 2008). These modules are constructed by calculating a correlation network analysis of large, high-dimensional datasets, which are based on pairwise correlations between genes due to their similar expression profile, and can correlate with different stages of clinical traits (Langfelder and Horvath, 2008). The R package for WGCNA has been successfully applied in various biological contexts, e.g., cancer (Heiland D. et al., 2017; Sun et al., 2017), mouse genetics (Savas et al., 2017), and analysis of brain imaging data (Heiland D. H. et al., 2017), which can also be used to describe the correlation structure between gene expression profiles, image data, genetic marker data, proteomics data, and other high-dimensional data (Langfelder and Horvath, 2008). The R package along with its source code and additional material are freely available at https://cran.r-project.org/web/packages/WGCNA/WGCNA.pdf. Even though, WGCNA approach has provided a comprehensive characterization of the transcriptomic changes for disease's functional interpretation and led to new insights into the molecular aspects of clinical-pathological factors, there are very few reports applying WGCNA to identify gene co-expression networks associated with normal brain aging. To fulfill this gap, we conduct a WGCNA method by calculating module-trait correlations based on GSE53890 public microarray dataset, which include 41 samples and 24,455 genes. This approach identifies six meaningful co-expression modules significantly related to normal brain aging and highly preserved in other brain aging datasets. Besides, hub genes contributing to normal brain aging are also verified. Herein, this paper is devoted to discovering novel gene signatures that greatly impact the progression of normal brain aging by WGCNA approach.

MATERIALS AND METHODS

mRNA Expression Data

First, the microarray-based expression dataset GSE53890 provided by Lu et al. (2014) was downloaded from the NCBI Gene Expression Omnibus (GEO[1]). This dataset contained quantile normalized genome-wide expression profiles of adult human brain samples from prefrontal cortical regions, including samples from 12 young (<40 years), 9 middle aged (40–70 years), 16 normal aged (70–94 years), and 4 extremely aged (95–106 years). And these postmortem brain tissue samples used in this study were neuropathologically normal for age, and were derived from non-demented individuals (**Supplementary File 1, Table S16**). The dataset was produced using Affymetrix Human Genome U133 plus 2.0 arrays, which allowed the expression analysis of over 47,000 transcripts. The other microarray datasets referenced during the study (GSE1572, GSE71620, GSE30272, GSE21779, and GSE11882) were also available in the public repository from NCBI GEO datasets. All the other datasets supporting the findings of this study were available within the article and provided it as **Supplementary File 2, Table S1**. For the public datasets, its detailed experimental methods and descriptions could be found in the original references. Notably, only the human normal brain aging samples in these datasets were included in our study.

Microarray Data Analysis

After the raw data of GSE53890 was downloaded in CEL format, it was pre-processed identically with the R package affy by using the Robust Multichip Average (RMA) function for background correction, normalization, and summarization with the quantiles method (Irizarry et al., 2003; Giulietti et al., 2016). For this purpose, a cross-platform common identifier, the array annotation data hgu133plus2.db was used to transform the array probes to the respective Entrez Gene ID. Probes matching multiple genes were removed from the dataset, and then we calculated the average expression values of genes matching multiple probes. A proper threshold was set based on the amount of genes filtered out.

Gene Co-expression Network Construction

Co-expression networks were constructed using WGCNA (v1.47) package in R (Langfelder and Horvath, 2008). After filtering genes, gene expression values were imported into WGCNA to construct co-expression modules using the automatic network construction with default settings. First, a matrix of adjacencies using the WGCNA function adjacency was constructed by calculating Pearson correlations between all pairs of genes across all selected samples, after which this matrix was computed into a Topological Overlap Matrix (TOM) using the function TOMsimilarity (Zhang and Horvath, 2005). The TOM, referred to the interconnection between two genes, was used as input for hierarchical clustering analysis, and a cluster of genes with high topological overlap was defined as a module. Finally, modules were identified on the dendrogram with the function cutreeHybrid from the R package dynamicTreeCut algorithm (Langfelder et al., 2008). The module eigengene (ME) was considered as a representation of the gene expression profiles in a module, which was defined as the basic component of

[1]https://www.ncbi.nlm.nih.gov/gds

a given module (Langfelder and Horvath, 2008). The module membership (MM) was calculated by the WGCNA function signedKME that correlated the ME with gene expression values, so it quantified how close a gene was to a given module (Langfelder and Horvath, 2008). Moreover, genes, which were infirmly correlated with all of the MEs ($|kME| < 0.7$), were assigned to none of the modules (Lou et al., 2017). Finally, the interesting module network was visualized by Cytoscape_3.3.0 (Demchak et al., 2014).

Calculation of Module-Trait Correlations and Module Preservation

Correlations among gene expression modules and phenotypic trait for GSE53890 were investigated; age and sex were chosen as our interesting trait. Modules having significant relationships with age trait were listed in **Supplementary File 1, Table S3**. Modules were labeled with a conventional color scheme. Besides, a WGCNA integrated function (modulePreservation) was applied to calculate module preservation statistics between two relevant datasets. And then, two composite preservation statistics for module preservation were delineated as follows: the definition of Zsummary was the average of *Z*-scores computed for density and connectivity measures, which represented the significance of observed statistics. Analogously to the definition of median rank, the statistic median rank was defined as the average calculation of median ranks for connectivity and density measures of each module (Langfelder et al., 2011; Lou et al., 2017). Eventually, median rank was useful for identifying relative preservation among multiple modules; if a module had a lower median rank, it tended to exhibit stronger observed preservation statistics than a higher one. Zsummary was used to assess the significance of observed statistics by distinguishing preserved from non-preserved modules via permutation testing 200 times (Langfelder et al., 2011; Lou et al., 2017).

Feature Vectors in WGCNA Network

The correlation between individual genes and biological trait (age and sex) was defined as the gene significance (GS). The summation of adjacency performed over all genes in a particular network was calculated as the intramodular connectivity (K.in). Generally, if GS and MM were highly associated, it implied that genes were the highly important elements for modules and were most significantly correlated with the trait. Meanwhile, if the MM was highly related to K.in, it indicated that a gene was more vital than the given module (Zhang and Horvath, 2005; Lou et al., 2017). From above, hub genes were usually characterized with high GS, high MM and high K.in in a module, which were highly connected with other genes and hence of high functional significance, as well as tended to be located in the center of a module network (Lou et al., 2017).

Functional Annotation of the Modules

For genes in each module, Gene Ontology (GO) and KEGG pathway enrichment analysis were conducted to analyze the biological functions of modules. Significantly enriched GO terms and pathways in genes in a module comparing to the background were defined by hypergeometric test and with a threshold of false discovery rate (FDR) less than 0.05. The Enrichr database[2] contained a large collection of gene set library; these libraries had been constructed from many sources such as published studies and major biological and biomedical online databases (Chen et al., 2013; Giulietti et al., 2016). Thus, we input the interesting modules into the Enrichr by comparing them to the annotated gene sets libraries. Enrichr implemented four scores to assess enrichment results: *p*-value, *q*-value, rank (*Z*-score), and combined score. The rank score or *Z*-score was computed to assess the deviation from an expected rank by using a modification to Fisher's exact test. Finally, the combined score was calculated by multiplying the two scores as follows: $C = \log(p)^{*}Z$. Where C is the combined score, p is the p-value computed using Fisher's exact test, and Z is the Z-score computed to assess the deviation from the expected rank (Chen et al., 2013; Giulietti et al., 2016; Lou et al., 2017).

Animal Study and Histological Analysis of Mouse Brain

Animal housing and experiments were carried out according to the guidelines of the Animal Ethics Committees of Jinan University and were performed under the standard biosecurity and institutional safety procedures. Male C57BL/6J mice (3-month-old and 12-month-old) were maintained in a 12-h light–dark-cycle at room temperature with access to food and water *ad libitum* in our animal facilities. The mice were divided into two groups (3-month-old and 12-months-old). At the end of the experiments, brains were fixed by intracardial perfusion with 4% (vol/vol) paraformaldehyde (PFA) in PBS, followed by the fixation in the same mixture overnight. Then, they were processed for paraffin embedding, according to standard procedures. A part of the brain tissue was homogenated in TRIzol® Plus RNA Purification Kit (Life Technologies), and one microgram of RNA was then reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Invitrogen) for the quantitative real-time PCR analysis. Formalin-fixed brain tissue was processed into 4 μm thick paraffin sections and stained with hematoxylin and eosin (HE) staining. For quantification of neuronal density, randomly selected areas within the hippocampus or the cortex were imaged at a magnification fluorescent microscope (Carl Zeiss, Axio Imager.A2, Germany).

MDA and SOD Determination

About 200 ± 50 mg brain tissues of prefrontal cortex (PFC) was taken and washed by precooled normal saline (NS) for at least three times. And then, they were converted to 100g/L of brain homogenates in a homogenizer filled with nine times the mass of precooled NS. The homogenates were centrifuged at 4°C for 20 min at a speed of 3500 r/min. The protein quantification of supernatant was estimated by BCA method. And then, proper amount (50–100 μg) of supernatant's lipid peroxidation levels (MDA) and SOD activity were measured

[2]http://amp.pharm.mssm.edu/Enrichr

according to the specifications of MDA kit (S0131, Beyotime, China) and SOD kit (S0101, Beyotime, China).

qPCR Analysis

Total RNA from PFC brain tissue was extracted by TRIzol (Invitrogen, United States). Synthesis of cDNA was performed by using 2 μg of total RNA with PrimeScript™ Reverse Transcriptase (Takara) according to the manufacturer's instructions. Specific primers used for PCR were listed as follow:

5′-GTAACCCGTTGAACCCCATT-3′ (18S rRNA-sense),
5′-CCATCCAATCGGTAGTAGCG-3′ (18S rRNA-anti-sense);
5′-CCTGAACACCGCACATAC-3′ (Ppp3cb-sense),
5′-CATCACCTTGGTCAACCC-3′ (Ppp3cb-anti-sense);
5′-GAAGGCCTGGCTTACCTACC-3′ (Camsap1-sense),
5′-AGACCCAAAGCAGCTACACC-3′ (Camsap1-anti-sense);
5′-CCAAAGGAGGGTGTTGAGAGG-3′ (Actr3b-sense),
5′-GCCATGTCGTATAGGCCACTT-3′ (Actr3b-anti-sense);
5′-GCACTATGAGTATTGGTCAAGCA-3′ (GNG3-sense),
5′-GTGGGCATCACAGTATGTCATC-3′ (GNG3-anti-sense).

The gel image was acquired in the Gel Doc 1000 system and analyzed using the Quantity One software (Bio-Rad Laboratories, Hercules, CA, United States). 18S rRNA was chosen as the endogenous control and cycle dependence was carried out to ensure that the PCR products fell within the linear range. Quantitative real-time PCR was performed using the SYBR® Premix Ex Taq Kit (Takara) in a 7900 Real Time PCR System (Applied Biosystems, United States) for at least three independent experiments. The relative quantification expression of each gene was normalized to 18S rRNA, and calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

All experiments were performed for at least three independent times, and the data were expressed as the mean ± standard deviation (SD). All statistical analysis was performed using GraphPad Prism 6 Software (GraphPad Software, San Diego, CA, United States). Comparison between two groups was conducted by using Student's *t*-test. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

Pre-processing of the Aging Human Prefrontal Cortex Datasets and Construction of Weighted Gene Co-expression Networks

The combined dataset (GSE53890) containing a total of 41 samples [12 young (<40 years), 9 middle aged (40–70 years), 10 normal aged (70–90 years), and 10 extremely aged (90–106 years)] with clear brain aging staging was applied into this study (**Supplementary File 2, Table S1 and Figure S1**). Raw data from each microarray dataset were then pre-processed identically for background correction and normalization. Firstly, probes matching multiple genes were removed out from these datasets, and secondly the average expression value of gene measured by multiple probes was calculated as the final expression value. Finally, we identified in total 24,455 genes that were expressed (**Supplementary File 1, Table S1**). Besides, constructing a WGCNA needed an optimal soft-thresholding power to which co-expression similarity was raised to calculate adjacency. Thus, we performed the analysis of network topology for various soft-thresholding powers in order to have relative balanced scale independence and mean connectivity of the WGCNA. As shown in **Figures 1A,B**, power 8, the lowest power for which the scale-free topology fit index reached 0.90, was chosen to produce a hierarchical clustering tree (dendrogram). Next, through dynamic tree cut and merged dynamic, 35 distinct gene modules were generated in the hierarchical clustering tree (dendrogram) from 41 samples and each module labeled by different colors was shown by the dendrogram (**Figure 1B**), in which each tree branch constituted a module and each leaf in the branch is one gene. The size of modules ranged from 64 (darkolivegreen module) to 9,296 (turquoise module) genes. As shown in **Figure 1C**, the module network dendrogram was constructed by clustering ME distances. Modules with high K.in were located at the tip of the branches since they exhibit the highest interconnectedness with the rest of the module. The horizontal line (blue and red line) represented the threshold (0.2) used for defining the meta-modules. Thus, 19 distinct gene modules were identified. To further quantify co-expression similarity of entire modules, we calculated their eigengenes adjacency on their correlation of the entire modules (**Supplementary File 2, Figure S2**) and 19 modules (**Figure 1D**) based on the heatmap, respectively. Each module showed independent validation to each other as well, and the progressively more saturated blue and red colors indicated the high co-expression interconnectedness (**Figure 1D**). All attributes of genes and samples were shown in **Supplementary File 1, Tables S2, S3**.

Identification of Meta-Modules Related to the Brain Aging

As we known, the ME is the first principal component of a given module and can be considered as a representative of the module's gene expression profile. The 19 MEs for the 19 distinct modules were each correlated with age trait, which has been shown in eigengenes trait-specific expression profiles (**Supplementary File 1, Table S4**). Next, we evaluated the relationship between each module and aging status by correlating the eigengenes of each module with age and sex traits. The age and sex traits include the whole age and sex range in 41 individuals (**Supplementary File 2, Table S2**). We found that, as expected, six modules (blue, darkolivegreen, darkturquoise, magenta, steelbule, midnightblue) exhibited similar characteristics in age trait (absolute $r > 0.5$, $P < 10^{-2}$; **Figure 2A**), while others were not preserved. Notably, among them, four modules (blue, darkolivegreen, magenta, steelbule) were negatively correlated with age ($r < -0.5$, $P < 10^{-2}$; **Figure 2A**), thereafter named anti-aging module. Two positively correlated modules (darkturquoise

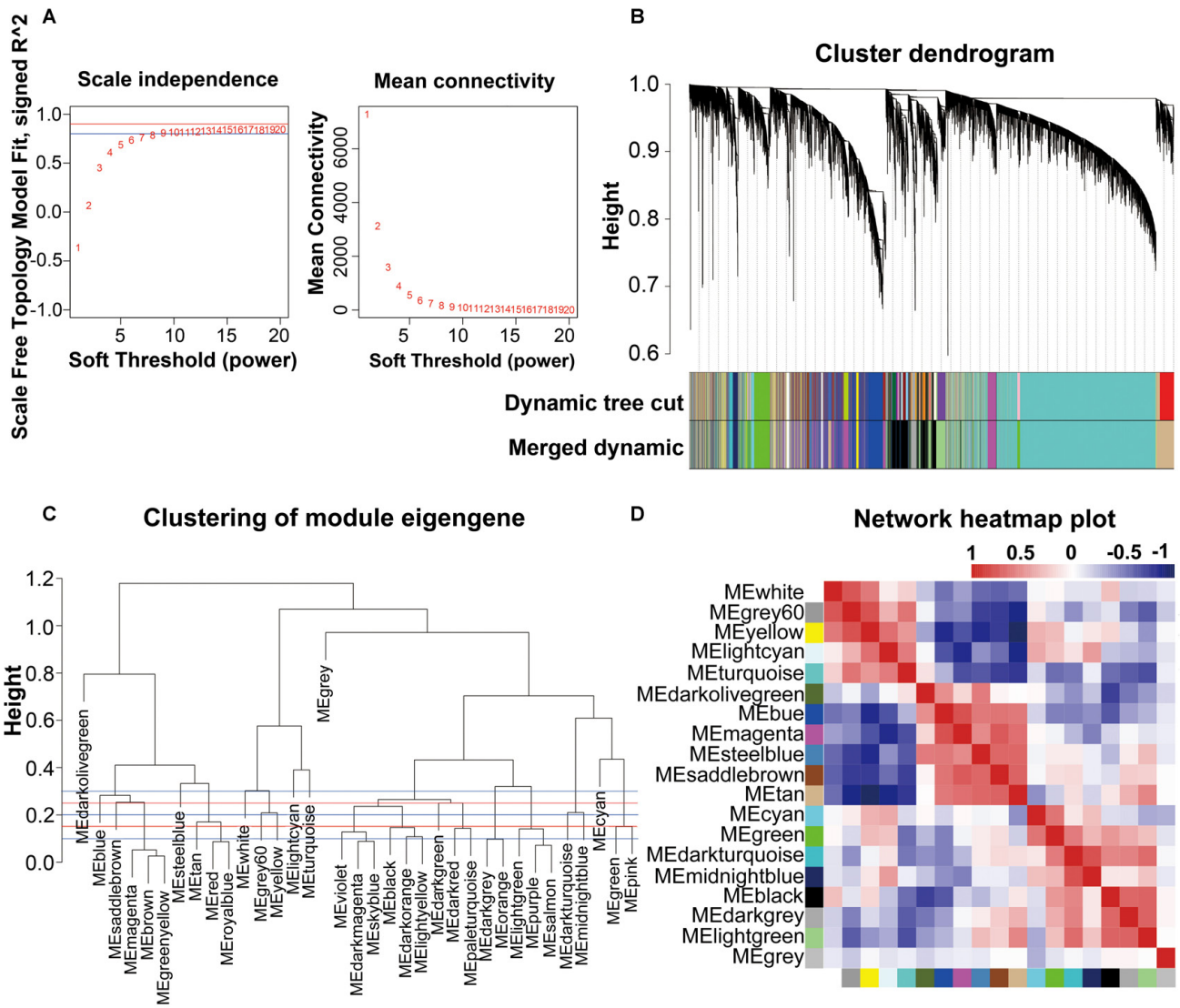

FIGURE 1 | WGCNA network and module detection. **(A)** Selection of the soft-thresholding powers. The left panel showed the scale-free fit index versus soft-thresholding power. The right panel displayed the mean connectivity versus soft-thresholding power. Power 8 was chose, for which the fit index curve flattens out upon reaching a high value (>0.9). **(B)** Cluster dendrogram and module assignment for modules from WGCNA. Genes were clustered based on a dissimilarity measure (1-TOM). The branches correspond to modules of highly interconnected groups of genes. Colors in the horizontal bar represent the modules. 35 modules with 24,455 transcripts were detected with WGCNA. **(C,D)** Meta-module identification and module-module relationship. The module network dendrogram was constructed by clustering module eigengene distances. The horizontal line (blue and red line) represents the threshold (0.2) used for defining the meta-modules. Thus, 19 distinct gene modules were identified. In the heatmap of module-module relationship, the progressively more saturated blue and red colors indicated the high co-expression interconnectedness.

and midnightblue) named aging module thereafter. Besides, in our study, we found that the sex trait had no significant relationship with the 19 distinct modules, so we just simply ignored it. Further, a consensus clustering also confirmed the two main group were clearly separated by the 41 aging samples from young to old (**Figure 2B**). Similarly, the six interesting modules based on ME expression profile and 41 samples with extract age trait from young to old were also displayed in **Figure 2C**. The module eigengene E in *Y*-value was defined as the first principal component of a given module. It can be considered a representative of the gene expression profiles in a module. The *X*-value of **Figure 2C** from young to old in exact ages was shown in **Supplementary File 2, Table S2**. Modules were labeled using a conventional color scheme.

Module Stability and Preservation Analysis

To test the stability of the indicated modules, a WGCNA integrated function (modulePreservation) was applied to calculate module preservation statistics and the Zsummary

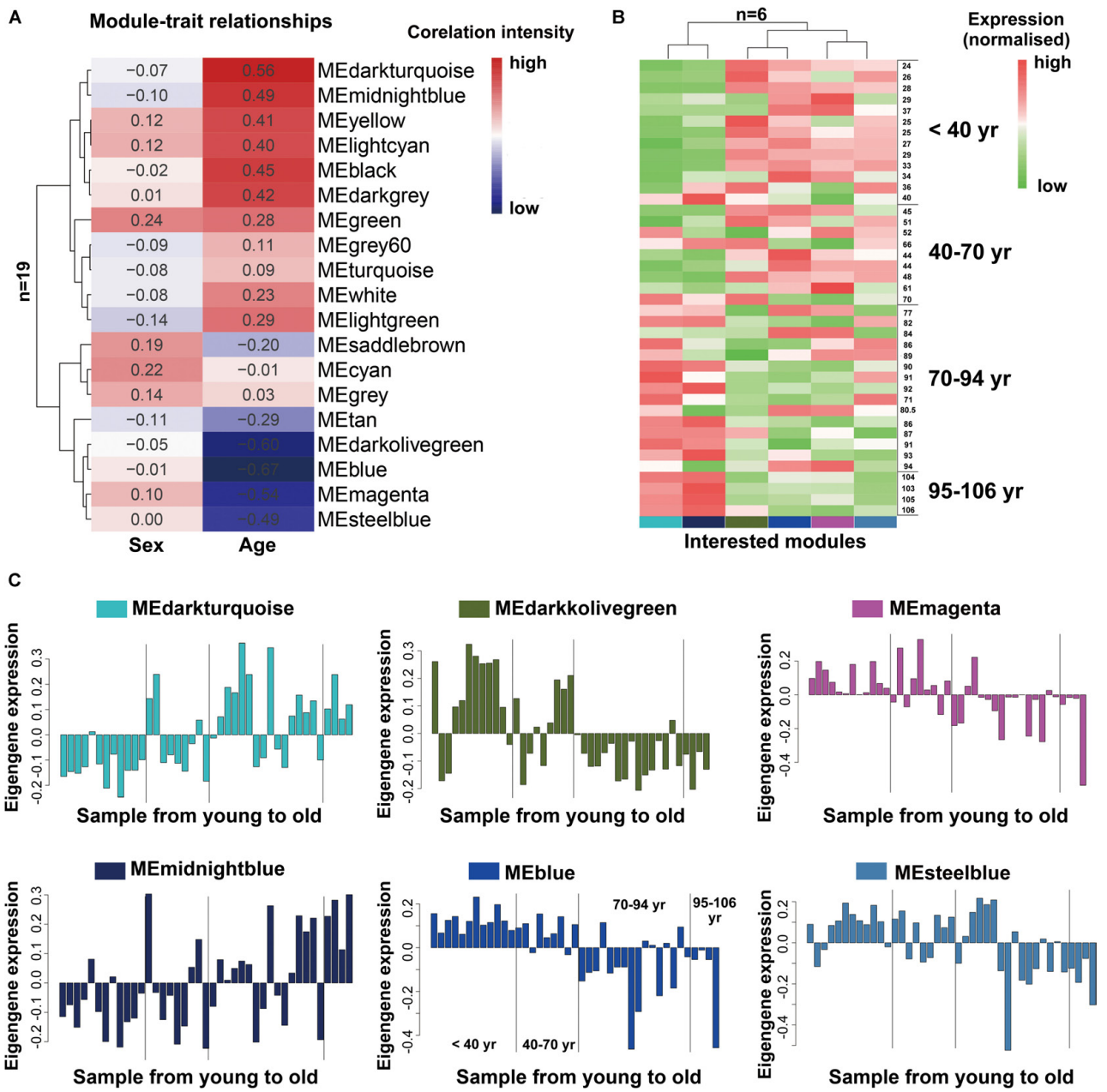

FIGURE 2 | Module-trait and the gene expression of interested modules in the aging human prefrontal cortex. **(A)** Supervised hierarchical cluster of each row correspond to a module eigengene (n = 19), column to a trait. Each cell contained the corresponding correlation. High correlations was colored in red, low correlation in blue. **(B)** Hierarchical cluster analysis of six interested modules, based on the module-trait's correlation and p value (absolute $r > 0.5$, $P < 10–2$), four modules (blue, darkolivegreen, magenta, steelbule) showed relatively high expression in young adults (red) and lower expression (green) in the aging population. Conversely, the other two modules (darkturquoise and midnightblue) showed the opposite result. Each lane represented an individual prefrontal cortical brain sample. **(C)** The histograms described the eigengene expression of each module from young to old.

score (Z-score) was used to evaluate whether a module was conserved or not. Modules with a Z-score > 10 were regarded as highly preserved. To ascertain if the identified modules were preserved in other different datasets, an independent validation was carrying out. We retrieved four datasets, which was relevant to brain aging and all samples were from human PFC. Results showed that the anti-aging modules (blue, magenta, darkolivegreen) were preserved stably in GSE11882, GSE30272, GSE71620, and GSE1572 datasets (**Figure 3**), while aging modules (darkturquoise, midnightblue) showed weak to none evidence for module preservation according to the summary preservation analysis (**Figure 3**). The blue and magenta modules were regarded as the highly representative aging-associated modules, because they both

made a higher conservation and consistent association with brain aging.

Functional Enrichment Analysis of the Gene Modules of Interest

To explore the biological functions of the anti-aging modules (blue, magenta, darkolivegreen), we performed GO term enrichment analysis, as well as pathway ontology analyses by using the Database for Annotation, Visualization and Integrated Discovery (DAVID[3]) (Huang da et al., 2009). Top biological processes and KEGG pathway in each module was shown in **Table 1**. For the blue module, the top two enriched terms in GO ontology were "transport" (FDR = 3.34E-15) or "establishment of localization" (FDR = 3.34E-15). For the KEGG pathway analysis, the top enriched terms were "Synaptic vesicle cycle" (FDR = 1.07E-09) and "cGMP – PKG signaling pathway" (FDR = 3.14E-08). For magenta module

[3]http://david.abcc.ncifcrf.gov/

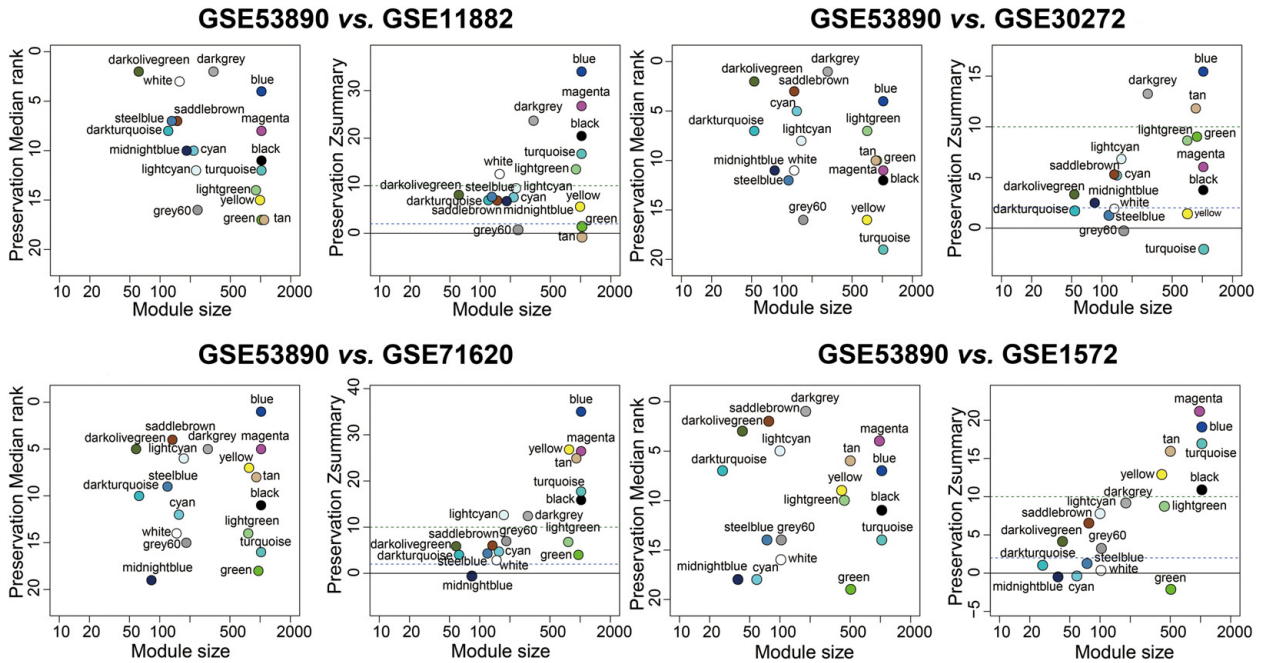

FIGURE 3 | Preservation analysis of GSE53890 network modules in different brain aging datasets. Each module was represented by its color-code and name. Left figure showed the composite statistic preservation median rank. This measure tended to be independent from module size with high median ranks indicating low preservation. Right figure showed preservation Zsummary statistic. The dashed blue and green lines indicated the thresholds $Z = 2$ and $Z = 10$, respectively. Zsummary < 2 implied no evidence for module preservation, 2 < Zsummary < 10 implies weak to moderate evidence, and Zsummary > 10 implies strong evidence for module preservation. The anti-aging modules (blue, magenta, darkolivegreen) showed high preservation statistics summary than expected by random chance using bootstrapping validation procedures.

TABLE 1 | Top GO and pathway enrichment in each module.

	Module	Category	Term	*P*-Value	FDR
Anti-aging module	Blue	GOTERM_BP	GO:0006810-transport; GO:0051234-establishment of localization	6.54E-17	3.34E-15
	Darkolivegreen	GOTERM_BP	GO:0007267-cell–cell signaling	1.69E-10	2.12E-08
	Magenta	GOTERM_BP	GO:0007005-mitochondrion organization	2.06E-41	1.50E-39
	Steelbule	GOTERM_BP	GO:0007267-cell–cell signaling	3.11E-03	4.86E-01
Aging module	Darkturquoise	GOTERM_BP	GO:0016070-RNA metabolic process	1.29E-02	3.38E-01
	Midnightblue	GOTERM_BP	GO:0090304-nucleic acid metabolic process	5.64E-02	9.83E-01
	Module	**Category**	**Term**	***P*-Value**	**FDR**
Anti-aging module	Blue	KEGG_PATHWAY	ko04721:Synaptic vesicle cycle	3.69E-12	1.07E-09
	Darkolivegreen	KEGG_PATHWAY	ko04713:Circadian entrainment	4.48E-03	1.76E-01
	Magenta	KEGG_PATHWAY	ko00190:Oxidative phosphorylation	1.09E-21	2.98E-19
	Steelbule	KEGG_PATHWAY	ko05032:Morphine addiction	7.74E-09	6.89E-07
Aging module	Darkturquoise	KEGG_PATHWAY	ko04914:Progesterone-mediated oocyte maturation	1.26E-02	3.13E-01
	Midnightblue	KEGG_PATHWAY	ko03022:Basal transcription factors	6.82E-04	4.70E-02

genes, the top enriched terms in the GO and KEGG pathway databases were "mitochondrion organization" (FDR = 1.50E-39) and "Oxidative phosphorylation" (FDR = 2.98E-19). Moreover, genes in darkolivegreen module were found to be significantly enriched in cell–cell signaling of the GO term and circadian entrainment signaling pathway. The complete annotation for each module was provided in **Supplementary File 1, Tables S5, S6**. These findings together with previous research implied that extensive oxidative phosphorylation and accelerated mitochondrion organization were the fundamental characteristics of brain aging.

Network Analysis of the Gene Modules of Interest

To further investigated the gene constitution of particular modules which were most related with the brain aging, three network unique properties such as GS, MM, and K.in were carried out. Abstractly speaking, if a gene is higher with GS, MM, and K.in, it is more meaningful with the clinical trait (Langfelder et al., 2013; Lou et al., 2017). Thus, a specific module whose MM, K.in or GS were significantly connected and associated with the brain aging trait; it implied that this module may play a more important biological role on aging progression (Lou et al., 2017). Of the six interesting modules, blue, magenta and steelblue modules showed significant correlations between MM and GS. Similarly, there were also a markedly correlation between GS and K.in in the blue, magenta, and steelblue modules (**Figure 4**). Overall, module blue were observed as the best meaningful module by its strongly positive correlations ($r = 0.71$, $p <$ E-200 in GS vs. MM; $r = 0.63$, $p <$ E-200 in GS vs. K.in). These results indicated that blue module was closely involved in human brain aging progression.

Characterization of the Blue Module Content and Hub Genes

To explore the blue module's gene expression profiles and its distribution in the 41 samples, a hierarchical cluster analysis was carried out, and result showed higher expression in young adults (red) and lower expression (blue) in the 41 aging population (**Figure 5A**). In the following, we focused on the core genes of the blue module; the core genes usually characterized by a high GS for aging status, as well as high MM and K.in. Thus, network top interesting genes (top125) of the blue module based on the above three indexes were listed in the Venn diagram and 12 genes were the intersections (**Figure 5B** and **Supplementary File 1, Table S10**). Similarly, we modeled a network view of blue module by cytoscape with TOM $\geq$ 0.25 and the 12 hub genes of blue module was depicted in **Figure 5C** and **Supplementary File 1, Tables S11, S12**. The values of each gene in the network view of blue module based on the three parameters were as follow: The K.in count ranged from 74.34 to 595.30, with an average of 366.11 $\pm$ 115.46; The GS score ranged from -0.82 to 0.54, with an average of -0.59 ± 0.087; The MM count ranged from -0.91 to 0.98, with an average of 0.81 $\pm$ 0.35. Further, applying GeneMANIA18 database to simulate the blue network gave the similar results (**Supplementary File 2, Figure S4**). PPP3CB and CAMSAP1, based on MM and K.in indexes, were the two top network hub genes and another two top genes (ACTR3B and GNG3) ranked on GS were also disclosed. Specifically, gene microarray in animal study has identified in region CA3, the catalytic and regulatory subunits for the phosphatase calcineurin (PPP3CB) are up-regulated by caloric restriction influences (Zeier et al., 2011). The homologene of PPP3CB in *C. elegans*, tax6 (C02F4.2), has been reported to regulate *C. elegans*' lifespan through DAF-16 (Tao et al., 2013), and it also has a multiple functions in its development, fertility, proliferation, and behavior (Lee et al., 2013). To the best of our knowledge, there has been nothing directly implicating CAMSAP1, ACTR3B, and GNG3 reported to be associated with aging. However, ACTR3B has been showed involved in age-associated cognitive dysfunction in the rat hippocampus (Ottis et al., 2013). CAMSAP1 (Calmodulin Regulated Spectrin Associated Protein 1) is probably a microtubule-binding protein that plays a role in the regulation of cell morphology and cytoskeletal organization. Through interaction with spectrin, CAMSAP1 may regulate neurite outgrowth and GO annotations related to this gene include microtubule binding and spectrin binding. The following gene GNG3 (G Protein Subunit Gamma 3) has been shown to have GTPase activity and G-protein coupled receptor binding activity from the GO annotations. Among its related pathways are GABAergic synapse and p75 NTR receptor-mediated signaling. All these four genes were significantly down-regulated in advanced aging-brain (GSE53890). Significantly lower expression of these genes was also validated in the aging-brain in other cohorts (GSE71620, GSE30272, and GSE11882, **Figure 5D**). These data suggested that PPP3CB, CAMSAP1, ACTR3B, and GNG3 might function as the novel candidate biomarkers for the normal brain aging.

Hub Genes Were Significantly Down-Regulated in the Front Cortex From Aging Mices

To further investigated whether hub genes expressed differentially across the progressive stages of brain aging, the 3-month-old and 12-month-old male C57BL/6 mices were used. HE staining of brain tissue with different age stages was shown to assess aging severity. The expression level of SOD and MDA were also tested in the 3-month-old and 12-month-old male C57BL/6 mices' PFC ($n = 6$ in each group, **Figures 6A,B**), revealing that aged mices compared to young mices showed low level of SOD enzyme activity and high level of MDA. Then, to explore if hub genes were modified in the different stage of brain aging, we measured PPP3CB, CAMSAP1, ACTR3B, and GNG3 mRNA levels in extracts of PFC from young adult (3 months) and aged (12 months) individuals. Similarly, the mRNA level of PPP3CB, CAMSAP1, ACTR3B, and GNG3 were both remarkably down-regulated in the aging mice's PFC, as verified by quantitative real time RT-PCR (qRT-PCR) ($n = 3$ in each group, **Figure 6C**). The data *in vivo* above indicates a rather close relationship between hub genes and normal brain aging progression.

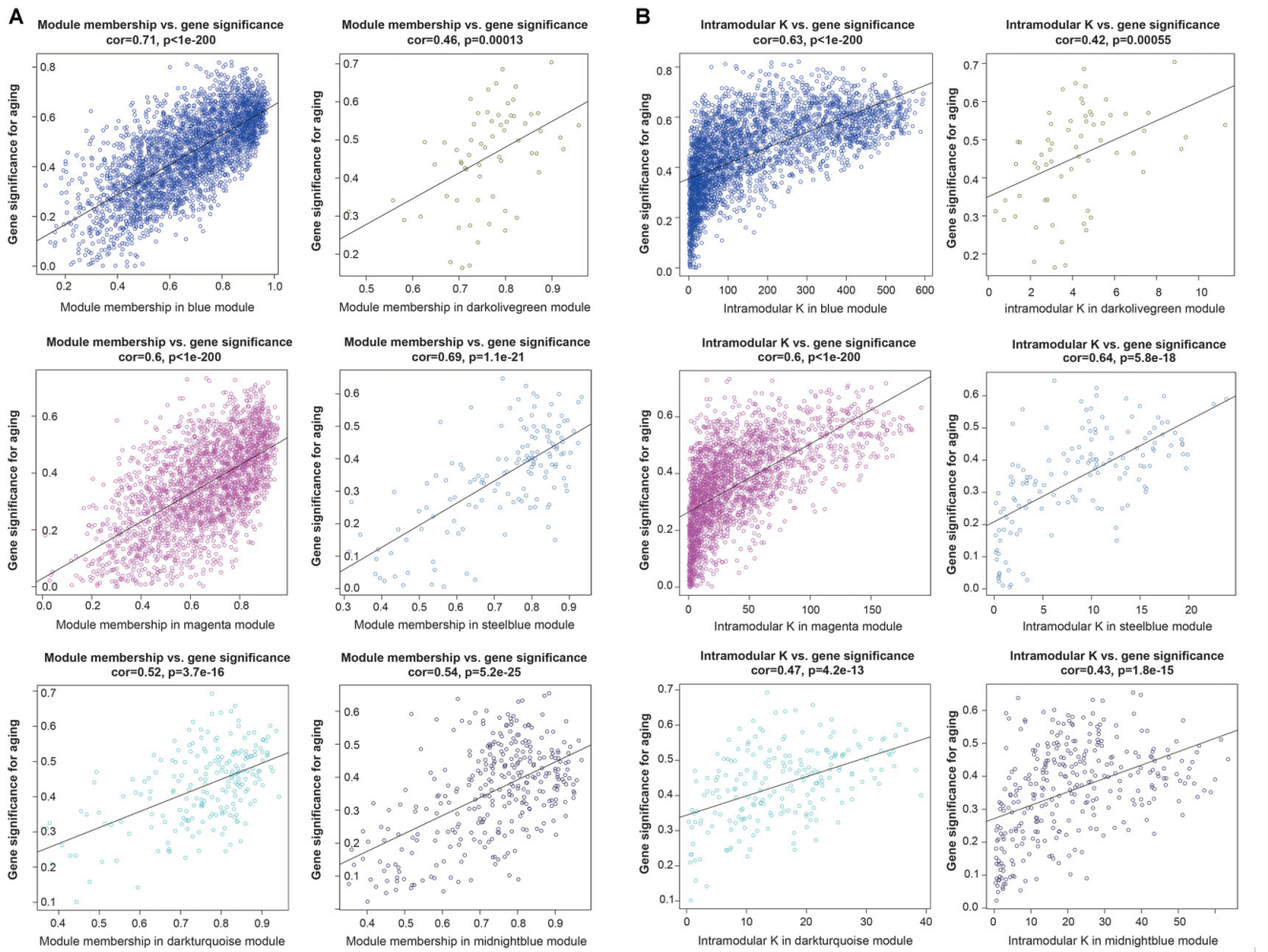

FIGURE 4 | Module features of GS, MM and K.in. **(A)** Modules significantly correlated with aging status (young versus aged cases). Each point represented an individual gene within each module, which were plotted by GS on the *y*-axis and MM on the *x*-axis. The regression line, correlation value, and *p*-value were shown for each plot. **(B)** Correlation of the K.in (*x*-axis) and the GS (*y*-axis).

The Main Functional Organization of the Blue Module

Next, for a more intuitive depiction of interesting modules, the OmicShare tools, a free online platform for data analysis[4], was used to re-annotated the functional relevance of blue and magenta module. With the cutoff set as Q-value < 0.05, synaptic vesicle cycle, cGMP-PKG, and dopaminergic synapse signaling pathway made up the main KEGG signaling pathways in blue module and the top of three oxidative phosphorylation, Huntington's disease and Parkinson's disease pathways constituted the main KEGG signaling pathways in magenta module, which were both depicted in bubble plots (**Supplementary File 2, Figure S3B**). For the blue module, the GO term of "transport" and "establishment of localization" were significantly enriched. The top enriched GO terms for magenta module were "mitochondrion organization" and "gene expression" (**Supplementary File 2, Figure S3A**). Moreover, there was a widespread consensus that co-expressed genes may be co-regulated by the common transcription factors (TFs), histone modification and microRNAs, so we performed a gene-set enrichment analysis by using ChEA, Encode, and TargetScan database (Lachmann et al., 2010; Mouse et al., 2012; Agarwal et al., 2015) for blue module. Thus, the top of significantly enriched TFs were observed for REST (RE1-Silencing Transcription factor), SUZ12 (SUZ12 polycomb repressive complex 2 subunit), CREB1 (CAMP Responsive Element Binding Protein 1), AR (androgen receptor), etc. (**Figure 7A** and **Supplementary File 1, Table S7**). Consistently, several studies showed that those TFs were functionally associated with brain aging. For instance, the elevated REST levels were closely related with increased longevity in aging humans by regulating a neuroprotective stress response during aging (Lu et al., 2014). For SUZ12, reports showed SUZ12 expression may regulate the transition from proliferation to

[4]www.omicshare.com/tools

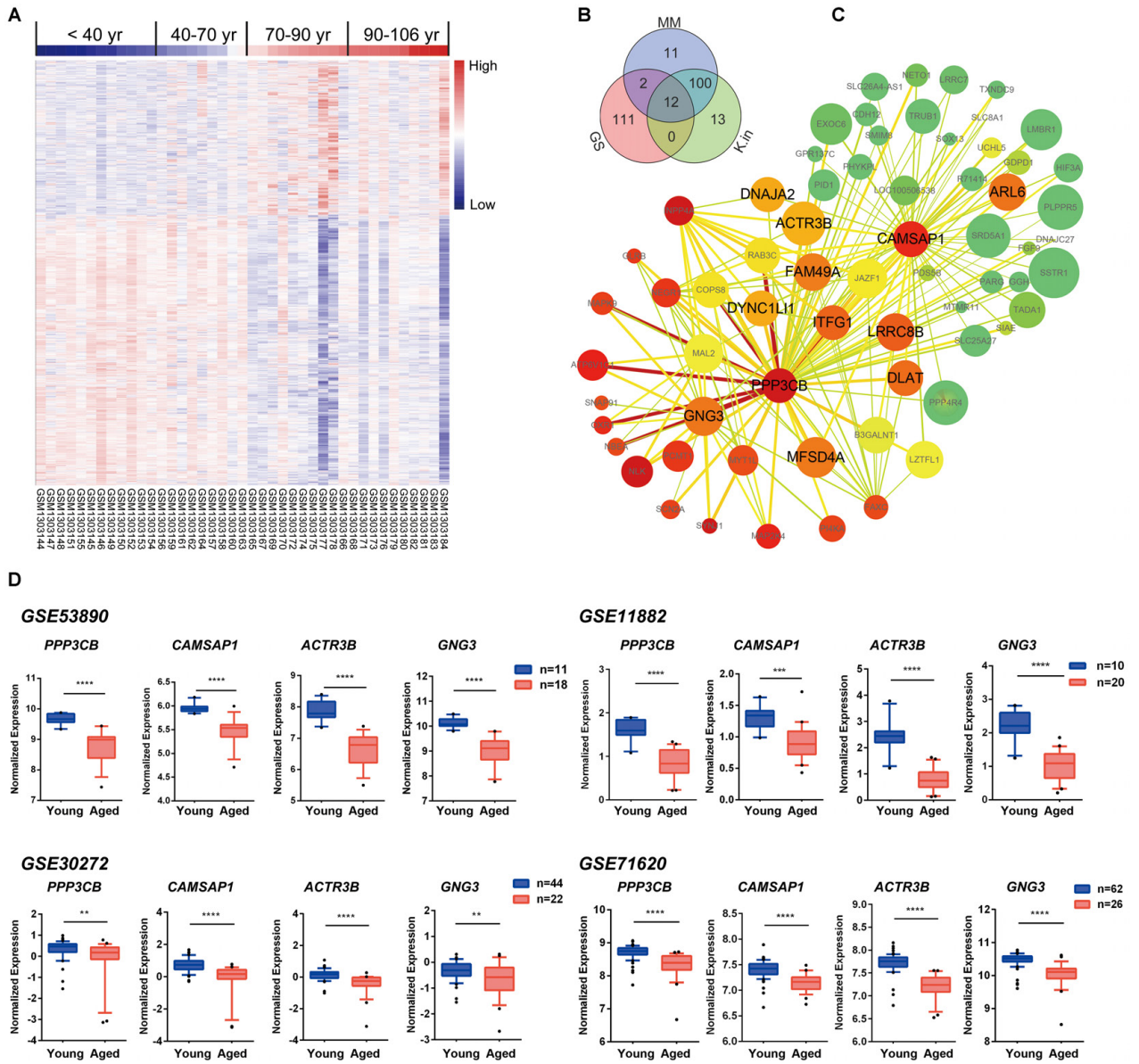

FIGURE 5 | Characterization of the blue module. **(A)** Heat map showing hierarchical clustering of each samples based on the expression of the blue module genes. **(B)** Venn diagram of blue module genes in the top of 125 based on high gene significance (GS), high module membership (MM), and high intramodular connectivity (K.in). **(C)** Interaction of gene co-expression patterns in the blue module. The module was visualized using Cytoscape_3.3.0 software. The node colors coded from green to red (low to high) indicated the K.in level when compared young with advanced brain aging state. The node size was proportional to the GS with age trait. The higher of the GS, the bigger of the node size. **(D)** Four hub genes expression pattern in brain tissues according to GSE53890, GSE71620, GSE30272, and GSE11882 cohort. Data were shown as box and whisker plot. Student's *t*-test was used for statistical analysis. $^{**}p < 0.0$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

cellular senescence (Overhoff et al., 2014). Specifically, in brain, the cyclic AMP responsive element binding protein1 (CREB1) TF was found to be involved in CREB signaling leading to cognitive deficits as observed in normal aging and neurodegenerative diseases by regulating specific genes (Paramanik and Thakur, 2013). Most recently, study showed that CREB1 was activated by nutrient deprivation in adult neurons and mediated the improved cognitive, electrophysiological, and pro-survival effects of low calorie intake (Fusco et al., 2016). Meanwhile, in the rat liver, AR expression might predict liver aging (Song et al., 1991; Supakar et al., 1993). As we known, dietary calorie restriction could retard age-related diseases and extends the invertebrate and vertebrate lifespan; interestingly, reversed loss of AR expression and restored androgen sensitivity in the aging liver were also observed during dietary calorie restriction (Song et al., 1991; Roy et al., 1996). Meanwhile, H3 lysine 27 trimethylation (H3K27me3) got a strongly enrichment for most of the genes in blue module (**Figure 7B** and **Supplementary File 1, Table S8**).

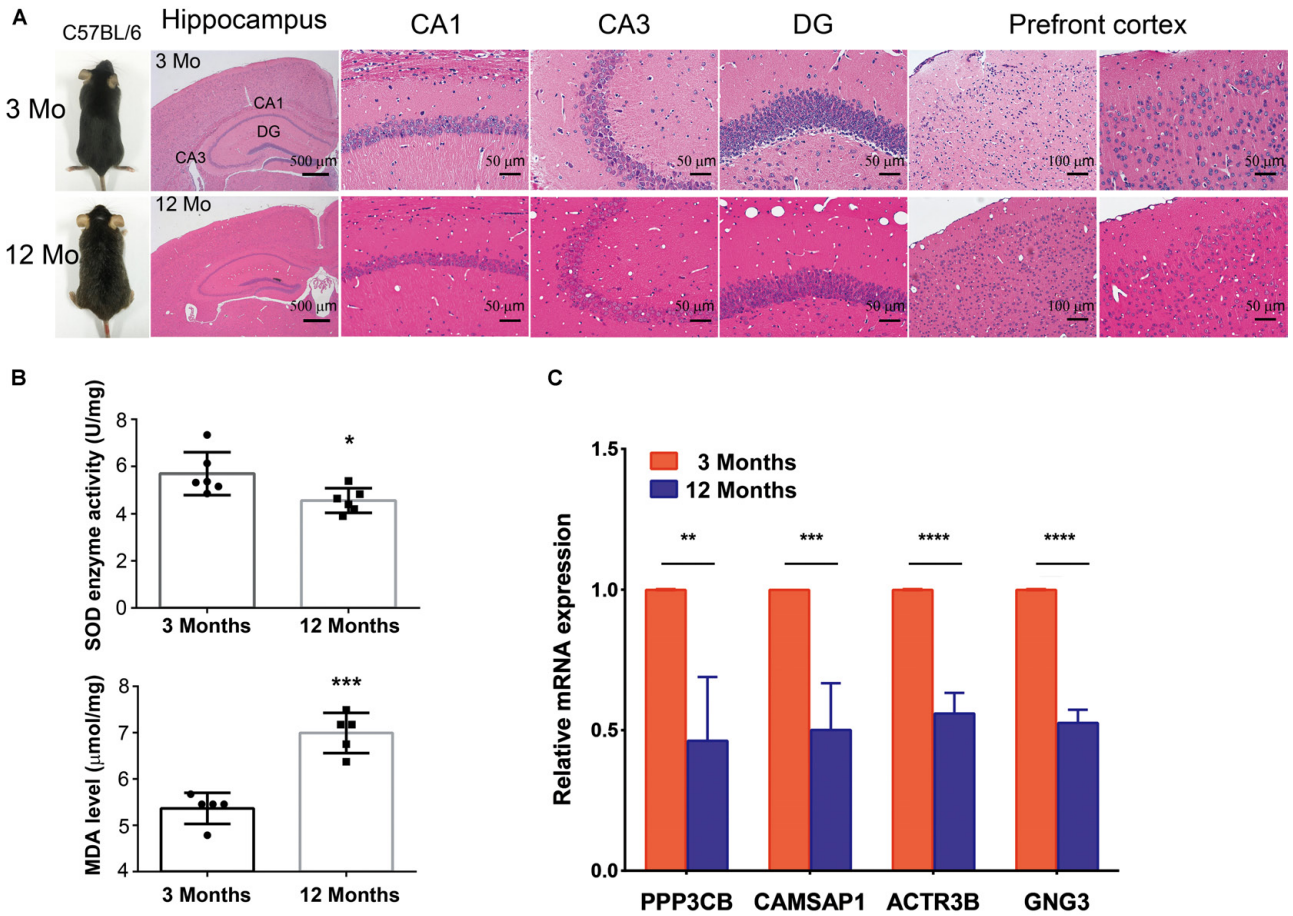

FIGURE 6 | Expression of hub genes in different aging stage of C57BL/6J mice brain. **(A,B)** The representative HE staining of hippocampus and prefrontal cortex with different age stages were shown. The production of SOD and MDA in different age of prefrontal cortex was presented. **(C)** Quantification of hub genes was confirmed and presented. *$p < 0.05$, **$p < 0.0$, ***$p < 0.001$, ****$p < 0.0001$.

It had shown that H3K27me3 was remodeled during early development, and H3K27me3 was a repressive epigenetic mark that changed dynamically during pre-implantation development in mice, bovine and pig embryos (Bogliotti and Ross, 2012). Finally, the most enriched miRNAs were observed for hsa-miR-16-5p, hsa-miR-26b-5p, hsa-miR-15b-5p, hsa-miR-15a-5p (**Figure 7C** and **Supplementary File 1, Table S9**). Study had indicated that the miR-15 family (miR-15a, miR-15b) was significantly down-regulated in the stress-induced premature senescence (SIPS) of the human diploid fibroblast (HDF) and human trabecular meshwork (HTM) cells (Li et al., 2009). In addition, miR-15b was a negative regulator of stress-induced SIRT4 expression thereby counteracting senescence associated mitochondrial dysfunction and regulating the senescence-associated secretory phenotype (SASP) and possibly organ aging, such as photoaging of human skin (Lang et al., 2016). Another study had shown forced expression of miR-16 could enhance p21 expression via down-regulation of the polycomb group protein Bmi1, thereby inducing cellular senescence (Kitadate et al., 2016). However, There were still no evidences whether the expression of hsa-miR-16-5p, hsa-miR-26b-5p, hsa-miR-15b-5p, hsa-miR-15a-5p changed with human brain aging.

DISCUSSION

The declining of cognitive function during aging has emerged as one of the major medical challenges of the 21st century. Earlier studies have demonstrated that neuronal loss is an integral feature of the aging brain. More recently, it is becoming clear that neuronal cell number is largely preserved and keeps their cognitive function relatively intact in the neocortex and hippocampus of the aging human brain, declining only in the setting of neurodegenerative disease (Gomez-Isla et al., 1996; Peters et al., 1998; Yankner et al., 2008; Lu et al., 2014). So, investigating how genes jointly preserve neurons and cognitive function relatively well during human brain aging is important, yet challenging. Recently increasing studies focus on high-throughput sequencing approach to investigated the regulation of normal brain aging and WGCNA is characterized effectively and systematically to find modules and gene signatures highly

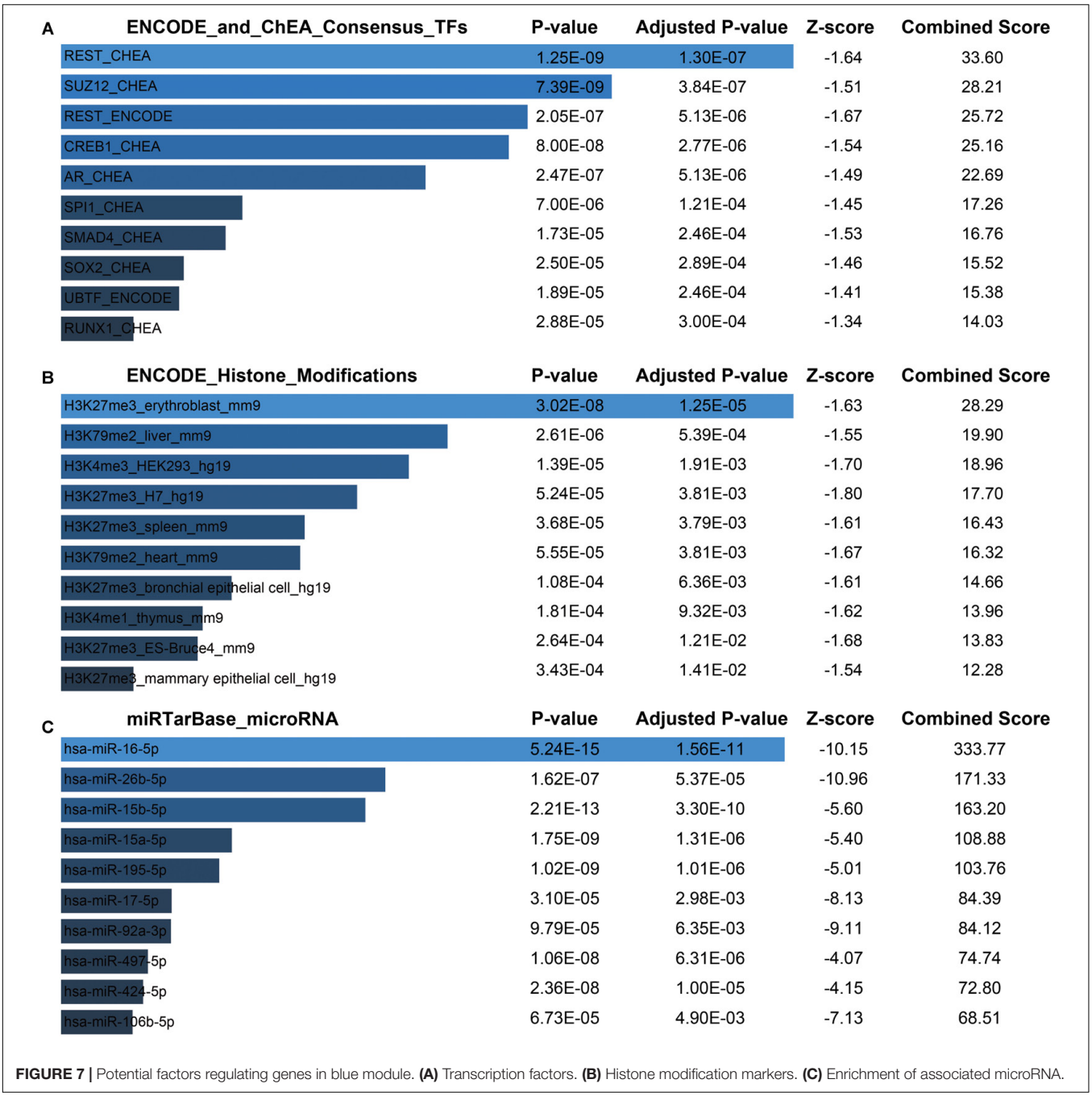

FIGURE 7 | Potential factors regulating genes in blue module. **(A)** Transcription factors. **(B)** Histone modification markers. **(C)** Enrichment of associated microRNA.

related with the clinical trait, such as the trait of brain aging. In our study, the modules and hub genes identified here are biologically rational. By using this analytical approach, 19 brain aging related modules were identified from the 41 human brain aging samples by reducing the complexity of the expression profiles. Among them, six modules were found to be significantly associated with brain aging progression. Moreover, the conservation of six modules among different datasets were also extensively studied. Further, we confirmed that the blue and magenta modules might serve as the main driver of brain aging based on the WGCNA meta-module, and further through the network feature (GS, MM, and K.in) analysis. Meanwhile, the enrichment of GO terms or pathway for blue and magenta module was also highly concordant. In particular, pathway analysis of these modules revealed that synaptic vesicle cycle, cGMP-PKG signaling pathway and oxidative phosphorylation were the top core gene sets of the blue and magenta module in human brain aging. The effect of oxidative phosphorylation on brain aging had been supported by lots of researches which report the aging of mammalian brain was associated with a continuous decrease of the capacity to produce ATP by oxidative phosphorylation (Ferrandiz et al., 1994;

Boveris and Navarro, 2008; Chakrabarti et al., 2011). Correspondingly, reports showed that cGMP-PKG signaling pathway might have a relatively relationship with the procedures of brain aging. For example, study reported that the effect of aging (4-, 12-, and 24-month-old animals) on the glutamate-cyclic GMP-PKG could modulate alpha1, alpha(2/3)-Na, K-ATPase activity in rat cerebellum and stimulate the glutamate-cyclic GMP–PKG pathway at different levels by progressively decreased of cyclic GMP levels, PKG basal activity and alpha(2/3)-Na, K-ATPase activity (Scavone et al., 2005). In addition, we found synaptic vesicle cycle signaling pathway was highly associated with brain aging. However, few studies had reported synaptic vesicle cycle could affect the normal brain aging or neurodegenerative diseases. Thus, whether synaptic vesicle cycle signaling pathway was related to aging requires further validation. Besides, to make full use of blue module in the development of efficient anti-brain aging strategies, small compounds derived from the Library of Integrated Network-based Cellular Signatures (LINCS) L1000 platform (Vempati et al., 2014; Lou et al., 2017) affecting the blue module's gene expression was shown in **Supplementary File 1, Tables S13–S15** and **Supplementary File 2, Figure S5**. And these candidate compounds might offer new drug interfere strategies in the development of brain aging. Next, novel potential biomarkers including PPP3CB, CAMSAP1, ACTR3B, and MFSD4A were confirmed in blue module, after extensive cross-validation. Interestingly, the co-expression mode of genes in blue module and its regulators (TFs and epigenetic markers), which might regulate the circuit during normal human brain aging progression, were noteworthy. Our study also has some limitations. First, there are a number of genes in blue module, we only select the top of 125 interesting genes in the blue module based on the indexes of GS, as well as MM and K.in, which may be biased in investigating the hub genes regulating the brain aging to some extent. Second, even though the hub genes in blue module are implicated in aging as validated by the mRNA expression of different GEO datasets and aging mices, as well as reported by some literature annotations, there are still a lot of experiments needed to validate these discovery clues. Recently studies suggest that almost all aged brains show characteristic changes that are linked to neurodegeneration. Therefore, this raises the question whether these characteristic changes represent lesser aspects of brain aging that do not considerably affect function or whether they are the harbingers of neurodegenerative diseases (Wyss-Coray, 2016). However, in our study, the postmortem brain tissue samples were neuropathologically normal and non-demented from the NCBI Gene Expression Omnibus. And only the transcriptomic profile from cognitively normal individuals at their certain ages were studied in WGCNA analysis. Besides, to test the stability of the indicated modules, we retrieved four datasets, which was also relevant to normal brain aging. Results showed that the anti-aging modules (blue, magenta, darkolivegreen) were preserved stably in GSE11882, GSE30272, GSE71620, and GSE1572 datasets (**Figure 3**). Taken together, this study generated a systematic and unbiased view of brain aging related modules and genes. In particular, blue module and genes regulating normal brain aging progression deserved further attention, which might be exploited as a novel biomarker for the evaluation of anti-aging interventions and highlight potential new targets for the prevention or treatment of age-associated brain disorders such as Alzheimer's disease.

AUTHOR CONTRIBUTIONS

The specific work of each author in this study was as follows: HL: perception and final approval of the version to be published. YH: participation in the whole work, drafting of the article, and data analysis, JP, YX, and JW: feeding the animals and responsible for the brain samples collection. XM and QG: RT-PCR data acquisition and assessment.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2018.00259/full#supplementary-material

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Visual Attention Performances and Related Cerebral Microstructural Integrity Among Subjects With Subjective Cognitive Decline and Mild Cognitive Impairment

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Objective: To compare visual attention performances and diffusion tensor imaging (DTI) between subjects with subjective cognitive decline (SCD) and mild cognitive impairment (MCI), and to discover neuronal substrates related to visual attention performances.

Methods: Thirty-nine subjects with SCD and 15 with MCI, diagnosed following neuropsychological tests and conventional brain magnetic resonance imaging, were recruited. All subjects were further examined by the Conners Continuous Performance Test 3 (CPT3) and DTI including fractional anisotropy (FA) and mean diffusivity (MD), in which group comparisons and stepwise linear regression were made.

Results: Subjects with MCI had a worse performance in all retrieval indices of verbal/nonverbal memory tests than those with SCD in the context of comparable general cognition and demographic status. In the CPT3, subjects with MCI had a significant longer hit reaction time (HRT) by univariate but not multivariate comparisons. Further analysis suggested that a longer HRT across all interstimuli intervals and at the point of fourth to sixth blocks were noted among MCI subjects. In DTI evaluations, FA value within the left forceps major was the only hotspot with significant between-group differences after the Bonferroni correction of FA and MD values. On the basis that HRT had significant inverse correlations with FA value within the genu of the corpus callosum and left forceps minor, regression analysis was conducted, showing HRT was best predicted by the FA value within the left forceps minor. Area under receiver operative characteristic curve was 0.70; the optimum cut-off for HRT was 515.8 ms, with a sensitivity of 85% but specificity of 47%.

Conclusions: Our report suggested that impaired sustained attention and vigilance to be an early cognitive marker in differentiating MCI from SCD, where MCI subjects had a longer HRT across all interstimuli intervals and more profoundly in later blocks. FA measures appeared to be more sensitive DTI parameters than MD values in detecting microstructural changes between SCD and MCI. The role of the anterior

interhemispheric fibers in sustained attention implementation during visual signal detection task was highlighted.

Keywords: diffusion tensor imaging, subjective cognitive decline, mild cognitive impairment, attention, cognition, continuous performance test

INTRODUCTION

Mild cognitive impairment (MCI) is a syndrome defined as cognitive decline beyond that anticipated from age-related changes (Gauthier et al., 2006), yet the affected individual does not meet the criteria for dementia (Albert et al., 2011). Current opinion has labeled MCI to be a risk state for dementia on the basis of 5%–15% annual dementia conversion rate (Mitchell and Shiri-Feshki, 2009). The concept of MCI underpins the importance in screening high risk groups by identifying factors that predict dementia onset within specific time periods. However, some people who are diagnosed having MCI may remain stable and even normalize in cognition over time (Gauthier et al., 2006), reflecting considerable heterogeneity of underlying pathogenesis under current diagnostic repertoire. In addition, the elderly with high education levels appear to be a group with high risk of being underdiagnosed. In their young adulthood, the cognitive performances of these individuals are often far above the average norm. In older age, they might report subjective cognitive decline (SCD) relative to their own baseline, while objectively still perform within the age-matched standards. Although subjects with SCD typically appear normal on standardized neuropsychological testing, this population has been gaining increasing attention as a pre-MCI condition based onto its close temporal relationship with dementia (Jessen et al., 2010). Of note, the risk of dementia conversion appeared to be more profound among those with worry or concerns (Jessen et al., 2014b). There are several lines of evidence supporting the unique role of SCD. In one epidemiology study, a high dementia conversion rate was concluded from longitudinal follow-up of a short period (2.6%–11.3% in 3 years; Jessen et al., 2010). In an autopsy report, there was a concrete relationship between memory complaints among non-demented elderly and Alzheimer's disease (AD) pathology including amyloid plaques and neurofibrillary tangles (Barnes et al., 2006). Consistently, a longitudinal neuroimaging study has also identified independent association between SCD and subsequent hippocampal volume loss (Stewart et al., 2011). A clinician shall be aware that patients may present cognitive complaints at various timing points in the unfolding of dementia. Therefore, to better characterize either SCD or MCI through identifying potential markers is critically important, in hope that early intervention may slow down the progression of the disease (Sperling et al., 2011).

In cognition perspective, memory impairment has been proposed as one of the hallmarks in MCI, especially for those due to AD pathology (Albert et al., 2011). However, the cognitive deficits of non-memory domains also present, either in isolation or conjunction with memory impairment (McKhann et al., 2011). For example, attentional control deficits have been identified to progress among MCI and AD continuum, indicating impaired attention are highly likely to develop during the preclinical phase of AD (Belleville et al., 2007). Moreover, lowered attention among subjects with MCI not only led profound daily function disturbance (Klekociuk and Summers, 2014) but also was reported to be early indicators of possible transition to dementia (Saunders and Summers, 2011). Several researches have also implied that compromised attention is the early sign among subjects with SCD. For example, a novel neuroimaging study revealed compensatory functional activation changes during divided attention task among SCD individuals (Rodda et al., 2011). Subjects with SCD also appeared to have a P3 event-related potential different from the normal control in response to an attention control task (Smart et al., 2014). These findings indicated possible changes of neuronal substrates related to information processing among subjects with SCD. However, the kernel of the SCD diagnosis remained to be debatable, as some other researchers reported that depression and neuroticism accounted for considerable variance in cognitive complaints (Kliegel and Zimprich, 2005). In clinical practice, the individuals' perceptions of cognitive problems are sometimes influenced by their affective status, which leads challenge in identifying the harbinger of ongoing neurodegeneration. Therefore, to identify a sensitive neuropsychological test in discerning MCI from SCD would further concretize the role of cognitive complaints, as the diagnosis of MCI carries a more imminent risk of dementia conversion.

In neuroimaging aspects, researchers have identified diffusion tensor image (DTI) to be an useful tool to discern microstructural changes between patients with MCI/AD continuum and the normal ageing (Teipel et al., 2011; Bosch et al., 2012; Tu et al., 2017). Due to the fact that DTI parameter changes might precede gray matter atrophy (Ibrahim et al., 2009; Selnes et al., 2012) and have significant correlation with cognitive performances (Grambaite et al., 2010), its application has been proven useful in predicting dementia conversion among MCI subjects (Haller et al., 2010). Furthermore, a novel study incorporating DTI and neurobiochemistry has identified a significant correlation between white matter microstructural changes and levels of cerebrospinal fluid biomarkers pathognomonic to AD (e.g., β-amyloid42 and total tau protein; Li et al., 2014). Studies recruiting subject with SCD further examined whether DTI has potential value to be an early marker for pre-MCI stage. However, the results have been inconsistent. Some DTI studies identified widespread white matter microstructural changes in SCD subjects evidenced by certain DTI parameters modification (Selnes et al., 2012). Others concluded that although SCD subjects displayed a trend of intermediate stage between normal ageing and MCI, the structural network during SCD stage was relatively preserved

(Wang X. N. et al., 2016). Interestingly, still some other study revealed no significant changes of white matter integrity between normal ageing and SCD (Kiuchi et al., 2014). Regarding DTI clinical relevancy, there has been valuable information in delineating visual attention network in the context of lesion-based study. Two cohorts with post-stroke neglect indicated that microstructural derangement within contra-lesional frontoparietal connections (e.g., inferior/superior parietal lobe and left corpus callosum/cingulum) and bilateral occipital connections were important for visual attention system processing (Umarova et al., 2014, 2017). Some other research also identified dissociable circuits, mostly confined within the superior longitudinal fasciculus, inferior fronto-occipital fasciculus, and corpus callosum, responsible for post-stroke neglect (Vaessen et al., 2016). In a study incorporating DTI with positron emission tomography, associations between microstructural changes within the superior longitudinal fasciculus and metabolic changes within the inferior parietal and frontal eye field regions were regarded to be responsible for visuospatial dysfunction among subjects with posterior cortical atrophy (Cerami et al., 2015). Research focusing associations between DTI modifications and attention performances among subjects with MCI/SCD, however, is limited.

Although the importance of comprehensive assessment in both memory and non-memory functions in categorizing MCI and SCD has been widely advocated, controversy remains due to the fact that study design using same batteries might create a discussion obsessed by circular reasoning. If individuals with cognitive complaints were classified according to specific neuropsychological tests, then it would be less surprising that cross-sectional analysis of their profiles reveals lowered performances within the same domains. Likewise, differentiation subjects with SCD from those with MCI based onto neuropsychological tests would also affect the results of neuroimaging comparisons. To deploy a sensitive neuroimaging tool and neuropsychological tests distinct from those primarily used for classification may further clarify the differences of cognitive profiles between SCD and MCI. Additionally, cerebral microstructural changes expected to be associated with AD pathology could be further clarified through neuroimaging and neuropsychological characterization. Therefore, our study was aimed to: (i) compare visual attention test performances and whole-brain DTI profiles between subjects with SCD and MCI diagnosed by standardized neuropsychological tests and (ii) examine the clinical relevancy between visual attention performances and DTI parameters.

MATERIALS AND METHODS

Participants

Thirty-nine subjects with SCD and 15 with MCI were enrolled from the Neurology outpatient clinic of our hospital. Data on demographics, serology tests, general cognitive function assessments, the CPT, and brain MRI (including DTI) studies were recorded for each subjects. This study was approved and carried out in accordance with the recommendations of Research Ethics Committee of the Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (REC 104-05), with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Inclusion and Exclusion Criteria

All subjects were of age $\geq$50 years-old and formal education $\geq$6 years. The criteria specific for MCI were organized based onto the conservative criteria proposed by Jak et al. (2009) in order to maintain diagnostic stability, including: (i) subjective memory complaints yet preserved autonomy in activities of daily living; (ii) normal general cognitive function, as evidenced by the Taiwanese Mini-Mental State Examination (MMSE) $\geq$26 (Shyu and Yip, 2001); and (iii) at least two test performances within a cognitive domain falling 1.5 *SD* below the average level with respect to age- and education-adjusted normative data. The inclusion criteria of SCD followed (i) and (ii), but not (iii). In other words, subjects with SCD should have less cognitive deficits and they did not fulfill criteria (iii) for MCI. The exclusion criteria were: (1) not demented (McKhann et al., 2011); (2) Hachinski Ischemic Score >4 (Hachinski et al., 1975); (3) known history of psychiatric or other neurological illness (e.g., major depressive disorder, substance use, traumatic brain injury, neurodegenerative disease, or delirium); (4) severe loss of vision, hearing, or communicative ability; (5) intolerability on taking neuropsychiatric assessment and MRI examination, and known; (6) derangements in serology tests that may have contributed to cognitive impairment such as abnormal levels of free T4, cortisol, folic acid, vitamin B12, or rapid plasma reagin; (7) remarkable white matter T2-hyperintensities on MRI, defined as the Fazekas scale >2 (Fazekas et al., 1987). All subjects were free from psychotropic agents, and were required to refrain from the use of alcohol and drinks containing caffeine for at least 12 h before neuropsychological and visual attention tests.

Demographic Data Registry

In addition to basic demographic data, the cognitive symptoms were detailed according to framework proposed by Jessen et al. (2014a). Briefly, this structural evaluation mainly focused onto categorizing cognitive symptom (e.g., frequent missing an appointment, forgetfulness, poor efficiency on work, etc.), the severity and duration of targeted symptoms, associated daily function impairment and confirmatory collateral information, if any. In addition, the Functional Activities Questionnaire was measured in order to rate these subjects' abilities. Cut-point of nine in sum scores indicates impaired function, and has been applied for distinguishing demented individuals from normal aging (Pfeffer et al., 1982).

Serology Test

Antecubital venous blood samples were collected after an 8-h fast for hemogram, serum creatinine, folate, vitamin B12, free T4, thyroid stimulating hormones, cortisol, and rapid plasma reagin measurements. Samples were collected in evacuated tubes containing EDTA, centrifuged within 10 min and stored below −20°C until analysis.

Neuropsychological Tests

Neuropsychological tests were administrated by licensed clinical psychologists. In general cognitive function, the Taiwanese MMSE (Shyu and Yip, 2001), Cognitive Abilities Screening Instrument (CASI; Lin et al., 2012) and CDR- sum of box (Morris, 1993) were scored. According to the comprehensive criteria for MCI diagnosis (Jak et al., 2009), at least two test indices were used for judging a deficit in any given cognitive domain. Memory function was assessed by three indices of the Chinese version of the Verbal Learning Test (Chang et al., 2010), including the total score of immediate recall, the 10-min delay free recall, and the recognition score. Executive function was assessed by the Trail Making Test part B (Reitan, 1958), the backward span of the Digit Span subtest of the Chinese version of the Wechsler Adult Intelligence Scale (WAIS)—the 3rd edition (Chen and Chen, 2002), and two indices of the modified Cart Sorting Test (Nelson, 1976), including the number of category completed and the number of perseveration. Attention function was assessed by the Trail Making Test part A (Reitan, 1958), the forward span of the Digit Span (Chen and Chen, 2002), and the Digit Symbol Coding subtest of the WAIS (Chen and Chen, 2002). Language function was evaluated by the Neurosensory Center Comprehensive Examination for Aphasia (NCCEA) Object Naming (Hua et al., 1997), as well as the 30-s animal fluency task and the Language indices of the CASI (Lin et al., 2012). Additional tests were also given to gather more information of participants' cognitive status, including the Visual Reproduction subtest of the Chinese version of the Wechsler Memory Scale (Hua et al., 2005), the Taiwan version of the Frontal Assessment Battery (FAB; Wang T. L. et al., 2016), and the Judgment of Line Orientation (Benton et al., 1994). The Beck Anxiety Inventory (Julian, 2011) and Beck Depression Inventory (Beck et al., 1988) were assessed for the severity of anxiety and depression symptoms of our subjects. Higher total scores in both inventories indicate more severe symptoms.

Visual Attention Tests

The Conners Continuous Performance Test 3 (CPT 3; Conners, 2002) was administered in a quiet testing room. The test takes around 15 to 20 min, and subjects are asked to click a mouse button in response to seeing target stimuli that appear on a screen as soon as possible anytime when any letter appears with the exception of letter X. When X appears, the examinee is instructed to refrain from responding to non-target stimulus. The interstimuli intervals (ISIs) are 1 s, 2 s, 4 s with a display time of 250 ms. The test includes six different blocks, with three sub-block each consisting of 20 trials. Several following raw scores are derived after subject completed the entire administration. Generally, higher raw scores indicate worse performance.

The test encompasses the following parameters:

(i) Detectability: a measure representative the ability to discriminate non-targets (i.e., the letter X) from targets (i.e., all other letters).
(ii) Omissions: missed targets.
(iii) Commissions: incorrect responses to non-targets.
(iv) Perseverations: responses that are made in less than 100 ms following the presentation of stimulus.
(v) Hit reaction time (HRT): the response speed measured in milliseconds. Longer HRT indicates slowing of response speed. HRT serves to be one of the measures of both sustained attention and vigilance.
(vi) Hit reaction time standard deviation (HRTSD): the consistency of response speed to targets for the entire administration.
(vii) Variability: the response speed consistency within respondent.

Error measurements (i.e., omissions, commissions and perseverations) were presented with a percentage value to all stimuli. HRT and HRTSD were also analyzed according to each individual block change (BC) and ISI changes (e.g., HRT_BC, HRT_ISI), in order to explicitly assess sustained attention and vigilance, respectively.

Brain Magnetic Resonance Imaging (MRI) and Diffusion Tensor Imaging (DTI)

All patients received brain MRI using a 3.0 T scanner (Discovery MR750, GE Medical System, Milwaukee, WI, USA) with an 8-channel phased array head coil in accordance with same imaging protocol, including axial T1-weighted imaging, T2 fluid-attenuated inversion recovery (T2-FLAIR), diffusion weighted imaging, and MR angiography for the circle of Willis. DTI data were acquired using a single-shot spin-echo echo-planar imaging sequence. The diffusion-sensitizing gradients were applied along 20 non-collinear directions with diffusion weighting factor b = 1,000 s/mm^2, plus one b = 0 image. Parallel imaging technique Array Spatial Sensitivity Encoding Technique (ASSET) was utilized for reducing susceptibility to geometric artifacts and the option of dual spin-echo was applied to alleviate the eddy-current induced distortion. The remaining imaging parameters were: TR/TE = 8,000/82 ms, matrix size = 128 × 128, field of view (FOV) = 240 mm, slice thickness = 3 mm with no intersection gap, number of excitations = 2, number of slices = 67, scan time = 5 min and 58 s. All of the DTI images were sent to a workstation Advantage Windows 4.4 and processed using the FuncTool software (GE Healthcare, Milwaukee, WI, USA). Before DTI parametric maps of fractional anisotropy (FA) and mean diffusivity (MD) were generated, all diffusion data were with the affine and rigid body registrations to the image (b = 0) for reducing the motion and distortion.

FA and MD values were measured from 19 circular priori-defined subcortical regions of interest (ROI) as the template defined by Tu et al. (2017). The ROI size was kept consistent (30–35 mm^2) in all of the subjects in order to obtain a stable number of voxels and decrease variance in the DTI parameters. In brief, midline ROIs included the genu, body (three portions) and splenium of the corpus callosum. Other ROIs including the superior longitudinal fasciculus, forceps minor, forceps major, anterior thalamic radiation, uncinate fasciculus, inferior longitudinal fasciculus, and cingulum, were evaluated symmetrically within bilateral hemispheres. The ROIs were

manually drawn by a single rater (Min-Chien Tu), and the DTI parameters of each white matter tract were obtained from the averaged ROIs of two adjacent slices.

Statistical Analysis

Comparisons between these two groups were made. The Shapiro-Wilk test was used to test normality of each variable. The independent *T*-test was used for those who were normally distributed, while the Mann Whitney *U* tests were used for those examined to be nonparametric variables. The χ^2 test was used to detect group differences in demographic data. Cohen's *d* was used to determine the effect size on comparing two groups of similar size and standard deviation. A Cohen's *d* value of 0.20, 0.50 and 0.80 corresponds to the effect that could be described as small, median, and large, respectively (Levene, 1960). To determine the reliability of DTI measurements, the same rater repeated ROI selections on all subjects in this study. The intra-observer reliability was assessed using the averages of intra-class correlation coefficients (ICCs) with absolute agreement. In current study, we used a "Two-Way Random" effects model with absolute agreement. This model fits the condition that the dependent variables (DTI parameters) are assessed by the same rater, where both an effect of rater and of ratee (i.e., two effects) is considered and both rater and ratee are drawn randomly from larger populations (i.e., a random effects model). The ICCs values were considered to indicate excellent agreement and substantial agreement if they were greater than 0.8 and 0.60–0.79, respectively (Shrout and Fleiss, 1979). Three types of variance were derived from the ICCs analysis: Var (β) is variability due to differences in the subjects; Var (ε) is variability due to differences in the evaluations of the subjects by the judges; Var (α) is variability due to differences in the rating scale used by the judges. Pearson correlation was applied to determine the association between visual attention tests and DTI parameters; partial correlation analysis was used to further examine the results with the aim of controlling for confounding factors including age and education (Conners, 2002). In addition, to examine the effect of microstructural changes within ROIs on the performances of visual attention tests, stepwise linear regressions between targeted attention function and DTI parameters of all ROIs were tested. Receiver operating characteristic (ROC) curve was created to test the clinical utility of target CPT3 parameter, and to set the cut-off score. Optimum cut-off score for the target CPT3 parameter was determined at the maximum Youden index (J = sensitivity + specificity − 1) level (Akobeng, 2007). The area under the curves (AUC) was examined to show sensitivity and specificity of the target CPT3 parameter for identifying MCI. It is generally accepted that an AUC value greater than 0.9 indicates high accuracy, 0.7–0.9 indicates moderate accuracy, and 0.5–0.6 indicates low accuracy (Akobeng, 2007). All statistical tests were performed using SPSS software version 19 (IBM, Armonk, NY, USA). A *p* value less than 0.05 was considered to be statistically significant. The Bonferroni correction would be applied for statistical tests with multiple comparisons problems (Armstrong, 2014).

RESULTS

Table 1 shows basic information of subjects with SCD and MCI. Both groups showed comparable demographic profiles including age, age of onset, symptom duration, education, and gender (p = 0.183–0.716). Regarding descriptions of cognitive symptoms, most subjects reported remarkable concern (n = 47; 87%), and their medical help seeking behavior had associations with their cognitive problems (n = 54; 100%). A considerable portion of subjects (n = 45; 83%) reported a feeling that their performance to be worse than those of similar ages. Overall, there were eighty-nine and eighty percent of subjects who reported memory (n = 48) and non-memory (n = 43) complaints, respectively. Executive complaints represented the majority of non-memory complaints, accounting for forty-nine and forty-seven percent among subjects with SCD and MCI, respectively. The Functional Activities Questionnaire in both groups ranged from 0 to 7, and their average scores in subjects with SCD and MCI were 1.1 and 1.6, respectively. There was no differences of scores in the Beck Anxiety Inventory and Beck Depression Inventory (p = 0.857–0.945). Regarding performances of cognitive tests, both groups had a comparable general cognition status (p = 0.832–0.876). Ten, three and two subjects were categorized as single-domain amnestic, multi-domain amnestic and non-amnestic MCI, respectively. Among tests specific for individual cognitive domains, only the CVVLT and Wechsler Memory Scale showed significant between-group differences, in which subjects with MCI had worse performance in all subsets of recall than those with SCD (p = 0.043∼ <0.001).

Table 2 shows comparisons of CPT3 tests. Of note, subjects with MCI had a significant longer HRT (p = 0.013; Cohen's d = 0.76) and wider HRTSD (p = 0.010; Cohen's d = 0.70) than those with SCD by univariate comparisons. No significant between-group differences were noted after the Bonferroni corrections, with a threshold selected to be 0.05/9 = 0.0055. There was a positive correlation between HRT and HRTSD (ρ = 0.651; $p < 0.001$), indicating HRTSD turned to be wider in line with longer HRT.

To further delineate the profiles of HRT, changes within each ISI and block were analyzed. In **Figure 1A**, subjects with MCI showed a longer HRT across all ISI (p = 0.012–0.036; Cohen's d = 0.68–0.74). In **Figure 1B**, subjects with MCI showed a wider HRTSD than those with SCD at the 2 s ISI (p = 0.009; Cohen's d = 0.73). In SCD subjects, the HRT differed significantly across each ISI (p = < 0.001–0.030). In MCI subjects, the HRT differed on comparing values between 1 s ISI to 4 s ISI (p = 0.007), 2 s ISI to 4 s ISI (p = 0.033), but not 1 s ISI to 2 s ISI (p = 0.240). In **Figure 1C**, subjects with MCI developed a longer HRT than those with SCD at the point of fourth to sixth blocks (p = 0.001–0.006; Cohen's d = 0.83–0.99). In **Figure 1D**, the HRTSD of MCI subjects at the point of fifth block also was larger than that of SCD subjects (p = 0.001; Cohen's d = 0.93). There was no interaction between SCD/MCI categorization with variables including ISI, BC, and their standard deviation (p = 0.055–0.949).

Figure 2 shows ICCs of DTI results, indicating substantial or excellent agreement for diffusion parameters (i.e., FA and MD) on the basis of generally high ICCs (all $p < 0.05$).

TABLE 1 | Basic information of patients with SCD and MCI.

	SCD (*n* = 39)	MCI (*n* = 15)	*P* value
Demographic data			
Age (years–old)	62.7 (7.61)	61.7 (7.75)	0.666
Age of onset (years–old)	61.5 (7.77)	60.7 (7.52)	0.716
Duration (years)	1.2 (1.39)	1.1 (0.94)	0.691
Education (years)	10.3 (3.10)	9.0 (3.40)	0.183
Gender (Male/Female)	14/25	7/8	0.467
Handedness (Right/Left)	38/1	15/0	-
Cognitive symptoms (*n*)			
Association with medical help seeking	39	15	-
Memory	35	13	0.747
Nonmemory	31	12	0.966
Execution	19	7	0.892
Attention	13	2	0.141
Visuospatial construct	4	3	0.339
Language	13	4	0.636
Concerns	33	14	0.392
Feeling worse than others	34	11	0.221
Impairment of daily function	36	14	0.897
Functional Activities Questionnaire	1.1 (1.8)	1.6 (2.1)	0.480
Neuropsychological tests			
Mini-Mental State Examination	28.2 (1.17)	28.2 (1.58)	0.876
Cognitive Abilities Screening Instrument	88.5 (13.80)	87.8 (6.16)	0.832
Clinical Dementia Rating—sum of box	0.7 (0.75)	1.0 (0.88)	0.188
Chinese Version Verbal Learning Test—total immediate recall	27.5 (3.44)	23.5 (2.82)	***<0.001***
—recall after 30 s	7.5 (1.56)	6.4 (1.40)	***0.015***
—recall after 10 min	7.2 (1.11)	5.4 (1.50)	***<0.001***
Visual Reproduction subtest of the —immediate recall	72.7 (13.75)	59.4 (21.87)	***0.010***
Wechsler Memory Scale —delayed recall	47.6 (19.68)	34.6 (22.55)	***0.043***
Frontal Assessment Battery	13.8 (1.74)	13.3 (2.32)	0.389
Object naming	15.7 (0.53)	15.3 (0.97)	0.109
Digit span	19.4 (4.03)	16.9 (5.49)	0.066
Judgement of Line Orientation	21.2 (4.25)	18.9 (7.02)	0.158
Beck Anxiety Inventory	11.7 (9.69)	11.6 (7.71)	0.945
Beck Depression Inventory	11.1 (8.72)	11.6 (7.13)	0.857

MCI, mild cognitive impairment; SCD, subjective cognitive decline. Data presented as Mean (Standard deviation) unless stated elsewhere. No significant differences were noted by using the independent T-test and Chi-square test where appropriate. P values < 0.05 are in bold and italicized.

Overall, the value of each variance follows as the order "Var (β) > Var (ε) > Var (α)," indicating the fact that the majority of variability derived majorly from the differences among the subjects, and a negligible effect from DTI parameters itself. This implies that differences in ICC observed between these two groups are driven by the between-subject variation rather than by the scanning technique being less reliable in one group than the other. For FA in subjects with SCD (**Figure 2A**), 18 (95%) atlas ROIs had an ICC of 0.8 or above. In subject with MCI, 10 (53%) ROIs had an ICC of 0.8 or above. The rest ROIs were all above 0.6, indicating substantial agreement from test-retest reliability. For MD (**Figure 2B**), 17 (89%) and 2 (11%) ROIs had ICCs above 0.8 and 0.6–0.8 in the SCD subjects; the corresponding number was 14 (74%) and 5 (26%)

TABLE 2 | Comparisons of the continuous performance test (CPT) between patients with SCD and MCI.

	SCD (*n* = 39)	MCI (*n* = 15)	*P* value
CPT raw scores			
Detectability	−3.1 (0.77)	−3.0 (1.00)	0.717
†Omissions (%)	1.9 (3.72)	2.7 (4.80)	0.526
†Commissions (%)	21.2 (15.33)	22.5 (15.06)	0.780
†Perseverations (%)	0.1 (0.27)	0.2 (0.41)	0.299
HRT (ms)	471.0 (55.29)	516.3 (63.78)	***0.013***
†HRTSD	92.3 (27.28)	121.6 (52.99)	***0.010***
†Variability	26.4 (17.29)	37.5 (39.78)	0.312
HRT Block Change	0.6 (10.50)	3.2 (8.67)	0.403
HRT Inter Stimulus Interval Change	13.2 (13.30)	14.5 (16.84)	0.773

MCI, mild cognitive impairment; SCD, subjective cognitive decline; HRT, hit reaction time; HRTSD, hit reaction time standard deviation. Data presented as Mean (Standard deviation). †Comparisons were conducted using the Mann-Whitney U Test. Items without labels were conducted by the independent T-tests. P values < 0.05 are in bold and italicized.

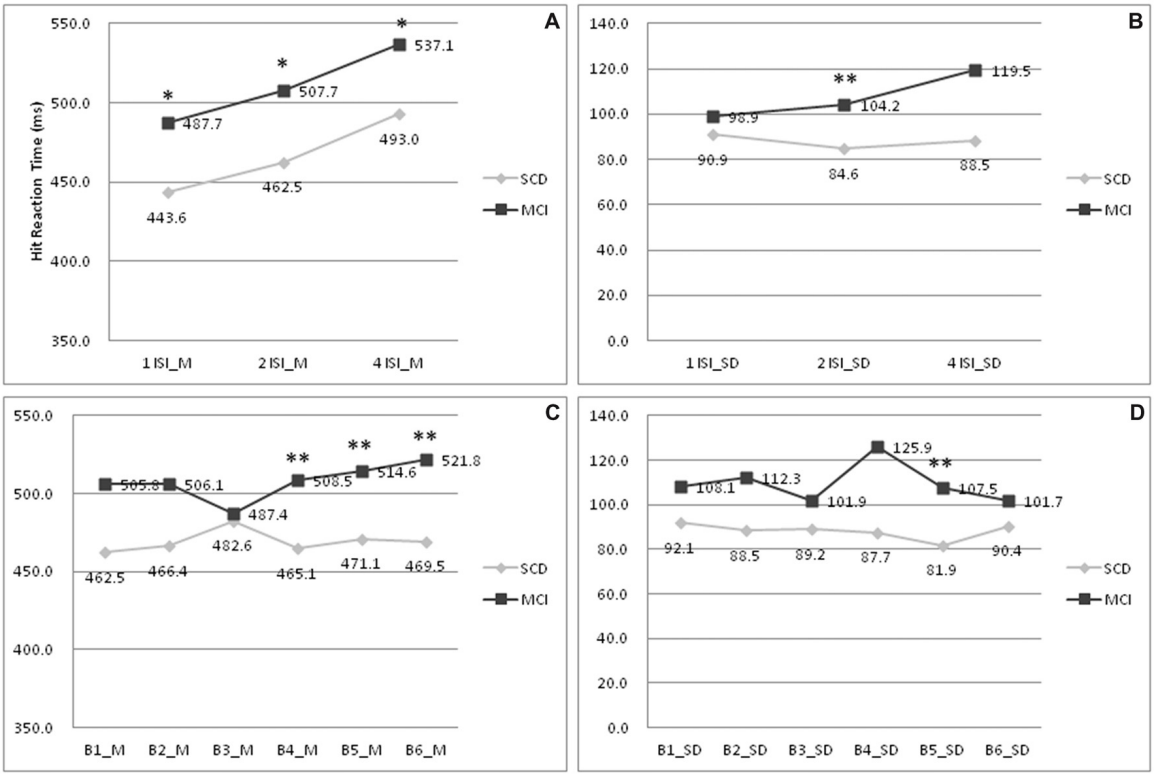

FIGURE 1 | Comparisons of hit reaction time (HRT) profiles between patients with SCD and MCI. **(A)** Mean of HRT under 1/2/4 ISI; **(B)** Standard deviation of HRT under 1/2/4 ISI; **(C)** Mean of HRT under B1-6; **(D)** Standard deviation of HRT under B1–6. MCI, mild cognitive impairment; SCD, subjective cognitive decline; M, mean; SD, standard deviation; 1/2/4 ISI, 1/2/4 s interstimuli interval; B1–6:1st to 6th block. *$p < 0.05$ on comparisons between subjects with MCI and SCD. **$p < 0.01$ on comparisons between subjects with MCI and SCD.

in the MCI subjects. In both SCD and MCI subjects, mean ICCs were very similar across metrics (SCD: FA mean ICC = 0.90; MD mean ICC = 0.89; MCI: FA mean ICC = 0.81; MD mean ICC = 0.82).

Table 3 shows comparisons of CPT3 parameters between subjects with SCD and MCI. Subjects with MCI had lower FA values within the left forceps major ($p = 0.001$; Cohen's $d = 0.87$) and the left uncinate fasciculus ($p = 0.029$), yet higher FA values

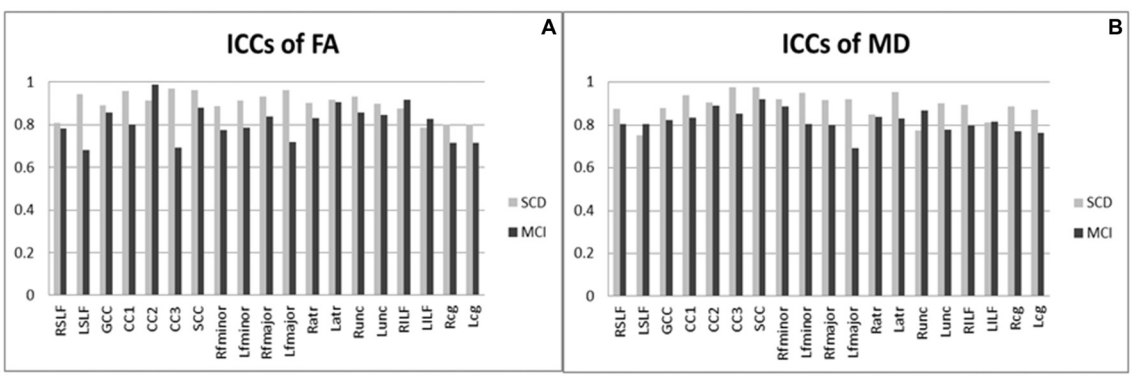

FIGURE 2 | Intra-class correlation coefficients of diffusion tensor imaging results (DTI) among patients with SCD and MCI. **(A)** ICCs of FA; **(B)** ICCs of MD. MCI, mild cognitive impairment; SCD, subjective cognitive decline; ICCs, intra-class correlation coefficients; FA, fractional anisotropy; MD, mean diffusivity; R/L, right/left; SLF, the superior longitudinal fasciculus; GCC, the genu of the corpus callosum; CC1/2/3, the body of the corpus callosum (portion 1/2/3); SCC, the splenium of the corpus callosum; fminor, the forceps minor; fmajor, the forceps major; atr, the anterior thalamic radiations; unc, the uncinate fasciculus; ILF, the inferior longitudinal fasciculus; cg, the cingulum. All ICC were of *P* values < 0.05.

within the right inferior longitudinal fasciculus (p = 0.037) on comparing to those from SCD. There were significantly higher MD values within the bilateral forceps minors in subjects with MCI than those with SCD (p = 0.031–0.046). To solve the multiple comparisons problems, an appropriate threshold was selected to be 0.05/19 = 0.0026, leaving the FA values within the left forcep major to be the only region with significant between-group differences.

Table 4 shows correlation analysis between DTI parameters and HRT/HRTSD. Among significant correlations related to HRT, FA values within the genu of the corpus callosum and left forceps minor had inverse correlations. On controlling age and education, FA value within the left forceps minor remained to be significant (correlation coefficient = −0.378; p = 0.006). We didn't identify significant correlations between MD values and HRT. Among significant correlations related to HRTSD, MD values within the body of corpus callosum had positive correlations. On controlling age and education, MD value within the body of corpus callosum remained to be significant (correlation coefficient = 0.353; p = 0.010).

To further pinpoint the neuronal substrates of HRT in CPT3, data of all SCD/MCI subjects were entered into stepwise linear regression analysis. The independent variables included: (i) FA values within the genu of the corpus callosum; (ii) FA values within the left forceps minor; (iii) demographic data including age, education, gender; and (iv) total scores of CASI.

Regarding HRT, the analysis found that FA value within the left forceps minor (Lfminor_FA; β = −0.378, t = −2.947, p = 0.005) significantly predicted its value (R^2 = 0.143, $F_{(1,52)}$ = 8.68, p = 0.005). Overall, the model accounted for 14.3% of the variance. The regression model was listed as below.

HRT = 639.2 − 382.1 * Lfminor_FA

R^2 = 0.143, p = 0.005

Age, education, gender and total scores of CASI didn't change the results of those regression models.

ROC curve was shown in **Figure 3**. The AUC was 0.70 (p = 0.028). Based on the maximum Youden index, the optimum cut-off point for HRT was 515.8 ms, with a sensitivity of 85% and specificity of 47%.

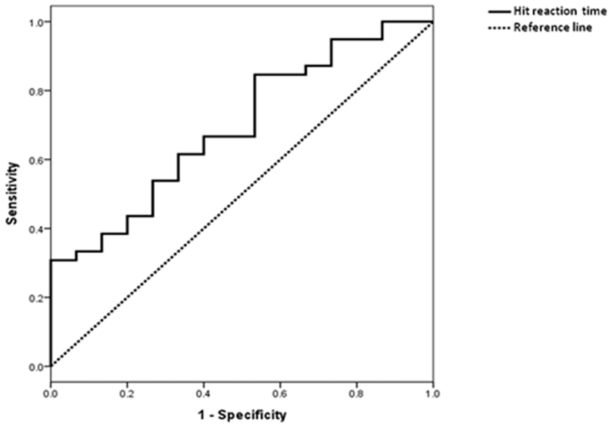

FIGURE 3 | Receiver operating characteristic (ROC) curve of HRT in differentiating subjects with MCI from those with SCD.

DISCUSSION

Our study identified that subjects with SCD and MCI presented differing visual attention performances in the presence of subtle microstructural changes. The significant correlations between HRT and DTI parameters provide valuable information in delineating sustained attention network. On the basis of no significant group—HRT interaction, the independent role of CPT3 was confirmed, and its application may serve as part of references for designing protocol in identifying subjects who are at risk of future cognitive decline.

The current study identified that MCI subjects had a significantly longer HRT in CPT3 than those with SCD, a result that was distinct and independent from neuropsychological tests primarily applied in standard diagnostic paradigm. Such discernible changes existed in overall comparisons and appeared to be more evident at the point of fourth to sixth blocks, suggesting a phasic change in alertness and defective sustained attention developed around 12 min since the start of test among subjects with MCI. It was attributed mainly onto sustained attention deficits due to the fact that such between-group differences were getting more robust as the CPT3 continued on. In another viewpoint, impaired vigilance may also contribute findings similar to our results. A significant correlation between HRT and HRTSD suggested the possibility that our respondents demonstrated a considerable variability within each individual block. Moreover, those with MCI also expressed a longer HRT across variable ISIs. This indicated MCI subjects would tend to have slower responses regardless varying levels of stimulus frequency. Although we didn't observe distinct pattern of omission/commission errors, vigilance in MCI subjects was likely to be impaired as they were unable to maintain performances during variable lagging period between signal appearance and internal expectation. We therefore proposed that sustained attention and vigilance were highly intercalated as examined in CPT3. Our current findings provided interesting contrast with a research aiming on longitudinal assessment of neuropsychological tests, in which highly-educated elderly are prone to demonstrated reduced performances in tests with eminent components of executive functions, memory, but not attention (Elkana et al., 2016). Another longitudinal study also stressed that declines in episodic memory and executive function accelerated 7 and 2–3 years before diagnosis of AD, respectively (Grober et al., 2008). While a prevailing body of evidences pointed out impaired memory and/or execution among SCD/MCI subjects (Grober et al., 2008; Sinai et al., 2010; Elkana et al., 2016), some literatures underpinned attention deficits to be one of the non-memory domains to be involved than traditionally conceptualized (Saunders and Summers, 2010, 2011). In a cross-section study, Saunders and Summers (2010) reported that SCD subjects showed better performance than MCI patients in sustained and selective attention. In their subsequent longitudinal study, it was furthermore concluded that the decline in simple sustained attention in amnestic MCI and non-amnestic MCI groups and

TABLE 3 | Comparisons of diffusion tensor imaging parameters between patients with SCD and MCI.

Regions of interest	SCD (n = 39)		MCI (n = 15)		P value for FA comparison	P value for MD comparison
	FA	MD	FA	MD		
Superior longitudinal fasciculus—Rt	0.47 (0.060)	0.77 (0.046)	0.47 (0.066)	0.78 (0.025)	0.878	0.243
—Lt	0.50 (0.073)	0.74 (0.022)	0.50 (0.053)	0.74 (0.036)	0.858	0.808
Corpus callosum—Genu	0.78 (0.058)	0.83 (0.073)	0.78 (0.052)	0.82 (0.064)	0.870	0.798
—Body 1	0.70 (0.074)	0.85 (0.112)	0.71 (0.053)	0.87 (0.124)	0.727	0.562
—Body 2	0.70 (0.051)	0.83 (0.064)	0.66 (0.088)	0.88 (0.110)	0.144	0.107
—Body 3	0.70 (0.064)	0.87 (0.084)	0.72 (0.052)	0.86 (0.057)	0.179	0.726
—Splenium	0.75 (0.102)	0.89 (0.151)	0.71 (0.108)	0.90 (0.157)	0.236	0.761
Forceps minor—Rt	0.44 (0.068)	0.82 (0.057)	0.44 (0.059)	0.86 (0.048)	0.960	***0.031***
—Lt	0.41 (0.064)	0.81 (0.067)	0.39 (0.044)	0.85 (0.053)	0.189	***0.046***
Forceps major—Rt	0.49 (0.115)	0.82 (0.091)	0.46 (0.050)	0.80 (0.063)	0.189	0.566
—Lt	0.52 (0.120)	0.84 (0.127)	0.44 (0.046)	0.81 (0.053)	***0.001***†	0.398
Anterior thalamic radiation—Rt	0.45 (0.061)	0.78 (0.045)	0.47 (0.075)	0.79 (0.067)	0.325	0.920
—Lt	0.46 (0.072)	0.77 (0.075)	0.47 (0.096)	0.78 (0.129)	0.446	0.609
Uncinate fasciculus—Rt	0.36 (0.093)	0.88 (0.053)	0.33 (0.063)	0.89 (0.095)	0.216	0.440
—Lt	0.42 (0.075)	0.83 (0.047)	0.37 (0.071)	0.85 (0.055)	***0.029***	0.179
Inferior longitudinal fasciculus—Rt	0.49 (0.048)	0.95 (0.132)	0.52 (0.046)	0.94 (0.117)	***0.037***	0.857
—Lt	0.55 (0.055)	0.86 (0.075)	0.56 (0.048)	0.86 (0.051)	0.473	0.903
Cingulum—Rt	0.45 (0.052)	0.84 (0.083)	0.45 (0.032)	0.82 (0.040)	0.892	0.465
—Lt	0.43 (0.057)	0.80 (0.056)	0.42 (0.032)	0.80 (0.035)	0.541	0.696

MCI, mild cognitive impairment; SCD, subjective cognitive decline; FA, fractional anisotropy; MD, mean diffusivity; Rt, right; Lt, left. FA Data presented as Mean (Standard deviation). MD Data presented as Mean (Standard deviation) expressed in units of $m^2s^{-1} \times 10^{-9}$. Comparisons were all conducted by independent T-tests. P values < 0.05 are in bold and italicized. †Significance after the Bonferroni correction.

divided attention in amnestic MCI may be early indicators of dementia conversion (Saunders and Summers, 2011) The aforementioned evidences were in line with our main findings that the complex attention may be one unattended cognitive domain vulnerable to early neurodegenerative process. As sustained attention is defined as the ability to maintain the focus of cognitive activity over time on a given task or stimulation (Sarter et al., 2001), it is expected to highly interact with encoding process of memory implement (Chun and Turk-Browne, 2007). Therefore, attention deficits would be expected along with memory degradation. Our clinical observations also supported this hypothesis, as SCD subjects who complained "memory problems" may frequently report frustration during handling work with more cognitive demands in other non-memory function, such as attention or information processing. Hence, a reliable tool in measuring attention function (e.g., CPT3) would be warranted for subjects with cognitive complaints.

TABLE 4 | Correlation analysis between diffusion tensor imaging parameters and HRT performances.

	HRT				HRTSD			
	FA		MD		FA		MD	
Regions of interest	r	P value	r	P value	r	P value	r	P value
Superior longitudinal fasciculus—Rt	−0.138	0.321	−0.140	0.312	−0.059	0.669	−0.086	0.535
—Lt	−0.046	0.741	−0.214	0.121	−0.038	0.784	−0.068	0.625
Corpus callosum—Genu	−0.279	***0.041***	0.090	0.515	−0.102	0.464	−0.078	0.573
—Body 1	−0.017	0.905	0.093	0.505	0.012	0.931	0.074	0.597
—Body 2	−0.027	0.847	0.200	0.147	−0.263	0.054	0.386	***0.004***‡
—Body 3	0.076	0.583	0.036	0.797	0.060	0.665	−0.055	0.690
—Splenium	−0.028	0.842	0.012	0.934	−0.067	0.632	0.032	0.819
Forceps minor—Rt	−0.259	0.059	0.241	0.079	−0.219	0.111	0.164	0.235
—Lt	−0.378	***0.005***‡	0.073	0.602	−0.104	0.456	−0.058	0.677
Forceps major—Rt	0.220	0.110‡	0.045	0.746	0.061	0.664	−0.204	0.139
—Lt	−0.027	0.849	0.026	0.853	−0.191	0.167	−0.122	0.380
Anterior thalamic radiation—Rt	0.239	0.082	−0.112	0.420	0.186	0.179	0.089	0.522
—Lt	−0.038	0.784	−0.005	0.969	0.096	0.489	−0.062	0.657
Uncinate fasciculus—Rt	0.011	0.937	−0.048	0.969	−0.021	0.879	−0.012	0.934
—Lt	−0.034	0.806	0.025	0.860	−0.010	0.942	0.076	0.538
Inferior longitudinal fasciculus—Rt	0.146	0.293	0.179	0.196	0.201	0.145‡	−0.062	0.658
—Lt	0.139	0.316	0.111	0.423	0.160	0.249	0.151	0.277
Cingulum—Rt	0.051	0.712	0.177	0.201	0.198	0.151	0.017	0.904
—Lt	−0.135	0.331	0.054	0.699	0.040	0.772	0.011	0.937

HRT: hit reaction time; HRTSD, hit reaction time standard deviation; FA, fractional anisotropy; MD, mean diffusivity; Rt, right; Lt, left. FA. P values <0.05 are in bold and italicized. r, Pearson correlation coefficient. ‡P < 0.05 on the partial correlations on controlling of age and education.

Previous literatures aiming on DTI evaluations among subjects with SCD or MCI are inconsistent. This is highly likely due to variable approaches of neuropsychological tests in addition to their presumed heterogeneous pathologies. In a research where cognitive deficits of SCD subjects were confined within verbal memory tests but with intact global cognition, there were widespread WM alterations on comparing to the normal control (Li et al., 2016). In another two cohorts where cognitive evaluation focused on global cognition and memory function, there was limited or no difference from comprehensive DTI measures (Kiuchi et al., 2014; Wang X. N. et al., 2016). Moreover, some other study depicted an intermediate pattern of DTI parameter modification between normal controls and MCI, in whom their cognitive profiles were diagnosed by comprehensive neuropsychological tests (Hong et al., 2015). Each subject in our current study had received a neuropsychological test aiming on major cognitive domains, therefore stringently confining their cognitive performance and relevant cerebral degenerative process within a very early stage. Compared with SCD subjects, those with MCI had a lower FA value within the left forceps major and a trend of lower FA values within the left uncinate fasciculus but higher FA values within the right inferior longitudinal fasciculus. Subtle changes within these tracts might indicate coexistence of both Wallerian degeneration and retrogenesis progression. While a lowered FA value within the left forceps major and uncinate fasciculus appeared to be consistent with previous report (Stenset et al., 2011) a paradoxical elevation of FA value within the right inferior longitudinal fasciculus could be the consequence related to a micro-inflammation state and/or their crossing fiber property. FA value elevation during cerebral degenerative process was proposed as compensatory glial activation in response to inflammatory effects of amyloid deposition (Racine et al., 2014). Such hypothesis was also supported by the models of brain abscess (Nath et al., 2007). Another possible explanation was hypothesized that selective degeneration of short interneurons but spared long projection neurons might happen alongside crossing fibers; a loss of collateral projections would therefore contribute FA value elevation (Racine et al., 2014).

Another interesting main finding is that FA values appeared to be more sensitive than MD values by between-group comparisons of DTI parameters. Theoretically, FA measures the overall directionality of water diffusion and reflects the axonal membrane and myelin integrity, whereas MD represents barriers to free water diffusion such as axon damages during neurodegenerative process (Alves et al., 2015). Previous studies indicated that MD and FA differences usually coexisted by comparing DTI parameter of subjects with SCD and MCI (Kiuchi et al., 2014; Hong et al., 2015). Some study also indicated a prevailing role of MD than FA regarding its greater spatial involvement and clinical relevancy (Kiuchi et al., 2014). However, some other researchers highlighted FA value to be a unique biological predictor for memory performances among subjects with SCD and MCI (Grambaite et al., 2010). A wider distribution of FA modifications than MD was also reported (Wang et al., 2012). In addition, some other studies also addressed that FA was a more sensitive measure than other diffusion parameters in differentiating patterns of white matter changes (Tu et al., 2017) and the subtype of MCI (Haller et al., 2013). Regarding clinical relevancy, our study supported that both FA and MD values can serve as useful biological determinants in terms of cognitive performances, as those relevant microstructural integrity of the genu/body of the corpus callosum and left forceps minor correlated with HRT or HRTSD. The results highlighted these neuronal substrates related with implement of sustained attention among SCD/MCI subjects, and were consistent to current opinion related to alerting network. In one line of evidences, experimental drug studies with humans and monkeys showed that norepinephrine governed alerting network (Morrison and Foote, 1986; Melasch et al., 2016). The cortical norepineprhine pathway includes the anterior insula/frontal operculum, medial orbitofrontal cortex (Melasch et al., 2016). In another way, several important regions including thalamus, anterior cingulate, and supplementary motor cortex were proven to be modulated by the contingent negative variation, an important negative potential observed by electroencephalography during response anticipation (Nagai et al., 2004).

The result of correlation analysis also indicated that subjects' reaction time might be modulated by later attentional processes more than that primarily reflected in reaction time. Our study showed an important observation that there were remarkable correlations between HRT and anterior interhemispheric fibers. As HRT performances of MCI subjects slowed during later blocks of CPT3, it is reasonable to infer that the expectancy of stimulus emergence may be modified by the executive network, in which the anterior cingulate cortex and associated projecting fibers governs the heading of target detection (Petersen and Posner, 2012; Neta et al., 2014). Consistently, previous literatures suggested the association between CPT performances and FA modifications within several regions, including corpus callosum, cingulum, frontostriatal tracts, and superior longitudinal fasciculus (Segura et al., 2010; Takahashi et al., 2010; Chiang et al., 2015). In another aspect of considerations, a slower HRT across all ISIs may reflect impaired alerting network. While reaction time, deemed to be the major component of alerting network, was once regarded to be heavily associated with the right hemisphere, a growing body of evidences supported the lateralization effect may vary in terms of the warning signal effects and/or temporal expectancies (Fan et al., 2005). Interestingly, our report suggested that microstructural integrity within the left forceps minor best predicted the performances of HRT. As subjects who underwent CPT3 are unable to predict the timing of target appearance, the results were consistent with the findings reported by Coull et al. (2000) where activation of the left frontal and bilateral orbitofrontal cortex was noted during the condition of breached temporal expectancy. Several lines of experiments may provide additional information regarding lateralization effect (Parasuraman et al., 1998; Fan et al., 2005; Petersen and Posner, 2012). The tonic alertness, a relatively sustained activation level with a slower effect, often is associated with right lateralization process (Parasuraman et al., 1998). The phasic alertness, a short-lived activation with higher temporal frequencies, is more likely

to be associated with left hemisphere implement (Fan et al., 2005; Petersen and Posner, 2012). Taken together, neuronal substrates related to HRT in CPT3 suggested the involvement of frontal-predominant processes with a left lateralization effect that may be attributed by the unpredicted temporal expectancy property of the task.

Strength of the current study included the study design, which compared and incorporated findings from comprehensive neuropsychological tests and neuroimaging assessment. The results were derived from a cohort in which subjects were properly categorized through stringent standard neuropsychological tests. Subjects in our cohort were free from remarkable white matter hyperintensities, making the possibility of vascular cognitive impairment less likely. The majority of our MCI cases were categorized as amnestic type, indicating our observation would fit into current concept of pathology related to AD rather than frontotemporal lobar degeneration. Therefore, homogeneity of cognitive status was expected to be purified by current diagnostic repertoire. However, several limitations should be considered in our research work. In subject recruitment, this study was limited by a lack of a control group; as such, the independent influence of "spontaneous report of cognitive deficits" cannot be ascertained. The subject number was limited, therefore raising concern that whether the results might be biased by the small sample size or the compensatory cognitive function as expected to happen during the early stage of SCD/MCI. The heterogeneity of underlying pathology remained to exist, as the speed of cognitive decline and genetic associations may vary according to age-of-onset (Jessen et al., 2014a). It is therefore incorporating biomarkers related to AD should be considered in the future studies. However, we are convinced that our report to be of clinical significance due to the fact that the majority of SCD subjects showed cognitive concern and most MCI subjects were identified to be amnestic type, which have been regarded typical for AD related pathology (McKhann et al., 2011). We were also aware that our current results were derived from a cross-sectional study; a longitudinal follow-up study would be necessary to re-examine the value of CPT3 in predicting underlying neurodegenerative pathology. In neuropsychological aspects, there might be considerations that CPT3 performances were affected by memory retrieval deficits in most of our MCI cases. We therefore clarified group—HRT interaction to be insignificant and confirmed the independent role of CPT3. Finally, as CPT3 may not fully clarify phenotypes associated with other complex attention (e.g., selective or divided attention), nor can it explore attention process in terms of individual network (e.g., alerting, executive and orienting networks), deployment of other attention measurement, such as the Attention Network Test (Fan et al., 2002), may be considered in the future study.

CONCLUSION

Our report suggested the independent role of the CPT3, which showed a discernible visual attention performance between subjects with SCD and MCI. The MCI subjects had a longer HRT across all ISIs more profoundly in later BC, corroborating that impaired vigilance and sustained attention are early cognitive markers in differentiating MCI from SCD. FA measures appeared to be a DTI parameter more sensitive than MD values in detecting microstructural changes, as significant FA modifications within the left forceps major was observed. The neuronal substrates related with HRT highlighted the role of anterior interhemispheric fibers in sustained attention implementation, and the left lateralization effect reflected unpredicted temporal expectancy property in CPT3.

AUTHOR CONTRIBUTIONS

M-CT and Y-HH: study concept and design, analysis, interpretation and drafting the manuscript. W-HH, JFD and Y-HH: neuropsychological test assessment and interpretation. C-PL and C-FH: study concept and critical review. All authors contributed in writing the manuscript.

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Potential Role of OERP as Early Marker of Mild Cognitive Impairment

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Olfactory impairment is present in up to 90% of patients with Alzheimer's disease (AD) and is present in certain cases of mild cognitive impairment (MCI), a transient phase between normal aging and dementia. Subjects affected by MCI have a higher risk of developing dementia compared to the general population, and studies have found that olfactory deficits could be an indicator of whether such a conversion might happen. Following these assumptions, aim of this study was to investigate olfactory perception in MCI patients. We recruited 12 MCI subjects (mean age 70 ± 6.7 years) through the Alzheimer Assessment Unit (UVA Unite) of ASL Lecce (Italy), and 12 healthy geriatric volunteers (HS) as the control group (mean age 64 ± 6.0 years), all of whom were first evaluated via a panel of neuropsychological tests. Subjects were asked to perform an olfactory recognition task involving two scents: rose and eucalyptus, administrated in the context of an oddball task during EEG recordings. Olfactory event-related potential (OERP) components N1 and Late Positive Potential (LPC) were then analyzed as measures of the sensorial and perceptive aspects of the olfactory response, respectively. It was determined that, in the MCI group, both the N1 and LPC components were significantly different compared to those of the HS group during the execution of the oddball task. In particular, the N1 amplitude, was reduced, while the LPC amplitude was increased, indicating that a degree of perceptive compensation can occur when sensorial function is impaired. Further, a correlation analysis, involving OERP components and neuropsychological battery scores, indicated that impairment of olfactory perception may share common pathways with impairments of the spatial system and long-term memory processing.

Keywords: CSERP, olfactory perception, MCI, neurodegenerative processes, aging, OERP

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INTRODUCTION

Mild cognitive impairment (MCI) refers to a clinical state marking the transitional phase between normal cognitive function and pathogenic Alzheimer's disease (AD) (Gauthier et al., 2006), characterized by deficits relating to memory, attention span, language, visuospatial ability, the speed of perception, and the performance of executive functions (Saunders and Summers, 2010; Petersen, 2011). Recent literature includes MCI due to AD (Albert et al., 2011) adding also the concept of prodromal AD (Dubois and Albert, 2004; Suk and Shen, 2013; Green et al., 2015),

and differentiation between amnestic MCI (aMCI), non-amnestic MCI (naMCI) (Dubois and Albert, 2004; Csukly et al., 2016). In aMCI the memory loss is predominant and it is associated with high risk to further conversion to AD (Grundman et al., 2004). Patients with naMCI do not show memory impairment, but do display loss in other cognitive domains and have a higher risk of developing the disease in other dementia forms (e.g., Lewy Body dementia) (Csukly et al., 2016). Both categories can be further assessed to single-domain and multi-domain according to the involvement of one or more cognitive deficit (memory, language, visuospatial ability, speed of mental processing, or executive function) (Libon et al., 2010; Albert et al., 2011). The impairment of neuropsychological and psychophysiological functions can be assessed at behavioral level through neuropsychological tests and can be related with data obtained through different neuroimaging tools (MRI, PET, or SPECT) (Albert et al., 2011). One of these clinical aspects is the atrophy of the hippocampus and the entorhinal cortex, structures involved in the sense of smell (Chételat et al., 2005; Devanand et al., 2007; Shi et al., 2009; Mueller et al., 2010). Clinical studies have shown that olfactory deficits often appear very early in patients at early stages of AD and/or MCI, often before the manifestation of cognitive symptoms (Masurkar and Devanand, 2014; Roberts et al., 2016). Olfactory impairment is significantly associated with aMCI and may improve the accuracy of the model used fit to predict MCI. Furthermore it is associated with progression from MCI to dementia, and from aMCI to AD (Roberts et al., 2016). So, detection of olfactory impairment could be particularly important when investigating for cue at this neurodegenerative level, given the fact that pathological changes will have already reached the neocortex by the time AD presents itself at the clinical level (De Santi et al., 2001; Chételat et al., 2005; Roberts et al., 2016). Early detection of AD-related developments in patients with MCI provides a window of opportunity within which disease progression may be delayed or forestalled through lifestyle changes, as well as preventative measures such as enriched environment, memory and cognitive training and neurocognitive enhancement (Hinrichs et al., 2011).

During the AD progression, a progressive disruption of the cortical association areas, involved in information retrieval, and a reduction of subcortical processing, leads to a rapid loss of neurons and synapses (Tampellini, 2015). Even in the early stages of the disease, there is a sharp decline in cognitive and memory functions. Thus, prodromal AD or MCI due to AD, broadly map onto the pattern of neurofibrillary tangle spreading and is characterized by an atrophy in medial perirhinal cortex, entorhinal cortex and lateral perirhinal cortex (Braak et al., 2006; Krumm et al., 2016), cortical structures closely related to olfactory discrimination (Van Groen and Wyss, 1990; Haberly, 2001; Poellinger et al., 2001; Chapuis et al., 2013). MRI and Fludeoxyglucose-PET (FDG-PET) studies have demonstrated that olfactory performance is significantly reduced in AD patients compared to control subjects (Wesson et al., 2010; Masurkar and Devanand, 2014). In MCI difference between early and advanced stages, are seen at both functional and anatomical levels due to atrophy in several areas, such as the para-hippocampal gyrus, the medial temporal lobe, the entorhinal cortex of the cingulum, the insula, and the thalamus (Apostolova et al., 2010; Davatzikos et al., 2011), while in AD, atrophy was seen in the entire hippocampus and the neighboring regions, as well as in the temporal lobe, the cingulum, the precuneus, the insular cortex, in the caudate nucleus, and in the frontal cortex (Chételat et al., 2005; Manning et al., 2014).

Olfactory deficits have also been identified in subjects possessing ApoE e4 allele (the best-known genetic risk factor for the development of AD) (Bertram et al., 2007), independent of their current cognitive function (Manning et al., 2014) or their short-term risk of developing AD (Olofsson et al., 2010), which suggests that this gene plays an important role in olfactory identification. It should be noted that gradual anosmia can also occur in healthy elderly subjects (Bahar-Fuchs et al., 2010). Moreover, a study conducted using the University of Pennsylvania Smell Identification Test (UPSIT), a behavioral assessment of olfactory memory, concluded that olfactory deficits may be a useful biomarker of AD progression (Kirkpatrick et al., 2006), while an MRI study of the olfactory bulb and olfactory tract atrophy in MCI and AD patients has indicated that olfactory bulb atrophy could be a surrogate biomarker of AD (Graves et al., 1999). Similarly, while several groups have observed AD-specific neuropathology occurring within the olfactory epithelium (Crino et al., 1995; Masurkar and Devanand, 2014), others, using a different set of markers, have reported seeing the same in the olfactory tissues of healthy and non-AD subjects (Yamagishi et al., 1994). Furthermore, other studies have failed to identify any marker present in the olfactory epithelium that would allow AD to be reliably distinguished from other conditions, such as Parkinson's disease or vascular dementia (Crino et al., 1995).

While several studies have described changes in event-related potentials (ERP) in MCI (Olichney et al., 2008; van Deursen et al., 2009; Chapman et al., 2011; Laskaris et al., 2013; Green et al., 2015), none so far have evaluated the use of olfactory event-related potentials (OERP) or chemosensory event-related potentials (CSERP) as tools for the investigation of the functional response to controlled chemical stimulation in MCI. We contend that the peculiarities of these ERPs could be exploited to gain a better understanding of the brain areas associated with the processing of various stimuli (e.g., visual, auditory, nociceptive, etc.), and the temporal latencies that are involved. In the present study, we investigate the OERP latencies and amplitudes relating to the sequential activation of different brain areas, starting with the olfactory bulbs, and progressing through the frontal and insular orbital cortex and the middle-rostral regions of the temporal lobe.

Olfactory event-related potentials consist of an early negative N1 component, followed by a positive phase termed P1, or the Late Positive Component (LPC) (Hummel et al., 1998; Kobal and Hummel, 1998; Pause and Krauel, 2000; Gudziol et al., 2014). When an odoriferous molecule activates the olfactory cells, a negative potential is generated, which is followed by a rebound potential which can be measured by placing electrodes near the olfactory epithelium (Lötsch and Hummel, 2006); the dimension of the track that reproduces the potentials generated change with the variation of the stimulus concentration and shows some

evidence of the adaptation phenomenon (Pause and Krauel, 2000; Wang et al., 2002).

Usually, during OERP recordings subject had to perform a simple tracking task or a simple olfactory recognition task (Pause et al., 1996; Lötsch and Hummel, 2006; Invitto et al., 2018). The nasal stimulation can be left or right lateralized (Pause et al., 1996) or frontal (with the stimulation of both the nostrils) (Invitto et al., 2018). As usually is for other ERP component early negative component (i.e., N1) is an indicator of the cortical sensorial response, and late components (i.e., LPC) are indicators of the cortical perceptive and cognitive responses to the stimuli. The present research aims to assess if there are changes in OERP that can be correlated with perceptual impairments in MCI patients, and if these changes can be helpful to investigate impaired multi-domain cognitive components.

MATERIALS AND METHODS

This research was conducted in the Neurology Unit of the Vito Fazzi Hospital (Lecce, Italy). Data collection was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), and written informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of Vito Fazzi Hospital, Lecce (Report No.01 – 30-01-17).

Subjects

This study involved 12 geriatric MCI patients and 12 healthy geriatric subjects (HS) recruited between February 2017 and January 2018, matched by age, gender and education. Patients (mean age 70.25 years; SD ± 7.74) were enrolled with a clinical suspicion of MCI and admitted to the Alzheimer Assessment Unit (UVA Unite; Lecce, Italy) of the ASL Lecce (Italy), where neuropsychological and olfactory psychophysiological evaluations were conducted. MCI diagnosis was confirmed by neurological and neuropsychological assessment, according to NINCDS-ADRDA (McKhann et al., 1984; Dubois et al., 2007), DSM-V guidelines (American Psychiatric Association, 2013) and according to the latest guidelines and recommendation of National Institute on Aging Alzheimer's Association (NIA-AA) (Albert et al., 2011). According to NIA-AA guidelines our MCI sample can be linked to an intermediated likelihood that MCI syndrome is due to AD. Infact, all patients showed positive biomarkers for neuronal injury (i.e., hippocampal or medial temporal lobe atrophy, diffuse cortical atrophy on MRI and so on). Furthermore, the standard hematochemical tests carried out on these patients, excluded, together with the MRI, the presence of other pathologies and or co-morbidities (Musicco et al., 2004). During the recruitment phase, the patients with other forms or causes of dementia (Babiloni et al., 2016), determined by anamnestic analysis, were excluded from the study.

HS (mean age 66.41 years; SD ± 5.71) were enrolled as volunteers through recruitment notice provided through university students. The HS did not report any current or past psychopathology, neurological illness, or substance abuse and did not report any impairment in normal daily activities.

Neuropsychological Assessment

Subjects (HS and MCI) were scored through the Mini-Mental State Examination (MMSE), Trial Making Test (TMT), Corsi Test (CT), Digit Span (DS), and Rey Auditory Verbal Learning Test (AVLT) according to NIA-AA guidelines (Albert et al., 2011) and to the guideline of Italian Society of Neurology (Musicco et al., 2004) (see neuropsychological scores in **Table 1**). Via *t*-tests, it was determined that while the two groups were not appreciably dissimilar in their ages and gender compositions ($p > 0.05$), their MMSE scores were significantly different, as was to be expected ($p < 0.05$).

Olfactory Psychophysiological Assessment

Subjects performed an olfactory recognition task involving two scents: rose odor perception (β-PEA, 2-Phenylethanol, CAS Number: 60-12-8, Number W285803 Sigma-Aldrich) and eucalyptus odor perception (1,3,3-Trimethyl-2-oxabicyclo [2.2.2] octane, CAS Number: 470-82-6, Number C80601 Sigma-Aldrich) chosen according to Hummel et al. (2009). Scents were administered via US2017127971 (A1) 2017-05-11 (Invitto et al., 2014), with 20 μL PEA provided in 10 mL of Vaseline oil, and both odorous solutions were presented in 20 mL transparent glass vials. Both scents were sealed with plastic film and stored in a darkened cabinet.

The presentation paradigm was an oddball task (Squires et al., 1975) adapted to olfactory stimulation (Invitto et al., 2018). Oddball olfactory task consisted in a pseudorandom administration of different smells (i.e., two different odorants) one of which is the rare stimulus (target stimulus) and the other is the frequent (non-target) stimulus. Usually the percentage of presentation of the rare stimulus is 25%. The presentation of the stimuli is pseudo-randomized so that the subject cannot predict the sequence of stimuli administration. Each stimulation had a duration of 450 ms, with an interstimulus interval (ISI) of 60 s, to avoid olfactory habituation (Pause and Krauel, 2000). The task ended after 40 min.

The device used to record odorous stimuli presentation allows the CSERPs evoked by olfactory stimuli to be measured in a controlled, automated fashion, synchronized to the acquisition of the EEG signal. This method additionally allowed for the blind presentation of smells (Invitto et al., 2014, 2017b).

OERP Recording

EEG signals were recorded using a 16-channel amplifier (Brain Products V-Amp), mounted on an electrode cap equipped with Ag/AgCl electrodes. Brain Vision Recorder and Brain Vision Analyzer (Brain Products GmbH) analysis software were used for the OERP study. Electrode impedance was kept below 15 kΩ, and the EEG recording sampling rate was 500 Hz. Electrodes were online referenced to FCz (Luck, 2005), and offline re-referenced with a common offline reference over all electrodes (Luck, 2005). One electrode was placed at the outer canthus of the right eye and used to monitor eye movements. Trials contaminated by eye movements and other artifacts were rejected. The signal was filtered offline (0.01–50 Hz, 24 dB),

TABLE 1 | MMSE, Rey Auditory Verbal Learning Test (AVLT), Digit Span Test, Corsi Spatial Test, and Trial Making Test (TMT) scores.

SUBJ	AGE range	MMSE	AVLTa	AVLTb	AVLTc	Digit Span	Corsi Test	TMT	TMT-B	TMT-AB	ADL	IADL
MCI	75–80	<22	0	0	0	3	1	0	0	0	6	8
MCI	55–60	<22	3	3	15	4	4	1	1	1	6	8
MCI	75–80	<22	3	4	15	3	3	2	1	4	6	8
MCI	70–75	<22	0	0	7	4	4	0	1	2	6	8
MCI	65–70	<22	0	0	7	3	3	0	1	4	6	8
MCI	75–80	<22	1	1	6	4	3	0	0	1	6	8
MCI	60–65	<22	1	0	0	4	2	4	3	3	6	8
MCI	70–75	<22	1	0	0	4	1	0	1	4	6	8
MCI	60–65	<22	0	2	7	3	4	0	1	4	6	8
MCI	65–70	<22	0	0	7	2	2	2	1	1	6	8
MCI	80–85	<22	3	3	15	1	4	1	1	1	6	7
MCI	70–75	<22	1	0	15	1	1	0	0	0	6	8
HS	60–65	>24	25	5	15	6	5	4	4	4	6	8
HS	65–70	>24	27	13	15	5	6	4	4	4	6	8
HS	70–75	>24	46	11	15	6	4	4	4	4	6	8
HS	65–70	>24	50	15	15	7	5	4	4	4	6	8
HS	75–80	>24	48	14	15	6	6	4	4	4	6	8
HS	65–70	>24	32	15	15	5	5	4	4	4	6	8
HS	65–70	>24	30	12	15	6	7	4	4	4	6	8
HS	70–75	>24	41	11	15	5	6	4	4	4	6	8
HS	60–65	>24	25	15	15	6	7	4	4	4	6	8
HS	60–65	>24	21	14	15	5	7	4	4	4	6	8
HS	55–60	>24	41	11	15	6	6	4	4	4	6	8
HS	70–75	>24	22	12	15	5	5	4	4	4	6	8

and the threshold for artifact rejection was set at $>|125|$ μV (Pause et al., 1996, 1997). Ocular rejection was performed through independent component analysis (ICA). ERP epochs included a 100 milliseconds pre-stimulus baseline period and a 500 milliseconds post-stimulus segment. Separate averages were calculated for each odorant segmentation (rose and eucalyptus). The detection of peaks was performed as previously described (Invitto et al., 2017a). OERP components were labeled N1 and LPC according to Pause et al. (1996). Latency windows were set to 150–300 ms for the N1, and 300–500 ms for the LPC (Pause et al., 1996; Pause and Krauel, 2000). These values correspond to the onset of CSERP negative and positive peaks components estimated from grand average waveforms (see **Figure 1**).

Moreover, we performed a Current Source Density (CSD), a topographic representation of EEG voltage values across the scalp. CSD is the results of mathematical algorithms, in this research conducted through the Brain Vision Analyzer software, that directly transform the scalp-recorded EEG into estimates of radial current flow at scalp. So, in EEG-CSD topography, the positive values identify the direction between the brain source to the scalp, and negative values represent current flow from the scalp to the brain source (Kayser et al., 2010; Kamarajan et al., 2016). To obtain CSD images, grand average OERP waveforms at each electrode were transformed into reference-free CSD estimates using a spherical spline surface Laplacian (interpolation of Spherical Splines – order of Splines 4; Max degree of Legendre Polynomial: 10; Default Lambda 1e-5) (Perrin et al., 1989; Kayser et al., 2010). The number of two representation Maps were chosen in the Interpolation window and the interval between Maps (ms/Hz) was defined as indicated in the default value (1000). The selected range of maps visualization was set between 100 and 500 ms (i.e., the temporal range in which the two OERP components were detected).

Then a method comparison operation was carried out in a subtractive way (i.e., differences between different datasets), by subtracting between frequent and infrequent stimulus, to topographically view the generic attentional effect rather than the olfactory oddball task due to the administration of two different odors.

Statistical Analysis

All statistical analyses were performed using IBM SPSS version 20. Further details regarding statistical tests are described in the following section.

RESULTS

Shapiro-Wilk and Kolmogorov-Smirnov tests showed that the HS and MCI groups behaved differently from each other with regards to the amplitudes of both the N1 and LPC components. Consequently a separate non-parametric one way ANOVA (i.e., Kruskal-Wallis Test for independent samples) was used for group comparisons (Maris and Oostenveld, 2007). We first analyzed the amplitudes of the N1 components elicited by the rose odorant using the Kruskal-Wallis test. Significant differences

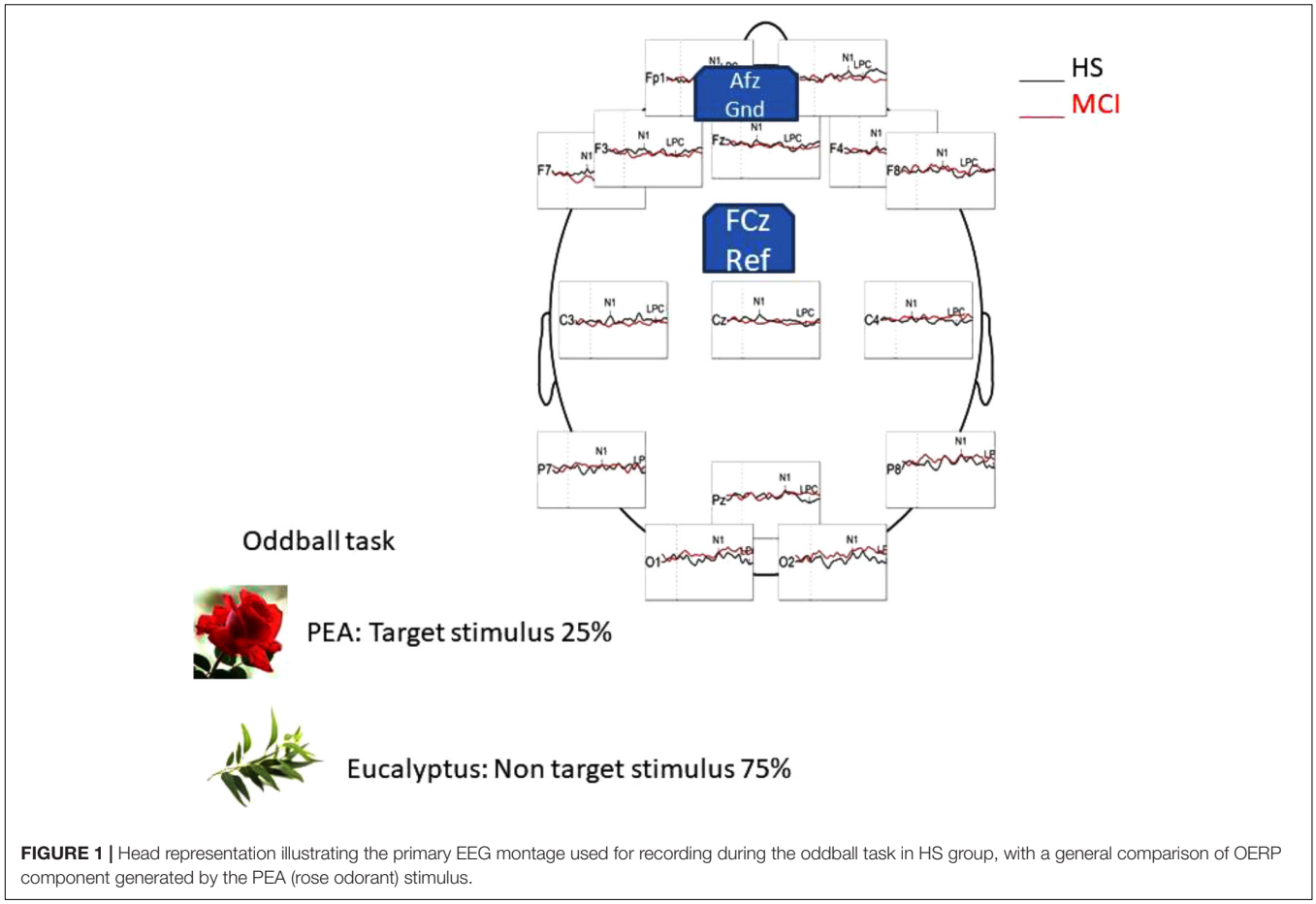

FIGURE 1 | Head representation illustrating the primary EEG montage used for recording during the oddball task in HS group, with a general comparison of OERP component generated by the PEA (rose odorant) stimulus.

were observed at the F3 position (z = 4.452; p = 0.03), with the MCI group showing decreased N1 amplitudes [MCI mean = −2.85 μV (SD = 3.39) vs. HS mean = −5.02 μV (SD = 3.13)], at F7 (z = 9.200; p = 0.002), where N1 was also smaller in the MCI group [MCI mean = −2.63 μV (SD = 1.60) vs. HS mean = −6.49 μV (SD = 3.94)] (see **Figure 2**) and at F8 (z = 5.635; p = 0.018), where N1 amplitude was higher in the MCI group [MCI Mean = −5.72 μV (SD = 3.96) vs. HS mean = −1.96 μV (SD = 3.23)]. There was, however, no significant difference in the N1 latencies of the two groups. Analysis of LPC amplitudes for the rose odorant identified a significant difference at the F7 (z = 4.278; p = 0.04) and F8 (z = −4.839; p = 0.028) positions, with the MCI group exhibiting enhanced amplitudes at the former [MCI mean = 8.74 μV (SD = 3.89) vs. HS mean = 3.79 μV (SD = 3.89)], and decreased amplitudes at the latter [MCI mean = 5.51 μV (SD = 2.50) vs. HS mean = 9.72 μV (SD = 4.52)] (a comparison of OERP components is shown in **Figure 1**). The mean amplitudes (±sSD) of OERPs elicited by the rose odorant for HS and MCI groups are shown in **Table 2**.

To investigate overall trends in olfactory perception of frequently encountered stimuli in an oddball setting, we analyzed the OERPs elicited by eucalyptus. The Kruskal-Wallis test for independent samples revealed significant differences in N1 amplitudes at C4 (z = 7.934; p = 0.005), where values were lower in the MCI group [MCI mean = −1.45 μV (SD = 0.76) compared to HS mean = −3.30 μV (SD = 1.75)], F7 (z = −5.078; p = 0.024) [MCI mean = −4.29 μV (SD = 2.40) vs. HS mean = −1.91 μV (SD = 2.15)], and in N1 latencies at Fp2 (z = 5.084; p = 0.024) [MCI mean = 157 ms (SD = 60) vs. HS = 215 ms (SD = 45)] and F7 (z = 5.081; p = 0.024) [MCI mean = 211 ms (SD = 48) vs. HS mean = 162 ms (SD = 63)]. Detailed features of the N1 and the LPC are shown in **Table 3**, and a comparison of HS and MCI OERPs recorded at the C4 electrode is shown in **Figure 3**. Our analysis also indicated that there were no significant differences in the LPC components elicited by the eucalyptus scent.

Figure 4 illustrates the CSD in HS and in MCI groups for the rose odorant, during the N1 and LPC phases. Images show how the two samples allocate the attention differently. In general, the range in voltage is lower in the MCI (MCI = ± 2.03 μV vs. HS = ± 2.45 μV) and the negative component seems to be more frontal and lateralized on the left, while the positive component seems more lateralized on the right occipito-temporal region.

The association between the results of the neuropsychological test and the OERP amplitudes that had been found to be significantly different between MCI and HS groups via the analyses described above (i.e., those recorded at F3, F7, and F8 for the N1 component and F7 and F8 for the LPC component) was further investigated via Kendall's tau-b (τb) correlation coefficient analysis, for which detailed values are shown in

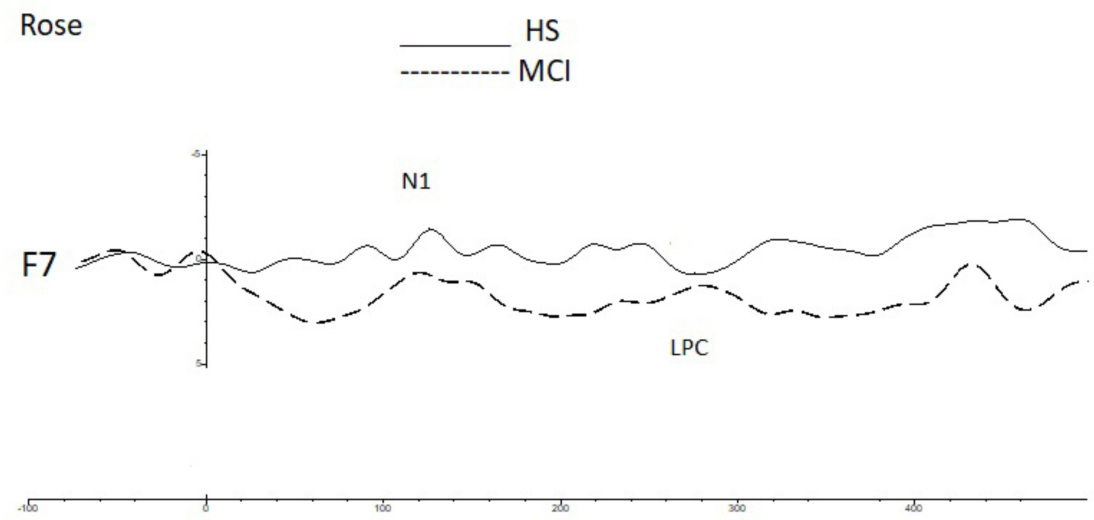

FIGURE 2 | A comparison of the grand averages of OERP waveforms (recorded at the F7 electrode) elicited by the rose odorant for the MCI (dashed line) and HS (continuous line) groups. The N1 and LPC components are labeled.

TABLE 2 | Means of N1 and LPC amplitudes (μV) elicited by the rose odorant for MCI and HS groups.

Electrode	N1 Rose					LPC Rose				
	HS		MCI			HS		MCI		
	Mean	*SD*	Mean	*SD*	*p*	Mean	*SD*	Mean	*SD*	*p*
Fp1	−5.58	6.63	−3.55	4.12	0.42	3.91	6.29	5.54	2.45	0.53
Fp2	−5.12	7.24	−4.11	2.83	0.60	5.31	6.66	4.75	2.67	0.77
F3	−5.02	3.13	−2.85	3.39	0.03*	4.13	5.53	5.54	3.98	0.22
F4	−3.53	1.52	−4.42	2.10	0.21	5.39	2.61	4.51	2.69	0.45
C3	−4.29	3.58	−2.47	1.88	0.29	2.69	3.43	4.05	3.49	0.77
C4	−3.34	2.35	−4.56	3.41	0.74	3.55	3.03	3.00	2.89	0.58
P7	−6.22	5.59	−5.04	3.01	0.64	6.23	3.69	6.25	2.60	0.77
P8	−6.45	3.73	−4.95	3.84	0.26	6.74	5.09	5.70	3.30	0.20
O1	−6.24	4.92	−6.42	2.99	1.00	5.67	3.69	5.12	3.38	0.87
O2	−5.88	4.53	−5.96	3.03	0.89	6.86	2.88	5.37	3.35	0.25
F7	−6.49	3.94	−2.63	1.60	0.00*	3.97	3.89	8.74	6.79	0.04*
F8	−1.96	3.23	−5.72	3.96	0.01*	9.72	4.52	5.51	2.50	0.03*
Cz	−3.76	2.91	−2.37	0.79	0.13	2.49	3.36	3.13	2.68	0.77
Pz	−3.74	3.44	−3.60	1.98	0.64	4.01	3.00	4.61	2.91	0.92
Fz	−2.48	1.99	−2.36	2.91	0.94	4.77	3.87	3.97	2.64	0.72

SD, standard deviation; p, p-value.

Table 4. A negative correlation between the N1 amplitudes of F7 and F3 was seen. Additionally, F7 N1 amplitude was found to be positively correlated with high neuropsychological test scores, and an association between the CORSI and TMTs was also seen (A-B-AB). Moreover, OERP amplitudes were found to be related to spatial scores. While left frontotemporal components were determined to be negatively correlated with high scores in spatial memory tasks, right frontotemporal components were, by contrast, positively correlated with the same. Finally, we also observed that subject scores for the Rey AVLT, which tests for episodic declarative memory, are particularly highly correlated with scores achieved in the b version of the test, designed to evaluate long-term episodic memory.

DISCUSSION AND CONCLUSION

Olfaction can serve as a useful biomarker in processes such as AD and Parkinson's disease, as it is often the first sense to be affected by neurodegeneration. It is additionally known that subjects afflicted with MCI and/or who possess the e4 allele

TABLE 3 | Means of N1 and LPC amplitudes (μV) elicited by the eucalyptus odorant for MCI and HS groups.

Electrode	N1 eucalyptus					LPC eucalyptus				
	HS		MCI			HS		MCI		
	Mean	*SD*	Mean	*SD*	*p*	Mean	*SD*	Mean	*SD*	*p*
Fp1	−3.58	4.36	−2.82	2.41	0.89	3.21	4.47	3.45	2.84	0.57
Fp2	−4.49	4.85	−2.44	3.01	0.14	3.15	3.38	3.72	2.12	0.62
F3	−2.43	1.71	−3.56	1.46	0.14	3.45	2.26	2.53	1.15	0.57
F4	−2.98	1.07	−1.87	1.52	0.91	2.76	1.86	4.52	2.53	0.12
C3	−2.13	0.91	−2.61	1.11	0.26	2.17	1.16	2.22	0.96	0.72
C4	−3.30	1.75	−1.45	0.76	0.00*	1.97	1.45	2.76	1.87	0.29
P7	−2.90	2.76	−3.83	1.90	0.44	5.24	2.95	3.37	2.23	0.18
P8	−3.64	3.46	−2.99	1.86	0.78	2.89	3.16	4.47	3.25	0.26
O1	−3.11	3.27	−5.60	2.98	0.08	5.50	4.49	4.30	5.41	0.48
O2	−3.45	3.52	−4.61	3.07	0.53	4.46	3.83	5.18	3.86	0.67
F7	−1.91	2.15	−4.29	2.41	0.02*	4.35	3.19	3.17	2.83	0.52
F8	−3.17	2.07	−2.72	3.18	0.48	4.01	2.41	5.15	2.16	0.12
Cz	−2.37	0.91	−1.66	1.31	0.16	2.37	1.15	2.83	1.81	0.52
Pz	−2.03	1.96	−2.83	1.48	0.08	3.55	2.22	3.18	2.54	0.29
Fz	−2.82	2.78	−2.31	1.88	0.89	2.67	2.11	2.89	1.82	0.94

SD, standard deviation; p, p-value.

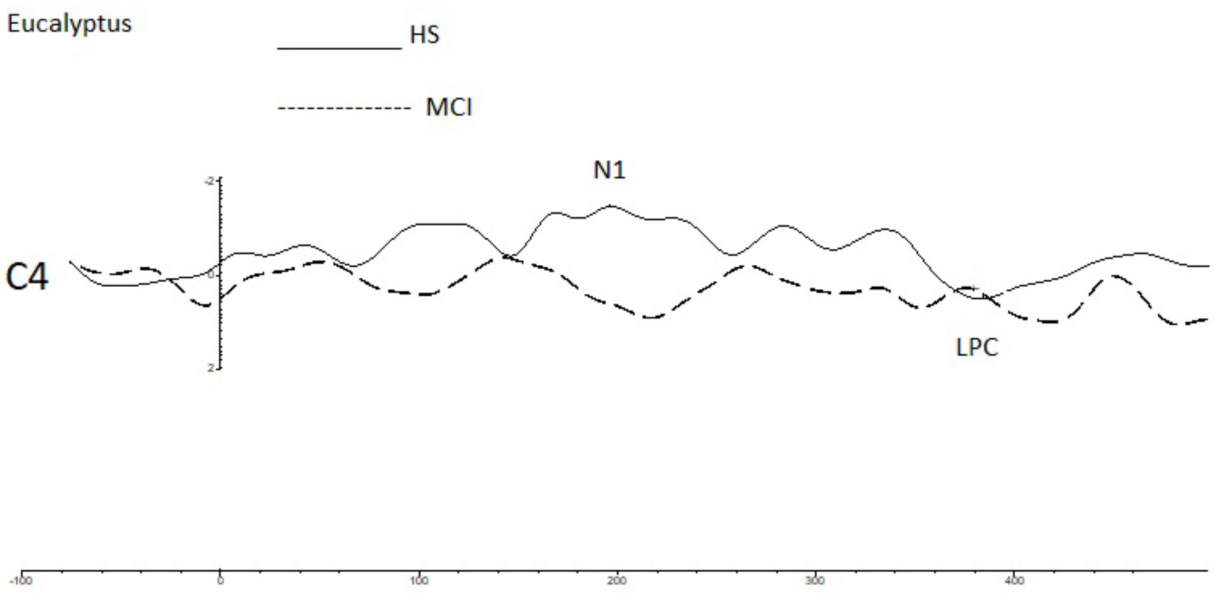

FIGURE 3 | A comparison of the grand averages of OERP waveforms (recorded at the C4 electrode) elicited by the rose eucalyptus for the MCI (dashed line) and HS (continuous line) groups. The N1 and LPC components are labeled.

of the ApoE gene, which has been linked to an increased risk for the development of AD, have severe anosmia (Olofsson et al., 2010). Further, MRI studies have suggested that the olfactory bulb is impaired in patients with MCI and AD (Wesson et al., 2010). Finally, several groups have described changes in ERP in MCI (Olichney et al., 2008; van Deursen et al., 2009; Chapman et al., 2011; Laskaris et al., 2013; Green et al., 2015). However, studies relating to OERPs in MCI or AD are currently lacking.

Compared to techniques such as MRI or fMRI, OERP measurements also have a higher temporal resolution, and can be conducted at lower cost with a lower degree of invasiveness. We have, for these reasons, developed a research protocol designed to assess both the sensorial and perceptual aspects of the olfactory cortical response in MCI patients (i.e., sample with intermediate probability that MCI is due to AD). In this study, we evaluated various facets of both the early (N1) and slow potentials (LPC) of the OERP in MCI patients and HS to determine if significant

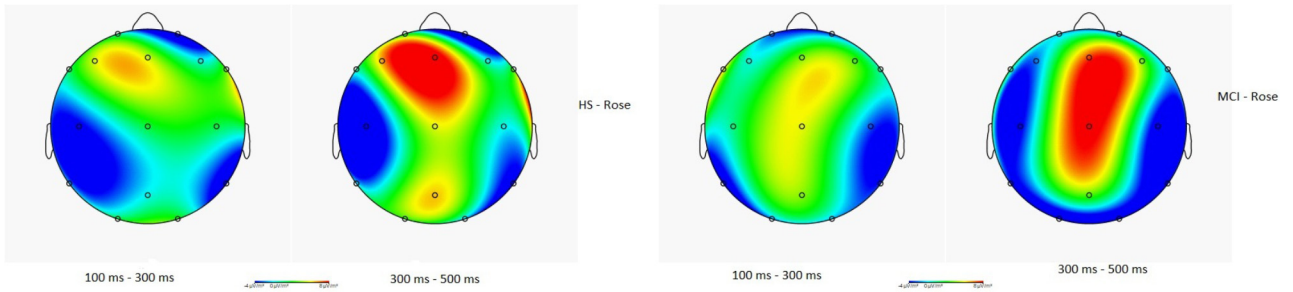

FIGURE 4 | Topographical map of current source density in the N1 and LPC intervals in the HS and MCI group when presented with the rose odorant.

TABLE 4 | Kendall's tau-b correlation coefficients relating neuropsychological test scores and OERP amplitudes significantly affected in the MCI group (F3, F7, and F8 in N1 component and F7 and F8 in LPC component).

	KENDALL'S TAU-B									
	F3 (N1)		F7 (N1)		F8 (N1)		F7 (LPC)		F8 (LPC)	
	Cor.	*p*	Cor.	*p*	Cor.	*p*	Cor.	*p*	Cor.	*p*
MMSE	−0.23	0.15	−0.35	0.03*	0.24	0.14	−0.22	0.17	0.31	0.05*
AVLTa	−0.21	0.20	−0.33	0.05*	0.31	0.06	−0.17	0.29	0.25	0.13
AVLTb	−0.34	0.05*	−0.54	0.00**	0.44	0.01*	−0.40	0.02*	0.38	0.03*
AVLTc	−0.32	0.05*	−0.33	0.04*	0.19	0.26	−0.26	0.11	0.35	0.03*
Digit Span	−0.14	0.39	−0.38	0.02*	0.42	0.01*	−0.32	0.06	0.26	0.12
Corsi	−0.25	0.12	−0.46	0.00**	0.31	0.06	−0.38	0.02*	0.37	0.02*
TMT	−0.33	0.06	−0.52	0.00**	0.31	0.09	−0.29	0.10	0.40	0.02
TMT-B	−0.33	0.05*	−0.52	0.00**	0.36	0.04*	−0.41	0.02	0.43	0.01*
TMT-AB	−0.26	0.13	−0.40	0.02*	0.36	0.04*	−0.45	0.01**	0.29	0.10

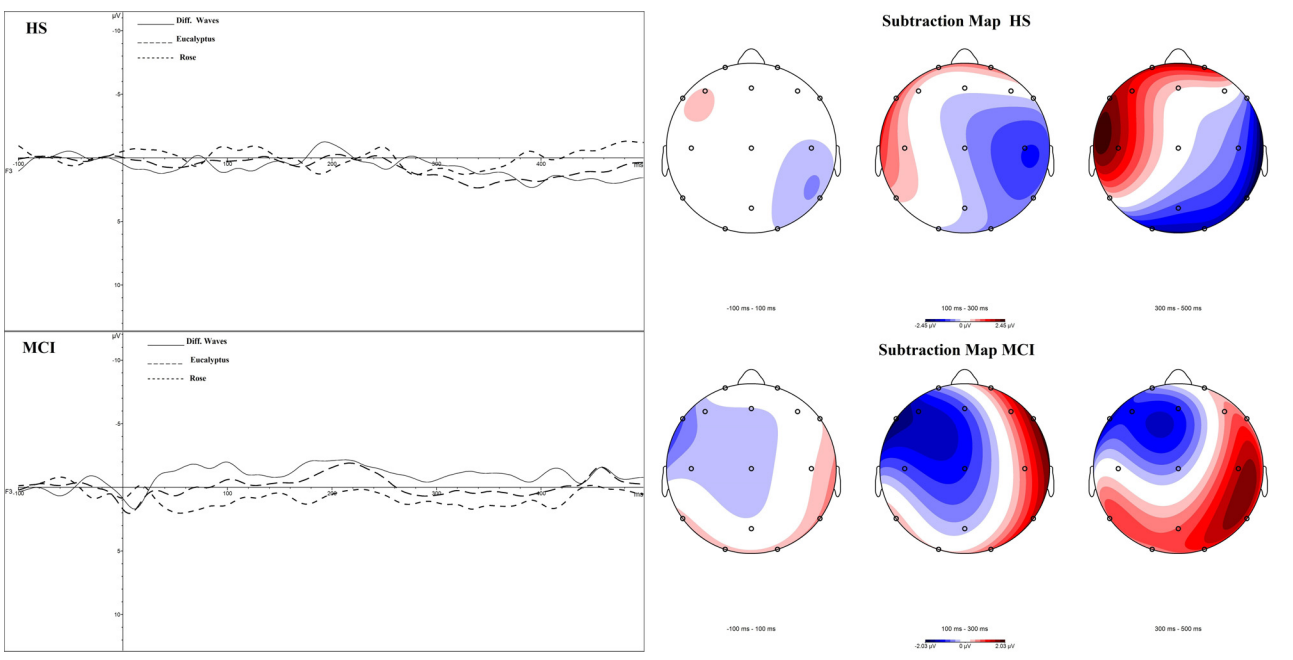

FIGURE 5 | Subtraction OERP components and Map in HS and MCI patients.

differences exist between the two groups (**Figure 5**). Our results showed a clear deficit in the early sensory N1 component in the MCI group, which exhibited reduced amplitudes in the left frontal and right centroparietal lobes compared with healthy controls, which may indicate reduced olfactory discrimination. Topographic mapping of OERP intensities across the scalp showed that the negative components are more compromised in the left orbitofrontal and frontotemporal areas and in the right centroparietal area, which are particularly closely associated with olfactory OERPs (Schriever et al., 2014). Also, slow positive potentials in the left prefrontal cortex in the MCI group had greater amplitudes, and longer latencies. This brain area is involved in olfactory perception and memory, and is specifically activated during the administration of pleasant scents (Soudry et al., 2011). The increased amplitude could be a mechanism compensating for impairments in the olfactory-sensorial components (i.e., N1), indicating the existence of a feedback system capable of activating more arousing resources to better detect the olfactory stimulus (e.g., LPC could be a component that can covert cognitive resources).

A similar mechanism was observed in a previous study involving obstructive sleep apnea syndrome (Invitto et al., 2018). However, the greater activation of early OERP components in the right frontoparietal area and the reduction of LPC in MCI patients seen here can be extremely important in supporting the previous results. Studies have demonstrated that the right frontotemporal area is responsible for the recruitment of olfactory memory rather than its sensorial perception. Also this activation can be the consequence of a compensation of a sensory deficit which, to be efficient, relies on more mnestic than perceptive components (Jones-Gotman and Zatorre, 1993). Further specifications can be due to the correlation between neuropsychological test and OERP results. The best area that fit with olfactory responses and neuropsychological scores is the left frontotemporal area (high scores to neuropsychological tests are related with greater negative amplitude in N1). This area is strongly associated with odor discrimination (Rami et al., 2007). Another interesting result from this study is the correlation between CORSI and TMT scores. OERP amplitudes are related to spatial scores. Left frontotemporal components have negative correlations with high scores in spatial memory tasks, whereas, right frontotemporal components have a positive correlation. These observations support the existing paradigm that the spatial and olfactory systems are closely associated at the level of the cortical pathways (Jacobs et al., 2015). The Rey AVLT, a test for episodic declarative memory, is highly correlated, in particular, the scores of the section linked to long-term episodic memory.

Our data demonstrate the usefulness of OERPs in the study of the early stages of neurodegenerative processes, as there was a clear defect in olfactory function in MCI patients, confirming the findings of others. One particular area that still needs further investigation is to clarify the extent that MCI samples are caused by AD. In fact, our sample was not recruited after genetic or CSF diagnosis, which would confirm AD pathology underlying the disease or the prodromal AD (Dubois and Albert, 2004). On the other hand, this study also did reveal the existence of compensatory processes that are activated to balance these deficits during the early stages of multi-domain aMCI, involving not only the sensory system, but also the spatial system. This process seems linked to the recognition process through long-term memory, which, in the specifics of olfactory perception, seems to be activated to recognize the odors no longer through its sensorial components, but through long-term recognition features. We intend to further develop this research by reassessing the same functions within the same cohort of subjects, over the next year, in order to determine if MCI has developed into AD in any of the cases. Our follow up will be conducted after adequate tests assessment, including neuroimaging, biomarkers and genetic assessment of ApoE gene. So we will examine how the olfactory components have been affected by the neurodegenerative process. This will allow us to understand whether these electrophysiological findings found in the multimodal amnesic MCI (with intermediate probability that MCI is due to AD) remain constant or progress; we will also understand if the subjects enter a diagnostic phase of MCI due to AD or we may actually find direct evidence in AD.

In any case, the electrophysiological data produced by olfactory stimulation shows us that in MCI there are complex alterations that also provide compensatory mechanisms with respect to the physiological responses of geriatric control subjects. These compensatory mechanisms can be considered as further cues in a diagnostic border picture, such as that of MCI with intermediate connection to AD, which shows altered responses on various levels. The assessment of a low-cost, relatively fast and non-invasive test such as that of OERPs may be an additional data to be integrated into this particular diagnostic frame.

AUTHOR CONTRIBUTIONS

SI contributed to research conception, protocol design, subjects EEG recording, EEG and neuropsychological data analysis, paper writing, and paper review. GP Neuropsychological Tests Administration and scoring. MC, LC, and GN MCI assessment and recruitment. VC technical support to olfactometer. GT Head of Neurology Unite – supervision on patients assessment and recruitment. SDN neuropsychological scoring analysis. MB theoretical suggestions in the paper review.

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Radiomic Features of Hippocampal Subregions in Alzheimer's Disease and Amnestic Mild Cognitive Impairment

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Alzheimer's disease (AD) is characterized by progressive dementia, especially in episodic memory, and amnestic mild cognitive impairment (aMCI) is associated with a high risk of developing AD. Hippocampal atrophy/shape changes are believed to be the most robust magnetic resonance imaging (MRI) markers for AD and aMCI. Radiomics, a method of texture analysis, can quantitatively examine a large set of features and has previously been successfully applied to evaluate imaging biomarkers for AD. To test whether radiomic features in the hippocampus can be employed for early classification of AD and aMCI, 1692 features from the caudal and head parts of the bilateral hippocampus were extracted from 38 AD patients, 33 aMCI patients and 45 normal controls (NCs). One way analysis of variance (ANOVA) showed that 111 features exhibited statistically significant group differences ($P < 0.01$, Bonferroni corrected). Among these features, 98 were significantly correlated with Mini-Mental State Examination (MMSE) scores in AD and aMCI subjects ($P < 0.01$). The support vector machine (SVM) model demonstrated that radiomic features allowed us to distinguish AD from NC with an accuracy of 86.75% (specificity = 88.89% and sensitivity = 84.21%) and an area under curve (AUC) of 0.93. In conclusion, these findings provide evidence showing that radiomic features are beneficial in detecting early cognitive decline, and SVM classification analysis provides encouraging evidence for using hippocampal radiomic features as a potential biomarker for clinical applications in AD.

Keywords: alzheimer's disease, amnestic mild cognitive impairment, hippocampal subregions, radiomic features, support vector machine

INTRODUCTION

As the leading cause of neurodegenerative dementia, Alzheimer's disease (AD) is characterized by a progressive deterioration in cognitive function, especially in episodic memory. Due to its influence on the normal lives of both patients and caregivers, AD has become a considerable burden on society (Reitz and Mayeux, 2014; Alzheimer's Association, 2017). Mild cognitive impairment (MCI) is generally defined as a transitional stage between the cognitive changes associated with normal aging and early dementia. Amnestic MCI (aMCI) means that while the memory ability of a patient has decreased, the patient does not fulfill the criteria for dementia, and aMCI is thought to be the prodromal stage of dementia due to AD and has a high risk of developing into AD (Petersen and Jack, 2009; Petersen, 2016).

Medial temporal atrophy is believed to be one of the magnetic resonance imaging (MRI) markers for progression to AD in a prodromal stage, and atrophy of the hippocampus, the most vulnerable structure in the medial temporal lobe, is one of its most robust markers (Ferreira et al., 2011; Yang et al., 2012; Ezzati et al., 2016). In the past decade, structural MRI has been widely used to quantify hippocampal atrophy for distinguishing MCI from AD (Pruessner et al., 2000; Shen et al., 2002; Apostolova et al., 2012). Evidence has also demonstrated that aMCI patients converting to AD show greater atrophy in the hippocampus than is found in those who do not convert to AD (Chételat et al., 2005; Apostolova et al., 2006; Leung et al., 2013). Beyond the decrease in volume, changes in the morphology of the hippocampus have also been found and may appear even earlier than atrophy in AD (Achterberg et al., 2014; Sørensen et al., 2016).

Radiomics, a morphological method for imaging analysis, can quantitatively examine a large set of texture features (Parmar et al., 2015) and is used in the classification of tumors and in predicting radiation therapy outcomes (Huynh et al., 2016). The implications of "texture" include many image properties, such as coarseness, rugosity, and smoothness. Recently, texture analysis has been successfully applied to produce imaging biomarkers for AD (de Oliveira et al., 2011; Anandh et al., 2015; Chincarini et al., 2015). For example, studies have shown that hippocampal texture abnormalities appear in MCI/AD, indicating that texture may serve as a prognostic neuroimaging biomarker for early cognitive impairment (Sørensen et al., 2016, 2017).

Hippocampal head (anterior) atrophy is the most obvious in AD (Raji et al., 2009) and has been reported as a predictive marker of conversion to AD (Costafreda et al., 2011). Convergence evidence has also demonstrated the existence of functional differences along the anterior-posterior axis of the hippocampus (Strange et al., 2014; Collin et al., 2015). The anterior-posterior discrepancies in the hippocampus have also been associated with neuropsychiatric symptoms in early AD (Lyketsos et al., 2011). Given that the posterior and anterior parts of the hippocampal are differentially vulnerable to neuropathology in AD (La Joie et al., 2014; Delli Pizzi et al., 2016; Zeidman and Maguire, 2016), evaluating imaging measurements in different subfields would provide more accurate and sensitive information for the early detection of AD (La Joie et al., 2013; Blanken et al., 2017; Mak et al., 2017). Thus, hippocampal morphological differentiation along the posterior-anterior axis of the hippocampus deserves more attention because they are relevant for the early diagnosis of AD.

Inspired by the above studies, we hypothesized that radiomic features in the hippocampus would be disrupted and that these changes might be employed in the early classification of AD and aMCI. To test this hypothesis, radiomic features that were used in previous studies (Aerts et al., 2014; Parmar et al., 2015) were calculated from hippocampal subregions based on the Brainnetome Atlas (Fan et al., 2016) from a structural MRI data of 38 AD patients, 33 aMCI patients and 45 normal controls (NCs). One-way ANOVA was then used to identify changes in the radiomic features among AD, aMCI and NC subjects. Then, correlation analyses between the identified radiomic features and Mini-Mental State Examination (MMSE) scores were calculated to evaluate the relationships between hippocampal textures and cognitive ability. In addition to these case-control comparisons, we employed the support vector machine (SVM) model to evaluate the diagnostic power of radiomic features (**Figure 1**).

MATERIALS AND METHODS

Ethics Statement, Subject Recruitment, and Neuropsychological Assessment

This study was approved by the Medical Ethics Committee of the Chinese PLA General Hospital. All subjects or their legal guardians (a family member) signed written informed consent forms. All subjects met identical methodological stringency criteria, and comprehensive clinical details can be found elsewhere in our previous studies (Zhang Z. et al., 2012, 2014; Yao et al., 2013; Zhou et al., 2013; Wang et al., 2015). To maintain the scientific integrity of the present study, herein we provide a brief introduction regarding the data inclusion and exclusion criteria, acquisition and processing.

The recruited AD patients fulfilled the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association) criteria for the diagnosis of probable AD (McKhann et al., 1984). The aMCI patients were required to conform to the criteria described by Petersen (Petersen, 2004). The AD and aMCI patients also met the core clinical criteria of the new diagnostic criteria for probable AD and aMCI due to AD (Albert et al., 2011; McKhann et al., 2011). The NCs included subjects lacking memory decline but matching with AD and aMCI patients in gender and age. All of the participants were 55 to 85 years old and were neurological inpatients and outpatients of the Chinese PLA General Hospital. Clinical, physical and neuropsychological assessments were performed before MRI examination. Subjects with neurological or psychiatric diseases or with a history of cerebrovascular attacks or other degenerative disorders were excluded.

All subjects underwent a battery of neuropsychological tests at the department of neurology of the Chinese PLA General Hospital. These tests included the MMSE, Clinical Dementia Rating, Auditory Verbal Learning Test (AVLT), Geriatric Depression Scale and Activities of Daily Living scale.

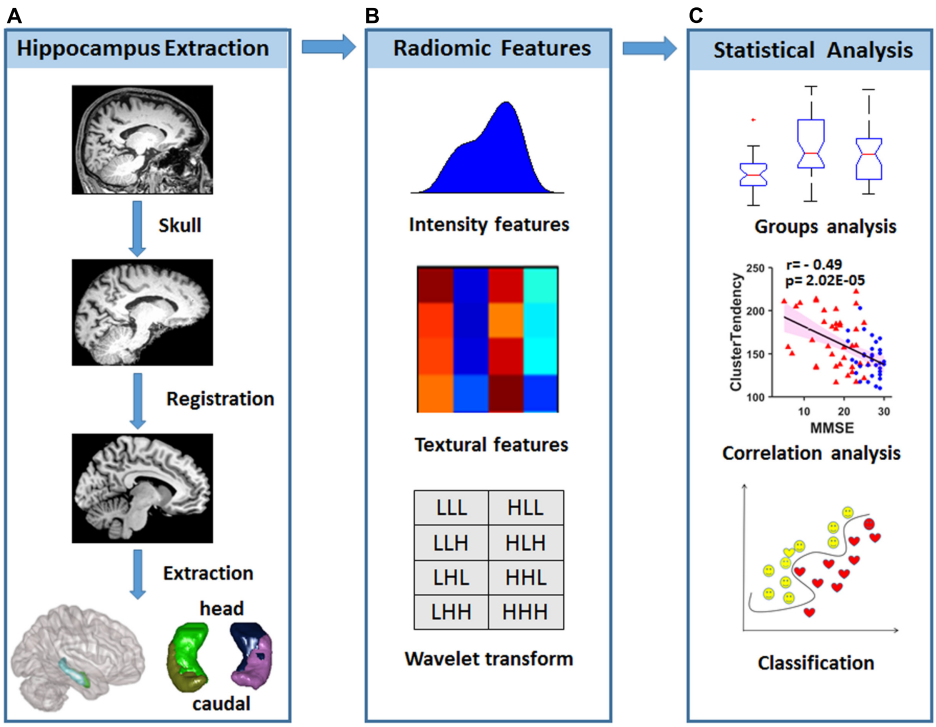

FIGURE 1 | Diagram of data processing and statistical analyses. **(A)** Strategy for hippocampal subregion extraction from high-resolution structural MRI. **(B)** Intensity features and textural features were extracted from the images, and wavelet transformation was achieved in each hippocampal subregion. **(C)** Statistical analysis was used to find radiomic features that were different among the groups, and correlations with the MMSE were performed to evaluate the relationship between radiomic features and cognitive performance. A classification analysis was performed to determine whether radiomic features could be used for diagnosis.

Structural MRI Data Acquisition

Discovery Data

Magnetic resonance imaging examinations were performed at the department of radiology of the Chinese PLA General Hospital using a 3.0 T Siemens MR system (Skyra, Siemens, Germany) with a 20-channel head coil. During the examinations, the subjects were given comfortable foam padding to minimize head motion and ear plugs to reduce the scanner noise. Before the structural MRI data were collected, T2-weighted images were collected and evaluated by two senior radiologists. Sagittal T1-weighted structural images (192 continuous slices) were acquired for each subject using a magnetization-prepared rapid gradient echo sequence with the following scan parameters: repetition time (TR) = 2,530 ms, echo time (TE) = 3.43 ms, inversion time (TI) = 1100 ms, field of view (FOV) = 256 mm × 256 mm, acquisition matrix = 256 × 256, flip angle (FA) = 7°, and slice thickness = 1 mm. The obtained three-dimensional images had a resolution of 1 mm × 1 mm × 1 mm.

Replicated Data

Magnetic resonance imaging examinations were further performed in the same department of radiology using another 3.0 T Siemens MR system (Skyra, Siemens, Germany) for other subjects, including AD and aMCI patients and NCs. The protocol and parameters used in the MRI examinations were all consistent with those used in the discovery data.

Hippocampal Radiomic Feature Extraction

All preprocessing steps were performed using statistical parametric mapping (SPM12)[1] and the Brainnetome fMRI Toolkit[2] (Xu et al., 2018). Briefly, each individual T1-weighted DICOM image of the brain was first converted to NIFTI data. Next, skull stripping was performed, and the obtained images were normalized to the Montreal Neurological Institute (MNI) standard T1 template (standard space 181 × 217 × 181 with a resolution of 1 mm × 1 mm × 1 mm). Meanwhile, we resliced the Brainnetome Atlas[3] to the standard MNI space with a resolution of 1 mm × 1 mm × 1 mm, and the caudal and head regions of the bilateral hippocampus were further extracted as masks. Lastly, for each subject, we obtained the subregions by point multiplication of the masks and the normalized T1 images.

Quantitative radiomic features were calculated using in-house MATLAB script as previously reported (Aerts et al., 2014; Huynh et al., 2016), and detailed descriptions of each feature are listed in the supplemental information to maintain the scientific integrity of the present study. Briefly, the intensity features were calculated based on the histogram, which represented the distribution of voxel intensities within the images (14 features),

[1]http://www.fil.ion.ucl.ac.uk/spm
[2]http://brant.brainnetome.org
[3]http://atlas.brainnetome.org

the textural features on the gray level co-occurrence matrix (GLCM) and the gray level run-length matrix (GLRLM) (33 features). Wavelet transformation (Symlet wavelet filter, "sym4" were used here) in eight directions (LLL, LLH, LHL, LHH, HLL, HLH, HHL, and HHH) was performed for the four hippocampal subregions to combine the spatial and frequency characteristics (for more detailed information, please refer to the **Supplementary Material**). As a result, 423 radiomic features were obtained for each hippocampal subregion, resulting in the inclusion of a total of 1692 (423 × 4) features for further analyses (**Figure 1**).

Statistical Analysis

Radiomic features were adjusted using the linear regression method to control the age and gender effects and were then statistically tested to determine the number of indices with significant differences among groups and to perform the further correlation analysis.

One-way ANOVA was employed to evaluate the differences between the AD, aMCI and NC groups at each subregion. Hence, 423 × 4-fold comparisons were performed, and we then used the Bonferroni correction to control type 1 error with $P < 0.01/(N = 423 \times 4)$. A *post hoc* analysis was performed to verify the differences between any two groups.

To assess the association between radiomic texture and cognitive ability, Spearman's correlation coefficient was calculated to evaluate the relationships between the identified features and MMSE ($P < 0.01$). In addition, a detailed map was provided of the correlations between these features and the AVLT scores in immediate recall, delayed recall, recognition of primary and new words ($P < 0.05$). To evaluate the replication of the results related to radiomic features that showed significant differences and their correlations with MMSE, another dataset was further analyzed using the same statistical methods.

TABLE 1 | Demographic, clinical and neuropsychological discovery data for AD, aMCI, and NC subjects.

	NC (*n* = 45)	aMCI (*n* = 33)	AD (*n* = 38)	*P*
Age (years)	68.2 ± 6.9	70.6 ± 8.2	71.7 ± 8.3	0.102
Gender (M/F)	22/23	14/19	16/22	0.680
MMSE score	28.6 ± 1.4	26.6 ± 2.6[a]	17.6 ± 5.6[a,b]	<0.001
AVLT-Immediate Recall[c,e]	5.6 ± 1.2	4.2 ± 1.4[a]	3.0 ± 1.3[a,b]	<0.001
AVLT-Delayed Recall[d,e]	5.6 ± 1.9	2.5 ± 2.3[a]	0.6 ± 1.1[a,b]	<0.001
AVLT-Recognition (primary words)[e]	9.4 ± 1.1	8.4 ± 1.5[a]	6.2 ± 3.5[a,b]	<0.001
AVLT-Recognition (new words)[e]	9.8 ± 0.7	8.7 ± 2.1[a]	6.8 ± 3.2[a,b]	<0.001

A Chi-square test was used for gender comparisons, and ANOVA with Bonferroni's post hoc test was used for age and neuropsychological test comparisons. [a]Significant compared with NC.[b]Significant compared with aMCI. [c]The mean of three scores for every immediate recall. [d]The scores for delayed recall after five minutes. [e]Thirteen AD subjects, 2 aMCI subjects and 3 NC subjects could not or refused to complete this test. MMSE, Mini-Mental State Examination; AVLT, auditory verbal learning test.

Classification Analysis

To assess the multivariate performance of radiomic features, a classification model was established based on the SVM. A nonlinear SVM with a radial basis function (RBF) kernel was employed in LIBSVM.[4] The performance of the classifier was evaluated by the leave-one-out cross-validation (LOOCV) method, which has been widely used as a reliable estimating approach of true generalization performance. In the present study, the following univariate feature-ranking approach based on *t*-test was performed, as described in previous studies (Kamkar et al., 2015; Beheshti et al., 2016):

$$T = \frac{\mu_{c1} - \mu_{c2}}{\sqrt{\frac{\sigma_{c1}^2}{\eta_{c1}}} + \sqrt{\frac{\sigma_{c2}^2}{\eta_{c2}}}}$$

where μ_{ck} $\sigma^2{}_{ck}$ and n_{ck} (k = 1,2) are the mean, variance, and number of samples, respectively, in the two classes (c1 and c2: AD and NC). Each feature was normalized before the rank analysis was performed with the following method:

$$X = \frac{X_i - X_{\min}}{X_{\max} - X_{\min}} (i = 1.....N)$$

These features were ranked by the *t*-test, with a high value indicating large discrimination performance in the training dataset. To avoid over-filtering, only up to 200 features were selected for analysis.

In this model, there were two LOOCV procedures for grid search (Beheshti et al., 2016). In the inner and outer loop, there were both training data and testing data. First, the feature rankings with *t*-tests were employed in the inner loop's training data; the parameters c (regularization) and g (control the kernel width) obtained by LOOCV and grid search were also used. Then, the feature rankings with *t*-tests were further employed in the outer loop's training data. Lastly, the parameters c and g obtained from the inner loop's training data were used in the testing data to access the classification performance.

Input: radiomic features
for i ← 1 to *N* (the number of data)
do: testing data = data (i)
training data = data (*N*| i)
Ranking features with *t*-test among training data
for j ← 1 to *N*-1
do: LOOCV with different c and g.
Compute the accuracy for each c and g.
Choose the best c and g.
Compute the accuracy for classification.
Output: the best classification results

The classification performance was evaluated by means of accuracy (ACC), sensitivity (SEN), and specificity (SPE). The diagnostic capabilities of the radiomic features were evaluated using receiver operating characteristic (ROC) curves with the corresponding area under the ROC curve (AUC). Finally, to

[4]http://www.csie.ntu.edu.tw/~cjlin/libsvm/

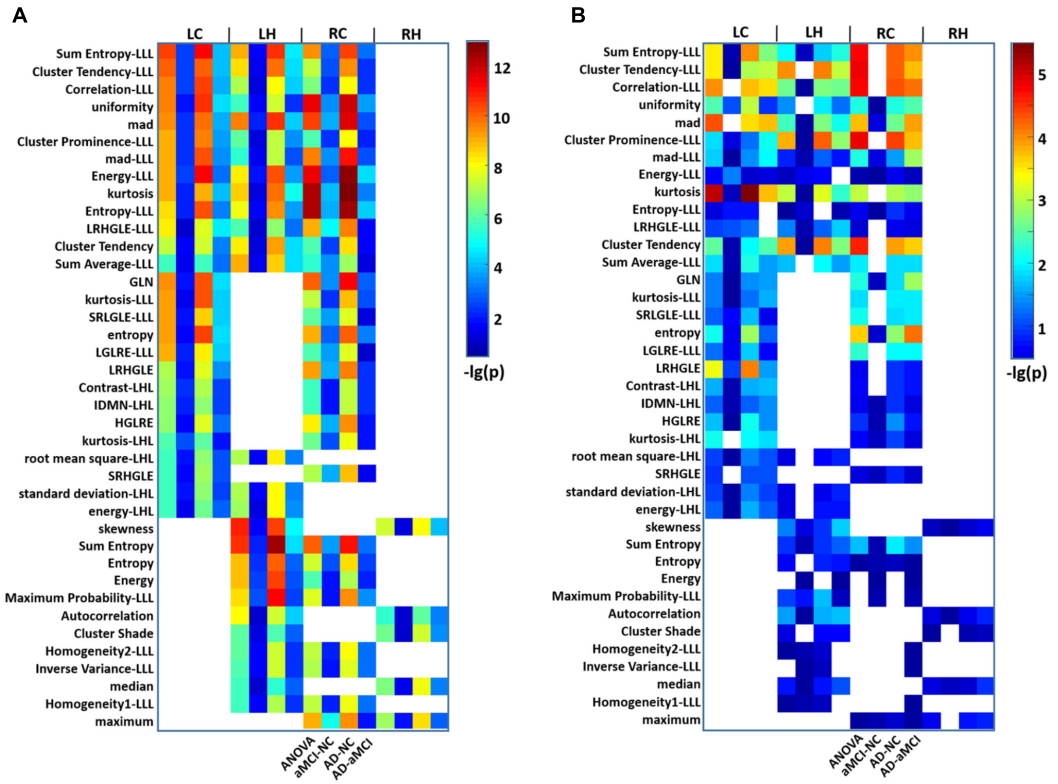

FIGURE 2 | Heat maps of radiomic features that were significantly different among groups in more than one hippocampal subregion in AD and aMCI subjects. **(A)** A total of 39 radiomic features showed significant differences among groups in more than one hippocampal subregion. Thirteen features were identified in the LC, LH, and RC subregions at the same time. For each feature observed in four subregions, there were four grids (*P*-values for ANOVA and *P*-values for the *t*-tests between aMCI and NC, AD and NC, and AD and aMCI). The color bar represents the –lg(*P*) values, a blank grid indicates there was no significant alteration in the related radiomic feature between AD and aMCI or AD and NC subjects ($P < 0.01$, Bonferroni corrected). **(B)** The replication results of another dataset were analyzed using the same procedure. LC, Left caudal; LH, Left head; RC, Right caudal; RH, Right head; mad, mean absolute deviation; LRHGLE, Long run, high gray level emphasis; GLN, Gray level non-uniformity; SRLGLE, Short run, low gray level emphasis; LGLRE, Low gray level run emphasis; IDMN, Inverse difference moment normalized; HGLRE, High gray level run emphasis; SRHGLE, Short run, high gray level emphasis.

assess the clinical relevance of this radiomic-based classification, we investigated correlations between the classifier output and cognitive ability scores (MMSE) in individual subjects.

Data Sharing

The patients' hippocampus nii image files, the texture features and the codes are available online at https://github.com/yongliulab.

RESULTS

Demographic Characteristics and Neuropsychological Assessment of Groups

In the present study, 116 subjects (38 AD patients, 33 aMCI patients and 45 NCs) were included in the discovery data. Mean age and gender ratio had no significant differences ($P > 0.05$). The MMSE score was remarkably and significantly different ($P < 0.001$), with AD patients having the lowest scores and subjects in the NC group having the highest scores. For the scores on the AVLT in immediate recall, delayed recall, and recognition of primary words and new words, the same sequence was observed among AD, aMCI and NC subjects ($P < 0.001$) (**Table 1**). The replicated dataset, which included 42 AD patients, 37 aMCI patients and 43 age- and gender-matched NCs, was used for a replication analysis of the identified feature changes (**Supplementary Table S1**).

Radiomic Features in AD, aMCI and NC Subjects

As a whole, 111 features showed significant differences ($P < 0.01$, Bonferroni corrected with $N = 423 \times 4$) among the three groups. Of these, 39 parameters were altered in more than one subregion (**Figure 2A**). The *post hoc* analysis further demonstrated that differences were more significant when comparing the AD and NC groups than that when comparing the AD and aMCI groups or the aMCI and NC groups (**Figure 2A**). Considering the category of these features, 8 of the 39 parameters belonged to the intensity features, 13 were textural features of the GLCM, 6 were from the GLRLM (**Table 2**). As shown in **Figure 2A**,

TABLE 2 | Summary of radiomic features with significant differences in hippocampal subregions.

Type of features	Detailed features
Intensity features (8/14)	uniformity, mad, kurtosis, entropy, root mean square, standard deviation, energy, skewness
Textural features of GLCM (13/22)	Sum Entropy, Cluster Tendency, Correlation, Cluster Prominence, Energy, Entropy, Sum Average, Contrast, IDMN, Maximum Probability, Autocorrelation, Cluster Shade, Homogeneity
Textural features of GLRLM (6/11)	GLN, LRHGLE, SRLGLE, LGLRE, HGLRE, SRHGLE

GLCM, Gray-Level Co-Occurrence Matrix; GLRLM, Gray-Level Run-Length Matrix; mad, mean absolute deviation; IDMN, Inverse difference moment normalized; GLN, Gray level non-uniformity; LRHGLE, long run, high gray level emphasis; SRLGLE, short run, low gray level emphasis; LGLRE, low gray level run emphasis; HGLRE, high gray level run emphasis; SRHGLE, short run, high gray level emphasis.

most of the 39 parameters were found in the left caudal, left head and right caudal regions of the hippocampus, whereas a small number of parameters were found in the right head region of the hippocampus. The features that indicated the most significant alterations among groups included kurtosis ($-\lg(P) = 12.97$), Energy-LLL ($-\lg(P) = 12.60$) and Entropy-LLL ($-\lg(P) = 12.54$) in the right caudal part and Sum Entropy ($-\lg(P) = 12.57$) in the left head part (**Figure 2A**). The same 39 parameters were evaluated in the replication dataset, in which they showed similar but weaker alterations (**Figure 2B**). Note that the most significant changes were identified in the caudal part of right hippocampus in the AD group in both datasets (**Figure 2**).

Correlations Between Radiomic Features and MMSE Scores

A total of 98 among the 111 identified features were significantly correlated with MMSE scores in AD and aMCI subjects ($P < 0.01$). Among these, 34 parameters in the bilateral subregions of the hippocampus were significantly correlated in two or three subregions (**Figure 3A** and **Table 3**). The other 64 features in one subregion are shown in **Supplementary Figure S2**. To construct a sketch map of these correlations, four typical features were selected for illustration in scatter diagrams (**Figure 3B**). Importantly, the same 34 parameters were significantly correlated with MMSE in more than one subregion in the replication dataset ($P < 0.05$, uncorrected) (**Supplementary Figure S3B**). For the bilateral caudal and left head subregions, approximately 50 parameters were significantly correlated with AVLT scores in more than one subregion, while 4 textural features were significantly correlated with AVLT scores in the right head region (**Supplementary Figure S4**).

Classification Performance

We introduced the SVM model to determine whether these textures were good features for classification analysis in the discovery data ($N = 116$, 38 AD patients, 33 aMCI patients and 45 NCs). To avoid over-filtering, up to 200 top features based on *t*-test rankings were selected for classification analysis. After training steps, we obtained the maximum classification accuracy with 163 features. **Table 4** presents the classifier performance results obtained using LOOCV for SVM classifiers in terms of ACC, SEN, SPE, and AUC. The accuracy of the feature vectors with 163 features for SVM classifiers was 86.75% (SPE = 88.89%, SEN = 84.21%, and AUC = 0.93) for distinguishing AD from NC. This rate was reduced by approximately 10% for all performance indicators (ACC, SEN, SPE, and AUC) when using the same features to distinguish aMCI from NC or AD from aMCI (**Figure 4**). In addition, we found that the distance from the hyperplane was highly correlated with MMSE scores in the AD plus NC ($r = 0.70$, $P < 0.001$) and AD ($r = 0.38$, $P = 0.020$) groups designated by the classification analysis (**Figure 4B**).

DISCUSSION

In the present study, we are the first to identify significantly different radiomic features in hippocampal subregions among AD and aMCI patients and NCs. These features demonstrated were significantly associated with cognitive ability in AD and/or aMCI subjects. More importantly, an SVM analysis demonstrated that these hippocampal textures are potential markers of AD.

In recent years, MRI-based biomarkers of AD that target gray matter atrophy or shape were found to be the most commonly used measures (Risacher et al., 2009; Ezzati et al., 2016; Caldwell et al., 2017). Reduced hippocampal volume has been well studied in AD and MCI individuals (for a review, see Shi et al., 2009). In the revised National Institute on Aging-Alzheimer's Association diagnostic criteria, hippocampal atrophy was one of the core markers for AD (Albert et al., 2011; Catani et al., 2013). Except for the reduced volume, abnormal metabolism levels, disrupted brain activity and microstructural properties within the hippocampus have been well reported (for a review, see Huijbers et al., 2015). The current results show that radiomic features are different in more than one subregion of the hippocampus, especially in the caudal parts. Among these identified to show altered texture features, three indices (kurtosis, Energy-LLL and Entropy-LLL) from the right caudal region exhibited the most significant alterations. In probability theory and statistics, kurtosis is a measure of flatness in probability distributions in brain images.[5] Entropy is a statistical measure of randomness that can be used to characterize the texture of an image. Significant differences in the above features indicate that cognitive impairments in AD and aMCI might result in complicated and changed distributions of voxel values within the hippocampus. This inference was supported by the significant correlation found between texture features and MMSE scores in the AD/aMCI groups and has also been confirmed by previous related studies (de Oliveira et al., 2011; Sørensen et al., 2016). Several investigators have reported that the shapes of brain structures can provide an additional dimension (structural morphology) when quantifying

[5]https://en.wikipedia.org/wiki/Kurtosis

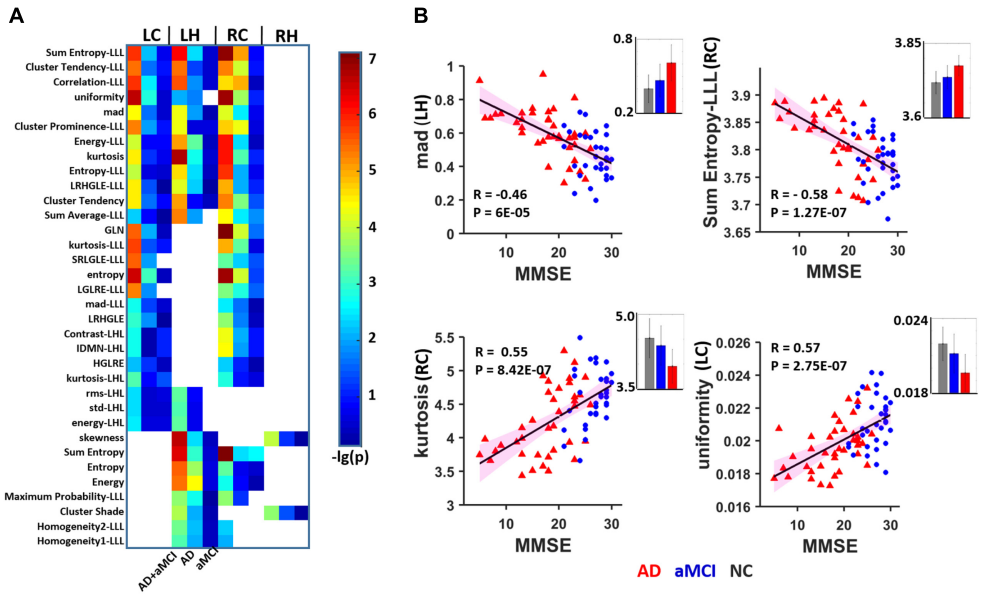

FIGURE 3 | Heat map of radiomic features correlating with MMSE in more than one hippocampal subregion. **(A)** A total of 16 features were significantly correlated with the MMSE in two or three subregions ($P < 0.01$, Bonferroni corrected). The color bar represents the –lg(P) values, a blank grid indicates there was no significant correlation between the related radiomic features and MMSE scores between AD and aMCI. **(B)** Four typical features were selected for illustration in scatter diagrams. The corresponding mean values are provided (gray: NC, blue: aMCI; red: AD). LC, Left caudal; LH, Left head; RC, Right caudal; mad, mean absolute deviation; LRHGLE, Long run, high gray level emphasis; GLN, Gray level non-uniformity; SRLGLE, Short run, low gray level emphasis; LGLRE, Low gray level run emphasis; IDMN, Inverse difference moment normalized; HGLRE, High gray level run emphasis.

TABLE 3 | Summary of radiomic features correlated with MMSE scores in hippocampal subregions.

Type of features	Detailed features
Intensity features (8/14)	uniformity, mad, kurtosis, entropy, energy, root mean square, standard deviation, skewness
Textural features of GLCM (13/22)	Sum Entropy, Cluster Tendency, Correlation, Cluster Prominence, Entropy, Energy, IDMN, Sum Average, Contrast, Maximum Probability, Cluster Shade, Homogeneity2, Homogeneity1
Textural features of GLRLM (5/11)	LRHGLE, GLN, SRLGLE, LGLRE, HGLRE

GLCM, Gray-Level Co-Occurrence Matrix; GLRLM, Gray-Level Run-Length Matrix; mad, mean absolute deviation; IDMN, inverse difference moment normalized; LRHGLE, Long run, high gray level emphasis; GLN, Gray level non-uniformity; SRLGLE, short run, low gray level emphasis; LGLRE, low gray level run emphasis; HGLRE, high gray level run emphasis.

TABLE 4 | Classification performance by the classification features (feature number = 163) between two groups.

	AD-NC	aMCI-NC	AD-aMCI
ACC	86.75%	70.51%	59.15%
SPE	88.89%	80.00%	63.16%
SEN	84.21%	57.58%	54.55%

*The detailed receiver operating characteristic curve (ROC) plot can be found in **Figure 4**. ACC, accuracy; SPE, specificity; SEN, sensitivity.*

alterations in cognitive ability in AD/MCI patients (Qiu et al., 2008; Tang et al., 2015, 2016). However, hippocampal shape abnormalities (Apostolova et al., 2006; Christensen et al., 2015) and texture features (Li et al., 2010; Zhang J. et al., 2012) have been reported to be associated with individual memory performance. Hence, the present study provides additional clues regarding the morphological alterations that occur in the hippocampus in AD.

As shown in the present study, several texture measures (such as the mean absolute deviation (mad), kurtosis, uniformity, entropy and Sum Entropy-LLL) showed a significant correlation with the MMSE score in AD/aMCI. Among these measures, mad reflects dispersion, kurtosis represents flatness, and both uniformity and entropy measure randomness of the intensity value distribution. For example, a positive kurtosis indicates a more peaked histogram than a Gaussian (normal) distribution of the selected image in AD than that in NC. This phenomenon might be caused by the atrophy of gray matter, which results in a less highly peaked histogram for the voxel intensity values similar to that due to atrophy. It should also be noted that the MMSE is limited in terms of its sensitivity to high and low levels of cognitive functioning (Tombaugh and McIntyre, 1992). Moreover, the correlation results between the textures and the AVLT scores immediate recall, delayed recall and recognition of primary words and new words provide further evidence suggesting that texture is an important and beneficial supplementary index, in addition to volume

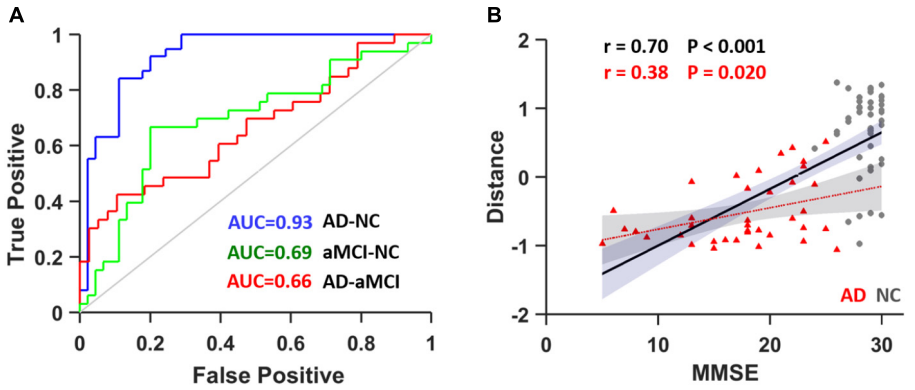

FIGURE 4 | (A) ROC curve for distinguishing between AD and NC subjects (blue), aMCI and NC subjects (green) and AD and aMCI subjects (red). **(B)** The correlation between the distance from the hyperplane and MMSE scores in AD plus NCs (black line) as well as in AD (red line). AUC, area under curve.

measurements, for understanding impaired cognitive ability in patients. Although the pathological features of AD, such as neurofibrillary tangles (NFTs) and amyloid-β (Aβ) plaques, cannot be detected on MRI, these microstructural changes might lead to altered textural patterns (Castellano et al., 2004) and might be manifested by texture analysis (Csernansky et al., 2005; Manning et al., 2015; Hwang et al., 2016). Indeed, a negative correlation between image textures and FDG-PET metabolism was identified in a recent excellent study (Shi et al., 2009). Regions with the highest atrophy rates were demonstrated to be located in the anterolateral hippocampus, which is also the region with the highest tau deposition (Franko and Joly, 2013). The anterior (head) hippocampus is thus thought to be the brain region in which the majority of volume differences are found in aMCI and AD patients.

Despite the identification of significantly different features and their correlation with MMSE scores, the application of these hippocampal features as a biomarker still needs to be confirmed. Using simple and common models of nonlinear (RBF) SVM, we obtained an accuracy of 86.75% (SPE: 88.89%; SEN: 84.21%) for distinguishing AD from NC by LOOCV. This finding is consistent with many previous imaging studies (Mak et al., 2011; Zhang Z. et al., 2012; Guo et al., 2014) suggesting that the hippocampal textures might be potential imaging markers for AD. The performance in terms of ACC, SEN, SPE, and AUC was competitive with several state-of-the-art results reported in previous studies (Jie et al., 2015; Ritter et al., 2015; Beheshti et al., 2016; Schouten et al., 2016). In addition, by analyzing the frequencies of the features selected in each LOOCV run, we found that the most powerful feature was the "intensity" of the hippocampus. More importantly, the individualized distance to the hyperplane was a neuroanatomical signature of AD and was significantly correlated with MMSE scores, indicating that the more severe AD becomes, the more likely it is to be identified using radiomic features based on the classifier's output. This result means that the atrophy and shape of the hippocampus play a very important role in distinguishing AD patients from healthy controls, as suggested by previous studies (Hampel et al., 2008; Beheshti et al., 2016; Sørensen et al., 2016).

LIMITATIONS

There were several limitations in this study. First, the hippocampal subregions segmentation was based on the atlas, which resulted in the same shape features in different subregions and, thus, were excluded from the analysis. Because only the hippocampus was included, some other important regions, such as the parahippocampus, amygdala and ventricles, should be investigated in future work. The hippocampus is also divided into subfields, including the Cornu Ammonis (CA1–4), the dentate gyrus and the subiculum, each of which has distinctive histological characteristics and specialized functions (Bird and Burgess, 2008; Delli Pizzi et al., 2016; Zeidman and Maguire, 2016; Blanken et al., 2017). However, radiomic feature extractions are unsuitable for such small regions as the above-described parcellation. Second, the results of the present study demonstrate that radiomic features have potential use in clinical diagnosis; meanwhile, we should also admit that the LOOCV might also have potentially overestimating performance (Varoquaux, 2017; Varoquaux et al., 2017). To validate if the result was robust, we performed a leave-four-subjects-out cross-validation and simulation 1000 times, and the results showed that we could obtain an accuracy of 83.55% (SPE: 84.66%; SEN: 82.66%) for distinguishing AD from NC. Using larger multi-center datasets is a solution to future challenges in reproducibility and statistical power by taking testing data from independent centers. Furthermore, a prospective longitudinal study with a large multi-center sample size is needed to detect the earlier stages of AD (Dubois et al., 2016). Lastly, although we believe this approach will increase our understanding of the multiple levels of hippocampal alterations observed over the course of AD, the radiomic features is much less widespread and less well developed than are other imaging approaches, therefore, combining texture with other markers (for example brain volume, cortical thickness, functional connectivity, and CSF, etc.) to achieve a powerful

biomarker of sufficient quality to be considered for clinical applications is needed for future studies.

CONCLUSION

In conclusion, in the present study, we found that hippocampal radiomic features exhibit significant disease-severity-related alterations in AD. We specifically investigated hippocampal textures as an MRI-based biomarker of AD. The results showed that hippocampal textures could be used as potential MRI markers for the early detection of AD from NC, with a relatively high correction ratio (ACC = 86.75%, specificity = 88.89%, and sensitivity = 84.21%) with the LOOCV method. The results of the present study highlight the importance of hippocampal texture abnormalities in AD and support the possibility that textures may serve as a neuroimaging biomarker for the early detection of AD and aMCI.

AUTHOR CONTRIBUTIONS

FF, PW, BZ, HY, QM, ZZ, LNW, LW, and NA collected the data. YL, KZ, and YD analyzed the data and performed the measurements. FF, KZ, and YL had the major responsibility of preparing the paper. YL and XZ supervised the project.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2018.00290/full#supplementary-material

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Increased Functional Brain Network Efficiency During Audiovisual Temporal Asynchrony Integration Task in Aging

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Audiovisual integration significantly changes over the lifespan, but age-related functional connectivity in audiovisual temporal asynchrony integration tasks remains underexplored. In the present study, electroencephalograms (EEGs) of 27 young adults (22–25 years) and 25 old adults (61–76 years) were recorded during an audiovisual temporal asynchrony integration task with seven conditions [auditory (A), visual (V), AV, A50V, A100V, V50A and V100A]. We calculated the phase lag index (PLI)-weighted connectivity networks modulated by the audiovisual tasks and found that the PLI connections showed obvious dynamic changes after stimulus onset. In the theta (4–7 Hz) and alpha (8–13 Hz) bands, the AV and V50A conditions induced stronger functional connections and higher global and local efficiencies, reflecting a stronger audiovisual integration effect, which was attributed to the auditory information arriving at the primary auditory cortex earlier than the visual information reaching the primary visual cortex. Importantly, the functional connectivity and network efficiencies of old adults revealed higher global and local efficiencies and higher degree in both the theta and alpha bands. These larger network efficiencies indicated that old adults might experience more difficulties in attention and cognitive control during the audiovisual integration task with temporal asynchrony than young adults. There were significant associations between network efficiencies and peak time of integration only in young adults. We propose that an audiovisual task with multiple conditions might arouse the appropriate attention in young adults but would lead to a ceiling effect in old adults. Our findings provide new insights into the network topography of old adults during audiovisual integration and highlight higher functional connectivity and network efficiencies due to greater cognitive demand.

Keywords: functional connectivity, EEG, audiovisual integration, aging, theta and alpha bands

INTRODUCTION

Humans use various modalities of sensory information (e.g., visual information, auditory information, olfactory information, and tactile information) in everyday life to perceive the outside world. Stimuli from multiple sensory channels are integrated into common perceptual states, which is a process known as multisensory integration (Ernst and Bülthoff, 2004; Senkowski et al., 2008). Among multisensory integration modalities, the most typical is audiovisual integration in humans and animals, which has been represented in several previous studies (Molholm et al., 2002, 2006; Roach et al., 2006; Doehrmann and Naumer, 2008). Recently, our event-related potential (ERP) studies have indicated that audiovisual integration is regulated by temporal factors (Yang et al., 2014; Ren et al., 2017b). The temporal structure of auditory and visual stimuli can intervene in human audiovisual processing. One example from among life experiences is that we generally see lightning and then hear the thunderclap, although the lightning and thunderclap occur simultaneously. This phenomenon indicates that the integration of multiple forms of sensory information obeys the temporal principle (Stein, 2012; Ren et al., 2017a). When auditory and visual signals occur at the same time and location, they tend to be integrated, and temporal proximity is always a necessary factor for the occurrence of audiovisual integration (Van der Burg et al., 2008; Ren et al., 2017b). To allow precise perception of the environment, a temporal window appears in the human brain. When auditory and visual signals fall within this temporal window, the brain will integrate the stimuli. A previous study showed that when the time between the occurrence of auditory and visual stimuli is less than 100 ms, there will be a strong multisensory integration effect (Ren et al., 2017b).

Growing evidence has demonstrated that audiovisual integration can be influenced by development, aging, attention, training and listening experience (Lippert et al., 2007; McNorgan and Booth, 2015; Paraskevopoulos et al., 2015; Yu et al., 2017). For example, Koelewijn et al. (2010) focused on the question of whether multisensory integration is an automatic process, suggesting that multisensory integration is accompanied by attentional processes and that the two can interact in multiple areas of the brain. Moreover, multisensory integration is related to attention and can be modulated by attention; bottom-up mechanisms induced by cross-modal interactions can automatically capture attention to multisensory events (Koelewijn et al., 2010; Talsma et al., 2010). Old adults have been demonstrated to have abnormalities in audiovisual integration (Laurienti et al., 2006; Peiffer et al., 2007). For example, the presentation of audiovisual stimuli accelerated response times in both old and young adults, with a significantly greater gain in old adults (Laurienti et al., 2006; Tye-Murray et al., 2010; Wu et al., 2012). More recently, abnormal audiovisual integration in old adults was also represented by the delayed integration time window and peak time measured by race model analysis, which also reflected a greater effect of audiovisual integration in old adults (Wu et al., 2012; Ren et al., 2017b). It is well known that old adults experience deficits in visual and auditory abilities, such as a decrease in visual acuity (Bäckman et al., 2006; Liu and Yan, 2007) and an increase in auditory threshold (hearing loss). The greater effect of audiovisual integration in old adults has been explained as an effective compensatory strategy to overcome visual and auditory deficits (Peiffer et al., 2007).

Many studies have shown that neural oscillatory responses in the theta, alpha, beta and gamma bands are involved in sensory processing (Yordanova et al., 1998; Sakowitz et al., 2005; Donner and Siegel, 2011). Previous studies have demonstrated that these frequency bands play a role in multisensory attention tasks with different stages and locations (Sakowitz et al., 2005; Friese et al., 2016). Particularly, both theta and alpha bands are organized in fronto-centro-parietal sites (Sakowitz et al., 2005). We found that multisensory attention led to decreased lower-frequency theta and alpha activity in early sensory cortex areas and to increased low-frequency phase synchronization in the frontal cortex (Friese et al., 2016). The theta band appears to be predominantly implicated in cognitive control and short-term memory in audiovisual integration (Demiralp and Başar, 1992; Sakowitz et al., 2000; Keller et al., 2017), while the alpha band seems to be mainly related to the maintenance of sensory information, cognitive control and the suppression of distractions (Masahiro et al., 2010; Başar, 2012). Recently, van Driel et al. (2014) determined the functional role of the coupling of alpha phase dynamics between sensory cortices in integrating cross-modal information over time. Furthermore, previous studies have reported that healthy aging people show lower spontaneous electroencephalogram (EEG) amplitude in the theta and alpha bands than young people (Cummins and Finnigan, 2007; Gaál et al., 2010). Cummins and Finnigan (2007) found a significant decrease in old adults' mean theta power of resting EEG. Spontaneous EEG measurements from normal aging have demonstrated significantly reduced alpha power at each electrode (Yordanova et al., 1998; Kolev et al., 2002). We speculate that there are significant age differences in audiovisual integration in the low-frequency (theta and alpha) bands.

Audiovisual integration is involved in multiple brain regions, including the occipital, parietal, temporal, and frontal regions (Shams et al., 2005). Previous functional magnetic resonance imaging (fMRI) and EEG studies have yielded evidence of audiovisual integration effect occurrence in regions traditionally considered sensory specific (e.g., primary visual cortex) (Calvert et al., 2000; Macaluso, 2006; Chan et al., 2017; Ren et al., 2017a). Moreover, direct anatomical connections between the occipital regions (visual processing) and superior temporal regions (auditory processing) have confirmed that these regions may play key roles in audiovisual integration (Falchier et al., 2002). These brain regions synergistically complete audiovisual integration. A central question in audiovisual integration is how perceptive functions depend on the integration and coordination of widely distributed brain regions. To answer this question, a key concept is functional connectivity, which involves temporal correlations or synchronization in physiological signals recorded from different brain regions (Lee et al., 2003; Fingelkurts et al., 2005; Stam et al., 2009; Bhattacharya et al., 2018; Wang et al., 2018). Recent studies have shown that the functional connectivity of the brain network is organized in a highly efficient manner, which implies a high level of local efficiency combined with

global efficiency of information transfer (Stam, 2004; Bullmore and Sporns, 2009; Heuvel et al., 2009). Numerous studies have applied graph theoretical analysis to evaluate functional connectivity in EEG and MEG data (Smit et al., 2008; Stam et al., 2008; Vecchio et al., 2014). Our previous research first combined the phase synchrony of neuronal oscillations and graph theoretical analysis of network topography to investigate age-related audiovisual integration in EEG data and found that old adults had stronger functional connectivity and higher brain network efficiencies to execute synchronous audiovisual integration in the beta band (Wang et al., 2017). However, it is unclear how changes in functional connectivity and network efficiencies during audiovisual integration tasks with temporal asynchronous stimuli occur in aging.

To clarify the changes in functional connectivity and brain network efficiencies during audiovisual temporal asynchrony integration tasks in old adults, we designed an auditory or visual stimuli discrimination task consisting of three types of stimuli: unimodal visual (V), unimodal auditory (A) and bimodal audiovisual (AV) stimuli (in synchrony and asynchrony). Two subject groups participated in our experiment, and we recorded EEG signals from different brain regions for all tasks. The phase lag index (PLI), a synchronization measure, was used to construct the brain network in the theta, alpha, beta, and gamma frequency bands. We further used graph theoretical analysis to investigate functional connectivity and network efficiency during these audiovisual conditions.

MATERIALS AND METHODS

Participants

Twenty-five old individuals [61–76 years, mean age ± standard deviation (SD), 68.8 ± 0.90] and 27 young individuals (21–25 years, mean age ± SD, 23.1 ± 0.19) participated in this study. All subjects, who were naive to the purpose of the experiment, had normal hearing and normal or corrected-to-normal vision capabilities. All subjects had no history of cognitive impairment and had normal Mini-Mental State Examination (MMSE) scores (>24) adjusted for age and education (Bravo and Hébert, 1997). Moreover, all subjects provided written informed consent for this experiment, which was approved by the ethics committee of Okayama University.

Stimuli and Task

The visual non-target stimulus was a black and white checkerboard image, and the visual target stimulus was a black and white checkerboard image containing two black dots (52 mm × 52 mm, with a visual angle of 5°). The auditory non-target stimulus was a 1000 Hz sinusoidal tone, and the auditory target stimulus was white noise. The visual stimuli (V) were randomly presented to the lower left or lower right quadrant of the black screen on a 21-inch computer monitor located 60 cm in front of the participant's eyes for 150 ms (**Figure 1A**). The auditory stimuli (A) were randomly presented to the left or right ear through earphones at 60 dB SPL for 150 ms. The audiovisual stimuli (AV) were presented in the following conditions, which were a combination of the visual and auditory stimuli with different periods of stimulus onset asynchrony (SOA) of 0 ms, ± 50 ms, or ± 100 ms: simultaneous AV; auditory lag visual 50 ms, 100 ms (V50A, V100A); or auditory leading visual 50 ms, 100 ms (A50V, A100V; **Figure 1B**). Moreover, the non-target stimuli comprised 80% of the total stimuli, and the duration of each trial of each stimulus was between 150 and 250 ms, depending on the SOA that was chosen according to previous behavioral studies (Bolognini et al., 2005; Yang et al., 2014).

All participants were instructed to perform an auditory or visual stimuli discrimination task consisting of visual (V), auditory (A), and audiovisual (AV) stimuli (in synchrony and asynchrony) in this experiment. Eight sessions were conducted for each participant, and each session started with a 3000 ms fixation period and then randomly presented 25 visual stimuli, 25 auditory stimuli and 125 audiovisual stimuli (AV, A50V, A100V, V50A, and V100A). In total, there were 80 non-target stimuli and 20 target stimuli for each condition on the left or right side. All the stimuli were presented with an ISI that varied from 1300 to 1800 ms (**Figure 1C**). The participants were instructed to determine as quickly and accurately as possible whether the targets appeared on the left or right side.

EEG Data Collection

The EEG was recorded from 32 scalp electrodes mounted within an electrode cap (Easy cap, Germany), which were placed according to the International 10–20 electrode placement standards, and 2 electrooculogram electrodes that were referenced to the earlobes. The EEG signals were amplified by BrainAmp amplifiers (Brain Products, Munich, Germany) using a 0.05 to 100 Hz bandpass and were digitized at a sampling rate of 500 Hz.

Behavioral Performance and Race Model Analysis

Behavioral performance analyses were performed on the trials with target stimuli. The hit rate (HR) was the correct responses to target stimuli divided by the total number of target stimuli, and the false alarm (FA) rate was the responses to non-target stimuli divided by the total number of non-target stimuli. No subject had behavioral performance with HRs below 70% and FA rates above 30%. The use of cumulative distribution functions (CDFs) to evaluate the race model ensures that multisensory enhancement is identified using the entire response time distribution rather than a single central tendency score (i.e., the mean response time) (Miller, 1982, 1986). To complete this analysis, the CDFs of each condition were performed on the response times by using 10 ms time bins. Unimodal CDFs were used to calculate the race distribution at each time bin with the following formula: [P (A) + P (V)] − [P (A) * P (V)]. Each participant's race model curve was then subtracted from their audiovisual CDFs. The peak time point of each probability difference curve was recorded and represented the time at which the audiovisual integration most likely occurred. For more details, please refer to our previous behavioral study with a similar design (Ren et al., 2017b).

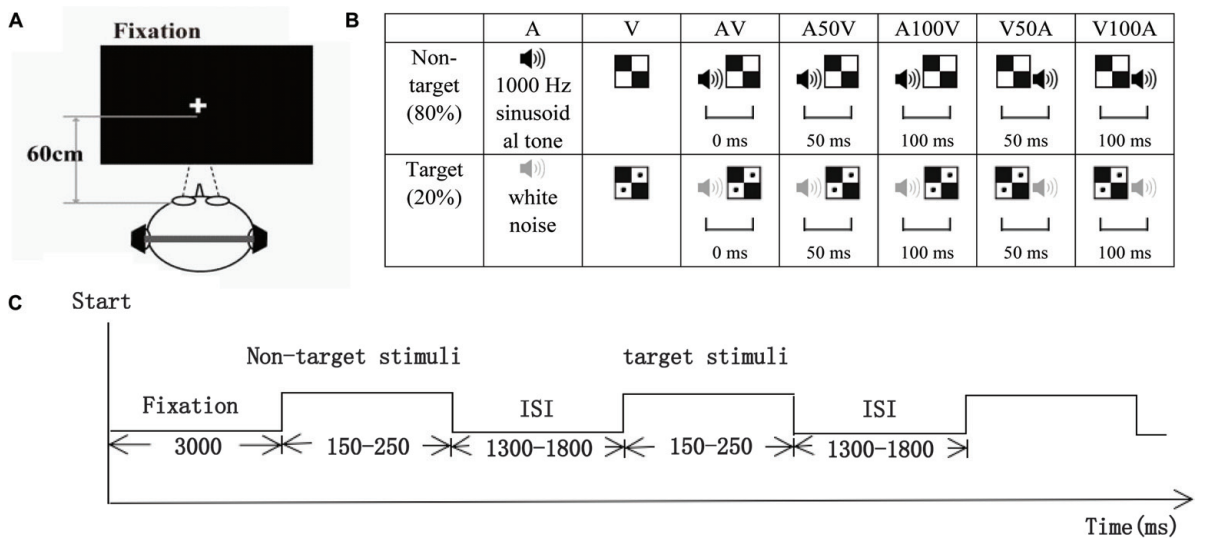

FIGURE 1 | Schematic of the experimental design. **(A)** The subject was approximately 60 cm away from the computer screen used to present visual stimuli. **(B)** The visual non-target stimulus was a black and white checkerboard image, and the visual target stimulus was a black and white checkerboard image containing two black dots. The auditory non-target stimulus was a 1000 Hz sinusoidal tone (black speaker), and the auditory target stimulus was white noise (gray speaker). The audiovisual stimuli included visual and auditory target or non-target stimuli. AV, simultaneous auditory and visual; V50A and V100A, auditory lag visual 50 ms and 100 ms, respectively; A50V and A100V, auditory leading visual 50 and 100 ms, respectively. **(C)** The timeline of one session. Each session started with a 3000 ms fixation period, and then visual, auditory, and audiovisual target or non-target stimuli with a duration of 150–250 ms were randomly presented. All stimuli were presented with an ISI that varied from 1300 to 1800 ms.

Preprocessing of EEG Data

The EEG data were preprocessed using Brain Vision Analyzer software (version 2.02, Brain Products GmbH, Munich, Bavaria, Germany) and MATLAB R2014a (MathWorks Inc., Natick, MA, United States) with the following open-source toolboxes: EEGLAB[1] (Swartz Center for Computational Neuroscience, La Jolla, CA, United States). Brain Vision Analyzer software was used to automatically reject trials with electrocardiographic activity or eye and muscle artifacts from the data for all electrodes. The EEG data were divided into epochs with 700 time points, which were from 500 ms before the stimulus onset to 900 ms after the stimulus onset, followed by baseline correction for each epoch. In the present study, we analyzed only non-target stimuli to avoid effects of motor responses. Some studies have reported the same results for responses to both non-target and target stimuli (Missonnier et al., 2007; Kukleta et al., 2009). The epochs of non-target stimuli were filtered into theta (4–7 Hz), alpha (8–13 Hz), beta (14–30 Hz), and gamma (31–50 Hz) frequency ranges. The network synchronization of these four bands was investigated, as these rhythms are considered particularly relevant for interregional communication (Lewis, 2005; Sakowitz et al., 2005; Palva and Palva, 2007; Donner and Siegel, 2011).

Interregional Phase Synchronization

Interregional phase synchronization was evaluated by calculating the PLI and was analyzed by an in-house program in MATLAB. First, the Hilbert transform was employed to obtain time series of instantaneous phase measures for each trial epoch and each electrode. Then, the locked-phase synchrony was indexed by PLI, which measures the asymmetry of the phase difference distribution between two electrodes at a given time point across trials.

$$\mathrm{PLI} = \left|sign\left(\Delta\phi\left(t_{\mathrm{n}}\right)\right)\right| = \left|\frac{1}{N}\sum_{\mathrm{n}=1}^{\mathrm{N}} sign\left(\Delta\phi\left(t_{\mathrm{n}}\right)\right)\right| \qquad (1)$$

The PLI analysis produces an electrode-by-electrode adjacency matrix (30 × 30) across trials (700 time points). We did not display the first and last 300 ms (150 sample points) in the synchrony analyses of epochs because of distortions involved in calculating the Hilbert transform at the edges of the analyzed epochs (Doesburg et al., 2008; Leung et al., 2014; Wang et al., 2017; Yan et al., 2017). Thus, we reported only the adjacency matrix for reduced epochs (400 time points) from 200 ms before to 600 ms after stimulus onset. The PLI analyses were performed on each frequency band, each stimulus condition, and each subject.

Functional Connectivity

The adjacency matrices at each time point were then averaged for each trial condition. The average network connectivity time series was obtained to investigate functional connectivity dynamics. Permutation tests with 10,000 repeats were employed at each time point to compare the differences in the mean PLI values between the two groups. A time point with significant group differences (permutation test, $p < 0.05$, corrected)

[1]http://sccn.ucsd.edu/eeglab/

reflected a difference in the strength of functional connectivity when processing the stimuli. To easily compare differences in group and condition, the time windows with significant group differences were determined across all conditions. To avoid double dipping, we determined the time windows of each subject by the leave-one-out method and obtained individual time windows. The individual time windows were approximately 0–370 ms after the stimulus for the theta band and approximately 40–220 ms for the alpha band. The averaged adjacency matrices within the time windows represented the functional connectivity for each subject and each condition and were further used in analyses of functional connectivity and network topology.

After the functional connectivity was obtained, the mean weight (PLI) of all connectivities in the network was used to characterize the global network strength. The network strength was measured for each condition and subject. To further identify specific functional connectivities that were different between young and old adults, the connectivity matrices were entered as repeated-measures dependent variables into the network-based statistic (NBS) toolbox. The initial univariate *t*-test for between-group comparisons of interregional connectivity was adapted for the data distributions being analyzed to $T = 3$ ($p < 0.01$) in the 30 × 30 adjacency matrix (Zalesky et al., 2012; Leung et al., 2014; Wang et al., 2017). A surrogate statistical approach with 5,000 repeats was used to determine the statistical significance of connectivity components reflecting group differences in network synchronization.

Analysis of Network Topologies

To characterize the network topologies, we constructed a weighted network from connectivity matrices (30 × 30) for each condition, frequency band, and subject. To compare group differences in topological metrics between brain functional networks regardless of the selection of specific thresholds, we calculated the g topological metrics of the networks with a range of sparsity thresholds from the top 5–40% of individual subject connections. The network metrics, including global efficiency (Eg), local efficiency (Eloc) and degree, were calculated by GRETNA (Wang et al., 2015). Global efficiency (Eg) measures the capacity to integrate information across the network. This metric is the inverse of the average shortest path length from one node to all other nodes (Sporns, 2011; Barbey, 2017; Wig, 2017). Local efficiency (Eloc) measures the local information processing capabilities of the network and reflects the fault tolerance of the network. This metric is the inverse of average shortest path length of the neighbors of a node (Sporns, 2011; Wig, 2017). The degree measures the connectivity of each node (Burdette et al., 2010). Finally, we characterized the integrated metric for each network metric by calculating the area under the curve (AUC) for sparsity thresholds from 5 to 40% in order to provide a scalar that did not depend on the specific threshold selection.

Statistical Analysis

All statistical analyses were performed using SPSS, version 20.0 (SPSS, Inc., Chicago, IL, United States). For each frequency, a repeated-measures ANOVA was carried out separately for the behavioral performance and network metric. To examine the effects of age and conditions as well as their interaction, a 2 groups (old, young) × 7 conditions (A, V, AV, A50V, A100V, V50A, V100A) repeated-measures ANOVA was performed. For any difference with $p < 0.05$ (Mauchly's sphericity test), *post hoc* tests were performed by a permutation test (10,000 repeats) for group differences, and a paired-permutation test (10,000 repeats) was performed for condition differences. These multiple comparisons were corrected by the Bonferroni method at the condition level. To examine the relationship between topological properties and integration performance, a mixed-effects model was used to test the correlations between each network metric and peak time. The mixed-effects model reflected only the global relationship across all conditions. To evaluate correlations in each condition in greater detail, Spearman correlations were also used to measure the relationship between topological properties and integration performance.

RESULTS

Behavioral Performance

The average HRs of old adults and young adults were 93.7 and 95.2%, respectively. For detailed results of the race model, please refers to our previous behavioral study with a similar design (Ren et al., 2017b). In the present study, the results of CDFs of V, A, AV and the race model of all subjects are shown in **Figure 2A**. The distribution of CDFs revealed that the responses to the AV stimuli were faster than the response to V or A stimuli. Furthermore, to identify whether audiovisual integration occurred, we measured the probability difference by subtracting the CDFs of AV from the race model. **Figure 2B** shows the mean probability difference of all subjects. The peak time point of each probability difference curve reflects behavioral facilitation during audiovisual integration. Thus, the probability differences were calculated for each audiovisual condition and each subject. The peak time points of all audiovisual conditions are shown in **Figure 2C**. The repeated-measures ANOVA revealed that peak time had a significant main effect of group [$F(1,50) = 26.731$, $p < 0.001$]. Further *post hoc* tests showed that old adults had a longer peak time than young adults in all conditions (**Figure 2C**, paired-permutation test, $p < 0.001$, Bonferroni corrected). There were significant main effects of condition [$F(4,200) = 11.230$, $p < 0.001$] and interaction between group and condition [$F(4,200) = 4.076$, $p = 0.007$]. The pairwise comparison showed that the young adults had the shortest peak time in the V50A condition, and the old adults had the shortest peak time in AV (permutation test, $p < 0.05$, corrected).

Time Courses of Mean PLI

The PLI or network metrics of the left and right sides were averaged because there were no differences between them. The time courses of the PLI for old adults and young adults are shown in **Figure 3**. The results showed obvious dynamic changes after stimulus onset for all conditions, especially for the audiovisual conditions. We found differences in PLI between young and old

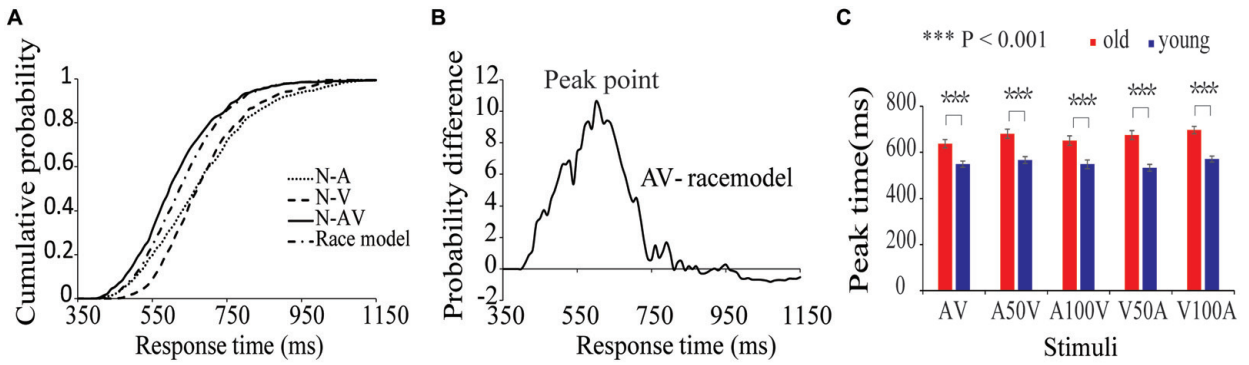

FIGURE 2 | Cumulative probability functions and race model. **(A)** Cumulative distribution functions (CDF) for response times to visual, auditory, and audiovisual stimuli in the two age groups. The summed probability of visual and auditory responses is shown by the race model curve (race model). **(B)** The curve of probability difference between the CDFs and race model. A positive difference indicates the existence of audiovisual integration in the behavioral performance. **(C)** The peak time point is the significant difference between the two groups in each audiovisual condition.

adults in the theta and alpha bands (**Figure 3**) but not in the beta or gamma bands. Significantly higher values of PLI curves were mainly found within the range of 0-370 ms for the theta band (permutation test, $p < 0.05$, corrected) and 40–220 ms for the alpha band (permutation test, $p < 0.05$, corrected).

Functional Connectivity

The network strengths were measured as the mean weight (value of PLI) of all connectivities (**Figure 4**), which were used to characterize the global weighted network during audiovisual tasks. By repeated-measures ANOVA, the significant main effects of the stimulus condition were determined in the network strengths of the theta [$F(6,300) = 39.965$, $p < 0.001$] and alpha bands [$F(6,300) = 12.346$, $p < 0.001$]. In the theta band, a pairwise comparison showed stronger connectivity strengths in the V50A and AV conditions than in all other conditions (paired-permutation test, $p < 0.01$, corrected). There were stronger connectivity strengths in multimodal stimulation conditions (AV, V50A, A100V, and V100A) than in unimodal stimulation (A or V, paired-permutation test, $p < 0.05$, corrected). For the alpha band, the V50A condition showed the strongest connectivity strengths among all conditions (paired-permutation test, $p < 0.05$, corrected). There were stronger connectivity strengths in the three types of multimodal stimulation conditions (AV, A50V, and V50A) than in the unimodal stimulation (A or V, paired-permutation test, $p < 0.05$, corrected). More details of the pairwise comparisons are shown in **Supplementary Tables S1, S2**.

In addition, the network strengths in the theta and alpha bands showed a significant main effect of group {theta: [$F(1,50) = 17.190$, $p < 0.001$], alpha: [$F(1,50) = 13.139$, $p = 0.001$]} and a significant interaction between group and condition [theta: $F(6,300) = 3.744$, $p = 0.010$, alpha: $F(6,300) = 3.838$, $p = 0.005$, **Figure 4**]. For the theta band, the *post hoc* tests showed that old adults had significantly stronger connectivity strengths than young adults in all conditions except the V condition (permutation test, $p < 0.05$, corrected, **Figure 4A**, permutation test), especially in audiovisual conditions ($p < 0.01$, corrected). In the alpha band, old adults had significantly stronger connectivity strengths than young adults in the AV, A50V, and V50A conditions (**Figure 4B**, permutation test, $p < 0.01$, corrected).

Connectivity alteration in the functional network is the foundation of a difference in network strength. Thus, we further used an NBS approach to localize the specific functional connectivity that was significantly enhanced in old adults compared with young adults (**Figure 5**). In the theta band, old adults had significantly stronger connections in all of the audiovisual conditions (AV, A50V, V50A, A100V, and V00A, **Figure 5A**, $p < 0.001$, NBS corrected). In addition, in the alpha band, old adults had significantly stronger connectivity in all of the audiovisual conditions, especially in the AV, A50V, and V50A conditions (**Figure 5B**, $p < 0.001$, NBS corrected). The significantly stronger connections were mainly located in the fronto-centro-parietal site and the superior temporal site (**Figure 5**). The specific functional connectivity was significantly altered in old adults, which was attributed to the differences in topological properties between young and old adults.

Statistical Results of Network Metrics

For the theta band, the global efficiency, local efficiency, and degree showed significant main effects of group [Eg: $F(1,50) = 20.259$, $p < 0.001$; Eloc: $F(1,50) = 23.465$, $p < 0.001$; degree: $F(1,50) = 20.688$, $p < 0.001$] and stimulus condition [Eg: $F(6,300) = 48.302$, $p < 0.001$; Eloc: $F(6,300) = 31.634$, $p < 0.001$; degree: $F(6,300) = 46.827$, $p < 0.001$]. There were significant interactions between group and condition [Eg: $F(6,300) = 6.214$, $p = 0.001$; Eloc: $F(6,300) = 5.385$, $p < 0.001$; degree: $F(6,300) = 4.116$, $p = 0.006$]. The pairwise comparison revealed that the AV and V50A conditions were significantly larger than all other conditions (paired-permutation test, $p < 0.001$, corrected). The global and local efficiencies and degree in unimodal stimulation conditions (A or V, paired-permutation test, $p < 0.001$) were smaller than those in multimodal stimulation conditions (AV, V50A, and A100V). More details of the pairwise comparison are shown in **Supplementary Table S1**.

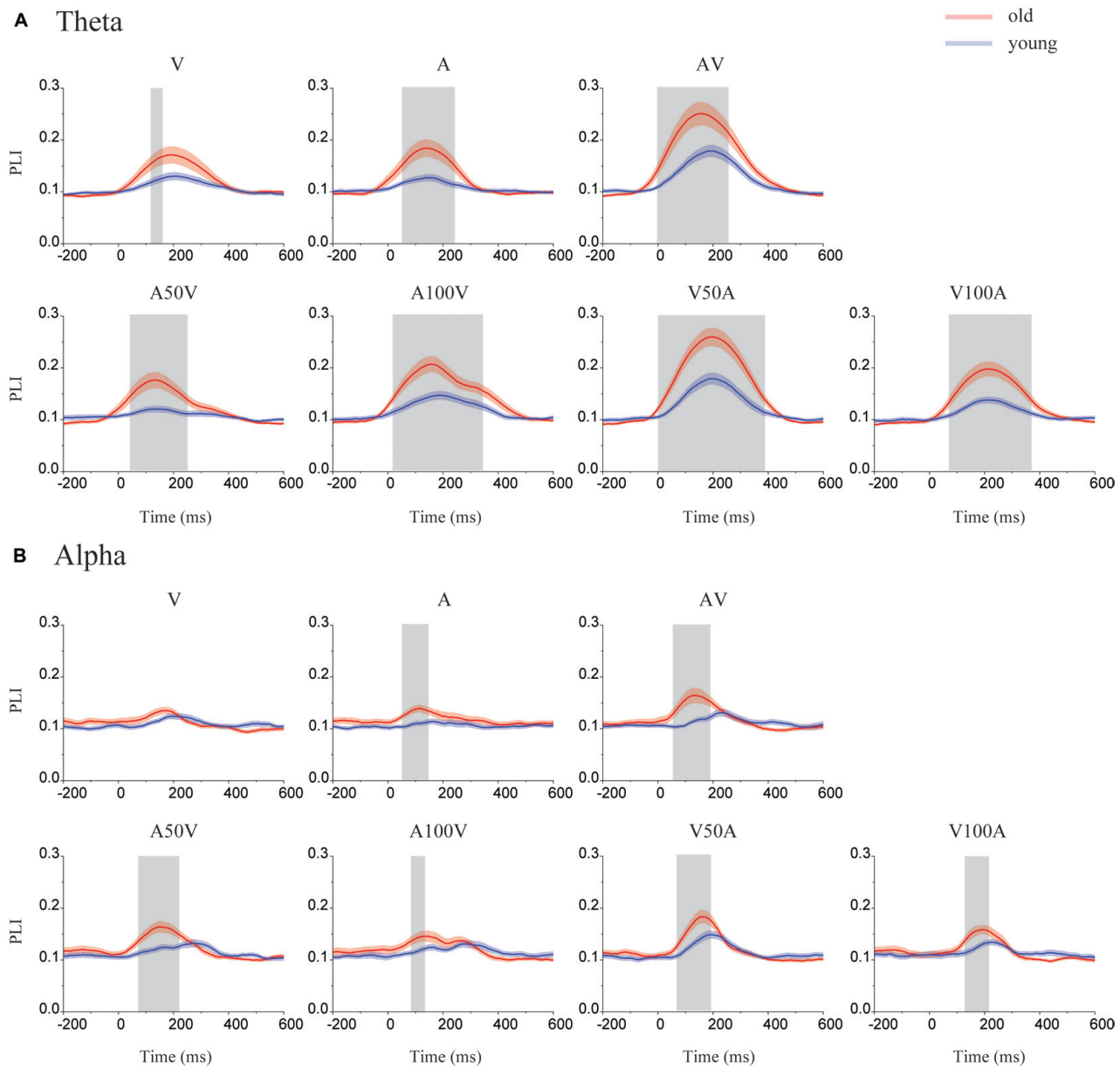

FIGURE 3 | Time courses of the mean PLI in the theta and alpha bands. **(A)** In the theta band, the time courses of the mean PLI for each group in each condition (including the A, V, AV, A50V, A100V, V50A, and V100A conditions). **(B)** In the alpha band, the time courses of the mean PLI for old (red line) and young adults (blue line) included time points of 200 ms before stimulus onset and 600 ms after stimulus onset. The shaded areas indicate the time periods with significant group differences (permutation test, $p < 0.05$, corrected).

Moreover, the *post hoc* tests showed that old adults had higher values of global efficiency, local efficiency and degree than young adults in all conditions (**Figure 6A**, permutation test, $p < 0.05$, corrected). There were more significant group differences in the multimodal conditions, especially in the AV, V50A, and V100A conditions (permutation test, $p < 0.01$, corrected), than in the unimodal conditions.

For the alpha band, the network metrics of global efficiency, local efficiency, and degree showed significant main effects of group [Eg: $F(1,50) = 11.824$, $p = 0.001$; Eloc: $F(1,50) = 12.812$, $p = 0.001$; degree: $F(1,50) = 15.345$, $p < 0.001$] and condition [Eg: $F(6,300) = 11.836$, $p < 0.001$; Eloc: $F(6,300) = 7.095$, $p < 0.001$; degree: $F(6,300) = 12.495$, $p < 0.001$]. Moreover, significant interactions between group and condition were observed only in global efficiency and degree [Eg: $F(6,300) = 3.321$, $p = 0.010$; Eloc: $F(6,300) = 0.366$, $p = 0.861$; degree: $F(6,300) = 2.872$, $p = 0.025$]. A pairwise comparison showed that the higher global and local efficiencies and degree in the V50A condition had the largest value among these conditions (paired-permutation test, $p < 0.05$, corrected). In addition, the A50V condition

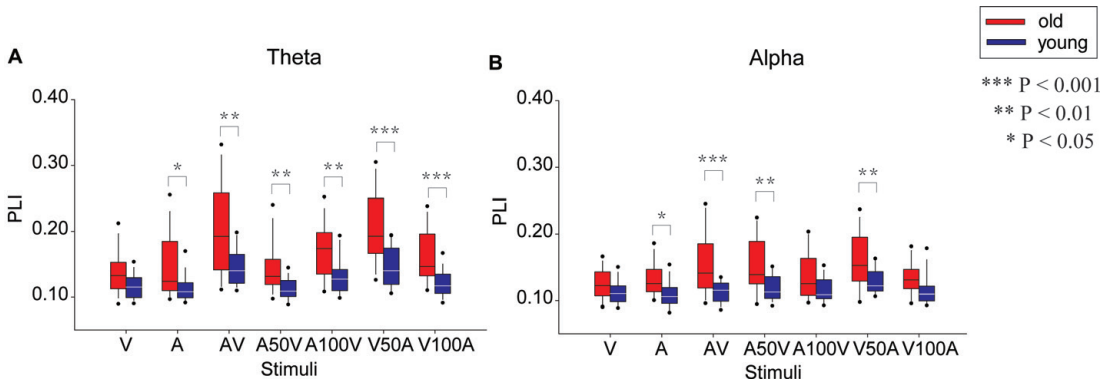

FIGURE 4 | Comparison of the mean PLI between old and young adults in the seven conditions (A, V, AV, A50V, A100V, V50A, and V100A) in the theta **(A)** and alpha **(B)** bands.

had a significantly higher global efficiency and degree than the A and V conditions (paired-permutation test, $p < 0.05$, corrected). More details of the pairwise comparison are shown in **Supplementary Table S2**. Compared with young adults, the old adults had significantly higher global efficiency in the AV, A50V, and V50A conditions and higher local efficiency and degree in all audiovisual conditions, especially in the AV, A50V, and V50A conditions (**Figure 6B**, permutation test, $p < 0.05$, corrected).

Relationship Between Network Metrics and Integration Performance

There were significant associations between network metrics and the peak time of the audiovisual conditions in young adults but not in old adults (**Figure 7A**). By mixed-effects model analysis, only young adults showed significant negative correlations between peak time and global efficiency ($F = 13.818$, $p = 0.000$), local efficiency ($F = 6.543$, $p = 0.012$) and degree ($F = 10.500$, $p = 0.002$) in the theta band (**Figure 7A**). To further determine the correlation in each condition, we performed Spearman correlations between the network metrics and peak time for each condition and each group. These network metrics in the AV, A50V, and V50A conditions showed significant correlations with peak time (Spearman correlations, $r > -0.416$, $p < 0.05$). Scatterplots for each condition with significant correlations are shown in **Supplementary Figure S1**. For the alpha band, global efficiency and degree had significant negative corrections with peak time in young adults (Eg: $F = 9.927.818$, $p = 0.002$; Eloc: $F = 2.815$, $p = 0.096$; degree: $F = 8.739$, $p = 0.004$, **Figure 7B**). The network metrics in the AV and V50A conditions were also determined to have significant correlations with peak time (Spearman correlations, $r > -0.397$, $p < 0.05$, **Supplementary Figure S2**).

DISCUSSION

The goal of the present study was to examine whether functional connectivity during an audiovisual temporal asynchrony integration task was influenced by aging. There were significant differences between the two age groups within 200 and 400 ms after stimulus onset in the alpha and theta bands, respectively. The old adults had stronger functional connectivity in the theta band for all audiovisual stimuli (AV, A50V, V50A, A100V, and V100A) and in the alpha band especially for some audiovisual stimuli (AV, A50V, and V50A). This result was confirmed by graph theoretical analysis, which showed that significantly higher global and local efficiencies and degree were found in old adults. In addition, the network efficiencies were significantly associated with the peak time of integration only in young adults. Together, our findings provide new insights into the network topography of old adults during audiovisual integration tasks with temporal asynchronous information.

Audiovisual Integration and Temporal Synchrony

Research on age-related audiovisual integration has attracted much interest in recent years, as sensory systems and cognitive functions undergo significant changes with age. Brain function is based on the current environment, and different sensory organs are responsible for filtering and screening input information, whereby limited attention is allocated to integrating useful information. In addition, it has been shown that the temporal asynchrony between sensory modalities is a key factor affecting multisensory integration (Durk et al., 2009). To precisely perceive the environment, the human brain defines a temporal window, and when a pair of audiovisual signals fall within this window, the two signals are integrated by the brain (Durk et al., 2009; Ren et al., 2017b). Furthermore, our previous behavioral and ERP studies revealed that integration occurs when the two stimuli fall within a temporal window ranging from 0 to 100 ms (Ren et al., 2017b).

It is worth noting that we found that both old and young adults had significantly higher functional connectivity and network metrics (including global and local efficiencies and degree) under the AV and V50A conditions than under other conditions. Our findings indicated that the strongest audiovisual integration effects occurred in the AV and V50A conditions, which might be due to the auditory information arriving at the primary auditory

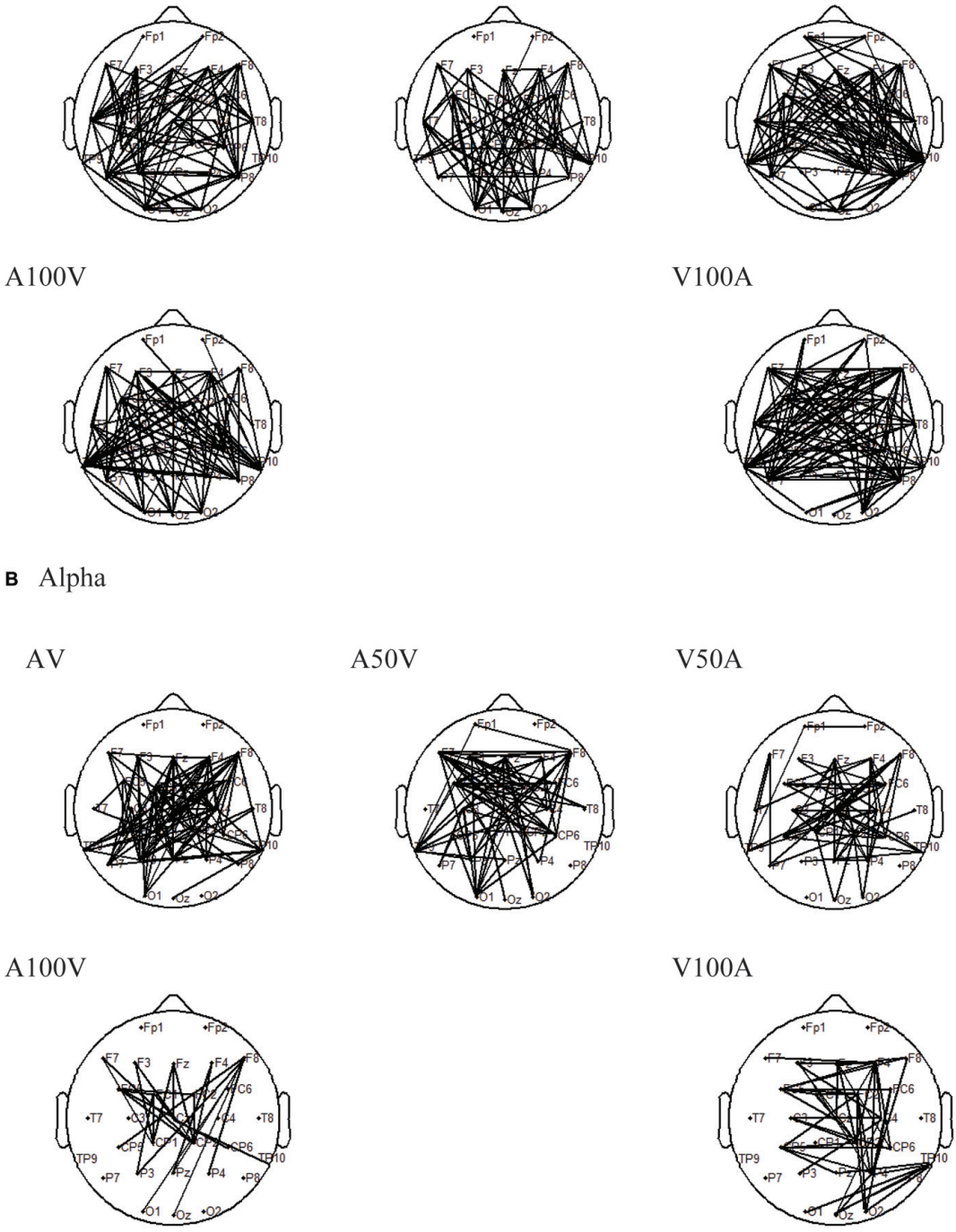

FIGURE 5 | Results of pairwise associations. We used the NBS to identify interregional connectivity that significantly differed between the groups for the theta and alpha bands. **(A)** In the theta band, old adults had significantly stronger connections in all of the audiovisual conditions. **(B)** In the alpha band, old adults had significantly stronger connections in all of the audiovisual conditions.

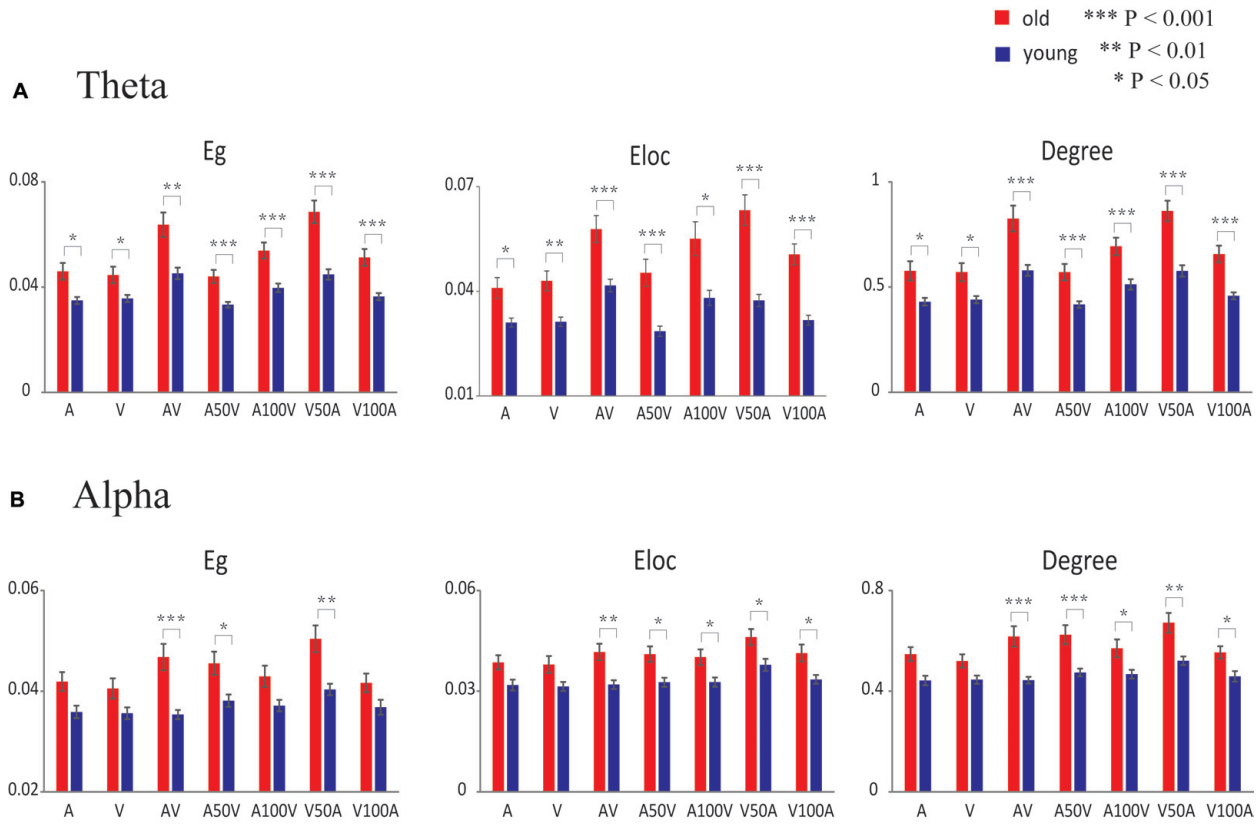

FIGURE 6 | Statistical results of network metrics for each condition. **(A)** In the theta band, the comparison of network metrics between the two groups in each condition. **(B)** In the alpha band, the comparison of network metrics between the two groups in each condition. The error bar indicates the standard error of the mean. Eg, global efficiency; Eloc, local efficiency.

cortex earlier than the visual information reaching the primary visual cortex (King and Palmer, 1985). Molholm et al. (2002) studied the onset of early audiovisual integration and found that the onset latency of visual stimuli was approximately 50 ms, whereas the onset latency of auditory stimuli was less than half that of visual stimuli (9–15 ms from stimulus presentation). The auditory and visual neural signals arrive at the brain at closer times, resulting in a larger integration effect.

Increased Phase Synchronization of Neural Oscillations in Aging

The theta and alpha bands play a major role in audiovisual integration (Sakowitz et al., 2005; Başar, 2012; van Driel et al., 2014; Friese et al., 2016; Keller et al., 2017). Both old and young adults showed increased PLIs in the alpha and theta bands after stimulus onset, especially for audiovisual conditions (**Figures 3**, **4**). Researchers have reported evidence that EEG oscillations in the alpha and theta bands particularly reflect attention and cognitive control (Klimesch, 1999; Sakowitz et al., 2005; Friese et al., 2016; Keller et al., 2017). In addition, an EEG revealed increased phase locking in the theta and alpha bands between regions of the human brain in a difficult working memory task (Halgren et al., 2002). We further found age-related differences only in the theta and alpha bands in audiovisual conditions. The significantly higher PLI for old adults during audiovisual conditions indicates the presence of stronger phase locking in old adults while performing tasks. Some studies have examined age-related differences in multisensory integration, demonstrating that old individuals exhibit greater multisensory integration than young individuals (DeLoss et al., 2013; Stevenson et al., 2015). The results of functional imaging studies have also suggested that there is increased activity in the brain network during motor tasks with aging (Seidler et al., 2010; Schmiedtfehr et al., 2016).

The difference in connection revealed by NBS also confirmed the mean PLI of global connections. We found significantly stronger connections, mainly located in the fronto-parietal site and superior temporal site, in old adults than in young adults in the audiovisual conditions only (**Figure 5**). Consistently, van Driel et al. (2014) determined the functional role of the coupling of alpha phase dynamics between sensory cortices in integrating cross-modal information over time. A recent report also found that multisensory attention leads to increased low-frequency phase synchronization in the frontal cortex (Friese et al., 2016). These connection patterns might imply that the theta band plays a role in cognitive control and short-term memory in audiovisual integration (Demiralp and Başar, 1992; Sakowitz et al., 2000; Keller et al., 2017), and the alpha band

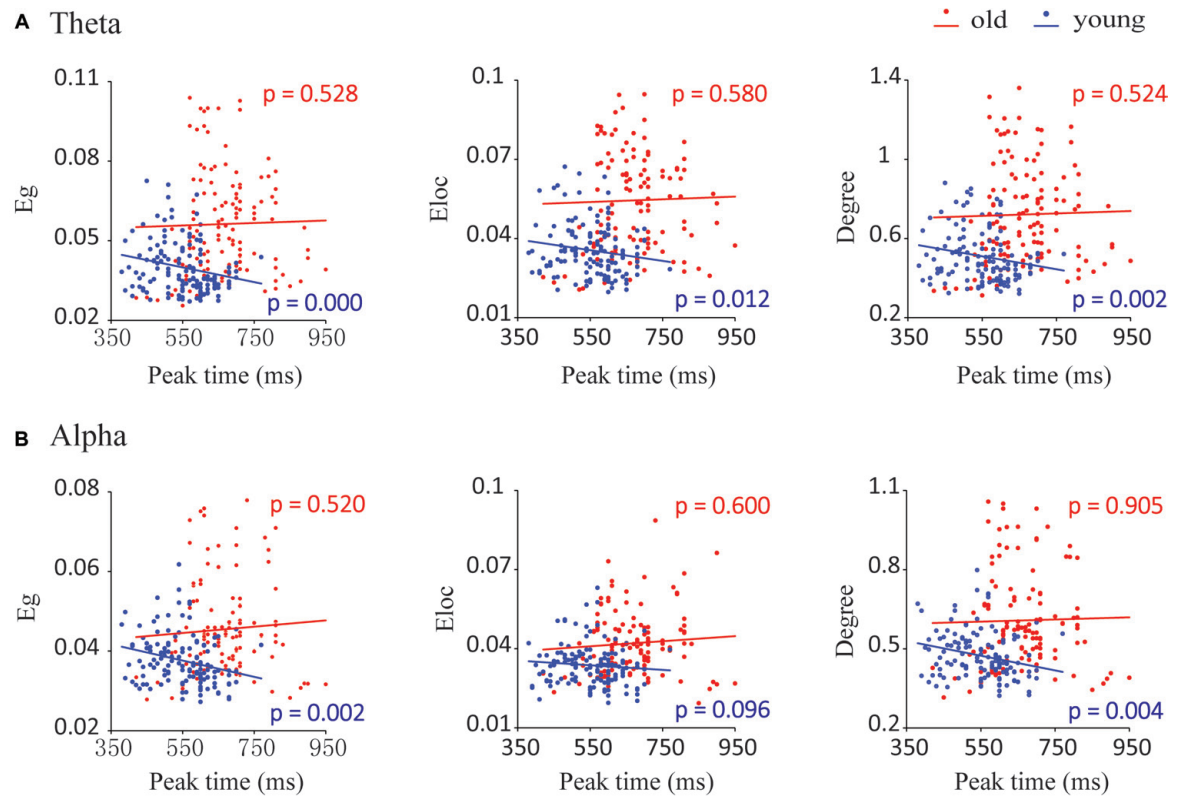

FIGURE 7 | Correlations between network parameters and the peak time point of cumulative probability difference curves. **(A)** In the theta band, global efficiency (Eg), local efficiency (Eloc), and degree had significant negative correlations with the peak time for young adults during audiovisual stimuli. **(B)** In the alpha band, we detected that global efficiency and degree had significant negative correlations with the peak time for young adults during audiovisual stimuli.

seems to be mainly related to the maintenance of sensory information, cognitive control and suppression of distractions (Masahiro et al., 2010; Başar, 2012). Thus, a possible explanation could be that the greater connections in the theta and alpha bands are likely due to cognitive demand and attention maintenance.

Age-Related Alteration in Network Efficiencies of the Theta Band

In the present study, we found a significant increase in theta band functional connectivity, which was mainly located in frontal, parietal, and temporal sites (**Figures 4A**, **5A**), as well as in the network metrics (global and local efficiencies and degree) during audiovisual stimuli in old adults (**Figure 6A**). Previous studies also showed that old adults had greater responses in the theta band during sensory processing (Yordanova et al., 1998; Dushanova and Christov, 2014). Theta activity is generally thought to be associated with a general brain integrative mechanism (Sauseng et al., 2007, 2010) and central executive functions during audiovisual integration (Masahiro et al., 2010). In working memory tasks, interregional theta synchronization has been shown to be related to integration mechanisms (Sauseng et al., 2010; Keller et al., 2017). The topological properties of whole-brain networks support the integrative role of the connection in the theta band. Higher global efficiency of the network corresponds to fast global information communication (Sporns, 2011; Wig, 2017), and local efficiency supports fast local information processing (Sporns, 2011; Wig, 2017). A previous analysis revealed more connectivity, including in the frontal and parietal cortices, during more difficult tasks, demonstrating that audiovisual integration requires not only local information processing within the auditory or visual systems but also global information communication for audiovisual integration and executive control (Sauseng et al., 2007). Recent studies have demonstrated that theta oscillations, mainly located in frontal and parietal regions, are associated with both attention and cognitive control (Friese et al., 2016; Keller et al., 2017). By using the time-window-of-integration model, Diederich et al. (2008) demonstrated that old adults had a smaller probability of integration and larger neural enhancement for neural integration and preparation of an oculomotor response. Moreover, the audiovisual condition showed a larger group difference, especially for the AV, V50A, and V100A conditions, which showed stronger audiovisual integration. Our findings suggest that the higher network efficiency of the theta band in old adults is associated with higher attention and cognitive control and reflects higher cognitive demand in old adults.

Age-Related Alteration in Network Efficiencies of the Alpha Band

In the alpha band, we found significantly higher functional connectivity, mainly in frontal and parietal sites (**Figures 4B**, **5B**) and in network metrics, in old adults than in young adults in the audiovisual condition (**Figure 6B**). These results indicate that old adults require higher functional connectivity to complete audiovisual integration. Moreover, old adults had significantly higher global and local efficiencies, especially in the AV, A50V, and V50A conditions. There are two potential interpretations of these differences in the alpha band. In general, alpha synchronization reflects an active attentional suppression mechanism or executive functions (Kelly et al., 2006; Masahiro et al., 2010), which are critical for the evocation of a multisensory response (Koelewijn et al., 2010). Diederich et al. (2008) demonstrated that old adults had greater neural enhancement for neural integration and preparation of an oculomotor response. Old adults were more likely to be distracted by irrelevant stimuli and show less efficient inhibitory function than young adults (Andrés et al., 2006; Li et al., 2013). Therefore, alpha phase synchrony was significantly enhanced in old adults during temporal audiovisual integration, supporting the idea that the observed enhancement may function to compensate for distraction and disinhibition in old adults (Li et al., 2013; van Driel et al., 2014). In addition, the alpha amplitudes also involved the maintenance periods in the temporal area for auditory information and the parietal area for visual information (Masahiro et al., 2010). Old adults experience an increase in auditory threshold (hearing loss) and a decrease in visual acuity (Bäckman et al., 2006; Liu and Yan, 2007). These deficits likely result in a smaller probability of integration even with a wider window of integration, produce more cognitive demand and manifest greater neural enhancement (Diederich et al., 2008), which may be attributed to higher functional connectivity and larger network efficiencies.

Furthermore, previous studies on a general audiovisual integration task did not find significant differences between groups in alpha band-related metrics. A possible reason is that under their experimental conditions, which incorporated fewer conditions and less difficulty than our study, old adults did not need greater attentional resources to complete the audiovisual integration task (Wang et al., 2017). Previous studies also demonstrated that the alpha band involves executive functions and the memory storage buffer for auditory and visual information (Masahiro et al., 2010). During the present audiovisual temporal asynchrony integration task, there were more conditions and a higher cognitive demand; therefore, the difference in network efficiencies of the alpha band between young and old adults was likely to become larger.

Relationship Between Network Metrics and Integration Performance

We found that the time window of behavioral integration in young adults was shorter and less delayed than that in old adults, which is consistent with the findings of our previous behavioral study (Ren et al., 2017b). The peak time point represents the likely occurrence of audiovisual integration. The peak time point of each probability difference curve was recorded for each participant in each group. In the theta and alpha bands, network metrics of audiovisual processing showed strong correlations with peak time points in young adults but not in old adults, especially in the AV, A50V, and V50A conditions. Interestingly, our recent report also demonstrated such correlations in the beta band in old adults but not in young adults (Wang et al., 2017). These findings indicate that the network efficiency reflects the efficient integration of information in the brain. A greater efficiency is associated with a shorter peak time of integration. Moreover, the different design of audiovisual integration of these two reports led to different associations in EEG bands. A previous study demonstrated that activities in the alpha and theta bands appeared as intersensory processing and could therefore participate in different stages of perception and executive functions (Sauseng et al., 2007; Masahiro et al., 2010).

Due to the confusion in temporal asynchrony and more conditions, the audiovisual integration task in the present study was much harder and required more cognitive resources than the standard audiovisual integration task, especially for old adults. The greater cognitive resources required to perform highly demanding tasks would lead to changes in communication within the cortical system (Sauseng et al., 2007). Young adults needed to maintain attention to achieve tasks that lead to associations with integration performance. However, such associations disappeared in old adults because the current task was too hard for them. Moreover, this assumption was also supported by the associations between network efficiency and integration performance only in old adults during a much simpler audiovisual integration task (Wang et al., 2017). There was a ceiling effect in old adults during the audiovisual integration task with temporal asynchrony and a floor effect in young adults during a simple audiovisual integration task.

Limitations

Our study has several limitations. One limitation is that we chose 32 scalp electrodes to construct the brain network; thus, the number of nodes in the network was relatively small. Second, we performed a PLI analysis and constructed the connection among electrodes. This type of connection has limited ability to reveal the connections among the center regions. However, with EEG source analysis or magnetoencephalography, the topological properties in cortex regions will benefit from investigations of the mechanism of an aging effect during audiovisual integration. Third, we determined the associations between peak time and network metrics only in young adults. The EEG analysis selected trials for non-target stimuli only. The attentional effects between target and non-target stimuli require further investigation, which may clarify the association between integration performance and network metrics. In the future, we will determine how to adjust low-frequency band functional connectivity or temporal asynchrony between inputs to different sensory modalities to improve sensory function, which gradually weakens during normal aging.

CONCLUSION

In the present study, for both the theta and alpha bands, the AV and V50A conditions induced stronger functional connections and higher global and local efficiencies, reflecting a stronger audiovisual integration effect, which was attributed to the auditory information arriving at the primary auditory cortex earlier than the visual information reaching the primary visual cortex. The old adults showed a stronger connectivity strength of the theta and alpha bands in the audiovisual temporal asynchrony integration task. The results of the network metrics of old adults also showed higher global efficiency, local efficiency and degree, especially in audiovisual conditions. These larger network efficiencies reflect more demand of attention and cognitive control in old adults, who found the current task more difficult than did young adults. The network efficiencies were significantly associated with the peak time of integration in young adults only, which implied that an audiovisual integration task with multiple conditions might arouse the appropriate attention in young adults but lead to a ceiling effect in old adults. Our findings provide new insights into the network topography of old adults during audiovisual integration tasks with temporal asynchronous information and highlight the higher functional connectivity and network efficiencies due to greater cognitive demand.

AUTHOR CONTRIBUTIONS

BW, TianyiY, and JX conceived and designed the experiments. PL, YN, and TL analyzed and interpreted the data, and wrote the paper. DL, RC, PY, YG, TingY, XL, and FW revised the paper. WY, YR, JW, and HZ performed the experiments.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2018.00316/full#supplementary-material

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Frailty and Cognitive Impairment in Predicting Mortality Among Oldest-Old People

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Backgrounds: Frailty and cognitive impairment are critical geriatric syndromes. In previous studies, both conditions have been identified in old-age adults as increased risk factors for mortality. However, the combined effect of these two syndromes in predicting mortality among people with advanced age is not well understood. Thus, we used Chinese community cohort to determine the impact of the combined syndromes on the oldest-old people.

Methods: Our present study is part of an ongoing project on Longevity and Aging in Dujiangyan, which is a community study on a 90+ year cohort in Sichuan Province in China. Participants were elderly people who completed baseline health assessment in 2005 followed by a collection of mortality data in 2009. Frailty and cognitive function were assessed with 34-item Rockwood Frailty Index and the Mini-Mental Status Examination, respectively, and the combined effect(s) of these two parameters on death was examined using the Cox proportional hazard regression model.

Results: This study consisted of a total of 705 participants (age = 93.6 ± 3.3 years; 67.4% females), of which 53.8% died during a four-year follow-up period. The prevalence of frailty, cognitive impairment, and the overlap of these two syndromes was 63.7, 74.2, and 50.3%, respectively. Our data showed that the subjects with combined frailty and cognitive impairment were associated with increased risk of death (age, gender, education level, and other potential confounders adjusted); the hazard ratio was 2.13 (95% confidence interval 1.39, 3.24), compared with the control group. However, neither frailty alone nor cognitive impairment alone increased the risk of death in these individuals.

Conclusion: The combined frailty and cognitive impairment, other than the independently measured syndromes (frailty or cognitive impairment alone), was a significant risk factor for death among the oldest-old Chinese people.

Keywords: frailty and cognitive impairment, frailty index, mortality, MMSE, oldest-old, cognitive frailty

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INTRODUCTION

Frailty is a common geriatric syndrome, presenting a clinical state of decreased physiological reserve and increased vulnerability to death and/or developing an increased dependency to even a small stressor (Morley et al., 2013). The prevalence of frailty is about 7.0% among community-dwelling people aged 65 years or more; it varies with different operational definitions and increasing age (Fried et al., 2001; Collard et al., 2012). Frailty is an emerging public problem with the advent of aging society worldwide, for it can increase the risk of adverse clinical outcomes, such as disability, delirium, falls, and death (Clegg et al., 2013; Cesari et al., 2016). Frailty is a transitional and reversible state, and therefore, it has provided us with an opportunity to carry out research which would provide insight into the occurrences and consequences "of adverse outcomes" among the elderly and to plan strategies to reduce the incidence of any non-reversible adverse outcomes (Michel et al., 2015). Currently, the specific pathophysiology of frailty is poorly understood, and the frailty state has generally been regarded as a disorder of several physiological systems, including the brain, skeletal muscle, endocrine system, and the immune system (Clegg et al., 2013).

Brain aging or frail brain plays an essential role in physical frailty (Malmstrom and Morley, 2013). More and more studies have shown frailty to be closely related to cognitive impairment in a prospective cohort study (Boyle et al., 2010; Sugimoto et al., 2018). In order to encourage combined research in frailty and cognitive impairment, the International Academy on Nutrition and Aging (IANA) and the International Association of Gerontology and Geriatrics (IAGG) organized an International Consensus Group (ICG) to propose the operational definition of cognitive frailty (Kelaiditi et al., 2013). After the consensus was published, researchers put more attention on these two critical geriatric syndromes. Although the prevalence of cognitive frailty in the community setting is low (1.0–1.8%), it has been associated with a high risk of disability, poor quality of life, and death (Sugimoto et al., 2018). Furthermore, researchers have also found a 50-item frailty index (FI) to be significantly associated with temporal and frontal cortical atrophy, detected by computerized axial tomography, which indicates that frailty and cognitive decline might share common pathophysiological mechanisms (Fougere et al., 2017; Gallucci et al., 2018). All of these findings show that frailty and cognitive impairment are closely related to each other. However, Shimada et al. (2013) found that only 2.7% participants displayed overlapping frailty and cognitive impairment, with the majority of the subjects (97.3%) devoid of the combined syndromes.

The use of frailty and cognitive impairment parameters in predicting mortality has previously been investigated (Jacobs et al., 2011; Matusik et al., 2012; Forti et al., 2014; Jha et al., 2016; Feng et al., 2017b; Lee et al., 2018). The results, however, revealed several discrepancies among various reports; some found combined physical frailty and cognitive function assessment to enhance the likelihood of the prediction of individual's risk of death than either measurement alone (Matusik et al., 2012; Jha et al., 2016; Feng et al., 2017b; Lee et al., 2018), while some researchers found no statistically significant enhancement of the combined effect (Jacobs et al., 2011; Forti et al., 2014). Furthermore, most participants in these studies were Caucasians and aged from 60 to 90 years. The characteristic of cognition or frailty among the oldest-old had been shown to be different with other age groups (Luo et al., 2013; Hao et al., 2016). Thus, the role of cognitive impairment, frailty or a combination of both in predicting adverse outcomes need to be further classified, primarily, among the oldest-old (aged 90 or more) and also other races. Based on the above-mentioned findings, we hypothesized that the combined effect(s) of cognitive impairment and frailty would be more capable of predicting mortality in very old Chinese people than the independent syndromes.

To date, no studies have focused on only cognitive impairment or frailty or the two syndromes combined in predicting mortality in advance late-life, and thus the combined effects remain unclear in the oldest-old population (90+ years or older). In 2005, we included 870 old-aged people (aged 90 years or older) in Dujiangyan (town level), Chengdu, and Sichuan in China, for the PLAD project explained in detail in the Methods section below. Four years later (in 2009) we collected the information on the death of the participants (4-year all-cause mortality and the time of death). This study provided us with the opportunity to explore the effects of frailty and cognitive impairment or a combination of both in predicting mortality in this elderly population.

MATERIALS AND METHODS

Study Population

The data from the Project of Longevity and Aging in Dujiangyan (PLAD) is a cross-sectional study conducted in Dujiangyan in 2005. Dujiangyan is a town of Chengdu located in southwestern China. PLAD was conducted to explore the relationship between age-related diseases, longevity, lifestyle, and other factors. The details regarding the PLAD research have been reported previously (Wu et al., 2007; Wang et al., 2010; Flaherty et al., 2011). Briefly, PLAD included 870 elderly people aged 90 years or older, based on the 2005 census in Dujiangyan region (total of 1115 community members, aged 90 years or older). Face-to-face interviews with trained volunteers were used to collect baseline data, using several validated scales of general questionnaires. Medical staff performed anthropometric measurements, physical examination, and collection of fasting blood samples for various analyses [22–24]. All the participants or their legal proxies were informed about the details of the study and gave formal written consent before the study was initiated. The Ethics Committee of Sichuan University approved the study protocol (Chengdu, Sichuan, China). The exclusion criteria for our current study were as follows: participants with missed data on mortality (n = 53), MMSE (n = 100), or >20% of the FI variables (n = 10), and participants with previous denoised dementia (n = 2), which resulted in a study population of 705 (males: 230 cases or 32.6%; females: 475 cases or 67.4%).

Construction of the Frailty Index

In this study, FI was constructed using 34 items available in the PLAD dataset, according to a standard procedure, which was similar to previous study reports (Searle et al., 2008; Hao et al., 2016). All selected variables meet the following criteria: associated with health status; increased with age (generally); not saturate too early; cover a range of important systems (Searle et al., 2008). The 34 variables in the construction of FI were Instrumental Activities Daily Living (IADL) and Activities Daily Living (ADL) disability items (n = 14), disease (n = 9), psychological problems (n = 1), symptoms (n = 5), and abnormality in the physical examination (n = 6). Items used to assess the cognitive function such as all items in MMSE were excluded. A binary variable was coded as present = 1 or absent = 0. For variables with 3–4 scale levels, the intermediate response was coded between 0 and 1. For each old-age person, the FI was calculated as the sum of all deficits present divided by the total number of whole considered variables (here it is 34), which made the FI a continuous variable, theoretically ranging between 0 and 1. We set FI = 0.21 as a cut-off point for diagnosis of frailty, in accordance with Hoover et al. (2013) study.

Evaluation of Cognitive Function

In this study, the 30-item Mini-Mental State Examination (MMSE) scale was used to evaluate cognitive function, as it is a reliable and widely used method of assessment of the condition, and it includes the measurements of the following parameters: attention and calculation, orientation, recall, language, and ability to follow simple commands (Tuijl et al., 2012). Visual and auditory abilities were basic requirements for most items of MMSE (Holtsberg et al., 1995). Our study excluded 100 participants (28 men and 72 women) who were unable to complete the MMSE test due to hearing or visual problems, in order to be able to address the influence of hearing and visual impairment on cognitive function. In Asian people, the cut-off point of MMSE is highly variated, ranging from 17 to 29 (Rosli et al., 2016). The educational level of most subjects in this study was low (illiterate or primary school; 97.4%), and cognitive impairment was defined as an MMSE score of 0–18. An MMSE score of 19–30 was defined as "without cognitive impairment," according to previous reports, and this cutoff point has been shown to be 80 to 100% specific and 80 to 90% sensitive for diagnosis of cognitive impairment (Katzman et al., 1988; Tombaugh and McIntyre, 1992; Zhu et al., 2006; Cui et al., 2011; Matusik et al., 2012). Furthermore, we performed several methods to promote the assessment quality and methodological reliability, which includes the following: (1) MMSE assessors were trained by experienced geriatricians in comprehensive geriatric assessment, provided research manually, and video for all researchers; (2) observed MMSE administrators performing the MMSE on standardized patients; (3) quality control researchers received and responded to feedback or questions, while conducting the MMSE on the participants.

Mortality Data and Other Co-variables

The mortality data, the status of survival (died or survived), and the time of death, were collected for all participants from local government records, relatives, or neighbors in 2009. There were about 48 (5.5%) participants lost to follow-up. Age, gender, educational levels (illiteracy, primary school, or secondary school and advanced), weight, height, waist circumference, systolic blood pressure, diastolic blood pressure, smoking, alcohol drinking, exercise, and comorbidity were collected as co-variables. Comorbidity was defined as two or more chronic illnesses occurring in the same participant. All chronic diseases were diagnosed by certified physicians in the local hospital.

Statistical Analysis

In this study, baseline characteristics of the participants were shown according to the status of frailty and cognitive impairment of the data types. Continuous variables were presented as means and standard deviations. Categorical variables were presented as numerals and percentages. The differences between groups were tested by Analysis of Variance (ANOVA) or unpaired Student's t-test for continuous variables or Chi-square test for categorical variables. Cox proportional hazard regression models were employed to estimate the hazard ratio (HR) and its 95% confidence interval (CI) of the status of frailty and cognitive impairment as a function of increased mortality. Age, gender, and educational levels were regarded as general covariates in adjusted Cox regression model 1. Lifestyle factors (smoking, alcohol consumption, and exercise) and chronic diseases were added in Cox regression model 2. Several other co-variables ($P < 0.1$, when compared among different groups for baseline variables), regarded as potential confounders, were adjusted further in model 3. Statistical Product and Service Solutions (SPSS) software package for Windows, version 17.0 (SPSS Inc., Chicago, IL, United States), was used in all statistical analyses. Two-tailed P-values of <0.05 were set as statistically significant.

RESULTS

Baseline Characteristics, Frailty, and Cognitive Impairment

Overall, we included 705 participants in this study. The percentage of females was 67.4%, and the mean age of the subjects was 93.6 ± 3.3 years, ranging from 90 to 108. The maximum, mean, and median FI scores of the participants were 0.62, 0.26, and 0.25, respectively. The standard deviation of FI is 0.10. The 99th percentile obtained for the FI was 0.53. The maximum, mean, and median MMSE scores of the participants were 28, 14.82, and 15, respectively. The standard deviation of MMSE was 5.68.

Women had significantly higher FI scores and lower MMSE scores than men (0.26 ± 0.11 vs. 0.24 ± 0.10; $t = -2.53$, $P = 0.012$; 13.70 ± 5.28 vs. 17.14 ± 5.79; $t = 7.86$, $P < 0.001$) and more females presented in the frailty and cognitive group than males (66.1 vs. 58.7%, $X^2 = 3.68$, $P = 0.055$; 83.6 vs. 54.8%, $X^2 = 67.01$, $P < 0.001$). The overall prevalence of frailty and cognitive impairment among the whole population were 63.7% (95% confidence interval (CI) = 60.1–67.2%) and 74.2% (95% CI = 70.8–77.3%), respectively.

The combined prevalence of frailty and cognitive impairment, frailty alone, cognitive impairment alone, and no frailty nor cognitive impairment (control group) were 50.1% (95% CI = 46.4–53.8%), 13.6% (95% CI = 11.3–16.4%), 24.1% (95% CI = 21.1–27.4%), and 12.2% (95% CI = 10.0–14.8%), respectively. Subjects with combined frailty and cognitive impairment were older with significantly higher percentage of female, illiteracy, comorbidity, and death, but significantly lower weight, height, and systolic blood pressure (SBP), compared with the control group. **Table 1** shows the characteristics of the study participants, according to their frailty and cognitive impairment status.

Baseline Characteristics and All-Cause Mortality

The 4-year death rate was 53.8% in these old-aged individuals. Those who died were slightly older than the survival group, but there was no statistical significance (93.8 ± 3.3 vs. 93.4 ± 3.4, $t = -1.86$, $P = 0.063$). Mortality was significantly enhanced in participants with higher FI but lower MMSE scores than the survival group (0.27 ± 0.11 vs. 0.24 ± 0.10, $t = -4.32$, $P < 0.001$; 13.86 ± 5.83 vs. 15.94 ± 5.31, $t = 4.93$, $P < 0.001$). The proportion of frailty and cognitive impairment was also higher in the death group than in the survival group (68.3 vs. 58.3%, $X^2 = 7.66$, $P = 0.006$; 78.6 vs. 69.0%, $X^2 = 8.45$, $P = 0.004$). Lifestyle habit, regular exercise, was also less common in the death group than in the survival group (33.0 vs. 47.2%, $X^2 = 14.63$, $P < 0.001$). There was no statistically significant difference between the death and the survival groups for comorbidity (57.3 vs. 56.4%, $X^2 = 0.047$, $P = 0.828$) and other co-variables (see **Table 2** for more details).

The Relationship Between Frailty, Cognitive Impairment, and All-Cause Mortality

Table 3 shows the results from unadjusted and adjusted Cox proportional hazard regression models for the frailty and cognitive impairment status, as a function of increased risk of death. Compared to the control group, subjects with combined frailty and cognitive impairment had a significantly higher risk of mortality [HR: 1.82, 95% CI (1.27, 2.61), $P = 0.001$] than those with the individual syndrome. Frailty only could not predict the risk of death in the study population [HR: 1.29, 95% CI (0.83, 2.00), $P = 0.256$] when compared with the control group. A similar result was yielded when participants with cognitive impairment alone were compared with the control group [HR: 1.31, 95% CI (0.88, 1.94), $P = 0.184$]. This model was stable after

TABLE 1 | Characteristics of the study population according to frailty and cognitive impairment status.

	Status of frailty and cognitive function				*P*-value
	Frailty and cognitive impairment jointly (*n* = 353)	**Frailty only (*n* = 96)**	**Cognitive impairment only (*n* = 170)**	**No frailty and no cognitive impairment (control group) (*n* = 86)**	
Age (years)	94.2 ± 3.5	93.1 ± 3.4	93.3 ± 3.1	92.5 ± 2.6	<0.001**
Female (%)	75.1	51.0	77.6	33.7	<0.001**
BMI (kg/m^2)	18.9 ± 3.5	19.4 ± 4.2	19.5 ± 2.9	20.2 ± 3.3	0.009**
Weight (kg)	39.7 ± 7.9	41.9 ± 9.4	41.3 ± 7.3	46.7 ± 9.3	<0.001**
Height (cm)	145.4 ± 10.5	147.9 ± 10.6	145.4 ± 8.6	152.5 ± 7.7	<0.001**
WC (cm)	76.7 ± 9.2	76.5 ± 11.1	77.1 ± 8.5	79.3 ± 9.8	0.131
SBP (mmHg)	137.3 ± 22.8	144.0 ± 22.6	144.4 ± 23.4	140.8 ± 23.0	0.003**
DBP (mmHg)	72.6 ± 12.0	73.4 ± 12.4	72.8 ± 12.1	73.0 ± 12.1	0.936
MMSE	11.9 ± 4.5	21.4 ± 2.0	13.5 ± 3.9	22.2 ± 2.4	<0.001**
Frailty index	0.32 ± 0.08	0.29 ± 0.06	0.15 ± 0.04	0.16 ± 0.04	<0.001**
Education level (%)					
Illiteracy	84.4	49.5	80.6	31.8	
Primary school	13.9	47.4	17.1	62.4	
Secondary school or advanced	1.7	3.2	2.4	5.9	<0.001**
Smoking (%)	40.3	50.0	46.2	44.2	0.311
Alcohol drinking (%)	21.7	22.9	29.6	41.9	0.001**
Having exercise habit (%)	36.0	36.8	42.5	51.2	0.056
Comorbidity (%)	77.3	76.0	17.6	29.1	<0.001**
Status of survival (%)					
Alive	39.7	52.1	50.0	59.3	
Death	60.3	47.9	50.0	40.7	0.002**

Data are the mean ± SD unless otherwise indicated. Comorbidity was defined as the presence of two or more chronic diseases (hypertension, cardiovascular disease, cerebrovascular disease, diabetes, respiratory disease, digestive disease, chronic renal disease, and osteoarthritis). Abbreviations: BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MMSE, mini-mental status examination.
***P < 0.01.*

TABLE 2 | Characteristics of the study population according to survival status.

	Status of survival		
	Alive (*n* = 326)	**Death (*n* = 379)**	***P*-value**
Age (years)	93.4 ± 3.4	93.8 ± 3.3	0.063
Female (%)	68.4	66.5	0.589
BMI (kg/m^2)	19.4 ± 3.3	19.2 ± 3.6	0.329
Weight (kg)	41.4 ± 8.2	41.2 ± 8.7	0.692
Height (cm)	146.6 ± 10.1	146.5 ± 10.0	0.891
WC (cm)	76.9 ± 9.7	77.2 ± 9.2	0.701
SBP (mmHg)	140.3 ± 22.5	140.4 ± 23.6	0.929
DBP (mmHg)	72.2 ± 11.4	73.3 ± 12.6	0.249
MMSE	15.9 ± 5.3	13.9 ± 5.8	<0.001**
Frailty index	0.24 ± 0.10	0.27 ± 0.11	<0.001**
Education level (%)			
Illiteracy	72.0	72.8	
Primary school	25.5	24.6	
Secondary school or advanced	2.5	2.6	0.952
Smoking (%)	45.7	41.6	0.279
Alcohol drinking (%)	28.0	24.7	0.327
Having exercise habit (%)	47.2	33.0	<0.001**
Comorbidity (%)	56.4	57.3	0.828
Frailty (%)	58.3	68.3	0.006**
Cognitive impairment (%)	69.0	78.6	0.004**

*Data are the mean ± SD unless otherwise indicated. Comorbidity was defined as the presence of two or more chronic diseases (hypertension, cardiovascular disease, cerebrovascular disease, diabetes, respiratory disease, digestive disease, chronic renal disease, and osteoarthritis). Abbreviations: BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MMSE, mini-mental status examination. **P < 0.01.*

adjusting for age, gender, education levels, lifestyles, and other potential confounding factors. Subjects with joint frailty and cognitive impairment had a significantly higher risk of mortality, compared to the control group after adjustment of these potential confounding factors (HR: 2.13, 95% CI (1.39, 3.24), $P < 0.001$). Neither frailty alone nor cognitive impairment alone was able to predict the risk of mortality, as compared to the control group. **Figure 1** shows the survival curves of the study population according to their frailty and cognitive impairment status at baseline.

DISCUSSION

In this report, we studied the relationship between frailty, cognitive impairment, and mortality in community-dwelling oldest-old people (90–108 years) in Dujiangyan, Chengdu, and Sichuan province in China. Our present study is the first to investigate the combined role of frailty and cognitive impairment in predicting mortality among old people with advanced age. We have shown that the combined syndromes, and not frailty alone or cognitive impairment alone, is a significant risk factor for death among the oldest-old Chinese people. This study indicates that it is critical to assess a combination of frailty and cognitive function than as separate entities to predict the risk of mortality among old-age people, and also to define the existence of "cognitive frailty," coined by the ICG in 2013.

The IANA and the IAGG organized the ICG in 2013 who first proposed the operational definition of cognitive frailty, described as the simultaneous presence of both physical frailty (Fried frailty phenotype) and cognitive impairment (clinical dementia rating [CDR] = 0.5) (Kelaiditi et al., 2013). The prevalence of cognitive frailty was estimated to be 1.0–1.8% among the community setting of old-age people without dementia or other neurodegenerative conditions, which suggested a limited clinical utility of cognitive frailty in the elderly (Sugimoto et al., 2018). Shimada et al. (2013) included 5104 older adults (mean age 71 years) in Japanese community studies and found that the prevalence of combined frailty and cognitive impairment was only 2.7%. However, our present study, which included Chinese non-agenarians and centenarians, revealed that the prevalence of combined frailty and cognitive impairment was 50.1% (95% CI = 46.4–53.8%), which indicated that cognitive frailty is more common in the very old population and supports the idea that the prevalence of the dual syndrome increases with age (Feng et al., 2017a). In other words, the simultaneous presence of both physical frailty and cognitive impairment is common among the oldest-old population.

The 34-item FI was employed to assess the presence of frailty in this study, while the frailty phenotype proposed by Fried et al. (2001) was most commonly used in previous studies (Zaslavsky et al., 2013). Additionally, the frailty phenotype was recommended to define cognitive frailty by the international consensus (Kelaiditi et al., 2013). To date, although multiple operational frailty assessment methods have been validated, frailty phenotype and FI are two most common measures of frailty (Cesari et al., 2014; Blodgett et al., 2015). Since we did not have data on grip strength and walking speed in our study, we could not use frailty phenotype to define frailty and to compare FI with frailty phenotype in predicting mortality. However, the operational definition of frailty in cognitive frailty also needed to be discussed. Although these two commonly used measures of frailty are different, both are associated with mortality and cognitive impairment (Cesari et al., 2014; Sugimoto et al., 2018). Furthermore, FI could be used to classify more people as frail, as it is based on a more comprehensive geriatric assessment, such as physical examinations, multi-functional measures, and diagnostic data, and hence, more capable of predicting mortality than frailty phenotype measurements (Theou et al., 2013; Cesari et al., 2014). Thus, it is rational to use FI to define frailty in the operation of cognitive frailty.

Although CDR = 0.5 was recommended to assess cognition in cognitive frailty by the international consensus (Kelaiditi et al., 2013), the majority of studies identified cognitive impairment according to global cognitive assessment scales, including MMSE and Montreal Cognitive Assessment (MoCA) (Sugimoto et al., 2018). Among these studies, MMSE was used to define cognitive impairment, focusing on the association of physical frailty with cognitive impairment, and the cut-off point varied from 18/30 to 26/30 (Matusik et al., 2012; Sugimoto et al., 2018). In contrast, our study, which focused specifically on very old cohort as most

TABLE 3 | Estimate of the effect of frailty and cognitive impairment mortality modeled with Cox regression model.

Models	Group	HR 95% CI	*P*-value
Unadjusted model	Frailty and cognitive impairment jointly	1.82 (1.27, 2.61)	0.001**
	Frailty only	1.29 (0.83, 2.00)	0.256
	Cognitive impairment only	1.31 (0.88, 1.94)	0.184
	No frailty and no Cognitive impairment	1 (Reference)	N/A
Adjusted model 1[a]	Frailty and cognitive impairment jointly	2.06 (1.39, 3.04)	<0.001**
	Frailty only	1.40 (0.90, 2.20)	0.136
	Cognitive impairment only	1.51 (0.99, 2.30)	0.058
	No frailty and no Cognitive impairment	1 (Reference)	N/A
Adjusted model 2[b]	Frailty and cognitive impairment jointly	2.00 (1.33, 3.00)	0.001**
	Frailty only	1.41 (0.88, 2.25)	0.152
	Cognitive impairment only	1.41 (0.93, 2.16)	0.109
	No frailty and no Cognitive impairment	1 (Reference)	N/A
Adjusted model 3[c]	Frailty and cognitive impairment jointly	2.13 (1.39, 3.24)	<0.001**
	Frailty only	1.49 (0.92, 2.42)	0.106
	Cognitive impairment only	1.43 (0.93, 2.20)	0.108
	No frailty and no Cognitive impairment	1 (Reference)	N/A

*[a]Adjusted for age, gender, and educational levels. [b]Adjusted for factors in adjusted model 1 plus smoking, alcohol drinking, exercise habit, and comorbidity. [c]Adjusted for factors in adjusted model 2 plus BMI, body mass index, height, weight, and systolic blood pressure (SBP). **P < 0.01.*

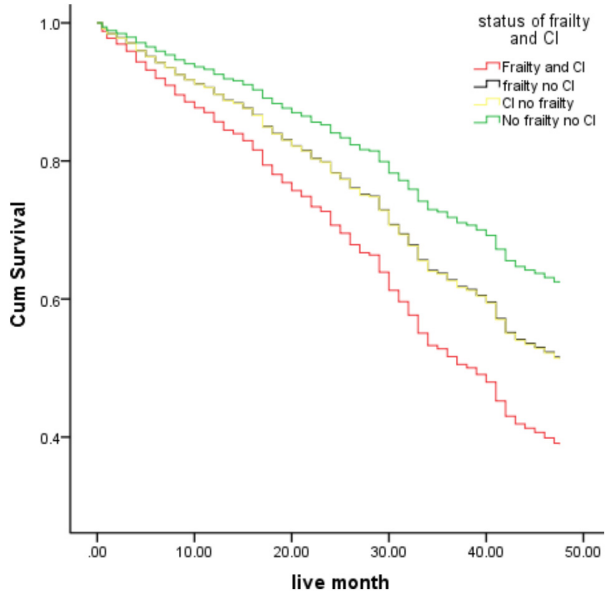

FIGURE 1 | Survival curves of the study population, according to frailty and cognitive impairment status at baseline (the survival curves significantly differs in the Cox model when compared between the joined frailty and cognitive impairment vs. control group). CI: Cognitive impairment.

of the participants (97.4%) with low educational level (illiterate or primary school), yielded mean and median scores of MMSE equal to 14.82 ± 5.68 and 15, respectively. We set 18 as the cut-off point for cognitive impairment, according to previous studies (Matusik et al., 2012). In the Chinese population, this value has been shown to effect acceptable sensitivity (80–90%) and specificity (80–100%) for diagnosis of cognitive impairment (Katzman et al., 1988; Tombaugh and McIntyre, 1992; Zhu et al., 2006; Cui et al., 2011). Had we used 26 as the cut-off point for the MMSE score in our present study, the cognitive impairment would have been 97.9%. Therefore, we considered the MMSE = 18 as an acceptable cut-off point for the determination of diagnosis of cognitive impairment among the very old people. However, only using MMSE to assess cognitive function would be considered as one of the limitations in the present study.

It is a well-known fact that the risk of death increases exponentially with age during the human lifespan (Searle and Rockwood, 2015) and mortality is high among very old people. We obtained a 4-year death rate of 53.8% with our study participants, and it is the first study to analyze the combined effect of frailty and cognitive impairment in the oldest-old people. Our results are consistent with those of the previous studies conducted among old people in nursing homes by Matusik et al. (2012). Their study included 86 old people, living in two nursing homes, with ages ranging from 66 to 101 years (mean age: 83.8 ± 8.3 years). They predicted mortality (50.0%) of the combined frailty and cognitive function in a 1-year follow up, but did not find statistical significance between mortality with the separated syndromes among the disabled geriatric patients (Matusik et al., 2012). Our study extends the funding to community-dwelling of very old Chinese people, which might be related to the inconsistencies in reports from other groups (Jacobs et al., 2011; Forti et al., 2014). Forti et al. (2014) found that a clock drawing test other than frailty phenotype might predict the 7-year risk of all-cause mortality, but combining these two syndromes (frailty and cognitive impairment) did not improve the prognostic abilities among 766 dementia-free Italian community dwellers (mean age: 73.6 ± 5.9 years). On the other hand, Jacobs et al. (2011) found frailty phenotype other than cognitive impairment (assessed by MMSE) to be significantly predictive of 5-year mortality among 840 community-dwelling

people with ages ranging from 85 to 90 years (Jacobs et al., 2011). In the present study we did not find blood pressure, smoking, or obesity to influence mortality, which did not support the evidence generated from other populations (Park et al., 2013; Pan et al., 2015). The differences of these observations might be explained by the differences in age groups, races, and follow-up periods. The potential mechanism of combining frailty and cognitive impairment in predicting mortality should be further investigated, using a large sample size, validated assessment methods, and reasonable follow-up period.

The results of our present study should be interpreted with caution for the following limitations. First, we included 705 participants (males: 230, females: 475) in this study, and the number of subjects is low in the control group (no frailty and no cognitive impairment group, n = 86). This might reduce the efficiency of statistical analysis and could limit the detection of the association of frailty or cognitive impairment and mortality. The small number of male participants also limited us from conducting subgroup analysis according to gender. However, the prevalence of frailty, cognitive impairment, and death ranked high in this specific cohort, which gave us the opportunity to examine these associations in a stable elderly population. Second, we only included Chinese Han oldest-old people, which is a good model to avoid major fatal diseases. However, this migh t have caused survival bias in our study, which could not be avoided. Moreover, we cannot extend the conclusion of our study to other races and it only could be extended to elderly people around the same age. Third, although we have adjusted age, gender, education level, and other potential confounding factors, all of the other potential confounders such as bilingualism, work life, neuropsychiatric or emotion issues, and family or social support may also play a role affecting both frailty and cognitive functioning with age. Fourth, most subjects (90%) in PLAD from a rural community were farmers who, now in their old age, would have usually had regular physical activities in their work age, limiting the extension of our study conclusion to the urban population. Fifth, we did not have data for the reason of death of our participants, so we cannot attribute only frailty and cognitive impairment to all-cause mortality in this study.

CONCLUSION

Both frailty and cognitive impairment are very common among non-agenarians and centenarians. The combined syndrome, and not frailty or cognitive impairment alone, is a significant risk factor for death among the oldest-old Chinese people. This indicates that frailty and cognitive function should be assessed jointly other than separately in predicting mortality among the elderly population and defining cognitive frailty as essential for the prediction of mortality and assisted caregiving decisions for the elderly. Additionally, prospective studies with large sample size starting in middle age, and following up the participants in early old age (60–65 years) and then every 4–5 years, are warranted to inform about the mechanistic relationship between frailty and cognitive functions.

AUTHOR CONTRIBUTIONS

QH conducted the data analysis and drafted the initial manuscript. MY and Biao D helped with results interpretation and gave critical comments for the manuscript. Birong D and YW secured funding for data collection and verified the analysis outcomes. All authors read and approved the final manuscript.

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Blood-Based Kinase Assessments in Alzheimer's Disease

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Alzheimer's disease (AD) is marked by memory disturbances followed by aphasia, apraxia and agnosia. Brain lesions include the accumulation of the amyloid peptide in extracellular plaques, neurofibrillary tangles with abnormally phosphorylated tau protein and synaptic and neuronal loss. New findings have suggested that brain lesions could occur one or two decades before the first clinical signs. This asymptomatic preclinical phase could be an opportunity to put in place a secondary prevention but the detection of these brain lesions can only be achieved so far by cerebrospinal fluid (CSF) evaluation or molecular amyloid and tau PET imaging. There is an urgent need to find out simple and easily accessible new biomarkers to set up an efficient screening in adult and aging population. Neuropathological and biochemical studies have revealed that abnormal accumulations of potentially toxic kinases are present in the brains of AD patients. Kinase activation leads to abnormal tau phosphorylation, amyloid production, apoptosis and neuroinflammation. Increased levels of these kinases are present in the CSF of mild cognitive impairment (MCI) and AD patients. Over the last years the search for abnormal kinase levels was performed in the blood of patients. Glycogen synthase kinase 3 (GSK 3), protein kinase R (PKR), mamalian target of rapamycin (mTOR), dual specificity tyrosine-phosphorylation-regulated kinase 1A (DIRK1A), c-Jun N-terminal kinase (JNK), protein 70 kD ribosomal protein S6 kinase (P70S6K), ERK2 and other kinase concentrations were evaluated and abnormal levels were found in many studies. For example, GSK3 levels are increased in MCI and AD patients. PKR levels are also augmented in peripheral blood mononuclear cells (PBMC) of AD patients. In the future, the assessment of several blood kinase levels in large cohorts of patients will be needed to confirm the usefulness of this test at an early phase of the disease.

Keywords: Alzheimer, kinase, blood, biomarkers, diagnosis

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INTRODUCTION

Neuropathological lesions in Alzheimer's disease (AD) include amyloid plaques made of Aβ peptides, neurofibrillary tangles composed of hyperphosphorylated tau proteins, amyloid angiopathy and synaptic and neuronal loss (Duyckaerts et al., 2009). Recent findings concerning the pathological evolution of AD have converged and proposed that abnormal biochemical modifications of the brain, including Aβ accumulation, could occur one or two decades before the

first clinical sign, reflecting the presence of a long clinically silent period of the disease (Jack et al., 2018). The amyloid cascade hypothesis proposes that Aβ oligomers could be toxic for neurons and could induce progressive neuroinflammation and neurodegeneration (Selkoe and Hardy, 2016). In this model, the activation of kinases by Aβ in neurons and other brain cell types could induce tau phosphorylation and the triggering of detrimental cellular pathways leading to neuronal demise and microglial activation (Iqbal et al., 2009). The current diagnosis of AD is now clearly facilitated by molecular imaging biomarkers or cerebrospinal fluid (CSF) biomarkers (Lista et al., 2015). Previous works have revealed that many kinase levels are modified in the brain and CSF of AD patients including glycogen synthase kinase 3 (GSK3; Pei et al., 1997), cyclin dependent kinase 5 (CDK 5; Baumann et al., 1993), c-Jun N-terminal kinase (JNK; Gourmaud et al., 2015), mamalian target of rapamycin (mTOR; Lafay-Chebassier et al., 2005), proapoptotic kinase R (PKR) (Chang et al., 2002b), protein kinase C (PKC; Masliah et al., 1990). Many of these kinases can phosphorylate tau but they are also pro-apoptotic and can lead to neurodegeneration (Fielder et al., 2017). The rational for assessing kinase levels or activities in blood is linked to the recent findings revealing that plasma Aβ levels could be associated with CSF Aβ concentrations in AD patients (Janelidze et al., 2016; Hanon et al., 2018). The links between kinase levels in the brain and in the blood or in blood cells are not fully understood but since kinase concentrations are enhanced or decreased in AD brains and CSF, it was proposed by several teams that evaluating blood kinase levels in AD patients and controls could be worth trying as a new search for easily accessible biomarkers. During the last years several studies have been performed in AD patients, mild cognitive impairment (MCI) and controls, comparing blood concentrations or activities of specific kinases involved in brain lesions. The major goal of these studies was to determine if the results of blood kinase levels could be utilized in the future as possible biomarkers in AD. It seems clear that evaluating on a large scale, individuals with or without cognitive symptoms using molecular imaging or CSF biomarkers is not realistic due to the cost or the invasive nature of the approaches. The screening of persons with putative AD brain lesions using a blood test seems an attainable goal. The justification of this preventive attitude will be fully justified when an appropriate and efficient disease modifying treatment for AD will be found. Large-scaled proteomics of the human kinome have been already developed but so far never extensively applied to possible AD patients (Oppermann et al., 2009). The use of kinase as biomarkers has already largely been assessed in oncology and has served as a validation for target engagements in drug discovery (Yu et al., 2007). The purpose of this brief review is to describe the major current results performed in AD or MCI due to AD patients using blood-based kinase evaluations. All studies published so far have not included the results of sensitivity, specificity and individual predictive values. In addition many studies have used semi-quantitative methods to assess kinase levels. The findings cannot be referred as real biomarkers but could only reflect abnormal blood kinase metabolisms occurring in AD patients.

GLYCOGEN SYNTHASE KINASE 3 (GSK 3)

Glycogen synthase kinase 3 (GSK 3) is widely expressed in many human tissues and is present in peripheral blood mononuclear cells (PBMC). GSK3 is expressed with two isoforms α and β. GSK3 is implicated in tau phosphorylation and in Aβ precursor protein (APP) processing linking the two pathological processes in AD (Hooper et al., 2008). In an initial clinical study, the levels of GSK3 α and β and their respective regulating phosphorylated epitopes (serine 9 and serine 21) have been assessed using western blots in PBMC from AD patients ($N = 60$), in MCI ($N = 33$) and from healthy aging individuals ($N = 20$; Hye et al., 2005). The findings revealed that total levels of GSK 3 α and β were increased in AD and MCI patients as compared to controls but the seine 9 phosphorylated epitope was not significantly different in the three groups. The authors concluded that measuring GSK3 PBMC levels could be useful as diagnostic biomarker in AD. Another report has mentioned different results in a cohort of patients suffering from AD, MCI, depression and in normal individuals (Marksteiner and Humpel, 2009). Using an ELISA method in PBMC, the authors revealed that GSK3-β levels were significantly decreased only in MCI patients and no differences were observed in other groups. It was proposed that both GSK3-β and PI3K, which regulates GSK3-β, could be similarly modulated in PBMC. In 2011, a new research also using western blots in PBMC has shown in a cohort of 20 AD, 25 Parkinson's disease patients and 30 healthy controls that the concentrations of total GSK3 α and β and their respective phosphorylated epitopes were significantly increased in AD as compared to controls, confirming the first results (Armentero et al., 2011). Further studies are needed in exploratory and confirmatory cohorts to determine the usefulness of GSK3 as a possible blood biomarker in MCI and AD patients.

CYCLIN DEPENDENT KINASE 5 (CDK5)

Cyclin dependent kinase 5 (CDK5) is a key enzyme controlling tau phosphorylation and has been implicated in the pathogenesis of AD (Cruz and Tsai, 2004). To the best of our knowledge, a recent survey of published data has not found any evaluation of cdk5 in PBMC or serum in MCI or AD patients. In contrast, the cdk5 gene polymorphism seems to be a risk factor for AD. A first report in 2009 has shown in a cohort of 549 AD patients and 5728 controls that the haplotype GG of the SNP rs2069442 of cdk5 gene increased the risk for AD in ApoE4 negative patients. In incident non-ApoE4 AD cases, the risk was augmented by 1.9-fold (Arias-Vásquez et al., 2008). This type of association was not observed in a Polish population of 257 AD patients and 80 controls concerning cdk5 polymorphisms and AD risk factor nor with several other blood biochemical parameters (Czapski et al., 2012). The evaluation of cdk5 levels and activities in PBMC of AD and MCI

patients could be worth trying to decipher putative abnormal cdk5 levels.

MAPK KINASES: C-JUN N-TERMINAL KINASE (JNK) AND p38 KINASE

JNK is a serine-threonine mitogen activated protein kinase (MAPK) coded by three genes, JNK1, JNK2 and JNK3. JNK1 and JNK2 isoforms are widely found in tissues, whereas JNK3 is mainly located in the brain (Davis, 2000). Activated JNK (pJNK) accumulations have been detected in AD brains and we have demonstrated that JNK3 levels were enhanced in AD CSF, and were linked to cognitive decline in patients (Gourmaud et al., 2015). JNK can induce enhanced Aβ production, through the phosphorylation of APP on threonine 668 (Thr668), leading to an exacerbated amyloïdogenic processing (Standen et al., 2009). P38 is also a MAPK kinase that has been implicated in neuroinflammation and tau phosphorylation and is the focus of new targeting therapies in this disease (Munoz and Ammit, 2010). A recent study has revealed that assessing total and activated JNK and p38 PBMC levels can lead to interesting results in AD (Wang et al., 2014). The authors found in a small cohort of 20 AD patients and 20 controls that PBMC levels of activated JNK and p38 were significantly increased in AD and correlated with the duration of evolution and MMSE scores. Patients with low MMSE scores had increased PBMC levels of both MAPK kinases. Another recent data has demonstrated that the EDTA plasma levels of two other MAPK kinases were associated with AD. The levels of MAPKAPK5 were positively associated with the cognitive tests assessed over a period of 10 years and the levels of MAP2K4 were negatively associated with the volume of the left entorhinal cortex (Kiddle et al., 2015). Further studies will be needed to confirm these results in large validated cohorts.

MAMALIAN TARGET OF RAPAMYCIN (mTOR)

The kinase mTOR has also been implicated in the pathogenesis of AD (Pei and Hugon, 2008). mTOR is a serine/threonine kinase originating from two genes TOR1 and TOR2. Only mTORC1 interferes with rapamycin. mTOR can control cell growth, cell proliferation, protein synthesis and autophagy. Among the mTOR downstream targets controlling translation, the protein 70 kD ribosomal protein S6 kinase (p70S6K) has also been studied in AD. Previous studies of mTOR and p70S6K levels in AD brains have found contradictory results with either decreased concentrations (Lafay-Chebassier et al., 2005) or increased levels (Caccamo et al., 2010). We have carried out, using western blots, a research in a cohort of 32 AD patients and 33 controls assessing the levels of mTOR and p70S6k in PBMC (Paccalin et al., 2005, 2006a,b). mTOR levels were significantly reduced in AD patients as compared to controls and these levels correlated with cognitive scores of the free and cued recall tests (FCRT), the MMSE scores and the reverse digit span tests. Similar findings were detected for p70S6k levels which correlated with FCRT, reverse digit span test an oral denomination test. AD patients at an early stage of the disease had increased levels of these kinases and rather preserved neuropsychological scores. In addition, p70S6k levels also correlated with the results of emotional memory tests. Although it is difficult to extrapolate these results to neurons and brain, it is known that stressed neurons can perhaps modulate protein translation and increase the expression of the APP (Lesort et al., 1997). The links between kinase levels in brain and PBMC need to be compared in future researches. Nevertheless assessing the PBMC levels of these kinases might be a useful way to determine new screening biomarkers in patients with memory complaints.

EUKARIOTIC INITIATION FACTOR KINASE 2 (PKR2)

PKR is a proapoptotic serine/threonine kinase activated by virus, inflammatory and toxic cellular signals and is responsible for translation initiation blockade via the phosphorylation of its downstream target, the eukaryotic initiation factor two α (eIf2α). In addition to triggering apoptosis, PKR is involved in innate immunity and the process of inflammation that are typical features observed in AD brains (Dabo and Meurs, 2012). Using immunohistochemistry, we have demonstrated in 2002 that the levels of PKR and eIf2α were increased in AD brains as compared to control individuals. PKR labeling was often but not always associated with neurofibrillary tangles and hyperphophorylated tau protein (Chang et al., 2002a,b). Later we have observed increased levels of PKR and phosphorylated PKR (pPKR) in the CSF of AD and MCI patients as compared to neurological controls (Mouton-Liger et al., 2012). CSF activated PKR levels were predictive of cognitive decline of the patients; high CSF concentrations of PKR were observed in rapidly declining patients over a 2-year period (Dumurgier et al., 2013). We have also assessed the levels of total PKR and activated PKR and eIf2α in PBMC from AD patients and control individuals. The results demonstrated significant increased levels of these two cellular signals in AD patients. These concentrations were correlated with MMSE scores, FCRT, and reverse digit span cognitive tests (Paccalin et al., 2006b). High levels of PKR and eIf2α were detected in the more severely affected AD patients. These findings associated with the previous ones suggest that mTOR levels in PBMC are higher at the onset on the clinical disease whereas PKR levels are higher over the course of the disease. How the levels of these biomarkers are during the silent and preclinical periods of AD remains to be explored in order to determine their usefulness as markers of the biological phase of the disease (Jack et al., 2018). Although the links between cerebral and PBMC levels of these kinases are not very well known, it is worthy to notice that the kinase mTOR favors cell growth whereas the kinase PKR is part of the cellular apoptotic process.

PROTEIN KINASE C (PKC)

The PKC family includes transmembrane serine/threonine kinases regulating signal transduction and encompasses in

mammals 12 isoenzymes including PKC-α, PKC-δ, and PKC-ζ. PKC deficit has been implicated in the pathogenesis of AD (Malik et al., 2015). It has been postulated that a PKC deficiency could lead to memory loss, increased Aβ levels, enhanced phosphorylated tau and augmented neuroinflammation. There are two studies reporting results of blood PKC levels in AD patients. In 2006, a manuscript showed abnormal PKC conformation in red blood cells of affected patients (Janoshazi et al., 2006). Using a new and specific fluorescent probe called Fim-1, the authors demonstrated that PKC conformation is modified in AD patients ($N = 33$) and not in Parkinson's patients ($N = 15$) as compared with healthy individuals ($N = 25$). This alteration was not linked to age or to duration of disease and the authors proposed that this test could be a screening method in patients with cognitive problems. In 2008 another report using flow cytometry analyzed three PKC isoforms PKC-α, PKC-δ, and PKC-ζ in T-lymphocytes extracted from PBMC and exposed to Aβ. AD patients ($N = 40$) and healthy controls ($N = 40$) were explored. The findings have shown that Aβ activated T-lymphocytes from AD patients revealed highly expressed cell subpopulation with phosphorylated PKC-δ and PKC- ζ. This result was not observed in PBMC from controls as well as in freshly purified non Aβ-exposed T-lymphocytes from both groups (Ciccocioppo et al., 2008). The assessment of PKC in PBMC or red blood cells could be performed in further research to validate this approach as a screening tool in patients with cognitive complaints.

OTHER KINASES OR PHOSPHATASES

Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DIRK1A) is able to phosphorylate many substrates and is implicated in tau phosphorylation via an interaction with GSK3β. A recent study has demonstrated that serum levels of DIRK1A were modified in AD. Using slot-blotting method in human serum to detect kinase relative expression, the results in 26 AD patients and 25 controls revealed a significant reduction of DIRK1A levels in AD patients as compared to controls. Serum levels correlated with CSF tau and phosphorylated tau concentrations (Janel et al., 2014).

Phosphatases have also been explored as putative AD biomarkers. For example, thiamine diphosphatase and thiamine monophosphatase are implicated in thiamine metabolism and their heparin plasma activities were recently measured in 45 AD patients and 38 control individuals. Significantly increased activities of both phosphatases were detected in AD patients as compared to controls suggesting that a disturbed thiamine diphosphate metabolism was present in AD patients (Pan et al., 2017).

G-protein-coupled receptor kinase-2 (GRK2) is largely involved in the regulation of the G-protein-coupled receptors at the cell membrane. GRK2 is increased in the brain of AD patients (Obrenovich et al., 2006). Using qPCR and western blots, GRK2 mRNA and protein levels were explored in blood lymphocytes of mild (MMSE: 18–24) and moderate to severe (MMSE < 18) AD patients. The findings revealed that GRK2 mRNA and protein levels were significantly increased in both groups of AD patients with a major augmentation in severely affected patients (Leosco et al., 2007).

MULTI-TARGET KINASE THERAPIES AND BIOMARKERS

There is so far no treatment able to block or attenuate AD brain lesions in humans and new disease modifying drugs are currently evaluated in many clinical trials. Kinase inhibitors have been already tested in AD without current success. For example, the GSK3 inhibitor tideglusib was assessed but no significant clinical outcomes were obtained (Lovestone et al., 2015). We have seen in this review that many kinases present in human brains are involved in signaling pathways implicated in AD brain lesions such as Aβ metabolism, tau phosphorylation, neuronal apoptosis and neuroinflammation. A new therapeutic approach targeting several kinases was recently highlighted (Tell et al., 2016). A pharmacological intervention in AD using this method will need blood companion biomarkers readily available to evaluate drug targeting of these new kinase inhibitors. In the future, blood-based kinase biomarkers could be included in diagnostic and therapeutic evaluations in preclinical patients or MCI due to AD patients.

CONCLUSION

We have seen that many results concerning blood-based kinase biomarkers in AD need new findings in lager confirmatory cohorts and in longitudinal follow-ups of patients. There are several limitations in the studies reported so far in AD. (1) Very few publications have reported the results in confirmatory cohorts with a large numbers of patients. (2) Most of the studies have focused the research on one or a few kinase levels or activities but large-scaled proteomics focalized on this type of proteins should be worth assessing in MCI, AD patients and controls. Phospho-peptides could also be assessed on a large scale. (3) The real signification of blood kinase changes described in this review is not so far perfectly elucidated. Further studies will be needed to assess if these modifications originate from a general disturbed kinase metabolism or from signals coming from the AD brain.

It is plausible to envisage in the future that several kinase levels could be simultaneously assessed in the blood (PBMC, RBC Proceed, plasma) of patients. The use of an algorithm incorporating this kinome analysis might be useful to screen MCI or pre-symptomatic patients and eventually to compare these results with those obtained in the CSF of patients as seen for PKR and JNK3. Finally specific blood-based kinase biomarkers could be incorporated as surrogate markers of target engagement in new clinical trial assessing kinase inhibitors.

AUTHOR CONTRIBUTIONS

JH, EC and CP made the review of the literature and wrote the manuscript. FM-L and JD performed some experiments. All authors read and corrected the manuscript.

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Regional Cerebral Perfusion and Cerebrovascular Reactivity in Elderly Controls With Subtle Cognitive Deficits

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Background: Recent studies suggested that arterial spin labeling (ASL)-based measures of cerebral blood flow (CBF) as well as cerebral vasoreactivity to CO_2 (CVR CO_2) show significant alterations mainly in posterior neocortical areas both in mild cognitive impairment (MCI) and Alzheimer disease. It remains, however, unknown whether similar changes occur in at risk healthy elders without clinically overt symptoms. This longitudinal study investigated patterns of ASL perfusion and CVR CO_2 as a function of the cognitive trajectories in asymptomatic elderly individuals.

Methods: Seventy-nine community-dwelling subjects (mean age: 78.7 years, 34 male) underwent three neuropsychological assessments during a subsequent 3-year period. Individuals were classified as stable-stable (SS), variable (V), or progressive-progressive (PP). Between-group comparisons were conducted for ASL CBF and transit-time delay maps and β-maps of CO_2 response. Spearman's rho maps assessed the correlation between ASL (respectively, CVR CO_2 measures) and Shapes test for working memory, as well as Verbal fluency test for executive functions. Three group-with-continuous-covariate-interaction designs were implemented to investigate group-based differences on the association between neuropsychological scores and ASL or CO_2 measures.

Results: Comparison of CBF maps demonstrates significantly lower perfusion in the V-group as to PP-cases predominantly in parietal regions, including the precuneus and, to a lesser degree, in temporal and frontal cortex. A stronger CVR CO_2 response was found in the PP-group in left parietal areas compared to the V-group. V-cases showed a stronger ASL-Shape value relationship than V-group in right temporoparietal junction and superior parietal lobule. CO_2-Shape value correlation was significantly higher in both SS and PP-groups compared to the V-group in right insular and superior perisylvian regions.

Conclusion: Our data indicate the presence of decreased ASL and CVR CO_2 values mainly in parietal and fronto-temporal areas in cases with the first signs of cognitive

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instability (V-group). Importantly, the PP-group, at high risk for MCI transition, displays an increase of both parameters in the same areas. Clinicoradiologic correlations also indicate a clear distinction between the V-group and both PP and SS-cases. These data imply the presence of an inverted U-shape pattern of regional blood flow and CVR in old age that might predict subsequent cognitive fate.

Keywords: arterial spin labeling, asymptomatic controls, cerebrovascular reactivity, brain perfusion, clinicoradiologic correlations, CO_2

INTRODUCTION

Age-related changes in cerebral blood flow (CBF) have been related to increased risk for cognitive decline and Alzheimer disease (AD), implying that cerebrovascular mechanisms play a pivotal role in brain health and sustenance of cognition (Wierenga et al., 2014; Montagne et al., 2015; Popa-Wagner et al., 2015; Sierra-Marcos, 2017). For the past three decades, the nuclear medicine techniques Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) have served as gold standards for perfusion and metabolism studies in brain aging and AD. These techniques, however, require use of radioactive tracers and are more expensive that the more recently developed perfusion-weighted MRI techniques. Among these latter, arterial spin labeling (ASL) uses magnetically labeled arterial blood water as a diffusible endogenous tracer and displays similar diagnostic ability to detect AD as fluoro-2-deoxy-D-glucose (FDG)-PET (Tosun et al., 2016). This technique revealed brain hypoperfusion mainly in bilateral parietal areas, precuneus, angular and posterior cingulate cortex in MCI and early AD, which overlaps with the patterns of hypometabolism on FDG-PET observed later in disease progression, indicating the potential to use ASL for early detection of cognitive decline (for review see Alsop et al., 2008; Chen et al., 2011; Haller et al., 2016; Fällmar et al., 2017; Riederer et al., 2018). The rare ASL studies showed a more diffuse hypoperfusion in posterior inferior and frontal aspects of the brain in at risk healthy controls (Tosun et al., 2016; de Vis et al., 2018). Higher ASL measured-CBF in medial frontal, lateral temporal, parietal cortex, insula, and basal ganglia was reported in APOE ε4 carriers with the worst cognitive performances (Zlatar et al., 2016).

The capacity of brain vasculature to enhance blood flow in response to challenging conditions is found to be a key parameter in very early stages of neurodegeneration, even prior to the development of clinically overt cognitive deficits. In fact, higher ASL values in these cases may be the consequence of the brain vasculature's efforts of adapting to a threatening cellular environment. Blood oxygenation level dependent (BOLD) functional MRI allows for assessment of this reactivity. Recent data has indicated that the cerebral vasoreactivity CO_2 (CVR CO_2) could detect significant dysfunctions both in MCI and AD cases. In contrast, whether or not similar changes occur in at risk healthy elders is still unknown (Cantin et al., 2011; Yezhuvath et al., 2012; Richiardi et al., 2015).

Within this study, both ASL perfusion and CVR CO_2 patterns were explored in 79 community-dwelling elderly individuals who were cognitively preserved at inclusion and had undergone two neuropsychological assessments during a subsequent 3-year period. The data revealed distinct patterns of brain perfusion and cerebrovascular reactivity as a function of the cognitive trajectories of elderly controls.

MATERIALS AND METHODS

Participants

The series used in our analysis is part of a population-based longitudinal study on healthy aging funded by the Swiss National Foundation of Research in Geneva. The research protocol was approved by the Ethics Committee of the University Hospitals of Geneva. All experimental procedures were carried out in accordance with the approved guidelines and with the principles of the Declaration of Helsinki. All participants were given written informed consent prior to inclusion. Participants were contacted via advertisements in local media to guarantee a community-based sample. Exclusion criteria included psychiatric or neurologic disorders, sustained head injury, history of major medical disorders (neoplasm or cardiac illness), alcohol or drug abuse, regular use of neuroleptics, antidepressants or psychostimulants and contraindications to MR imaging. To eliminate possible confounding effects of cardiovascular disease, individuals with subtle cardiovascular symptoms and a history of stroke and transient ischemic episodes were also excluded from the present study.

The final sample included 79 participants, classified as cognitively healthy controls (mean age 78.7 ± 3.5 years; 44 women) who underwent three neuropsychological evaluations (baseline 18 months and 36 months follow-up) and a MRI-T1 examination (only baseline). Clinical assessment included the Mini-Mental State Examination (MMSE, Folstein et al., 1975), the Lawton Instrumental Activities of Daily Living (IADL, Barberger-Gateau et al., 1992) and the Hospital Anxiety and Depression Scale (HAD, Zigmond and Snaith, 1983), the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery, Digit Span (Wechsler, 1955) and Corsi block (Milner, 1971) for verbal and visual working memory respectively, the Trail Making Test A and B (Reitan, 1958) for executive functioning, Digit-Symbol-Coding (Wechsler, 1977) for attention, Boston Naming (Kaplan et al., 1983) for language, Ghent Overlapping Figures (Schnider et al., 1997) for visual gnosis and RI-48 Cued Recall Test (RI-48) for episodic memory (Haller et al., 2017). Individuals meeting the DSM-IV criteria of dementia or for MCI on the basis of

clinical and neuropsychological assessments, were excluded from the study (Haller et al., 2017). In order to explore correlations between the neuropsychological performances and imaging variables, and given the limited sample size, we selected two main tests that encompass visual working memory (Shapes test, Baddley et al., 1994) and executive functions (Phonemic verbal fluency Bruyer and Tuyumbu, 1980). Descriptive statistics and statistical differences between the SS, V, and PP group on age, gender and the neuropsychological scores were calculated with separate one-way ANOVA's using IBM SPSS Statistics version 25.

Neuropsychological Follow-Up

For follow-up measurements, which took place 18 months after inclusion, the cognitively healthy individuals underwent full neuropsychological assessment once again. Individuals who obtained stable cognitive scores over the baseline and follow-up evaluation were classified as stable controls. The progressive control group obtained a follow-up evaluation of at least 0.5 standard deviations (SD) lower than measured at baseline, on a minimum of two cognitive tests. Two neuropsychologists clinically assessed all individuals independently. The final classification was determined by a trained neuropsychologist taking into account both the results of the neuropsychological tests and overall clinical assessment (Xekardaki et al., 2015). All of the cases were assessed once again 18 months later with the same neuropsychological battery. The participants were subsequently grouped as described above (−0.5 SD in at least two cognitive tests), with comparison of the scores of the latest assessment. Stable individuals showing no changes in the second assessment were classified in the stable-stable (SS) group and progressive individuals demonstrating a further decline as progressive-progressive (PP). The variable group (V) refers to participants demonstrating a fluctuating scoring pattern, incorporating stable-progressive, progressive-stable or progressive-improved individuals. The final sample included 24 SS, 33 V, and 22 PP cases.

ASL

MR Imaging

As described previously in more detail (van der Thiel et al., 2018), ASL imaging was performed on a 3T GE MR750w using a 32-channel head array coil. Perfusion images were acquired with a 3D stack-of-spiral fast spin echo sequence preceded by a Hadamard encoded Pseudo-Continuous ASL (PCASL) module with background suppression. A total label duration of 4 s. was encoded into seven sub-blocks. The label durations were 0.22, 0.26, 0.30, 0.37, 0.48, 0.68, and 1.18 s, post label delays were chosen to be 1.00, 1.22, 1.48, 1.78, 2.15, 2.62, and 3.32 s. The total scan time lasted for 4.02 min.

Images were created at all delay times. The combined delay map consists of the sum of the delay times per subject. Two CBF maps were used for subsequent analysis, the raw uncorrected flow maps and the transit time corrected flow maps adjusted for arterial transit time.

Key imaging parameters were: field of view (FOV) = 22.0 cm, slice thickness 4.0 mm, 32 slices, bandwidth ± 62.5 kHz, 4 arms with 640 points each. The PCASL images had a matrix size of 128 × 128 and a voxel size of 1.88 × 1.88 × 4.0. Images were acquired with an echo time of 10.5 ms and a repetition time of 5936 ms. Acquisition included a T1/PD weighted reference image with matched parameters for quantification. The T1/PD combination image was acquired with the same TR as the perfusion images (5936 ms) and was formed by saturation recovery sequence with a 2.0 s saturation time. This saturation time is the same as that of the ASL sequence. The T1 value for blood assumed in the CBF quantification was 1.60 s. The Image reconstruction was performed using IDL based recon code and reconstructed images were stored as DICOM images into the scanner's database.

Data Preprocessing ASL

The ASL data was processed using the fMRI utility of the Brain Software Library (FSL, Version 5.0.9[1]). The combined delay image was obtained per participant and non-brain tissue was removed using the Brain Extraction Tool (BET[2], part of FSL). The brain extracted combined delay maps were normalized to Montreal Neurological Institute (MNI) standard space using an Echo Planar Image (EPI) template from the Statistical Parametric Mapping (SPM8) toolbox, standard space using linear registration (FMRIB Linear Image Registration Tool[3], part of FSL). The concatenated transformation matrix of the transit delay maps was then applied to the uncorrected flow maps, the transit time corrected flow maps and the transit delay maps to spatially normalize the data to the EPI template.

The normalized ASL images were smoothed with a 5 mm FWHM Gaussian kernel using a dilated 2 mm brain extracted MNI mask (FSLUtils[4], part of FSL). Due to the low intrinsic signal to noise level of ASL, we have decided to apply spatial smoothing in order to improve detection of group differences since spatial normalization intrinsically includes a certain degree of smoothing. Furthermore, some statistical procedures require smoothed data.

ASL Group Comparisons

All of the following analysis were executed for the corrected, uncorrected and delay maps separately. The cognitive groups were compared using voxel-wise permutation-based testing (Randomize[5], part of FSL), with threshold-free cluster-enhancement correction for multiple comparisons applied (Smith and Nichols, 2009) and $p < 0.05$ considered significant. Five thousand permutations were executed per contrast. The MNI 2 mm brain extracted mask was used during randomization for masking of non-brain voxels. A triple T-test design was administered with age and gender as non-explanatory co-regressors (Liu et al., 2012). Age was defined as the difference in days between the date of birth and the day of scanning.

[1]http://fsl.fmrib.ox.ac.uk/fsl

[2]http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET

[3]http://www.fmrib.ox.ac.uk/fslwiki/FLIRT

[4]http://www.fmrib.ox.ac.uk/fslwiki/Fslutils

[5]http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomize

ASL-Based Clinicoradiologic Correlations

For each group, Spearman rho correlations were calculated between the Shapes test and the Verbal fluency test and the corrected, uncorrected and delay maps separately using MATLAB version R2017b, to assess clinicoradiologic correlations in each group.

Group differences in correlation between the neuropsychological scores on the Shapes test, and the Verbal fluency test and the ASL response were investigated with a continuous-covariate-interaction-design[6].

To see whether the relationship between the ASL response and the neuropsychological test differed between groups, a triple *T*-test with 6 contrasts was carried out per neuropsychological test, representing all possible differences in scores between groups. The cognitive groups were once again compared using voxel-wise permutation-based testing with 5000 permutations per contrast (Randomize; see text footnote[5], part of FSL), threshold-free cluster-enhancement correction for multiple comparisons applied (Smith and Nichols, 2009) masked with the 2 mm brain extracted mask and $p < 0.05$ considered significant.

CVR CO_2

CO_2 Admission

The CO_2 administration protocol is described in more details elsewhere (Richiardi et al., 2015). In short, the CO_2 challenge consisted of 9 min of CO_2 admission via a nasal cannula. A concentration of 7% CO_2 mixed with synthetic air was given with the sequence 1 min OFF, 2 min ON, 2 min OFF, 2 min ON, 2 min OFF. Subjects were asked to breathe normally through the nose. During admission, EPI covering the entire brain was acquired using a 32 multi-channel coil with the following parameters: FOV = 28.5 cm, 96 × 96 matrix, voxel size of $2.9688 \times 2.9688 \times 3$ mm^3, echo times of 30 ms, repetition time of 3000 ms, 45 repetitions.

Preprocessing MRI

The CO_2 data was processed using FSL Version 5.0.9 (Jenkinson et al., 2012). The functional sequences were realigned to the mean per sequence to correct for motion effects (Jenkinson et al., 2002) and non-brain tissue was removed using the Brain Extraction Tool (Smith, 2002). Individual structural images were skull-stripped and co-registered to a standard 2 mm MNI brain extracted by employing standard FLIRT procedure (Jenkinson et al., 2002).

The transformation matrices from the functional to the subject space were calculated using the mean functional images per subject with the epi_reg script provided by FSL (Jenkinson et al., 2002).

White matter (WM) and Cerebral Spinal Fluid (CSF) masks were calculated as follows; WM and CSF were segmented from the individual high-resolution 3D images with FMRIB's automated segmentation tool (Zhang et al., 2001). The resulting masks were compared with *a priori* tissue mask containing an average of MRI images of 152 subjects, as provided by the MNI and hereafter binarized with a threshold of 0.6 in subject space. For both WM and CSF masks, a mean time series of the functional data within the tissue specific mask was calculated and used to filter out the WM and CSF effects, and additionally the motion correction parameters with the FSL command-line tool. Smoothing was applied to the denoised functional data with a 5 mm FWHM Gaussian kernel using the brain-extracted mask of the mean functional image. As for the ASL data, we applied spatial smoothing to reduce the effects of noise on the group analysis. In addition, to ensure an analysis pipeline as similar as possible for both the CO2 and ASL data, the application of spatial smoothing was appropriate. In this manner, comparability of the two techniques was optimized.

The denoised, smoothed functional CO_2 data was normalized to the standard 2 mm MNI brain extracted template by usage of the FMRI Expert Analysis Tool v6.00, with the same normalization method employed as described earlier. In addition, a high pass filter of 270 s was applied to the data and pre-whitening was performed with FMRIB's Improved Linear Model.

First-Level Analysis CVR CO_2

The pre-processed functional CO_2 sequences were used to carry out the first-level analysis. A standard first-level FSL pipeline was employed to fit the CO_2 response to the subjects' response. Convolution of the ON/OFF response of the CO_2 admission was done using a simple square wave form, correcting for wash-in and wash-out effects in a straightforward and effective way (Mutch et al., 2012; Richiardi et al., 2015). The square wave form has shown to approximately model the increase and decrease of CO_2 levels during administration (Richiardi et al., 2015).

CVR CO_2 Response Group Comparisons

To investigate whether there was a difference in CVR CO_2 response between the SS, V and PP group, an *F*-test design was applied. To further specify which groups differentiated from one another on the CO_2 response, a triple *T*-test design was executed with a standard higher-level FSL pipeline.

CVR CO_2 Response-Based Clinicoradiologic Correlations

Spearman rho correlation was computed between the Shapes test and Verbal fluency test and CO_2 maps for each group, using MATLAB version R2017b. The relationship between the neuropsychological scores (the Shapes and the Verbal fluency tests) and the CVR CO_2 response was investigated by a three group with continuous-covariate-interaction-design see text footnote[6]. To see whether the relationship between the CVR CO_2 response and the neuropsychological test differed between groups, triple *T*-tests with 6 contrasts were carried out per neuropsychological test, covering all potential group-differences.

[6]https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM#Two_Groups_with_continuous_covariate_interaction

RESULTS

Demographic Data and Neuropsychological Test Scores

No statistically significant group differences were found in demographic variables (age and gender). Neuropsychological scores at baseline did not differ between the three groups (see **Table 1**).

ASL Group Comparisons

The quantitative CBF and CVR values of the included study groups are shown in **Table 2**. Comparison of the perfusion maps between the three groups demonstrated a significantly higher perfusion value in both the corrected and uncorrected maps of the PP group as compared to the V group. As can be seen in **Figure 1**, these regions largely overlap in both perfusion maps. Differences within the corrected flow maps between the groups can be clearly seen within the parietal regions, including the precuneus but also in temporal and frontal regions. Example corrected perfusion maps from the different groups emphasize the hypoperfusion within the precuneus of the V group once more, while in the example maps of both the SS and PP group perfusion in these regions remains preserved (**Figure 2**).

Group comparison of the uncorrected maps demonstrates a significantly higher perfusion in PP compared to the V group that was diffusely present in several neocortical areas. No other significant group differences were found in respect to ASL delay maps.

ASL-Based Clinicoradiologic Correlations

The Spearman correlation maps per group reveal distinct patterns as a function of the group of reference only for the Shapes test. In SS group, negative associations between ASL values and Shapes test performances were found in most areas with the exception of the frontal and cerebellar regions (**Figure 3**). A similar predominance of negative correlations was observed in PP group. Interestingly, the Spearman rho maps of

TABLE 1 | Demographical and neuropsychological data of the included study groups of the SS, V, and PP participants.

	Stable-Stable		Variable		Progressive-Progressive		*P*-value			
	Mean	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Group**	**SS vs. V**	**V vs. PP**	**PP vs. SS**
Gender (male)	11 M		13 M		10 M		$p = 0.967$, $\eta^2 = 0.000$	$p = 0.883$	$p = 0.900$	$p = 1.00$
Age (years)	78.86	4.122	78.96	4.074	78.19	2.158	$p = 0.728$, $\eta^2 = 0.091$	$p = 0.994$	$p = 0.727$	$p = 0.810$
Shapes	34.75	2.609	33.06	4.007	34.32	2.476	$p = 0.128$, $\eta^2 = 0.053$	$p = 0.134$	$p = 0.341$	$p = 0.894$
Verbal fluency	18.00	4.625	17.85	4.912	17.59	4.896	$p = 0.850$, $\eta^2 = 0.004$	$p = 0.878$	$p = 0.997$	$p = 0.866$
Trail A/B	2.53	0.814	2.71	0.982	2.72	0.668	$p = 0.668$, $\eta^2 = 0.011$	$p = 0.710$	$p = 0.997$	$p = 0.714$
MMSE	28.58	1.283	28.39	1.784	28.36	1.002	$p = 0.959$, $\eta^2 = 0.001$	$p = 0.992$	$p = 0.979$	$p = 0.956$
IADL	8.54	0.779	8.15	0.364	8.64	1.529	$p = 0.128$, $\eta^2 = 0.053$	$p = 0.276$	$p = 0.154$	$p = 0.938$
HAD	5.54	3.623	7.73	4.837	8.50	5.087	$p = 0.076$, $\eta^2 = 0.066$	$p = 0.184$	$p = 0.813$	$p = 0.080$
Digit span forward	7.96	1.732	9.03	2.243	8.23	1.974	$p = 0.119$, $\eta^2 = 0.054$	$p = 0.126$	$p = 0.326$	$p = 0.895$
Digit span backward	5.25	1.511	5.88	1.691	4.86	1.754	$p = 0.079$, $\eta^2 = 0.065$	$p = 0.338$	$p = 0.073$	$p = 0.710$
Corsi block forward	7.42	1.316	7.55	1.394	7.27	1.352	$p = 0.766$, $\eta^2 = 0.007$	$p = 0.934$	$p = 0.747$	$p = 0.932$
Corsi block backward	6.83	0.963	6.42	1.251	6.41	1.260	$p = 0.356$, $\eta^2 = 0.027$	$p = 0.400$	$p = 0.999$	$p = 0.443$
Boston naming	19.21	1.021	19.27	1.153	19.27	0.767	$p = 0.967$, $\eta^2 = 0.001$	$p = 0.970$	$p = 1.00$	$p = 0.975$
Ghent overlapping	4.96	0.204	4.94	0.348	5.00	0.000	$p = 0.682$, $\eta^2 = 0.010$	$p = 0.958$	$p = 0.659$	$p = 0.842$
CERAD	10.83	0.381	10.82	0.528	10.73	0.767	$p = 0.790$, $\eta^2 = 0.006$	$p = 0.995$	$p = 0.831$	$p = 0.803$
Digit-symbol-coding	55.38	12.521	55.18	8.658	51.95	11.090	$p = 0.466$, $\eta^2 = 0.020$	$p = 0.997$	$p = 0.515$	$p = 0.523$

No significant group-differences were found on age, gender or any of the neuropsychological tests.

TABLE 2 | Quantitative CBF and CVR values of the included study groups of the SS, V, and PP participants.

	Stable-stable		Variable		Progressive-progressive	
	Mean	***SD***	**Mean**	***SD***	**Mean**	***SD***
ASL CBF maps	36.180118	16.936111	34.896856	15.598446	34.043468	15.517011
ASL Delay maps	1535.553350	408.553332	1535.864498	410.159331	1523.346373	426.743474
CO_2 CVR maps	9.490050	25.768619	9.131709	25.240867	9.982506	25.039040

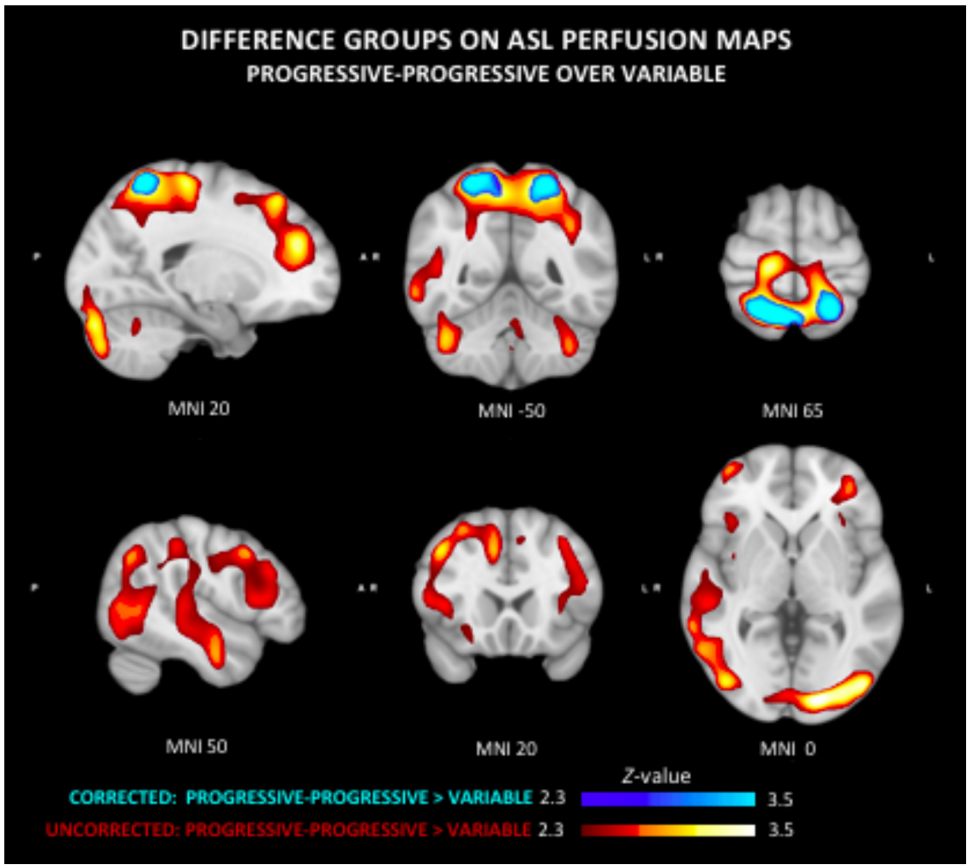

FIGURE 1 | Group differences in arterial spin labeling (ASL) perfusion measures. The progressive-progressive (PP) group shows regional higher perfusion values as compared to the variable (V) group in both the uncorrected and corrected flow maps. This difference can be clearly seen in the corrected flow maps in the parietal regions including the precuneus. The PP group similarly shows significantly higher perfusion than the V group within the parietal lobe, but also temporal and frontal regions.

the V group show prominent positive correlations in areas that show negative rho values in the SS group. Group comparisons showed significant differences in ASL-Shapes test correlation between the V and SS group in right temporo-parietal junction and superior parietal lobule (**Figure 4A**).

CVR CO_2 Response Group Comparisons

The *F*-test demonstrated that there were significant differences between groups on the CO_2 response (data not shown). The *T*-tests demonstrated a significantly stronger CO_2 response of the PP group in left parietal areas as compared to the V group (**Figure 5**). No regional differences in CO_2 response were found between the other groups.

CVR CO_2 Response-Based Clinicoradiologic Correlations

No group differences were identified in respect to the association between Verbal Fluency test performances and CVR CO_2 maps. **Figure 6** shows the Spearman's rho maps referring to the relationship between the CVR CO_2 maps and Shapes test. The SS and PP groups display a fairly similar pattern of correlation, showing a positive relationship between the CVR CO_2 response and Shapes test performance in medial brain regions with negative rho values in frontal cortex. In contrast, the V group demonstrates an opposite pattern with negative medial correlations, along with positive associations represented in parietal areas. CVR CO_2-Shapes test correlation was significantly higher in both the SS and PP groups compared to the V group in the right insular region and superior perisylvian areas (**Figure 4B**).

DISCUSSION

To our knowledge, this is the first study combining ASL perfusion and CVR CO_2 measures with a longitudinal follow-up of cognitive abilities in elderly individuals with preserved neuropsychological performances at baseline. Our data indicate that both imaging measures show a subtle decrease in cases with the first signs of cognitive instability (V group) suggesting the presence of cerebral hypoperfusion and decreased cerebrovascular reactivity mainly in parietal and fronto-temporal association areas in this particular group. Importantly, cases with

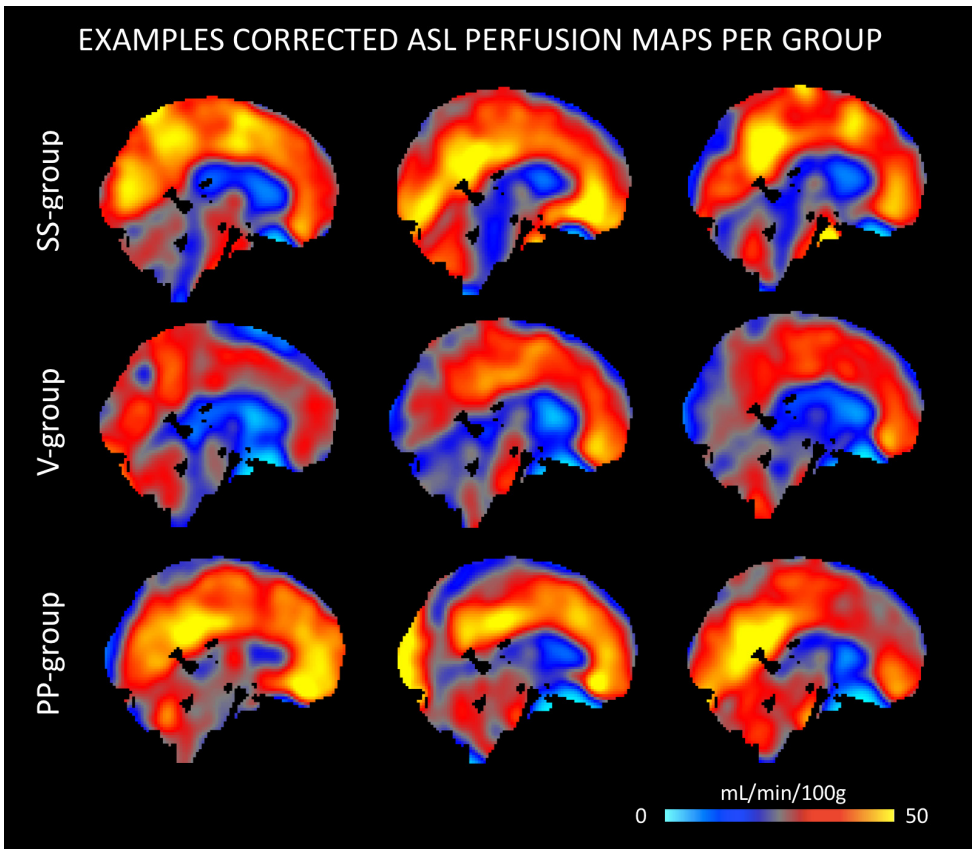

FIGURE 2 | Example ASL perfusion maps of stable-stable (SS), V, and PP group separately. The examples of the V group demonstrate a lower perfusion within the precuneus, while in the example maps of both the SS and PP group perfusion in these regions remains relatively preserved.

continuous cognitive decline (PP group) at high risk for MCI transition display an increase of both parameters in the same areas. The distinct profile of cognitively unstable cases compared to the two other groups of healthy controls is also documented by our clinicoradiologic correlations.

Early ASL-MRI contributions showed both hypoperfusion and hyperperfusion areas in MCI and AD cases stressing the brain efforts to compensate lesion invasion and cognitive loss. Despite controversial observations, two main patterns have been identified. A marked hypoperfusion in posterior cingulate cortex, precuneus and parietal cortex is already present in MCI cases (Johnson et al., 2005; Dai et al., 2009). In AD, a global decrease in blood flow is observed as compared to healthy controls, but region specific decrease in perfusion have also been detected (Austin et al., 2011). The hypoperfusion areas become more diffuse in clinically overt AD as to MCI including temporo-occipital and parieto-occipital cortices as well as orbitofrontal cortex (Alsop et al., 2000; Asllani et al., 2008; Fleisher et al., 2009; Austin et al., 2011; Bron et al., 2014; Haller et al., 2016). A hyperperfusion in hippocampus and basal ganglia was reported both in MCI and AD cases but also in non-symptomatic high risk APOE ε4 carriers (Alsop et al., 2008; Dai et al., 2009; Fleisher et al., 2009; Ding et al., 2014). ASL data on cognitively preserved elderly persons are very scarce. They showed both hypoperfusion in frontal, parietal and cingulate areas but also a strong negative association between diffuse hyperperfusion in neocortical association areas and cognitive performances in APOE ε4 carriers pointing to the presence of compensatory mechanisms explained by a pathological elevation of neural activity, inflammation or increased blood supply through vascular dilation or increased vascular density. In a longitudinal study, we first reported that decreased ASL values in posterior cingulate cortex were associated with subtle cognitive changes in cognitively intact elderly subjects (Xekardaki et al., 2015). More recently, ASL perfusion rates in medial frontal and anterior cingulate cortex predicted cognitive performances in a 4-year follow up of healthy elders (de Vis et al., 2018). Our longitudinal findings in carefully selected healthy controls shed some light into subtle changes of brain perfusion in the very initial stages of cognitive instability. In agreement with the observations made in MCI and AD cohorts, we found a subtle decrease of ASL values mainly in parietal cortex and precuneus (and to a lesser degree in temporal and frontal cortex) that is already present at inclusion in cases with cognitive fluctuations over a subsequent 3-year follow-up. PP cases showed increased ASL values in the same areas at inclusion (even higher than those in the SS group yet non-significant), but they deteriorate continuously suggesting that

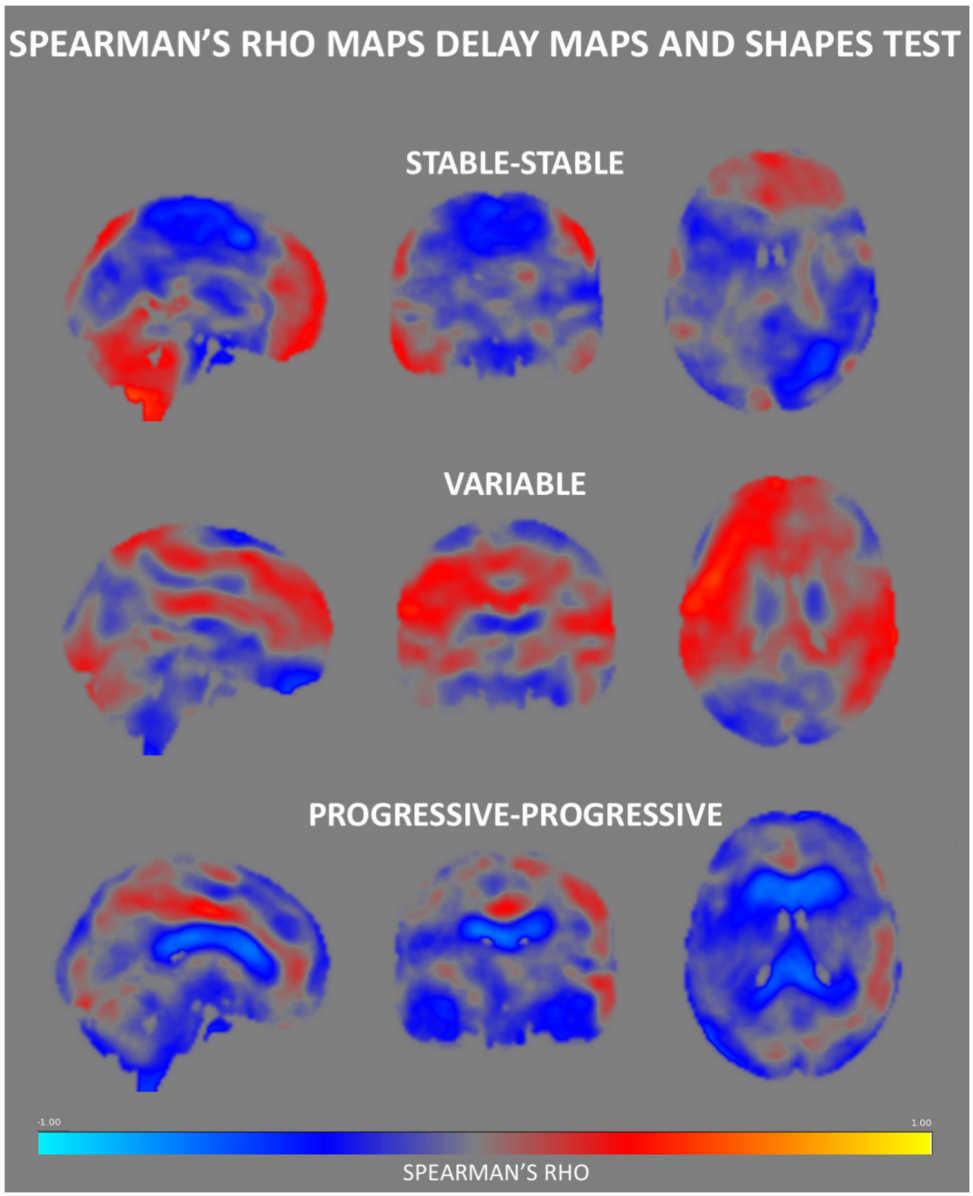

FIGURE 3 | Spearman's rho correlation maps between ASL values and the Shapes test performance. A predominance of negative associations was found in the SS group, with positive correlations residing in the frontal and cerebellar regions. The PP group also displays a negative association in most of the brain areas especially in regions surrounding the ventricles. Interestingly, the Spearman rho maps of the V group show global prominent positive correlations in areas that show negative rho values in the SS group, residing largely in the medial part of the brain.

hyperperfusion in these areas is not an efficient defense against the neurodegenerative process. This idea is further supported by the CVR CO_2 measures showing a slightly decreased brain cerebrovascular reactivity in V cases with a steady increase in PP cases mainly in parietal areas. As for ASL, early studies using BOLD functional MRI documented CVR CO_2 decrease mainly in temporal, parietal and posterior cingulate areas in AD cases but also in hippocampus in MCI cases (Cantin et al., 2011; Yezhuvath et al., 2012; Richiardi et al., 2015). Taken together, these data imply the presence of an inverted U-shape pattern of regional blood flow and cerebral vasoreactivity in parietal cortex in old age that might predict subsequent cognitive fate. In fact, the cerebral vasoreactivity to CO_2 slightly decreased in control cases with fluctuant cognitive performances, shows a compensatory increase in these with continuous decline prior to the MCI status before a marked decrease in MCI and AD cases. The clinico-radiologic correlations between Shapes test measures, an indicator of visual working memory, and both ASL and CVR CO_2 data point further to the different behavior of V cases compared to both PP and SS cases. Similar differences were not observed in respect to Verbal fluency possibly because of the absence of significant associations

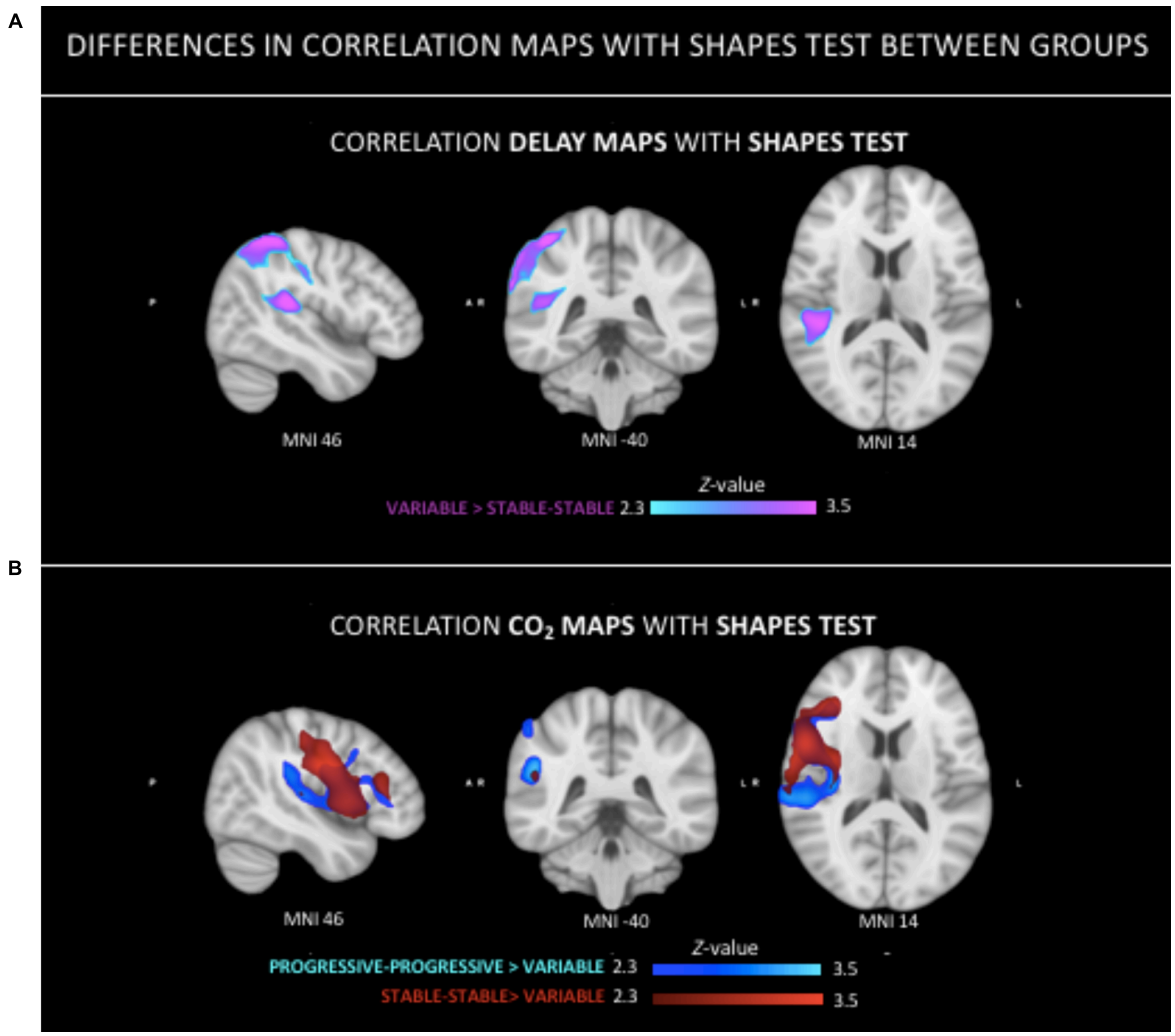

FIGURE 4 | Group differences in the relation between neuroimaging parameters (ASL, CVR-CO_2) and Shapes test performance. **(A)** Stronger positive relationship in the tempo-parietal junction and superior parietal lobe in V group as compared to the SS group. **(B)** Stronger positive correlation in both the PP and SS group as compared to the V group in right peri-sylvian and superior areas.

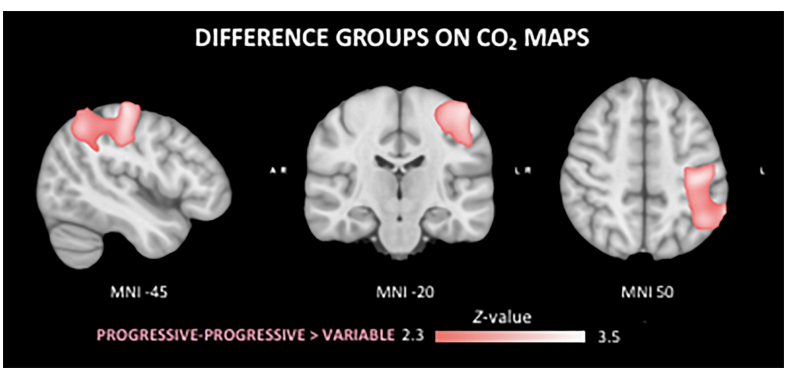

FIGURE 5 | Differences in the cerebral vasoreactivity to CO_2 (CVR CO_2) response between groups. The PP group shows a stronger CVR CO_2 response in comparison to the V group in the superior left parietal lobe.

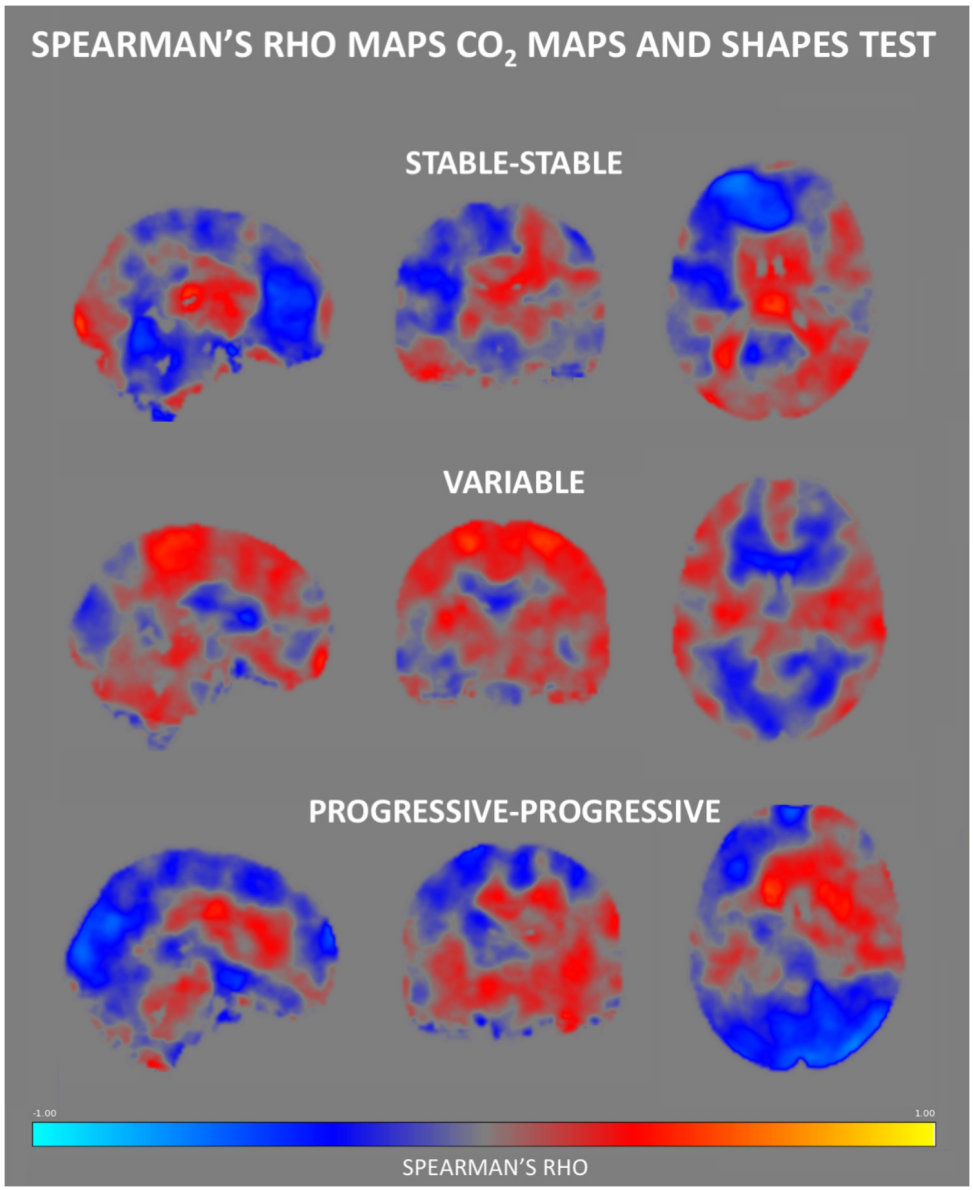

FIGURE 6 | Regional Spearman's rho correlation maps between CVR CO_2 response and the Shapes test performance. Both the SS and PP groups displayed a fairly similar pattern of correlation, showing a positive relationship in the medial brain, with a frontal display of negative rho values. In contrast, the V group demonstrates negative correlations in medial brain regions, along with positive ones in parietal areas.

between cognitive performance and imaging parameters for this test.

The biological significance of these observations remains matter of debate. Cortical hypometabolism is a core feature in preclinical AD and is associated with worst clinical evolution (Ewers et al., 2013; Besson et al., 2015; Mendes et al., 2018). However, increased glucose metabolism was also reported in amyloid-negative amnestic MCI cases and is thought to reflect compensatory mechanism to the neuronal damage occurring early in the disease process (for review see Ashraf et al., 2015). Whether the hypoperfusion observed mainly in parietal and posterior cingulate areas is causally related to AD or only its epiphenomenon remained unclear. Without bringing a definite answer, our data suggest that parietal and posterior cingulate cortex hypoperfusion is an early event in totally asymptomatic cases with fluctuations in cognitive performances within the normal range. It has been long thought that the hyperperfusion patterns in clinically overt AD and MCI may reflect increased neural activity as part of compensatory mechanisms aiming to counterbalance the cognitive decline. Alternatively, they may reflect alteration in brain vasculature due to increased angiogenesis or increased cerebrovascular reactivity possibly reflecting dysregulation of the neurovascular unit without any significant gain in terms of cognitive performance (for review

see Zlatar et al., 2016; Sierra-Marcos, 2017). The present observations combining ASL and CVR CO_2 measures clearly support the second hypothesis. In fact, our PP cases displayed higher ASL and CVR CO_2 values in parietal areas prior to their cognitive decline. These results parallel the findings of Zlatar et al. (2016) in APOE ε4 healthy carriers who reported a negative association between verbal memory function and ASL values in medial fronto-temporal and parietal cortex.

Strengths of the present study include the 3-year neuropsychological follow-up, careful exclusion of MCI and incipient AD cases, and combined use of ASL and CVR-CO_2 techniques. Several limitations should, however, be considered when interpreting these data. In the absence of longer follow-up, the cognitive fate of PP and V cases remains uncertain. No CSF measures of tau and Aβ protein were available in this work so that the real extent of AD pathology remains unknown. Most importantly, the small sample size may mask subtle imaging differences between SS and PP as well as V cases. Future studies including PET amyloid and tau assessment of AD pathology as well as longer follow-up are warranted to define better the role of cerebrovascular mechanisms in the prediction of cognitive deterioration in asymptomatic elderly individuals.

AUTHOR CONTRIBUTIONS

All authors contributed to the concept and preparation of the manuscript. MvdT, SH, and PG were responsible for data interpretation. CR, SH, and PG collected the data.

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Neurocompensatory Effects of the Default Network in Older Adults

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The hemispheric asymmetry reduction in older adults (HAROLD) is a neurocompensatory process that has been observed across several cognitive functions but has not yet been examined in relation to task-induced relative deactivations of the default mode network. The present study investigated the presence of HAROLD effects specific to neural activations and deactivations using a functional magnetic resonance imaging (fMRI) n-back paradigm. It was hypothesized that HAROLD effects would be identified in relative activations and deactivations during the paradigm, and that they would be associated with better 2-back performance. Forty-five older adults (*M* age = 63.8; range = 53–83) were administered a verbal n-back paradigm during fMRI. For each participant, the volume of brain response was summarized by left and right frontal regions of interest, and laterality indices (LI; i.e., left/right) were calculated to assess HAROLD effects. Group level results indicated that age was significantly and negatively correlated with LI (i.e., reduced left lateralization) for deactivations, but positively correlated with LI (i.e., increased left lateralization) for activations. The relationship between age and LI for deactivation was significantly moderated by performance level, revealing a stronger relationship between age and LI at higher levels of 2-back performance. Findings suggest that older adults may employ neurocompensatory processes specific to deactivations, and task-independent processes may be particularly sensitive to age-related neurocompensation.

Keywords: older adults, neurocompensation, fMRI, default mode network, HAROLD

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INTRODUCTION

The number of individuals aged 65 or older is projected to exceed 1.5 billion in 2050 (National Institute on Aging and World Health Organization, 2011) and will comprise approximately 30% of the population by 2060 (Parker et al., 2012). While this expansion is due in part to advancements in healthcare, the aging population will present new challenges for older adults (OAs) and their caretakers. In addition to OAs affected by neurodegenerative conditions, such as Alzheimer's disease (Alzheimer's Disease International, 2010), OAs without such conditions also exhibit well-studied patterns of neurocognitive decline. These include declines in processing speed (Salthouse, 1996; Charness, 2008), selective attention (Barr and Giambra, 1990; Madden, 1990), working memory (WM) (Balota et al., 2000; Zacks et al., 2000), and task-switching (Kray and Lindenberger, 2000; Kray et al., 2002). Underscoring their functional significance, these decrements have been linked to declines in independent living among healthy OAs (Suchy et al., 2011; Duda et al., 2014).

Consistent with cognitive findings, neuroimaging studies have identified patterns of neural changes in non-demented OAs. Structural neuroimaging studies have revealed age-related reductions in prefrontal and parietal white matter integrity (Persson et al., 2005; Grady, 2012) and hippocampal and prefrontal gray matter volumes (Haug and Eggers, 1991; Raz et al., 2004). Functional neuroimaging studies, using positron emission tomography (PET) and functional magnetic resonance (fMRI), have revealed age-related increases and decreases in task-related brain activity. These changes were initially thought to reflect poorer brain function underlying cognitive decline; decreased activation was thought to reflect a reduced allocation of neural resources, whereas increased activation was considered a marker of reduced efficiency and selectivity of neural responses, referred to as *dedifferentiation* (Li and Lindenberger, 1999; Li and Sikström, 2002; Grady, 2008). While dedifferentiation is still a viable explanation for some age-related neural changes, some patterns of alterations have been positively associated with cognitive function (Berlingeri et al., 2010) and have contributed to a general theory of *neural compensation* (Grady, 2012).

The theory of neurocompensation was initially invoked to explain observations of increased neural activity while OAs performed cognitive tasks as well as younger adults (YAs; Cabeza, 2002), or when increased activity positively correlated with performance only in OAs (McIntosh et al., 1999; Reuter-Lorenz et al., 2000). This view suggests that activation of alternate brain regions may serve to counteract cognitive decline (Cabeza, 2001, 2002; Cabeza et al., 2004). Evidence of age-related neurocompensation has been found using tests of episodic retrieval (Davis et al., 2008; McDonough et al., 2012), visuospatial skills (Lee et al., 2011), WM (Rypma et al., 2007; Carp et al., 2010), response inhibition (Hsieh and Fang, 2012; Sebastian et al., 2013), and selective attention skills (Davis et al., 2011; Ansado et al., 2012). These effects are frequently reported in the prefrontal cortex (PFC; Goh, 2011), which is consistent with prior literature of neurocompensatory processes in OAs (Cabeza et al., 1997; Madden et al., 1999a,b). These findings collectively support the age-related compensatory hypothesis, although several questions remain unanswered. For example, it is unclear which cognitive processes are supported by neurocompensatory mechanisms (Rajah and D'Esposito, 2005; Greenwood, 2007; Zarahn et al., 2007).

One prominent model of age-related neurocompensation is the hemispheric asymmetry reduction in OAs (HAROLD), which postulates that under similar circumstances, PFC activation during cognitive processes tends to be less lateralized in OAs than YAs (Cabeza, 2002). HAROLD and other age-related neurocompensatory theories offer different predictions of age-related neurocompensatory processes. For example, the compensation-related utilization of neural circuits hypothesis (CRUNCH) theory posits that the magnitude of network activation increases with task demand (Reuter-Lorenz and Cappell, 2008), while the scaffolding theory of aging and cognition (STAC) suggests a recruitment of additional networks when the primary network becomes inefficient (Park and Reuter-Lorenz, 2009). The HAROLD model is particularly attractive for studying age-related neurocompensation due to its focus on the PFC, which is consistent with the majority of age-related neurocompensatory findings (see Goh, 2011 for review). Moreover, patterns of activation consistent with HAROLD have been well-validated using a range of neuroimaging paradigms, from simple motor tasks (Mattay et al., 2002) to more complex tasks, such as verbal working memory (VWM) and episodic retrieval (Bäckman et al., 1997; Cabeza et al., 1997; Reuter-Lorenz et al., 2000; Morcom and Friston, 2012). HAROLD thus offers a useful framework for investigating age-related patterns of neurocompensation.

While the HAROLD model has been well-documented, several aspects of this model are not fully delineated. For example, some evidence suggests that HAROLD patterns observed in prefrontal regions may be specific to higher levels of task demand (Berlingeri et al., 2013). In addition, studies assessing the HAROLD activation pattern have not consistently evaluated or found support for the relation between neurocompensatory activity and behavioral performance. For example, using PET to investigate age-related neural changes in verbal and spatial WM, OAs showed an increased bilateral response in the PFC that was associated with slower reaction time but equivalent WM accuracy. There has also been some evidence to suggest the presence of HAROLD relative to functional connectivity (FC), though with limited evidence of cognitive change (Li et al., 2009).

Another aspect of the HAROLD model that has not been clarified is the distinction between relative activation and deactivation. Despite substantial evidence for HAROLD effects in task-related activation patterns, neurocompensation relative to task-independent neural processes have been understudied. The default mode network (DMN) has been defined by coactivation within a distributed network of cortical regions, which characterizes the resting state of the human brain (Greicius et al., 2004). Evidence suggests that several properties of the DMN are sensitive to the aging process (Spreng et al., 2016; Klaassens et al., 2017). First, reduced DMN coherence has been associated with declines in processing speed (Ng et al., 2016), WM (Hampson et al., 2006), and cognitive control (Turner and Spreng, 2015). Second, suppression of DMN regions (relative deactivation), which occurs during task engagement (Anticevic et al., 2012; Binder, 2012) is a marker of efficient executive functioning (EF) that becomes compromised in OAs (Duverne et al., 2008; Petrella et al., 2011; Spreng et al., 2016; Avelar-Pereira et al., 2017).

Evidence suggests that DMN deactivations may be particularly susceptible to aging and a potential contributor to neurocompensatory processes. For example, using fMRI and spatial judgment tasks, Park et al. (2010) reported significantly reduced deactivations in OAs, and importantly, faster reaction times were demonstrated by OAs who did effectively deactivate DMN regions. In addition, Miller et al. (2008) reported enhanced DMN deactivation in YAs that correlated with successful memory encoding; however, OAs did not show this pattern, and reduced deactivation was most evident among poorer performers. Using a verb generation task, Persson et al. (2007) also reported an age-related reduction in DMN deactivations that was associated with slower reaction time performance. Perhaps the strongest evidence of DMN-related neurocompensation is described by

Davis et al. (2008), who developed the posterior-anterior shift in aging (PASA) hypothesis to explain the observation that OAs showed a pattern of reduced deactivations of posterior midline cortex, with increased deactivations of medial frontal cortex during a verb generation task. When matching groups on performance, OAs showed greater DMN deactivation of the left anterior cingulate and right anterior insula, as well as increased activations in the left middle frontal gyrus and right supramarginal gyrus. Both deactivation and activation effects were seen bilaterally, which, in the context of the relation between the DMN and task-positive network functions, suggests that the deactivations may also support cross-hemispheric neurocompensatory processes. Importantly, given the inverse correlation between DMN and task-positive networks that supports cognitive functioning (Fox et al., 2005), these bilateral effects suggest that an age-related neurocompensatory process (i.e., HAROLD) of relative deactivations may support successful task-positive functioning.

Further investigations of task-independent processes are needed to determine how the DMN and task-independent networks may interact with other models of neurocompensation, such as the HAROLD pattern in OAs. Since HAROLD and PASA models do not have direct support from studies of relative deactivation and given a lack of clarity in prior literature whether effects included summation of activation and relative deactivation or only activation effects, we considered examination of activation and relative deactivation separately to have high theoretical and methodological importance. The present study provides the first examination of HAROLD lateralization effects in both brain activations and relative deactivations in healthy OAs. Using fMRI during a WM paradigm, we evaluated the presence of the HAROLD pattern in the task-dependent brain response because neural activation associated with VWM has previously demonstrated sensitivity to age-related neurocompensatory processes, including the HAROLD effects. Second, using a resting fixation baseline to isolate suppression of baseline DMN activity, we evaluated the presence of HAROLD among task-induced deactivations. Moderation analyses were conducted to address whether observed HAROLD effects were associated with cognitive performance (i.e., n-back accuracy). Two homologous frontal lobe regions of interest (ROI) were used to calculate the laterality indices (LI) and examine the expected HAROLD effects. It was hypothesized that (1) OAs would demonstrate HAROLD patterns of task-elicited relative brain activation and deactivations, and (2) effects would be significantly moderated by 2-back performance, as a reflection of successful compensation.

MATERIALS AND METHODS

Participants

Participants included 45 healthy, right handed English-speaking men and women over the age of 50 (25 women, age range 53–83, *M* age = 63.78 years, *SD* = 7.99) who comprised a healthy control group in a larger study of neurocognitive function in cardiovascular disease (Haley et al., 2009). These participants were recruited from the community via advertisements in the Providence, RI area. All participants underwent standard informed and written consent procedures. Assessments were conducted over three visits that spanned approximately 6 weeks and included a neuropsychological assessment, an echocardiogram, and an MRI scanning session. Exclusion criteria included left-hand dominance, corrected visual acuity poorer than 20:40, below 60% performance accuracy on the 2-back VWM task (i.e., < 1 *SD* from 50%), low global cognitive function (>1.5 *SD*s below the sample population on the Mini Mental Status Examination), or any MRI contraindications (e.g., metal implants). Significant medical (e.g., surgery, heart infarct), neurological (e.g., multiple sclerosis, traumatic brain injury with loss of consciousness), and psychiatric problems (e.g., substance abuse with hospitalization, diagnosis of any current psychiatric illness) were exclusion criteria that were assessed by interview, physical examination, review of medical records and self-report questionnaires. Participants were compensated for their participation. The study was approved and monitored by the university and hospital institutional review boards (IRB) where the research took place and conformed to the Helsinki Declaration on human subjects' protection.

Demographic characteristics, estimated intellectual functioning, and 2-back performance are displayed in **Table 1**. The study sample comprised OAs with right-handed dominance (*M* = 86.7, *SD* = 14.40; range = 55–100; on a scale of 0-100, with increasing values representative of increasing right-dominance) and above average intellectual functioning (WTAR range = 96–119, mean = 111.53, *SD* = 6.40) and years of educational attainment (range = 12–21 years; mean = 16.36; *SD* = 1.96). WM performance (i.e., 2-back accuracy) was consistent with prior 2-back literature (Braver et al., 1997; Smith and Jonides, 1999; Sweet et al., 2008).

Behavioral Measures

Verbal working memory paradigm. The n-back paradigm was employed to challenge VWM systems during two imaging runs. The n-back has been widely used in functional neuroimaging research for more than 20 years and has the advantage of a well-described fMRI neural response (e.g., Braver et al., 1997; Smith and Jonides, 1999; Owen et al., 2005; Sweet et al., 2008). During

TABLE 1 | Demographic and mean summary data.

Variable	Mean	SD	Min	Max
Age (years)	63.78	7.99	53.00	83.00
MMSE	29.40	0.70	28.00	30.00
Handedness (%R)	86.7	14.40	55.00	100.00
Education (years)	16.36	1.96	12.00	21.00
Predicted FSIQ	111.5	6.40	96.00	119.00
2-back accuracy	82.98	9.70	64.00	99.00

MMSE, Mini mental status exam; Handedness was measured on a scale of 1-100, with increasing values representative of right-handed dominance, and all subjects reported right-handedness; Education, years of formal education attained; FSIQ, Full-scale intelligence quotient from the Wechsler Test of Adult Reading; 2-back accuracy includes correct hits and rejections proportional to the number of trials.

the 2-back, six series of 15 consonants were presented visually for 500 ms each, with an interstimulus interval (ISI) of 2500 ms. Participants were asked to make a *yes* or *no* button-press response following each consonant to report whether or not it was the same as the consonant (irrespective of capitalization) presented two earlier in the series (e.g., underlined letters in the following sequence would be answered "yes": w, N, r, N, R, Q, r, q, N, W, n . . .). Six 0-Back control blocks of nine consonants each were presented with the same duration of letter presentation and ISI. 0-Back blocks preceded each 2-Back block during the first imaging run and followed the 2-Back during the second run. Participants responded *yes* when a predetermined target consonant ("H" or "h") appeared and *no* for other consonants. Consonant blocks of both conditions contained 33 percent "yes" targets in random locations within each series. Capitalization was randomized throughout to encourage verbal encoding. Two 27-s (27000 ms) blocks of resting fixation blocks were presented between the 0-Back/2-Back cycles. 2-back performance was calculated for each participant using the following formula: (number of correct positives + correct negatives)/ total number of letters presented. Two subjects were excluded from behavioral analyses due to near-chance 2-back performance (<60% accuracy). A diagram of the n-back task is presented in **Supplementary Materials**.

Neuroimaging Measures

MRI Acquisition. Whole-brain echo-planar fMRI was conducted using a Siemens TIM Trio 3 tesla scanner (TR = 2500 ms, TE = 28 ms, $FOV = 192^2$, matrix size = 64^2, in 42 3-mm-thick axial slices). This procedure yielded 116 whole-brain volumes for each of the two 288s imaging runs, yielding a spatial resolution of 3 mm^3 per voxel. Whole-brain high-resolution T1 images were also acquired in the sagittal plane for anatomical reference (TR = 1900 ms, TE = 2.98 ms, $FOV = 256^2$ mm, matrix size 256^2). Two conditions of the n-back paradigm (i.e., 2-back and 0-back) and a resting state "+" were presented using E-prime (Psychology Software Tools, Sharpsburg, MD, United States) and back-projected onto a screen visible to the participant via a mirror mounted to the head coil.

MRI analysis. MRI dataset processing and statistical analyses were performed with Analysis of Functional NeuroImages version 18.0.05 software (AFNI; Cox, 1996). Preprocessing of the functional runs included slice-time correction and registration of each volume to the third volume of the first imaging run to correct for head movement. Data from participants with head movement of > 3.0 mm in any direction (i.e., x, y, z, yaw, pitch, and roll) or movement greater than 0.3 mm on more than 25% of repetitions were omitted from analyses. The functional volumes were aligned to the anatomical volume in Talairach space. A 5-mm full-width half maximum Gaussian filter was applied and the raw time-series was scaled to a mean of 100. For each subject, the general linear model (GLM) was used to quantify 2-back activity relative to a resting state (i.e., fixation across) after controlling for 0-back active control task and other covariates (i.e., movement parameters) in order to facilitate examination of task-independent deactivations.

Individual activity maps. The resulting individual activity maps were thresholded (two-tailed $\alpha = 0.01$) and corrected for multiple comparisons using AFNI's false discovery rate (FDR; $q = 0.05$) procedure in order to provide a measure of volumetric activity, measured by significantly activated voxels beyond the threshold per ROI, for each participant.

Group level processing. For group level-analyses, two frontal ROIs were defined based on left or right hemisphere (i.e., relative to Talairach coordinate x-plane = 0) which extended to y-plane = 0. LIs, defined as left relative to right (left/right) frontal ROI response to the 2-back were calculated using each individual's volume of significant response in each ROI (i.e., voxels of significant activity beyond threshold). Consistent with prior literature (Deblaere et al., 2004; Baciu et al., 2005; Yuan et al., 2006), an LI was calculated by subtracting intensity effects within the right frontal ROI from intensity effects within the left frontal ROI, then dividing this value by the average of mean activity across the two frontal ROIs (i.e., (left frontal − right frontal) / (left + right frontal). Thus, the scaling of LI was such that positive values indicate left-lateralized function and negative values indicate right-lateralized function. This process was conducted separately for relative activation and deactivation and repeated for volumes of significant activation and deactivation.

Group level analyses. Qualitative procedures were first conducted to examine the validity of brain activation patterns exhibited by the sample. In order to generate relative group level activation and deactivation maps for comparison to prior literature, the whole-brain unthresholded voxel-wise effects were tested against a hypothetical mean of zero (i.e., no 2-back effect) using one-sample t-tests. These whole-brain analyses were conducted using an FDR-corrected threshold of $p = 10^{-9}$ (voxels greater than t = 8.42) with and a minimum of 10 contiguous voxels. Clusters of significant n-back response exhibited by our sample was compared to prior n-back literature (e.g., Smith and Jonides, 1999; Owen et al., 2005); in addition, the Neurosynth Image Decoder was used to quantify the concordance between the whole-brain results and neuroimaging studies included in the publicly available database. Next, hemispheric lateralization was examined. Consistent with prior literature, hemispheric dominance was determined by the size of the LI (Deblaere et al., 2004; Baciu et al., 2005; Yuan et al., 2006), following the criteria adopted by Yuan et al., 2006, in which an LI threshold of 0.10 (representative of 10 percent greater left relative to right hemispheric activity) was considered evidence of lateralization. Lastly, support for HAROLD effects were assessed by performing bivariate correlation analyses between age and each LI.

Behavioral analyses. Hierarchical multiple regression and moderation analyses were conducted using the Statistical Package for Social Sciences IBM SPSS Statistics 21.0 to test the influence of 2-back accuracy on HAROLD effects. Before conducting moderation analyses, assumptions of multiple linear regressions were examined, including homoscedasticity, independence of residuals and normality of residuals (Cohen et al., 2003). Multicollinearity between the independent variable and moderator were reduced through the use of centering (Aiken and West, 1991). For moderation analyses, the PROCESS SPSS macro plug-in (Hayes, 2012) was applied to examine the moderating influence of n-back performance on the relation

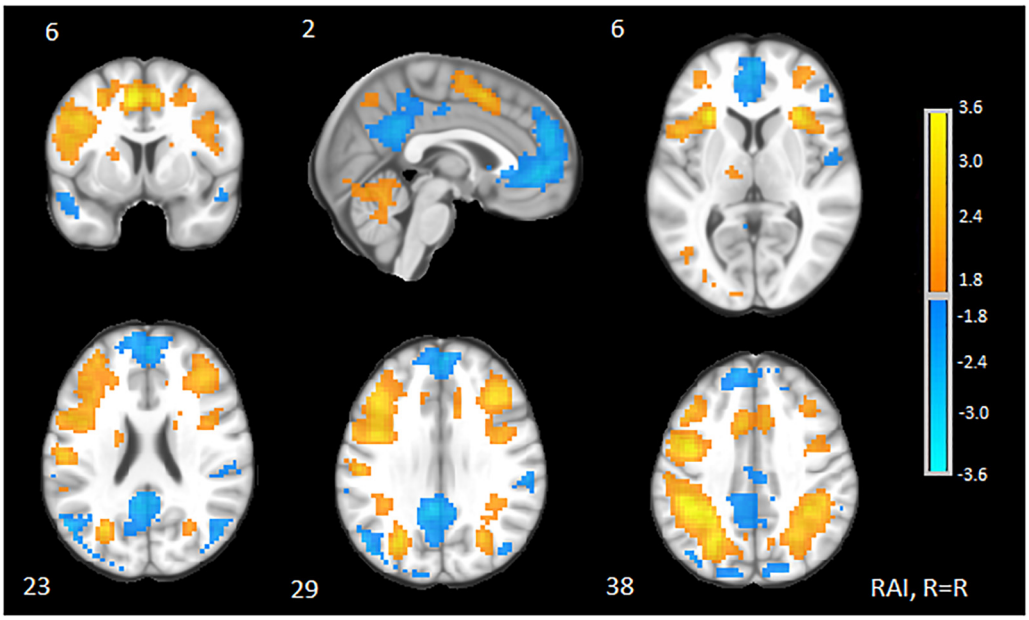

FIGURE 1 | Clusters of significant neural response to 2-back. Representative of 2-back task-based activations and deactivations relative to a resting state baseline ("+") from GLM analysis. Slices are provided in Talairach space and shown in RAI orientation (right = right). Yellow, activations; Blue, deactivations. Cluster centers of mass are reported in **Table 2**. Slice number (Z) is located above each slice. Color bars indicate magnitude effects for activations and deactivations.

between age and LI. For the present study, the interactions were visually probed in order to examine the nature of any moderation effects by examining conditional effects (i.e. simple slopes) at low (−1 *SD* below the mean) and high (+1 *SD* above the mean) levels of 2-back performance.

RESULTS

Whole Brain Voxelwise Analyses

The 2-back versus baseline contrast revealed widespread activation patterns commonly associated with n-back performance (Smith and Jonides, 1999; Owen et al., 2005), including the bilateral middle frontal gyrus, medial frontal gyrus, inferior parietal cortices, insula, and cerebellum. Relative deactivations were also consistent with prior literature (Anticevic et al., 2012; Binder, 2012) and overlapped substantially with regions associated with the DMN (Buckner et al., 2008), including the medial frontal gyrus/anterior cingulate, posterior cingulate, and superior frontal gyrus. Results from the Neurosynth Decoder, which was used to quantify the concordance of the present results to those in the publicly available database, indicated a value of 0.32 that was most consistent with task-specific measures of WM. See **Figure 1** and **Table 2** for neural patterns and related activity maps.

Laterality and HAROLD Effects

Volumetric effects of the 2-back were summarized by the two frontal ROIs for both activations and deactivations. LIs were then calculated from these values and averaged at the group level. The *activation LI* yielded left-lateralization of this VWM task

TABLE 2 | Clusters of significant neural response to 2-back versus resting state.

Region	Voxels	x	y	z
L inferior parietal lobule	239	34	50	41
B posterior cingulate	231	0	−47	10
R cerebellum	151	−26	53	−22
L medial frontal gyrus	57	5	−5	51
L precentral/postcentral gyrus	51	35	22	53
B medial frontal	46	2	48	30
L inferior frontal/precentral gyrus	45	42	−3	33
L insula	37	29	−22	9
R inferior parietal lobule	36	−42	44	41
R insula	34	−31	−23	6
R medial frontal gyrus	25	−9	−15	45
L cerebellum	21	32	50	−26
R middle frontal gyrus	21	−39	−29	29
R superior parietal lobule	20	−30	60	42
L middle frontal gyrus	14	25	6	50

L, Left; R, Right; B, Bilateral. Coordinates reported in center of mass Talairach space, RAI orientation. These clusters are shown in ***Figure 1****.*

(M = 0.15, SD = 0.19) as expected (Jonides et al., 1997). Similarly, the relative *deactivation LI* was left-lateralized (M = 0.17, SD = 0.19). Descriptive statistics and the computed LI values are presented in **Table 3**.

The presence of HAROLD was then examined via bivariate Pearson correlations between age and each LI. The LI of neural activations was significantly and positively associated with age for volumetric effects $r(42)$ = 0.34, p = 0.02 (see **Table 4**). Consistent with the HAROLD model predictions, the LI of neural

TABLE 3 | Neural effects of relative activations, deactivations, and laterality indices (LIs).

Regions	Mean	SD	LI
Activations			
Left Frontal	606	395	0.12*
Right Frontal	478	324	
Deactivations			
Left Frontal	511	310	0.17*
Right Frontal	363	233	

*ROIs, Regions of interest relative to task-induced activations and deactivations. Mean, Mean number of significantly active voxels within each frontal ROI; SD, Standard deviation of significantly active voxels within each frontal ROI. *Denotes left-lateralization of LI (Yuan et al., 2006).*

TABLE 4 | Correlations among demographics, cognition, and LI.

Variable	Age	Education	n-back
Activations			
Age			
Education	−0.02		
n-back	−0.18	0.05	
Laterality Index	0.34*	0.08	−0.15
Deactivations			
Age			
Education	−0.02		
n-back	−0.18	0.04	
Laterality Index	−0.31*	0.14	0.37*

$p < 0.05$. A significant relation between age and the laterality index of neural deactivations was interpreted as consistent with the HAROLD effects.

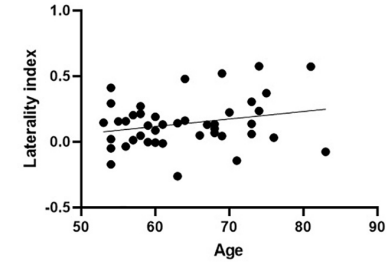

FIGURE 2 | Aging and increased left-lateralization of neural activations. A significant and positive relationship between age and laterality index of neural activations was not considered consistent with the neural pattern of the hemispheric asymmetry reduction in older adults (HAROLD) effect.

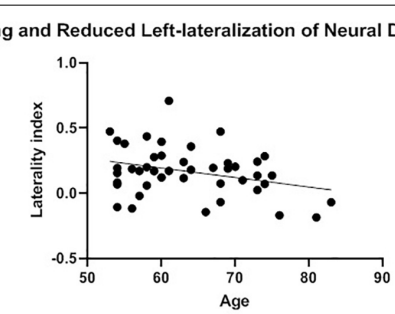

FIGURE 3 | Aging and reduced left-lateralization of neural deactivations. A significant and positive relationship between age and laterality index of neural deactivations was considered consistent with the neural pattern of the hemispheric asymmetry reduction in older adults (HAROLD) effect.

deactivations were significantly and negatively associated with age $r(42) = -0.31$, $p = 0.04$ (see **Table 4**). Scatter plots of the relation between age and LIs for relative activations and deactivations are presented in **Figures 2**, **3**, respectively, with residuals shown in **Supplementary Materials**.

Moderating Effect of 2-Back Accuracy

Hierarchical multiple regression and moderation analyses were conducted to examine the influence of 2-back performance on the relation between age and LIs. Two models were used to explore the four statistically significant relations between age and LIs of relative activations and deactivations.

For neural activations, age significantly predicted LI ($b = 0.01$, $p = 0.03$). However, 2-back accuracy ($b = 0.00$, $p = 0.98$), and the age × 2-back accuracy interaction term ($b = 0.00$, $p = 0.96$) were not significantly predictive of LI (total $R^2 = 0.13$, $p = 0.96$). Moderation analysis specific to neural deactivations, however, yielded significant effects. Over and above age ($b = -0.01$; $p = 0.02$), 2-back accuracy ($b = 0.01$; $p = 0.05$) was a significant predictor of unique variance in LI (total $R^2 = 0.28$; $F(3, 40) = 5.09$, $p < 0.001$). In addition, the interaction term of 2-back accuracy x age was a significant predictor of unique variance in LI over and above age and 2-back accuracy ($\Delta R^2 = 0.08$; $\Delta F(3,40) = 4.33$; $b = 0.00$; $p = 0.04$). Results of this moderation effect are presented in **Table 5**.

Given evidence of an interaction effect between age and 2-back accuracy on the relative deactivation LI, simple slopes analyses were conducted by estimating the conditional effect of age at specific values of 2-back performance (in this case, ± 1 SD from the sample mean, or −11.50 and 11.50, respectively) and tested whether the slopes were statistically significant from zero by a null hypothesis test (see Hayes, 2013). Results of these analyses revealed an association between age and LI, with age significantly related to LI for higher levels of 2-back performance ($b = -0.01$, $p = 0.01$, 95% *CI*s = −0.05, −0.00) but not significantly related to LI for lower levels ($b = -0.00$, $p = 0.82$, 95% *CI*s = −0.01, 0.01). In other words, high 2-back performers exhibited a stronger negative relationship between age and deactivation LI, or HAROLD effects. A plot of the simple slopes analyses is presented in **Figure 4**.

DISCUSSION

Hemispheric asymmetry reduction in older adults is a well-established neurocompensatory process that has been consistently demonstrated to support healthy OAs maintenance of cognitive function (Reuter-Lorenz et al., 2000; Cabeza, 2002; Morcom and Friston, 2012). However, to date, no studies have

TABLE 5 | 2-back accuracy moderates age and deactivation laterality.

Total Model	R^2	***SE***	**F**	***p***	ΔR^2	
	0.28	0.03	5.09	0.00	0.08	
Model	*b*	*SE*	t	*p*	LLCI	ULCI
Main Effects						
Age	−0.01	0.00	−2.33	0.02	−0.01	0.00
n-back	0.01	0.00	2.02	0.05	0.00	0.01
Interaction						
Age * n-back	0.00	0.00	−2.08	0.04	0.00	0.00

*$p < 0.05$.

investigated HAROLD effects on relative deactivations of the DMN. The present study sought to determine if (a) HAROLD would generalize to task-induced activations and deactivations using a verbal n-back paradigm, and (b) if HAROLD effects would be significantly and negatively associated with 2-back performance. Results of whole-brain voxelwise analyses revealed expected patterns of neural response associated with the 2-back vs. a resting state baseline (Owen et al., 2005), including deactivations in DMN regions (Buckner et al., 2008). LIs calculated from 2-back-associated neural activation indicated left-lateralization that was consistent with previous findings (Smith and Jonides, 1999; Owen et al., 2005). Extending this finding, the deactivation LI also indicated left-lateralization. Regarding neurocompensatory processes, OAs demonstrated an unexpected age-related increase in left-lateralized activity that was not overtly associated with 2-back performance. In contrast, and consistent with our second hypothesis, an age-related change in the deactivation LI reflected a HAROLD pattern that was influenced by 2-back performance, such that high- but not low-performers demonstrated this shift.

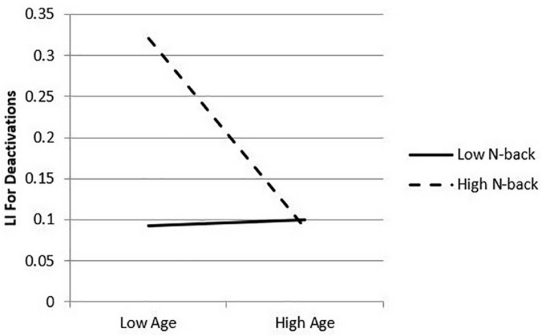

FIGURE 4 | Simple slopes plot of conditional effects of 2-back. 2-back performance moderates the relation between age and laterality index. Plot represents laterality index (greater number depicts greater left lateralization) as predicted by age at fixed values of the moderator, 2-back: +1 standard deviation (11.5) and –1 standard deviation (–11.5) from the sample mean.

The significant positive correlation between age and activation LI suggests that an age-related increase in left hemispheric brain response may have supported OAs' maintenance of cognitive functioning. While this finding appears to contradict the HAROLD model, it is consistent with evidence supporting the presence of other neurocognitive compensatory processes in OAs. For example, differences in capacity (i.e., the degree to which a brain network is maximally recruited to perform a task) has been associated with cognitive maintenance (Bosch et al., 2010). At the network level, the compensation-related utilization of neural circuits hypothesis (CRUNCH) theory predicts that increased cognitive demands will result in a concomitant increase in network function (Reuter-Lorenz and Cappell, 2008). In the same vein, evidence has supported the STAC, which posits that recruitment of additional neural circuits or even networks occur when the primary networks have become inefficient or damaged due to pathology (Park and Reuter-Lorenz, 2009). Thus, one or more broader theories of compensation may complement the HAROLD model as it relates to successful maintenance of VWM. The younger age range of the present sample, relative to the age of participants in prior HAROLD studies (Cabeza, 2002) is not inconsistent with more traditional compensatory models.

Uncertainty regarding the function of overactivation, however, has led to increased interest in cognitive performance correlates. For example, overactivation among OAs has been linked to both reduced (Logan et al., 2002; Milham et al., 2002; Johnson et al., 2004; Park et al., 2004; Thomsen et al., 2004) and maintained cognitive performance (Grady et al., 2003; Persson et al., 2004; Rossi et al., 2004; Gutchess et al., 2005) which have been interpreted to reflect dedifferentiation and compensation, respectively. In the present study, 2-back accuracy did not moderate the relation between age and LI for activations, suggesting that increased left-lateralized activity did not function to maintain performance. However, lack of an observable relation between overactivation and performance may not necessarily mean that these alterations are not compensatory in nature; for example, compensatory increases in bilateral recruitment may not be extensive enough to offset opposite-hemisphere reduction in efficiency (i.e., resulting in performance declines), or individual differences may not be related to performance on a specific task (see Grady, 2012 for review). Importantly, while higher performing OAs in the current study did not evidence a greater degree of overactivation, lower performing OAs also did not. It is therefore plausible that the lower performing OAs maintained previous levels of cognitive function prior to age-related decline via increased left-lateralized activation.

Findings of the present study support the notion that relative deactivations are sensitive to the aging process, lateralized similarly to task-induced activations, and associated with

successful task performance on a measure of VMW. A significant negative correlation between age and the deactivation LI, coupled with maintained 2-back performance (i.e., > 60% for each participant) suggests that reduced asymmetry of deactivations supported the OAs' maintenance of cognitive functioning. Therefore, the current results provide evidence of a novel HAROLD finding, extending previous findings to deactivations, and more specifically, via a within-group fMRI study of community-dwelling OAs. This finding is further strengthened by an observed moderation effect of 2-back accuracy, suggesting that reduced asymmetry of deactivations may support maintenance of baseline cognitive abilities. Results of simple slopes analyses, conducted to elucidate the nature of this effect, revealed that higher performing OAs demonstrated an increased reduction of asymmetry specific to deactivations, while lower performing OAs did not (see **Figure 2**). This is consistent with the growing expectation that neurocompensatory processes relate to an observable maintenance in cognition.

Due in part to observations of their importance in neurocognitive function, task-induced deactivations of the DMN have become the target of increased investigation (Anticevic et al., 2012; Binder, 2012; Gilbert et al., 2012; Spreng, 2012; Hansen et al., 2014; Spreng et al., 2016). Evidence suggests that deactivations are reduced in the aging process (Lustig et al., 2003; Rombouts et al., 2005; Grady et al., 2006) and associated with poorer task performance (Persson et al., 2007). The present findings suggest that DMN deactivations may also help to explain neurocompensatory processes. This notion is further substantiated by the relation between DMN and task-positive network functioning (Anticevic et al., 2012; Spreng et al., 2016); for example, cross-hemispheric neurocompensatory activations (e.g., HAROLD) may correspond with cross-hemispheric alterations in DMN deactivation patterns that are required for effective reallocation of neural resources.

Consistent with this notion, the PASA model has been used to explain an age-related posterior-anterior shift of task-related DMN deactivations that appear to support cognitive function, such as semantic fluency (Davis et al., 2008). Others have since identified general age-related reductions in DMN deactivation that were associated with slower reaction time on measures of spatial skills and higher scores on tests of EF (Persson et al., 2007; Park et al., 2010). Results of the present study provide further evidence that DMN deactivations may be particularly important for the maintenance of cognitive function in the face of age-related decline and warrant future investigations of neurocompensatory processes.

Limitations

Few studies have investigated HAROLD effects specific to WM among healthy OAs, and most were older PET studies. Prior literature has also focused almost exclusively on group differences (i.e., YA vs. OA) in HAROLD effects specific to task-dependent activations associated with WM. Interpretation of our findings by comparison to prior studies is therefore limited. A YA comparison group would have aided the interpretation of results. In addition, the sample of the present study was relatively young with respect to the aging literature (range = 53 to 83 years; mean = 63.78, *SD* = 7.99), as researchers often sample OA populations from 65 years and beyond. Interpretation of study findings are also limited by a relatively small sample size (i.e., not adequately powered to detect small effects). The sample was above average in intelligence, well-educated, and predominantly of Caucasian ethnicity (96%), which poses potential problems with generalizability.

Future Directions

As the first investigation of age-related task-independent neurocompensatory processes utilizing a within-group experimental design, findings of the current study warrant replication. Consistent with the CRUNCH model and a prior study of HAROLD (Berlingeri et al., 2013), results of the present study suggest that multiple compensatory processes may support cognitive function in old age. Given our identification of dual neurocompensatory processes (i.e., bilateralization of task-independent deactivations and increasing left-lateralization of task-related activations) during a VWM paradigm, future investigations may also benefit from consideration of both task-dependent and task-independent brain responses in the context of newer models of neurocompensation such as CRUNCH and STAC. Evidence of HAROLD effects among relative to deactivations suggest that baseline DMN processing may be altered in OAs. Therefore, decline in cognitive performance and associated task-related brain response may be explained, in part, by changes in baseline processing.

A better understanding of factors that influence neurocompensatory processes may aide healthy OAs in maintaining cognitive function and *aging gracefully*. For example, several sociological and cultural factors have been linked to maintenance of cognitive function in aging, such as education, occupational complexity, social activity, and physical exercise (Katzman, 1993; Valenzuela et al., 2008; Stern, 2012), and brain changes associated with these factors is a topic of growing interest (Barulli and Stern, 2013; Stern, 2016). Future research of age-related neurocompensatory processes may also aide researchers and clinicians in identifying problem areas in OAs (e.g., less efficient processing, overactivations and performance declines), and those at-risk of developing neurodegenerative conditions. Early identification of at-risk OAs may in turn inform developing cognitive training programs, which refer to a range of structured programs (e.g., computerized tasks engaging EF systems) intended to maintain cognition or ameliorate cognitive deficits (Bahar-Fuchs et al., 2013). A growing literature suggests that cognitive (Karbach and Verhaeghen, 2014) and physical exercise interventions (Bherer, 2015) induce neural changes associated with improved cognitive performance (for review, see Bamidis et al., 2014) and appear to be a promising future complement to pharmacological interventions for OAs and warrants continued research.

ETHICS STATEMENT

The study was approved and monitored by the university and hospital institutional review boards (IRB) where the research

took place and conformed to the Helsinki Declaration on human subjects' protection. All participants underwent standard informed and written consent procedures.

AUTHOR CONTRIBUTIONS

BD, MO, EH, and LS contributed to the design and implementation of the research, analysis of the results, and writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2019.00111/full#supplementary-material

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REVIEW
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Early Secure Attachment as a Protective Factor Against Later Cognitive Decline and Dementia

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The etiology of neurodegenerative disorders such as dementia is complex and incompletely understood. Interest in a developmental perspective to these pathologies is gaining momentum. An early supportive social environment seems to have important implications for social, affective and cognitive abilities across the lifespan. Attachment theory may help to explain the link between these early experiences and later outcomes. This theory considers early interactions between an infant and its caregiver to be crucial to shaping social behavior and emotion regulation strategies throughout adult life. Furthermore, research has demonstrated that such early attachment experiences can, potentially through epigenetic mechanisms, have profound neurobiological and cognitive consequences. Here we discuss how early attachment might influence the development of affective, cognitive, and neurobiological resources that could protect against cognitive decline and dementia. We argue that social relations, both early and late in life, are vital to ensuring cognitive and neurobiological health. The concepts of brain and cognitive reserve are crucial to understanding how environmental factors may impact cognitive decline. We examine the role that attachment might play in fostering brain and cognitive reserve in old age. Finally, we put forward the concept of affective reserve, to more directly frame the socio-affective consequences of early attachment as protectors against cognitive decline. We thereby aim to highlight that, in the study of aging, cognitive decline and dementia, it is crucial to consider the role of affective and social factors such as attachment.

Keywords: attachment, protective factor, aging, cognitive decline, dementia

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INTRODUCTION

As the prevalence of dementia continues to increase, the fear of cognitive decline is becoming a central preoccupation in the elderly population. Multiple genetic and environmental factors play a role in the development of dementia, and a great deal of scientific interest is currently focused on identifying relevant risk and protective factors. Various types of dementia exist, including Alzheimer's Disease, Vascular Dementia, Lewy Body Dementia, and Frontotemporal Dementia. Additionally, Mild Cognitive Impairment is used to describe an intermediate state between healthy

aging and dementia. Mild Cognitive Impairment is characterized by cognitive deficits and related dysfunction not severe enough to be diagnosed as dementia, though the presentation can vary considerably among individuals (Winblad et al., 2004). Even in healthy aging, it is normal to observe some decline in several but not all cognitive domains. Whereas working memory, episodic memory, processing speed, and some aspects of short-term memory are typically impacted, other abilities such as general language or basic conceptual functions tend to be spared.

In the search for protective factors against cognitive decline and dementia, the potential role of early attachment has been largely overlooked. Many studies have demonstrated that a supportive social environment in old age can protect against the progress of cognitive decline (Bennett et al., 2006; Gow et al., 2007). However, early social support may also have long lasting psychosocial, cognitive and neurobiological consequences. Furthermore, a safe early social environment could offer protection against cognitive decline through its effects on the establishment of particular emotions regulation strategies.

Early attachment refers to the quality of the interaction between the child and the primary caregiver. By repetition, the child progressively integrates these interactions into mental representations, which allow for the establishment of long-term attachment patterns. These patterns will then determine social behavior and emotional regulation strategies throughout adult life, and will be particularly useful in times of stress as they may help the individual to regain a feeling of comfort and well-being (Bretherton and Munholland, 1999; Cassidy, 2000; Waters and Waters, 2006). Prompted by multiple experiences of loss, separation, and vulnerability associated with aging, these established attachment patterns may pervade a person's perceptions, feelings and attitudes, both in healthy aging and in the presence of cognitive disorders (Bradley and Cafferty, 2001; Perren et al., 2007). Therefore, we believe that it is highly important to study attachment patterns in this particular population.

We will begin this review by introducing the concept of attachment and it's impact on an individual's social relations. We will then discuss how quality social relations may protect against cognitive decline. Next, we will examine how attachment might affect cognitive and neurobiological development of various brain structures and systems and the consequences this may have for later cognitive decline, as a vast literature has shown that some early environmental factors can significantly modulate and drive the interplay of numerous processes involved in brain maturation (Gluckman et al., 2008; Brummelte, 2017). Finally, we will discuss how early attachment might contribute to brain and cognitive reserve, which play a role in protecting against cognitive decline. We will consider evidence from different developmental fields of study including epigenetics, neurobiology and psychology.

ATTACHMENT, SOCIAL FACTORS, COGNITIVE DECLINE, AND DEMENTIA

"Man is by nature a social animal" Aristotle, *Ethique à Nicomaque*.

For many species, living in a group ensures a sustained food supply, protection against predators, reproduction and the opportunity to learn survival skills. Mammalian infants in particular are highly dependent on social interactions for their day-to-day needs. In humans, deprivation and neglect can have devastating and long-lasting consequences on children's intellectual and emotional development. One example of this is hospitalism syndrome, which is a form of developmental stunting first described by Spitz (1945). This syndrome refers to the severe physical and psychological retardation observed in children separated from their mothers for several months during their first year of life. Spitz (1947) observed that this deprivation of affection caused a progressive deterioration of the personality that eventually led, in more than one third of chronically hospitalized children, to marasmus and death by the end of the second year. This evidence demonstrates that social contact is fundamentally necessary to life itself and led Bowlby, who is considered the father of attachment theory, to assume that human beings have an innate need of interpersonal relations and social support (Bowlby, 1988).

Weak social ties and the experience of early maltreatment may affect both physical (Uchino, 2006; Reblin and Uchino, 2008) and mental health (Antonucci, 2001) across the lifespan. Studies in the elderly population have shown associations between early maltreatment or absent or weak social relations, both early and current, and higher incidence of cardiovascular diseases (Orth-Gomér et al., 1993; Rosengren et al., 2004; Burg et al., 2005), worse prognosis in breast cancer (Kroenke et al., 2006, 2013, 2017), increased risk of mortality (Seeman et al., 1993; Seeman, 2000; Santini et al., 2015; Kauppi et al., 2017), late-life depression and suicide risk (Dennis et al., 2005; Cacioppo et al., 2006; Fiori et al., 2006; Glass et al., 2006; Jardim et al., 2018, 2019; Novelo et al., 2018). Moreover, some studies suggest that, for elderly individuals, poverty or lack of social relations or integration may favor cognitive decline and increase the risk of developing dementias such as Alzheimer's disease (Tilvis et al., 2004; Bennett et al., 2006; Gow et al., 2007; James et al., 2011).

As early interactions with the caregiver may be the basis through which a person integrates and forges their social abilities, attachment theory is a valuable candidate to explain to which degree a person feels driven to seek proximity with others in various contexts. Furthermore, attachment theory could also predict the degree to which an individual is able to benefit from this social proximity, which could subsequently affect the preservation (or deterioration) of their cognitive functioning.

Attachment and Social Functioning

Based on the results of ethological studies, Bowlby (1969) argues that a child's attachment behaviors toward their caregiver are of vital necessity. Ainsworth (1979), Bowlby (1982) and subsequent authors have conceptualized attachment as a behavioral system. However, more recent evidence suggests that attachment is unlikely to rely on the functioning of any single identifiable neurological system, but probably results from the interplay of various social and motivational systems in the brain (Panksepp, 1998). Thus, throughout this review we will use the term "attachment system" to refer to behaviors, representations and

psychological processes related to attachment. To avoid any confusion, we also define the term "behavior" as the way in which one acts or conducts themselves, especially toward others.

Bowlby assumes that attachment behaviors are regulated by an innate motivational system whose main function is to establish a physical proximity with the attachment figure in case of real or perceived danger, or anxiety-provoking situations in general. Factors that trigger this need for safety can be either environmental (such as an unfamiliar stimulus or the rapid and threatening approach of an object) or directly related to the child's internal state (such as tiredness, hunger or illness) (Bowlby, 1969). Children adopt certain behaviors and signals to alert their caregivers to their needs. These signals, that include, among others, crying or calling the caregiver, essentially reflect the search for proximity with the attachment figure during the occurrence of stressful situations. These attempts to achieve proximity are called "primary strategies." The manner in which those close to the child respond to the child's behavior will guide the development of attachment styles; which will in turn form the basis for establishing effective internal models that will govern the feelings, expectations, and behavior of the individual in their relationships. Once the child's needs have been satisfied and eased, it resumes its activities (Ainsworth et al., 1978).

Since birth, the child begins to develop a repertoire of attachment behaviors that aim to catch or keep the attention of their caregiver. Depending on the effectiveness of these primary strategies the child will be more or less inclined to adapt their behavior and develop "secondary strategies." Secondary strategies include, on the one hand, avoidance of closeness and, on the other hand, energetic or exaggerated attempts to seek proximity or support. However, if the caregiver meets the child's needs in response to the primary strategies, the proximity search may cease as its goal has been achieved and the child can relax. If this positive and reassuring interaction consistently occurs throughout the early years of development, the child should become "securely attached." However, proximity-seeking behavior is not triggered exclusively by stressful or unpleasant states. For example, Trevarthen et al. (2006) emphasize the child's natural inclination for joyful social engagement, such as playing. Such positive interactions also contribute to the building and strengthening of secure attachment ties.

In the case of predominantly inconsistent or unavailable responses from the caregiver, the child will increasingly tend to adopt secondary strategies (Main, 1990). These strategies are constructed based on the child's assessment of whether reconciliation with the caretaker is possible, and how best to maintain a sustainable relationship with them. The use of secondary strategies reflects the development of "insecure attachment". Insecure attachment behavior may be triggered not only through the unavailability of the caregiver in times of need, but also in the case of inappropriate responses from them (Trevarthen et al., 2006).

As mentioned, secondary strategies can take two different forms. If the child is faced with an unavailable caregiver, they inhibit their primary strategies and adopt an avoidant attitude. A child who predominantly demonstrates secondary strategies of this type will be referred to as "avoidantly attached." If, on the contrary, access to the inconsistent caregiver seems possible, the child will respond by exaggerating and distorting attachment behaviors, resulting in crying and clinging. A child who predominantly adopts secondary strategies of this type will be referred to as "anxiously attached". Due to the difficulty of classifying all children into the three categories of attachment behavior discussed thus far, "disorganized" attachment style has been proposed as a fourth category (Main and Solomon, 1986). This type of insecure attachment is characterized by contradictory responses, oscillating between exaggerated and inhibited attachment behaviors.

The repetition of secure or insecure strategies will gradually be internalized and generate an interpersonal expectation of the attachment figure's availability toward the self and the availability of the self toward others. These internalized expectations, called Internal Working Models (IWMs), will generalize through various relationships and contribute to the establishment of internal regulation mechanisms. IWMs shape the representation one has of oneself and others, guiding behaviors, thoughts and coping strategies to be adopted in social interactions or in particularly stressful times (Main et al., 1985; Bretherton and Munholland, 1999; Cassidy, 2000). Thus, the attachment style defines an individual's emotion regulation abilities, which will in turn modulate their internal state and subsequent behaviors.

Despite their prototypical aspect (Sroufe, 1983; Collins and Read, 1994) and their influence on adult relationships (Carver, 1997; Miljkovitch and Cohin, 2007; Miljkovitch, 2009), IWM are not frozen representations. They can be modified through various life experiences and an individual's panel of behavioral or emotional responses can be enlarged (Vaughn et al., 1979; Bretherton, 1995). Nevertheless, some of the initial structure remains, and the first attachment experiences continue to steer individuals throughout adulthood (Waters et al., 2000; Carlson and Egeland, 2004; Grossmann et al., 2005; Groh et al., 2014).

More recent work by Panksepp (1998) highlights the neuro-affective mechanisms that may underlie the activation of attachment strategies. According to Panksepp, mammals are equipped with seven distinct but integrated neuro-emotional systems, i.e., FEAR, RAGE, PANIC/GRIEF, PLAY, SEEKING, LUST, and CARE. The SEEKING system has no direct object in the sense that it is considered to be a generalized motivational system, which "provides the arousal and energy that activates our interest in the world around us" and, as such, it drives the other six systems (Solms and Turnball, 2002, p. 115). Panksepp's theory assumes that social attachment is built on evolutionarily more ancient systems. For example, ancient pain mechanisms would underlie feelings of separation distress. Thus, various neuro-emotional systems described by Panksepp, such as the PANIC, CARE, and PLAY systems, are likely to be involved in the construction of attachment bonds.

Panksepp considers attachment bonds to be intrinsically related to the neural circuits of distress activated by separation, meaning that PANIC circuits are important for the development of social interactions. The distress triggered by real or felt separation will activate the PANIC system, which will induce

the need to seek proximity and social support. In this context, to restore the homeostatic balance, the SEEKING system will promote specific behaviors, including vocalizations, in order to favor social reunion. The behaviors or signals from a distressed child will in turn activate the parent's PANIC circuits, which will then activate the CARE system and lead the parent to provide protection and reassurance to their infant. However, the quality of the parent's CARE system depends on the caring experiences they have themselves gone through and internalized during their own childhood. Therefore, if the parent has experienced significant trauma, a state of anxiety or affective instability (disturbance) may remain, which will in turn influence the way in which the parent perceives their child's needs and responds to them. As previously discussed, the quality of the parent's response to their child will play a crucial role in shaping the child's attachment style (Panksepp, 1998).

The PLAY system is of particular importance as an early pro-social system due to the high levels of positive affect it evokes and its role in the refinement of social interactions (both by promoting the integration of social rules and by building empathy and trust) (Burgdorf et al., 2010; Watt, 2017). The first manifestation of social play arises as early as 2 months of age in humans. Through brief visual and auditory exchanges, the child and the primary caregiver experience their first social interactions by adjusting their attention and expressions based on one another's responses (Schore, 2001). Through these interactive situations and the joyful, pleasant feeling the child experiences, they progressively internalize the possibility of shared attention and practice adjusting their social behaviors and responses to one another (Kestly, 2014). In the section Influence of Early Attachment on Neurobiological and Cognitive Development, we will briefly examine the neurobiological links between separation distress, PLAY and attachment, in which neuropeptides such as endogenous opioids, oxytocin, vasopressin and prolactin are likely to play a critical role (Panksepp et al., 1997; Panksepp, 1998).

Effects of Social Relations on Cognitive Decline

Social context later in life is important in protecting against cognitive decline. Early attachment may be influential in determining both the availability of social support later in life as well as the degree to which an individual is able to benefit from such support. By maintaining social activities, a person will be engaged in stimulating and complex interactions, which require a variety of cognitive skills. Consequently, social interactions may in turn slow cognitive decline and the development of dementia (Seeman et al., 2001; Wang et al., 2002; Fratiglioni et al., 2004; Beland et al., 2005; Amieva et al., 2010; Qiu et al., 2010; Dickinson et al., 2011; Ellwardt et al., 2013). The influence of such psychosocial factors on cognitive abilities could be due to the internal feeling of comfort conveyed by social support, which may help to lower the level of stress and improve the capacity to face difficult life events (Wilson et al., 2011). Stress, anxiety, and/or depression may therefore induce or favor cognitive decline and the risk of developing later dementia (Beaudreau and O'Hara, 2008; Dotson et al., 2010; Gulpers et al., 2016; Freire et al., 2017).

Seeking proximity and support is a common coping strategy in the case of fear or stress (Zeidner and Endler, 1996; Mikulincer et al., 2003). Attachment style not only influences an individual's evaluation of a threat and moderates their need for social support, but it also shapes the strategies and effort they employ to seek the proximity needed to return to a feeling of well-being (Mikulincer and Florian, 1998; Collins and Feeney, 2000). For instance, as opposed to insecurely attached individuals, securely attached individuals tend to naturally and effectively seek proximity and rely on social support when facing a stressor (Larose and Bernier, 2001; Mikulincer and Florian, 2003), and experience positive effects and reduced stress when recalling the memories of a partner or an available attachment figure (McGowan, 2002; Rowe and Carnelley, 2003). In line with attachment theory, Siedlecki et al. (2014) assume that the feeling of contentment brought by satisfying relationships depends on the concrete sense of having people to turn to in case of need, but also on the expectation that relying on someone else is comforting.

Social relationships can be appraised from an objective or subjective point of view. For example, relational support can be objectively assessed by considering the size of the network, the frequency of contacts and the types of social ties available (marital, family, friends, and caretakers). From this perspective, different studies have revealed that living alone with no or few personal ties (Crooks et al., 2008) as well as being single or widowed (Helmer et al., 1999; Håkansson et al., 2009; Feng et al., 2014; Sundström et al., 2016) increases the risk of cognitive decline and dementia relative to people living with their spouse or partner. Attachment security has also been related to a larger social network in elderly individuals (Fiori et al., 2011).

Though some studies have found that a greater social network significantly reduces the risk of developing dementia (Tilvis et al., 2004; Wilson et al., 2007; Crooks et al., 2008), Amieva et al. (2010) showed that the quality of support impacts the occurrence of later dementia more than its quantity. This suggests that the subjective aspect of social support, i.e., the manner in which a person perceives the quality of the support they receive, is paramount. To distinguish the influence of objective and subjective social support on the onset of later dementia, Amieva et al. (2010) examined a variety of social network characteristics. They investigated six different aspects, namely marital status, number of ties, nature of the social network, satisfaction with network interactions, perception of being understood/misunderstood and reciprocity in the relationship. The results revealed that perceived social support variables had a more significant effect on the risk of developing later dementia than quantitative social support variables. Experiencing satisfaction in relationships reduced the risk of later developing dementia by 23% and by 55% when the participants reported that they received more support than they gave.

Perception of social support is likely to vary according to attachment style. Securely attached individuals demonstrate more optimistic life appraisal (Mikulincer and Florian, 1995; Berant et al., 2001; Shorey et al., 2003), more positive representations of others (Collins and Read, 1990; Simpson,

1990; Baldwin et al., 1996), more positive self-esteem and self-worth (Bartholomew and Horowitz, 1991; Brennan and Morris, 1997; Mikulincer et al., 2004), and more effective coping strategies (Cassidy, 1994; Simpson et al., 1996; Gross and John, 2003). Taken together, these factors may favor the maintenance of cognitive and affective availability, which may sustain an individual's capacity to invest themselves in daily life activities and in their social network. This investment will in turn protect against cognitive decline.

Individuals with insecure attachment profiles will be less able to access fruitful and supportive relationships (Simpson and Rholes, 2017). Insecure attachment has been connected to greater levels of depression, anxiety, psychosomatic illness and feeling of loneliness (Hazan and Shaver, 1990; Carnelley et al., 1994). Avoidant attachment is mainly characterized by self-reliance, as the other is perceived as dismissive and non-supportive. In order to maintain self-reliance, an avoidant individual will suppress painful memories and feelings associated with relationships from consciousness. This will help them maintain a low level of stress by avoiding threatening emotions, but this will also deprive the person from the emotional benefits another person can provide in times of stress. Therefore, when facing a threatening or emotional situation, an avoidant person will inhibit proximity needs and divert his attention toward other interests or goals (Mikulincer et al., 2003; Mikulincer and Shaver, 2007).

Conversely, an anxious attachment style is characterized by self-defeating representations and a pattern of anxiety-driven behaviors accompanied by pessimistic thoughts of others, considered as unable to provide sufficient support (Collins and Read, 1994). Anxious people tend to increase their thoughts and feelings of despair and unworthiness by focusing their attention on negative and painful aspects of themselves, their relationships, or situations (Kobak et al., 1993). Therefore, they display increased and possibly exaggerated attention and support-seeking behaviors (Cassidy and Berlin, 1994). These individuals rarely feel sufficiently reassured and an enduring feeling of dissatisfaction in their social relations remains.

Some studies have focused on the feeling of loneliness to explain the link between perceived social support and the occurrence of dementia. Loneliness is a subjective feeling of social isolation. It describes the distress a person experiences when their social relationships are perceived as unsatisfactory both in terms of quantity and especially quality. Consequently, some people may feel lonely even though they are socially engaged (Ayalon, 2016). Although social isolation and a lack of social engagement have been shown to increase the risk of cognitive decline and dementia (Bassuk et al., 1999; Helmer et al., 1999; Wang et al., 2002), loneliness appears to have even stronger effects on the emergence of these pathologies (Wilson et al., 2007; Holwerda et al., 2014). Thus, people who experience loneliness are twice as likely to develop Alzheimer's disease as those who do not feel lonely (Wilson et al., 2007). These results are consistent with those of Holwerda et al. (2014), who showed that the perceived absence of social relations and support were independently related to increased risk of cognitive decline over a 3-year follow-up. Furthermore, the effect of perceived social isolation on subsequent cognitive decline was significantly stronger than the effect of objective social isolation. Two longitudinal studies also showed that loneliness contributes to increased cognitive decline over periods of 10 and 4 years, respectively (Tilvis et al., 2004; Shankar et al., 2013).

Recent studies on young adults as well as elderly individuals also showed that insecurely attached people were more prone to experiencing loneliness than securely attached people, who express more satisfaction about the support they perceive and receive (Bernardon et al., 2011; Akdogan, 2017; Spence et al., 2018). Although a relationship between loneliness and insecure attachment has been demonstrated, the specific implications for anxious and avoidant attachment styles remain unclear. Loneliness and depression both contributed to worsening elderly people's cognitive abilities over the course of a 12-year longitudinal study (Donovan et al., 2017). The authors suppose that the feeling of loneliness may contribute to a state of emotional distress, which in turn may promote the emergence of a depressive syndrome.

Depression, both early and later in life, has been consistently linked to cognitive decline and later dementia (e.g., Jorm, 2000; Leonard, 2007; Byers and Yaffe, 2011; Da Silva et al., 2013; Zahodne et al., 2013; Donovan et al., 2014; Geda et al., 2014; Santos et al., 2016). Depression also seems to be fundamentally connected to attachment, with a vast body of work demonstrating that attachment-related early life stress can predispose an organism to depression (e.g., Heim and Nemeroff, 1999; Pryce et al., 2005; Heim and Binder, 2012; Nemeroff, 2016; Taillieu et al., 2016; Cecil et al., 2017). Indeed, Watt and Panksepp (2009) conceptualize depression as arising from an evolutionarily preserved "shutdown mechanism" resulting from protracted separation distress in early life. A comprehensive examination of the literature linking early attachment to depression, on the one hand, and depression to cognitive decline, on the other hand, is beyond the scope of this review. However, it is worth considering that depression may play a mediating role in the influence that early attachment could have on later cognitive decline. Furthermore, many of the neurobiological mechanisms which link attachment and separation distress to depression (see Watt and Panksepp, 2009 for a review), will also come forward in our discussion of the neurobiological links between attachment and cognitive performance and decline later in the text.

Thus far, we have examined the importance of social and affective relationships for psychological development and the maintenance of general well-being into old age. We have attempted to clarify this relationship through the lens of attachment theory. However, the impact of these social exchanges, and indeed of attachment processes, can also be observed at the neurobiological and cognitive level.

INFLUENCE OF EARLY ATTACHMENT ON NEUROBIOLOGICAL AND COGNITIVE DEVELOPMENT

It is now widely accepted that the early childhood environment plays a crucial role in neurobiological and cognitive development

(Brummelte, 2017). For infant mammals, the most meaningful aspect of their environment is their social context as it is through interactions with their caregivers that their needs are met (Kundakovic and Champagne, 2015; Chen and Baram, 2016). Early life social stress can therefore leave an enduring imprint on brain connectivity and, thus, cognition and behavior (Fareri and Tottenham, 2016). In this section we present data from both human and animal literature, as studies in animals are uniquely able to offer insights into the causal mechanisms whereby attachment and early life stress forge neurocognitive development. Indeed, Bowlby (1958) himself strongly recommended an ethological approach to the study of attachment.

The first years of life are characterized by remarkable cerebral plasticity (Diamond, 2013) during which an individual's experiences can greatly influence the development and specialization of synaptic networks (Fox et al., 2010; Kolb et al., 2012). Brain maturation over the course of childhood involves the development of connectivity patterns through synaptic stabilization, pruning and branching of dendrites and myelinisation (Bale et al., 2010; Regev and Baram, 2014). During this period of maturation, early attachment relations may have a significant impact on later cognitive abilities. Indeed, securely attached children appear to demonstrate better cognitive skills than insecure children do (De Ruiter and van IJzendoorn, 1993; Van IJzendoorn, 1995; Moss and St-Laurent, 2001; West et al., 2013).

As previously mentioned, the emotional, relational and cognitive development of the child is linked very early on to the quality of the investment of, and safety of its relationships with, its caregivers. The postnatal period appears as a moment of high sensitivity of brain development to stress. Especially if it is chronic and associated with prolonged secretion of cortisol, stress is likely to leave a neurobiological trace that can affect the entire life of the individual. Changes in brain architecture can lead to impaired intellectual, physical and affective development. Early toxic stress can cause subsequent hyper-reactivity to minor stresses with mental and physical consequences that persist into adulthood. Hence the importance of appropriate caregiver-child relationships that do not provoke excessive stress is clear.

In addition, Bowlby (1982) and Ainsworth et al. (1978) proposed that secure attachment would promote an individual's drive to explore their environment, a behavior which is critical to learning and cognitive development. This link with the exploration system may therefore constitute another mechanism by which early attachment can influence later cognitive abilities.

Epigenetic Processes: Mediators of Early Life Experiences on Neurobiological Function

Epigenetic processes may constitute mechanisms through which early attachment impacts later cognition as they allow environmental factors to long lastingly alter gene expression, and hence the phenotype, without altering the DNA sequence (Champagne, 2008).

Animal research has demonstrated that epigenetic regulators such as DNA methylation and acetylation of histones are crucial mechanisms by which the mother pup relationship can influence brain processes later in life (Gervai, 2009; Kundakovic and Champagne, 2015). DNA methylation refers to a chemical modification of the DNA bases, where higher levels of methylation usually lead to lower rates of gene transcription and consequently gene functioning (Allis and Jenuwein, 2016; Ein-Dor et al., 2018). Acetylation of histones, on the other hand, leads to greater levels of gene transcription (Zentner and Henikoff, 2013).

Weaver et al. (2004) provide a clear example of an epigenetic mechanism by which maternal care during early development can affect adult behavior. They demonstrated that differences in licking, grooming and nursing behaviors of rat mothers led to differences in the DNA methylation of the glucocorticoid receptor (GR) gene promotor in the hippocampus. In particular, the offspring of low licking and grooming (LG) mothers show increased DNA methylation of the GR gene lasting into adulthood, leading to reduced hippocampal GR expression which in turn leads to an elevated hypothalamic-pituitary-adrenal (HPA) axis response to stress (Weaver et al., 2004). This paper forms part of a body of work by Meaney and his colleagues which clearly demonstrates that variations in rodent maternal care have important consequences for HPA functioning, and subsequently also for various cognitive abilities (e.g., Liu et al., 1997, 2000; Caldji et al., 2000; Meaney, 2001; Champagne et al., 2003, 2008). For example, under conditions of stress, low LG pups demonstrate impaired spatial memory when compared with high LG pups (Liu et al., 2000). However, low LG pups show comparatively enhanced hippocampal long-term potentiation under conditions of stress, which has been linked to enhanced contextual fear conditioning (Champagne et al., 2008).

Subsequently, this same research group demonstrated a relationship between childhood abuse and epigenetic regulation of the human hippocampal glucocorticoid receptor (NR3C1) expression (McGowan et al., 2009). Increased methylation of NR3C1 has also been linked to attachment avoidance in humans (Ein-Dor et al., 2018), while Bosmans et al. (2018) showed a relationship between increased NR3C1 methylation and anxious attachment. NR3C1 methylation may lead to less efficient down-regulation of the HPA axis, thereby constituting a mechanism by which insecure attachment can affect emotion regulation and the stress response across the lifespan (Ein-Dor et al., 2018).

Brain Structures and Systems That Are Affected by Early Life Experiences

A number of studies have shown that early stress can lead to lasting changes in the activity, connectivity, and volume of various brain structures like the amygdala, hippocampus, and prefrontal cortex (PFC), as well as neuroendocrine, neurotransmitter and neuropeptide systems such as the hypothalamic-pituitary-adrenal (HPA) axis and the oxytocinergic system (see Chen and Baram, 2016, for a recent review). The functioning of these structures and systems is closely related. Thus, changes to any one of them can have direct

and indirect consequences for the functioning of other brain structures and systems relevant for the development of cognitive impairment in later life (Chen and Baram, 2016). Here, we focus particularly on those changes that may have consequences for the development of cognitive impairment in later life.

The Hippocampus

The hippocampus is a structure with a prolonged post-natal developmental trajectory. It is both highly sensitive to the effects of early-life stress and critical to later cognition as it plays a central role in memory processes (Chen and Baram, 2016). Research in rodents suggests that early life stress impacts hippocampal synaptic plasticity and impairs performance on hippocampus-driven memory tasks such as object recognition and object location into late adulthood (e.g., Brunson et al., 2005; Hulshof et al., 2011; Molet et al., 2016; Pillai et al., 2018; see Derks et al., 2017 for a recent review). Impoverished dendritic trees in the rodent hippocampus following early life stress have also been linked to impaired memory later in life (Ivy et al., 2010; Molet et al., 2016). These changes in dendritic trees likely lead to a reduced number of functional synapses and may progressively worsen with age (Brunson et al., 2005; Ivy et al., 2010). Furthermore, reduced hippocampal volume has been observed both in rodents exposed to early life stress (Molet et al., 2016) as well as in humans who experienced childhood adversity (Buss et al., 2007; Hanson et al., 2015; Teicher and Samson, 2016). Quirin et al. (2010) report reduced hippocampal cell density in insecurely attached individuals. In contrast, maternal support in early childhood has been positively associated with hippocampal volume (Kim et al., 2010; Luby et al., 2016). Rifkin-Graboi et al. (2015) also report a positive relationship between maternal support and hippocampal volume, and between maternal support and hippocampal connectivity to other limbic regions, most importantly the amygdala.

The Amygdala

In contrast to the typically observed reduced hippocampal volume following early life stress, severe childhood stress has been linked repeatedly with increased volume of the human amygdala (Mehta et al., 2009; Tottenham et al., 2010; Lupien et al., 2011; Tottenham, 2012; Davidson and McEwen, 2013; Pechtel et al., 2014). Furthermore, Lyons-Ruth et al. (2016) found that an insecure attachment in infancy predicted greater amygdala volume in adulthood and Coplan et al. (2014) found that early life stress was also associated with amygdala enlargement in macaques. The amygdala is a limbic structure that undergoes developmental changes throughout childhood and is critical to the expression and regulation of fear and anxiety. Thus, it is not surprising that early life stress and insecure attachment can impact its development (Tottenham, 2012; Fareri and Tottenham, 2016). In the rodent amygdala, early life stress leads to various changes including dendritic hypertrophy in the basolateral nuclei (Eiland et al., 2012), altered connectivity (Johnson et al., 2018), and increased activity in response to stress later in life (Sanders and Anticevic, 2007; Malter Cohen et al., 2013). In each case, these neurobiological changes are accompanied by enhanced anxiety and impaired fear regulation. Conversely, appropriate early maternal care (as indexed by high as opposed to low licking, grooming and nursing behaviors) has been associated with both differences in amygdala development and reduced fearfulness later in life (Caldji et al., 1998).

Altered amygdala functioning and connectivity has also been observed following early life stress in humans. Such alterations, which often involve increased amygdala reactivity as well as increased amygdala volume, have furthermore been associated with behavioral changes such as enhanced anxiety across the lifespan (Tottenham et al., 2010, 2011; McCrory et al., 2011; Pechtel and Pizzagalli, 2011; Burghy et al., 2012; Gee et al., 2013; Malter Cohen et al., 2013; Fan et al., 2015; McLaughlin et al., 2015; Lyons-Ruth et al., 2016). However, Hanson et al. (2015) report smaller amygdala volumes in children exposed to various types of early life stress. It is likely that amygdala responses to early life stress are non-linear, and differential outcomes later in life may be related to differences in the timing and severity of early life stress (Pechtel et al., 2014; Callaghan and Tottenham, 2015; Hanson et al., 2015).

The Prefrontal Cortex

The prefrontal cortex (PFC), which is critical to cognitive and behavioral control, can be significantly affected by early life stress (e.g., Van Harmelen et al., 2010, 2014; McEwen and Morrison, 2013; Yang et al., 2015; Demir-Lira et al., 2016). In humans, the PFC undergoes a particularly protracted maturation process, with certain time-windows during early infancy, childhood and adolescence being important for different aspects of this brain area's development (Diamond, 2002; Gogtay et al., 2004). Adverse life events and attachment experiences during any of one of these time-periods may therefore have a lasting impact on PFC functioning. In fact, numerous reports link attachment to the development of cerebral structures, and particularly areas of the PFC, since early stress interferes with brain maturation and, thus, cognition as well as the development of the attachment system (Kraemer, 1992; Schore, 1996; Gunnar and Quevedo, 2007; Belsky and de Haan, 2011). For example, early life stress has been shown to lead to changes in the dendritic density and morphology of medial PFC neurons and to corresponding functional deficits in rodents (Bock et al., 2005; Monroy et al., 2010; Chocyk et al., 2013; Yang et al., 2015; Soztutar et al., 2016). In both rodents and humans, early life stress has also been linked to altered connectivity of the PFC to limbic brain regions such as the hippocampus and the amygdala (e.g., Burghy et al., 2012; Demir-Lira et al., 2016; Reincke and Hanganu-Opatz, 2017; Johnson et al., 2018). Adults who experienced childhood emotional maltreatment show both reduced volume and reduced activation of the medial PFC (Van Harmelen et al., 2010, 2014). Correspondingly, severe early life stress can result in deficient executive control (Hostinar et al., 2012), to which the medial PFC seems to be key (Ridderinkhof et al., 2004).

Executive functions are cognitive processes that permit action initiation or inhibition and allow for adapted responses to new or problematic situations (Hughes, 2011). Executive functions such as working memory, inhibition and flexibility can be considered as cognitive self-regulation mechanisms (Zelazo et al., 2004; Diamond et al., 2007; Liew, 2012). The early family environment

can influence the development of executive functions (Bernier et al., 2010, 2012; Matte-Gagné and Bernier, 2011). Indeed, at first, a child relies on the caregiver for stimulation and regulation, but little by little, they internalize these processes to form their own self-regulation system (Calkins and Leerkes, 2004; McClelland et al., 2010). The quality of these first exchanges, paired with the maturation of cerebral structures and the developing capacity to self-regulate, work together to support the development of executive capabilities. By building the potential to control and inhibit impulses, to learn how to direct attention and to modulate emotions (Zimmerman and Schunk, 2001; Crugnola et al., 2011; Panfile and Laible, 2012), self-regulation allows the child to initiate voluntary and controlled actions (Calkins and Leerkes, 2004; Diamond et al., 2007; McClelland et al., 2010). Parental stimulation, encouragement, sensitivity, and support for autonomy all tend to enhance the development of subsequent working memory, flexibility and attention skills (Bibok et al., 2009; Bernier et al., 2010; Matte-Gagné and Bernier, 2011; Mezzacappa et al., 2011; Hammond et al., 2012; Clark et al., 2013; Hopkins et al., 2013).

The HPA Axis

Disruption of the hypothalamo-pituitary-adrenal axis (HPA axis) is likely to drive molecular mechanisms leading to altered hippocampal synaptic plasticity following early life stress (Ivy et al., 2010; Derks et al., 2017). The HPA axis drives a chain of neuroendocrine events in response to stress, starting with the release of corticotropin releasing factor (CRF or CRH) from the hypothalamus. CRF is subsequently the primary trigger for adrenocorticotropic hormone (ACTH) secretion by the anterior pituitary gland, which in turn triggers the systemic release of glucocorticoids by the adrenal gland (Bale and Vale, 2004). Changes in CRF release also appear to be implicated in the process whereby early life stress may impair the structural development of the PFC (Yang et al., 2015). The HPA axis is crucial for controlling the regulation of cortisol, the stress hormone, and therefore the behavioral stress response, throughout life (Rincón-Cortés and Sullivan, 2014). Dysregulation of this axis is a frequently observed consequence of early stress.

Corticosteroid hormones (mainly cortisol in humans and corticosterone in rodents) bind to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) that are expressed abundantly in limbic structures and are important for the transcriptional regulation of certain genes. Fluctuations in the levels of such hormones are thereby able to cause changes in gene expression (De Kloet et al., 2005). Such gene-environment interactions demonstrate how over-excitation of the HPA axis can lead to increased stress-susceptibility and how specific neurological changes can have important consequences for the development of other brain regions and systems (De Kloet et al., 2005).

Growth Hormone and the Insuline-Like-Growth Factor Axis (GH-IGF-1)

Various neurobiological signaling mechanisms that are likely to be influenced by early life stress may also play a role in the extent to which cognition is reduced later in life. For example, abnormally heightened HPA axis activity may lead to the suppression of the growth hormone-insulin-like growth factor axis (GH-IGF-1). Indeed, HPA axis dysregulation due to psychosocial causes in institutionalized children has been linked to suppression of the GH-IGF-1 axis and consequent growth failure (Johnson and Gunnar, 2011). Interestingly, IGF-1 is not only crucial to normal tissue growth, but also affects neuroplasticity and cognitive brain functioning throughout the lifespan (Aleman and Torres-Aleman, 2009; Dyer et al., 2016). A decrease in IGF-1 has been strongly implicated in age-related cognitive decline and has been identified as a potential risk factor for dementia (Sonntag et al., 2013; Ashpole et al., 2015; Doi et al., 2015; Quinlan et al., 2017; Frater et al., 2018). As social deprivation in childhood can lead to deficits in IGF-1 (see Johnson and Gunnar, 2011 for a review), suppression of IGF-1 may constitute a pathway whereby adverse attachment experience related to early life stress can exacerbate age-related cognitive decline. Furthermore, IGF-1 extracellular signaling genes are upregulated by juvenile rough-and-tumble play in rats (Burgdorf et al., 2010). Such rough-and-tumble play is considered a highly positive social interaction, and the underlying PLAY system is conceptually linked to early attachment (see section on Attachment and Social Functioning). Therefore, it seems that social deprivation could suppress IGF-1 signaling on the one hand, while on the other hand, positive social interaction could promote IGF-1 signaling, with potentially important consequences for cognitive functioning during aging.

Neuropeptide Signaling

Early attachment is likely to have important consequences for neuropeptide signaling throughout the course of life. For example, oxytocin (OT) seems to play a central role in the neurobiological basis of attachment across species. This hormone behaves like a neuropeptide in the brain and promotes the mother's protective behavior toward her young. In humans, oxytocin has been shown to impact empathy, generosity, sexuality, conjugal and social bonding, and stress reactivity (MacDonald and MacDonald, 2010). Despite this, it is not easy to determine the precise causal relationship between OT and the attachment system. Current evidence suggests a reciprocal and two-way relationship.

OT is synthesized in the hypothalamus, and OT signaling has been extensively linked to pro-social and attachment behavior (Galbally et al., 2011). In monogamous prairie voles, the oxytocinergic system promotes resilience to the effects of neonatal isolation on adult social attachment (Barrett et al., 2015). Early life stress and attachment profile can have epigenetic implications for the expression of the oxytocin receptor gene (OXTR) (Feldman et al., 2016; Pearce et al., 2017; Ein-Dor et al., 2018). Increased DNA methylation of the structural gene for oxytocin (OXT) has also been linked to higher levels of attachment insecurity in adults (Haas et al., 2016). Strathearn et al. (2009) and Pierrehumbert et al. (2012) report differential oxytocin responses to stressors based on differences in adult attachment styles, which are laid down chiefly during early childhood (see section Attachment and Social Functioning).

Many factors contribute to individual variations in the response to stressful experiences. Pierrehumbert et al. (2012) evaluated stress response patterns based on adult attachment style in a community sample as well as in subjects who had been exposed to traumatic events such as abuse or life-threatening diseases during childhood and/or adolescence. Subjects with an avoidant attachment style reported moderate subjective stress, high HPA response, and moderate oxytocin levels. Subjects with an anxious attachment style had moderate levels of subjective stress, HPA response, and relatively low levels of oxytocin. Finally, subjects with a disorganized attachment style reported high subjective stress; they had a suppressed HPA response and moderate levels of oxytocin. These data support the notion that attachment styles may affect stress responses and suggest a specific role for oxytocin in the attachment and stress systems.

However, it is unlikely that attachment is driven by the signaling of a single neuropeptide. The opioidergic, dopaminergic, prolactinergic, and vasopressinergic systems are all closely linked to the oxytocinergic system, and these systems are likely to drive attachment behavior in concert (Insel, 1997; Machin and Dunbar, 2011; Pearce et al., 2017). As put forward by the brain opioid theory of social attachment (Panksepp et al., 1978, 1980; Nelson and Panksepp, 1998; Machin and Dunbar, 2011; Loseth et al., 2014; Inagaki, 2018), the signaling of endogenous opioids, and specifically μ-opioids in the brain is critical both to feelings of social connection and social loss, i.e., separation distress. It is important to note that "social attachment" in terms of this theory refers to social bonds generally, and not specifically those formed during early interactions with the caregiver. Indeed, the developmental link between early attachment and the opioidergic system needs further investigation. However, differences in adult attachment style have been linked to differences in the expression of μ-opiod receptor genes (Troisi et al., 2012; Pearce et al., 2017) as well as to differences in the availability of μ-opioid receptors in the brain (Nummenmaa et al., 2015). In their work on the link between early attachment, separation distress and depression (see section Effects of Social Relations on Cognitive Decline), Watt and Panksepp (2009) also emphasize the importance of the opioidergic system, as well as that of the oxytocinergic and other neurotransmitter systems, the HPA axis, and immune responses. As social bonds can play an important role in maintaining cognitive abilities in old age, the potential impact of early attachment on the signaling of socially relevant neuropeptides provides another example of how early secure attachment could protect against cognitive decline later in life.

Neuroinflammation

It is also important to consider the role that neuroinflammation could play in mediating the impact of early attachment on cognitive capacity later in life. Neuroinflammation is thought to play an important role in Alzheimer's disease pathology (see Heneka et al., 2015 for a review), as well as in the pathology of other dementias (see Pasqualetti et al., 2015). Evidence suggests that early life stress can have lifelong consequences for susceptibility to neuroinflammation in rodents (Ganguly and Brenhouse, 2015; Roque et al., 2016; Hoeijmakers et al., 2017). Social stress and insecure early attachment have also been associated with inflammatory responses in humans (e.g., Gouin et al., 2009; Slavich et al., 2010; see Ehrlich, 2019, for a recent review of the links between adult attachment and psychoneuroimmunology, with a specific focus on inflammatory responses). Neuroinflammation is likely to constitute yet another mechanism through which stress can lead to cognitive decline (Hoeijmakers et al., 2018). Indeed, aside from the potential impact of early life stress on neuroinflammation, neuroinflammation may also mediate the link between later life stress and depression on the one hand, and cognitive decline and dementia on the other hand (Leonard, 2007; García-Bueno et al., 2008; Slavich and Irwin, 2014; Miller and Raison, 2016; Santos et al., 2016; Bisht et al., 2018; Justice, 2018).

Implications for Dementia Related Pathologies

Crucially, many of the brain structures and systems that are impacted by early adverse attachment experience and early life stress are also implicated in dementia-related neuropathology. For example, the two neuropathological hallmarks of Alzheimer's disease, neurofibrillary tangles (NFT) and amyloid containing senile plaques (SP) (alongside synaptic and neuronal loss), typically emerge in medio-temporal lobe areas such as the hippocampus, entorhinal cortex and amygdala, before spreading to areas of the neocortex (von Gunten et al., 2006; Giannakopoulos et al., 2009; Perl, 2010; Sperling et al., 2011; Nelson et al., 2012; Yang et al., 2012). Dysregulation in the HPA axis has been observed both in Alzheimer's disease and other dementias and has been linked to worsening cognition. Thus, HPA dysregulation constitutes one likely mechanism through which stress can lead to cognitive decline and possibly dementia (Lupien et al., 1998; Magri et al., 2006; Gil-Bea et al., 2010; Gupta and Morley, 2014; Popp et al., 2015; Pietrzak et al., 2017; Caruso et al., 2018). As brought forward above, neuroinflammation and changes in the signaling of neuropeptides and insulin-like growth factor are also likely to play a role in neurodegeneration and cognitive decline. Finally, although PFC damage can be observed in early stages of Alzheimer's disease (von Gunten et al., 2005, 2006), it may be more common in other types of dementia, such as frontotemporal dementia and vascular dementia (e.g., McPherson and Cummings, 1996; Rosen et al., 2002; Neary et al., 2005; Korczyn et al., 2012).

In animal models, current work is starting to link early life stress to the development of specific dementia-related pathologies more directly. For example, in mouse models of Alzheimer's disease, early life stress and maternal separation have been linked to increased amyloid accumulation in the hippocampus and to cognitive deficits (Hoeijmakers et al., 2017; Hui et al., 2017), whereas increased maternal care has been linked to delayed amyloid accumulation and delayed cognitive decline (Lesuis et al., 2017). Recently, Hoeijmakers et al. (2018) reviewed the evidence linking early life stress to enhanced risk for cognitive decline and Alzheimer's disease in rodent models.

Attachment and the Exploration System

The evidence outlined above points to a clear influence of early attachment experience on neurobiological development, with consequences for cognitive and social functioning across the lifespan. Furthermore, links between the attachment and exploration systems may promote cognitive development. Although the attachment and exploration systems are distinct, they are intrinsically linked, as, in addition to addressing needs of proximity and protection, attachment bonds also promote exploration behavior (Ainsworth and Wittig, 1969; Bowlby, 1969, 1980; Ainsworth et al., 1978). Such exploration is driven by what Panksepp calls the SEEKING system, which essentially compels an individual to explore the environment in response to appetitive needs (Ellis and Solms, 2017). Such exploration includes investigation of and engagement with the environment (Panksepp, 1998; Bergin and Bergin, 2009).

The level of attachment security is reflected in the balance between comfort seeking behaviors and the drive to explore the environment (Ainsworth, 1985; Weinfield et al., 1999). When the child feels sufficient confidence in their relationship with the caregiver, as well as confidence in the availability of the caregiver in case of need, this will allow the activation of the exploration system (Grossmann et al., 2008; Weinfield et al., 2008). When facing a threat, discomfort or challenging situation, children with a secure attachment profile have the ability to search for support and comfort from their caregiver. After being reassured and comforted, they may return to their exploratory activities. As they have the ability to internalize a representation of a positive and reliable caregiver, secure children tend to invest themselves in more challenging investigations, which may in turn induce greater cognitive stimulation (Bretherton, 1985; Bus et al., 1995).

Insecure children do not demonstrate the same balance between exploration and attachment. Anxious children maintain attachment behaviors even in the absence of threatening or harmful situations. As a result, they are unable to invest fully in the exploration of their environment (Ainsworth and Bell, 1970). On the contrary, when faced with threatening or stressful situations, avoidant infants suppress their attachment needs and appear to be able to maintain their exploratory activities without expressing the need for support. These children will therefore not experience the same beneficial interactions with the caregiver as secure children would (O'Connor and McCartney, 2007).

An increased ability to interact with the environment and social world will promote cognitive skills and favor the development of neural networks and cognitive functions central to self-regulation (Bernier et al., 2010; Stievenart et al., 2011). This is in line with animal research that has shown that frequent and diversified activity increases the number of neurons and synapses and positively influences brain and cognitive reserves (Churchill et al., 2002). Indeed, some studies have demonstrated that exploration mediates the link between attachment and later cognitive skills (O'Connor and McCartney, 2007; West et al., 2013; McCormick et al., 2016). O'Connor and McCartney (2007), observed that the effect of insecure attachment on cognitive skills in first grade children is attributable to various factors. Specifically, insecure children showed a low level of commitment to tasks, demonstrated reduced exploration, received poor quality maternal assistance, maintained poor quality relationships with teachers, and demonstrated low-level communication and attention skills, which were in turn associated with lower levels of cognitive abilities.

Therefore, in addition to the neurodevelopmental impact of early attachment, we have discussed how the attachment system may promote social interaction and cognitive development. Taken together, these processes could favor the development of brain and cognitive reserve and, thus, protect against later cognitive decline or dementia.

PROTECTIVE ASPECTS OF BRAIN AND COGNITIVE RESERVE

Aging may be associated with changes in cognitive performance as well as neurological changes on the chemical, structural and functional level. The concepts of brain and cognitive reserve (BCR) have been put forward as explanations for the frequent miss-match between the severity of neurodegeneration and the severity of its clinical manifestation. Inter-individual differences in available BCR may explain differences in the extent to which cognitive performance is preserved following neurodegeneration (Stern, 2002, 2009). BCR should protect both against the adverse consequences of decline due to normal aging, as well as against damage due to degenerative diseases or other pathological processes or events. We hypothesize that one of the mechanisms whereby early social interactions may promote the maintenance of cognitive abilities in later life is by contributing to the development of BCR.

The terms cognitive and brain reserve have been used somewhat interchangeably in the literature (Roe et al., 2007; Nithianantharajah and Hannan, 2011). Initially, the term brain reserve was used to describe inter-individual differences in certain quantitative properties of the brain, which might protect against the clinical manifestations of brain damage or degeneration (Satz, 1993; Stern, 2012). For example, individuals with larger brain size, or a higher number of neurons and synapses, may be able to sustain more extensive neurodegeneration before clinical manifestations emerge than individuals with lower levels of such "brain reserve" (Katzman et al., 1988; Katzman, 1993; Schofield et al., 1997; Van Loenhoud et al., 2018). According to this model, reserve was originally conceived as passive and predefined, and clinical symptoms should be observed once pathological alterations surpass a certain fixed threshold (Satz, 1993). By contrast, the concept of cognitive reserve was put forward to describe processes through which an individual might actively counteract or compensate for neuropathology, through the activation of cognitive systems and neural networks (Stern, 2002, 2009). Consequently, individuals with higher levels of education, intelligence, or occupational attainment may be better equipped to resist the clinical impact of brain damage, due to more efficient processing or the ability to recruit new neural networks when performing complex tasks (Stern, 2009). As such, cognitive reserve enhances the ability to make use of damaged resources in order to perform tasks successfully.

Although conceptually distinguishable, brain reserve and cognitive reserve are related one to the other (Nithianantharajah and Hannan, 2011; Stern, 2012). For example, cognitive reserve built up by education or general intelligence may be related to aspects of brain structure, such as increased synaptic density (Katzman, 1993). Brain reserve may not be as static as was originally proposed due, for example, to the potential for adult neurogenesis or enhanced neural plasticity as a result of upregulated BDNF (Stern, 2012; Van Loenhoud et al., 2018). For these reasons, we follow Nithianantharajah and Hannan (2009) in using the term BCR to refer to these ideas collectively.

Various lifestyle and environmental factors have been associated with BCR. Epidemiological studies have provided substantial evidence that factors such as linguistic ability in young adulthood, education (e.g., more years of formal schooling), intellectually stimulating work and engaging in leisure activities can slow cognitive decline and delay the onset of dementia (e.g., Snowdon et al., 1996; Fratiglioni and Wang, 2007; Sharp and Gatz, 2011; Pool et al., 2016; Soldan et al., 2017; Wang et al., 2017; Groot et al., 2018). A number of studies have focused specifically on the impact of early childhood education and socioeconomic environment on BCR. They suggest that this period may be critical for reducing the rate of cognitive decline and the risk of dementia later in life (Stern et al., 1994; De Ronchi et al., 1998; Moceri et al., 2000, 2001; Ravona-Springer et al., 2012; Dekhtyar et al., 2015; Zahodne et al., 2015). Early education may be important for BCR as it occurs during critical neurodevelopmental windows (Zahodne et al., 2015).

Recently, Lesuis et al. (2018) have argued for an important link between the early life environment and BCR. After reviewing evidence from rodent studies, they suggest that early life experiences may influence BCR, cognitive decline and the development of Alzheimer's pathology through a variety of mechanisms. These mechanisms may include altering dendritic and synaptic complexity and programming the HPA axis and the neuroinflammatory response. Correspondingly, we would like to argue that many of the mechanisms underlying the positive influence of early attachment on cognitive and neurobiological development (see section Influence of Early Attachment on Neurobiological and Cognitive Development) could protect against cognitive decline and dementia by acting on BCR. The socio-affective mechanisms whereby early attachment may protect against later cognitive decline (outlined in the section on Attachment, Social Factors, Cognitive Decline and Dementia) could be interpreted similarly. Indeed, a number of previous authors have highlighted the potential link between social factors such as network size, social support and social satisfaction with BCR (Glymour et al., 2008; Amieva et al., 2010; Stoykova et al., 2011). However, the empirical evidence remains limited, and further research is needed to test if, and how, early attachment contributes to BCR.

CONCLUSION AND FUTURE PERSPECTIVES

Although developmental psychology has traditionally focused on the progression from childhood into young adulthood, this review draws attention to the potentially long-lasting effects of early life experiences and early developmental processes into old age. For a schematic summary of the arguments put forward in this review, please see **Figure 1**. In line with Bowlby's words "from the cradle to the grave," we have offered evidence of the continuation of the effects of primary attachment relations from early childhood to old age. The quality of early life interactions influences neurobiological, cognitive, affective and social development and may thereby protect against later cognitive decline. On the one hand, early

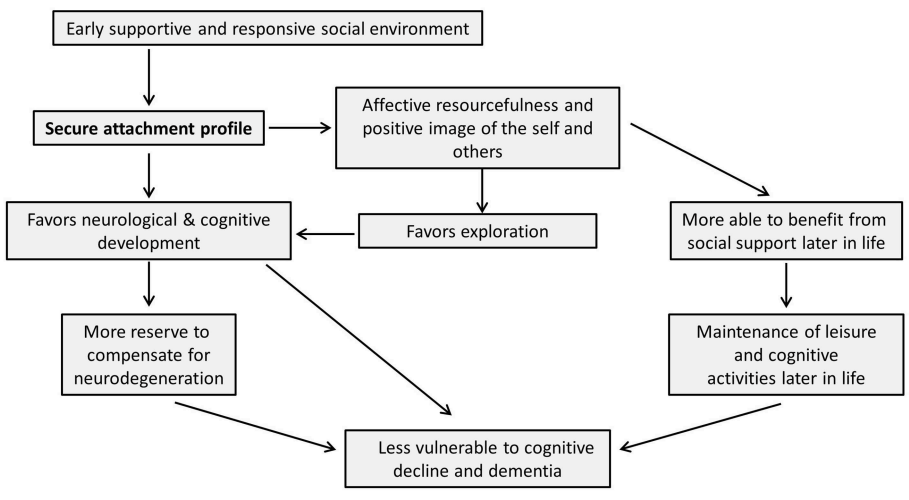

FIGURE 1 | Schematic summary of article. In this figure, we summarize the arguments put forward in this review. It is important to note that, while the arrows in this figure are unidirectional (thereby keeping with the main thrust of the article), it is likely that the influence between many of the nodes in this scheme could in fact run in both directions. However, we have chosen one-directional arrows as we believe these most accurately demonstrate the arguments brought forward in this review, which specifically emphasize the proposed impact of early secure attachment on later cognitive decline and dementia.

attachment experiences could influence the will to maintain social engagements and relationships later in life, as well as the perceived quality of social support. On the other hand, attachment experiences may influence—through their influence on neurobiological development and cognitive functioning—the development or availability of brain and cognitive reserve. Furthermore, affective and social consequences of attachment experiences may themselves be able to foster the successful functioning and maintenance of these reserves. Perhaps, alongside the notions of brain and cognitive reserve, we may want to introduce the idea of "affective reserve," which would explain how favorable affective resources might protect against cognitive decline.

Taken together, BCR and affective reserve could explain the differences observed in the way elderly people cope with age-related changes. In a similar vein, an increasing interest in the study of resilience in elderly people is currently emerging (MacLeod et al., 2016; Arenaza-Urquijo and Vemuri, 2018). Resilience refers to the capacity for positive adaptation in the face of life adversity, trauma or significant sources of stress. In older adults, resilience is mostly studied in the context of well-being, successful aging and preserved functioning (Ong et al., 2009). In line with the arguments brought forward in this review, early life experiences may favor the development of these adaptive processes, which may in turn help elderly people to cope successfully with significant somatic, psychological and environmental changes (Lesuis et al., 2018). Thus, resilience could be seen as similar to our notion of "affective reserve." A deeper understanding of affective and social contributors, and the manner in which they interact with more traditional ideas of BCR, could be crucial to elucidating how cognitive functioning may be maintained with age (Bartrés-Faz et al., 2018).

It is up to future work to test these ideas. The link between early attachment, BCR and affective reserve, and eventual cognitive decline and dementia needs to be investigated more directly and in greater detail. If such a link is confirmed, it may well-prove useful in the quest for early identification of individuals at risk of developing dementia and in suggesting new avenues for interventions.

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EW, YB, and AD equally contributed to the writing of the manuscript. All authors contributed to the conception and design of the review and/or revisions, manuscript revision, read and approved the submitted version.

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