NEUROPSYCHOLOGY AND NEUROPSYCHIATRY OF NEURODEGENERATIVE DISORDERS

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NEUROPSYCHOLOGY AND NEUROPSYCHIATRY OF NEURODEGENERATIVE DISORDERS

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This book compiles all articles within the Research Topic "Neuropsychology and neuropsychiatry of neurodegenerative disorders" published in the journal Frontiers in Aging Neuroscience.

The call was launched in 2014 and closed in 2015 with 21 articles published. Papers deal on several important topics of neuropsychology -such as language and visuospatial functions- and neuropsychiatry -such us the emotional or motivational spheres- , and the interphase between them.

There are also articles on psychometry, brain morphometry, brain connectivity, diagnostic tests and interventional studies. All these articles are focused on neurodegenerative conditions, mostly Alzheimer's disease and Parkinson's disease. Interestingly, several articles addressed the early stages of these diseases.

All together, this Research Topic provides a rich perspective of the research made today around neuropsychological and neuropsychiatric aspects of neurodegenerative diseases. We hope readers enjoy this collection of articles.

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EDITORIAL
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Editorial: Neuropsychology and Neuropsychiatry of Neurodegenerative Disorders

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Keywords: neuropsychology, neuropsychiatry, neurodegenerative diseases, mild cognitive impairment, parkinson disease, alzheimer disease, neuroimaging, neuropsychological tests

This Research Topic is published to gather some of the latest science around neuropsychology and neuropsychiatry in neurodegenerative disorders.The call was launched in 2014 and 20 articles were eventually accepted and published, including papers on language and visuospatial functions, emotion, psychometry, brain morphometry, brain connectivity, diagnostic tests, and interventional studies in different conditions, mostly Alzheimer's disease (AD) and Parkinson's disease (PD). Interestingly, several articles focused in the early stages of these diseases. This paper you are reading is the editorial article introducing these publications.

Let's start by the opinion article by Laurent and Noiret, elaborating on visual-motor embodiment of language and the implications for the neuropsychological evaluation in AD (Laurent and Noiret, 2015). They remind us how much cognition is situated, grounded and embodied in specific perceptual and perceptual-motor systems, which allow recursive processes, conceptual elaboration, and the enaction of modular "cognitive functions."

In a general commentary article, Moustafa commented on the paper "Effects of aging and involuntary capture of attention on event-related potentials associated with the processing of and the response to a target stimulus" by Cid-Fernández et al. (2014). Moustafa says Cid-Fernandez et al. findings have implications for the understanding of motor and cognitive problems associated with age-related neurodegenerative disorders, including PD and AD (Moustafa, 2014).

We are happy to say 17 original articles were published in this Research Topic. We want to start highlighting the one by Drummond et al., who showed deficits in narrative discourse elicited by visual stimuli are already present in patients with mild cognitive impairment (Drummond et al., 2015). This study evaluated parameters for investigating narrative discourse in patients with AD and amnestic Mild Cognitive Impairment (a-MCI) and a control group. The Control and AD groups differed in all parameters except narrative time and the total number of words recalled. The a-MCI group displayed mild discursive difficulties that were characterized as an intermediate stage between the Control and AD groups' performances. The a-MCI and AD groups were similar to one another but differed from the control group with respect to the type of words recalled, the repetition of words in the same sentence, the narrative structure and the inclusion of irrelevant propositions in the narrative. The narrative parameter that best distinguishes the three groups was the speech effectiveness index.

Continuing with visual stimuli, Yin et al., assessed the visuospatial characteristics of an elderly Chinese population using the Wechsler Adult Intelligence Scale-Revised (WAIS-R) Block Design Test (BDT) (Yin et al., 2015). They found that simple BDT task scores can distinguish demented patients from MCI, while difficult BDT task scores can ease discriminating between controls and

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MCI. Thus, normative data stratified by education and age for the Chinese elderly populations are provided to be used in the diagnosis of dementia and MCI in this population.

More on psychometric scales and populations; researchers from Brazil showed the psychometric properties of the Brazilian version of the FAQ (P-FAQ) has god ecological validity, reliability, internal consistency and construct validity. Therefore, this questionnaire is now ready to be used in the Brazilian population of older adults (Assis et al., 2014).

From psychometric to neuroimaging studies: Balardin et al., studied the differences in prefrontal cortex activation and deactivation in MCI using fMRI while encoding word lists (Balardin et al., 2015). MCI individuals showed reduced free recall scores when using self-initiated encoding strategies but they were increased to baseline controls' level after receiving directed instructions. Greater recruitment of front parietal regions was observed in both MCI and control groups during directed strategic encoding. In conclusion, this study provides evidence showing that differences of activity in these regions may be related to encoding deficits in MCI, possibly mediating executive functions during task performance.

Authors from Japan, studied how variations in WMH volume and cortical thickness relate to episodic memory, depressive state, and the presence of MCI (Fujishima et al., 2014). MCI participants exhibited thinner cortices in the inferior parietal and temporal lobes and greater WMH volumes in the semioval center and corona radiata than controls. Also, poor episodic memory was associated with increased WMH volume in the posterior periventricular regions and thinner cortices in the left entorhinal region in MCI participants. Compared with non-depressed MCI participants, depressed MCI participants showed greater WMH volume as well as reduced cortical thickness in the gyrus adjacent to the amygdala and in the anterior medial temporal lobe bilaterally. In MCI participants, a higher WMH volume was associated with cortical thinning in the frontal, temporal, and parietal regions. In conclusion, these results confirm that "depression and episodic memory are associated with both cortical thickness and WMH volume in MCI subjects."

A group of authors from California (USA), studied the interactive effects of vascular risk burden and advanced age on cerebral blood flow (Bangen et al., 2014). Authors conclude that "older adults with elevated vascular risk burden may be particularly vulnerable to cognitive change as a function of CBF reductions." Then CBF might be used as a potential biomarker in preclinical AD.

Graph theory is a mathematical approach to analyze relations between items and represents a promising tool to understand neuropsychological states. Graph analysis is even likely to become clinically relevant in neurology and psychiatry, being particularly useful for the differential diagnosis of different conditions. A couple of articles in this Research Topic used graph analyses. A group of authors from Brazil showed graph analysis of verbal fluency test discriminate between patients with AD, mild cognitive impairment and normal elderly controls (Bertola et al., 2014a). This research provides support for a new methodological frame to assess the strength of semantic memory through the verbal fluency task, with potential to amplify the predictive power of this test. The same group of authors also showed in a different article how impaired generation of new subcategories and switching is in a semantic verbal fluency test in older adults with mild cognitive impairment (Bertola et al., 2014b). This finding indicates that semantic memory impairment is a visible and recent deficit that occurs even in non-demented subjects with MCI.

It is well known that cognitive decline and dementia due to AD are associated with lifestyle, genetic and environmental factors. Several potentially modifiable risk factors should be considered for preventive or ameliorative interventions in AD. The list of such risk factors is still expanding. Researchers from Australia found bone mineral density (BMD) and body composition are two of such potentially modifiable risk factors (Sohrabi et al., 2015). Specifically, researchers found the List A learning from California Verbal Learning Test was significantly associated with lean mass and BMD. These findings indicate that "there is an association between BMD and lean body mass and episodic verbal learning."

Continuing with studies addressing the impact of cognition and emotional status on daily functioning, de Paula et al., cols investigated if depressive symptoms and specific cognitive domains affect different aspects of activities of daily living (ADL) (de Paula et al., 2015). They observed that depressive symptoms were predictive of ADL involving social contact and that different instrumental ADL have specific cognitive predictors. Authors conclude that there are specific patterns of influence depending on the specific instrumental ADL.

Iturria-Medina and Evans, from Canada, reviewed the role of brain connectivity in the progression of neurodegenerative diseases (Iturria-Medina and Evans, 2015), offering an overview on how connectivity dysfunctions mediate neurodegeneration, with a specific focus on how these dysfunctions are related to normal aging and the progression of neuropathologic changes.

This review article links with the original research article by Yi et al. (2015) who showed that differences in functional brain connectivity alterations are associated with cerebral amyloid deposition in a-MCI. Compared to controls, non-amnestic MCI showed atrophy in bilateral superior temporal gyri whereas a-MCI showed atrophy in right precuneus. The results indicate that "despite the similarity in cross-sectional cognitive features, non-amnestic MCI has quite different functional brain connectivity compared to a-MCI."

More on brain connectivity; researchers from China used a resting-state fMRI approach to compare connectivity in healthy controls with connectivity in PD with tremor (Zhang et al., 2015). Patients showed increased centrality in the occipital, parietal and frontal regions while decreased centrality in the thalamus and the cerebellum anterior lobe. Seeded at these regions, a distributed network was further identified that encompassed cortical and subcortical regions, as well as the brainstem and the cerebellum. Graph-based analyses of this network revealed "increased information transformation efficiency in the group of patients." Moreover, the identified network correlated with patients' clinical manifestations and this finding could distinguish controls from patients. Together, these results "provide a comprehensive view of network disorganization in PD with tremor and have

important implications for understanding neural substrates underlying this specific type of PD."

From imaging markers to biochemistry markers of PD: researcher found elevated levels of cerebrospinal fluid α-synuclein oligomers in healthy asymptomatic LRRK2 mutation carriers (Aasly et al., 2014). An inverse correlation between disease severity and duration and CSF levels of α- synuclein oligomers was observed. This study suggests that quantification of α-synuclein oligomers in CSF has potential value as a tool for PD diagnosis and presymptomatic screening of high-risk individuals.

PD is traditionally regarded as a neurodegenerative movement disorder, however, nigrostriatal dopaminergic degeneration is also thought to disrupt non-motor loops connecting basal ganglia to areas in frontal cortex involved in cognition and emotion processing. A couple of studies focused on the interface between motor, neuropsychological and neuropsychiatric features of PD. Researchers from Switzerland showed apathy in PD is related to executive function, gender and age but not to depression. Authors conclude that initiation dysfunction heralds apathy in PD. Even more, apathy is influenced by age and gender: older age correlates with apathy in men, whereas in women it seems to protect against it (Meyer et al., 2015).

In an interventional study, researchers from Nashville and Louisville (USA), assessed emotion recognition in early PD (EPD) undergoing deep brain stimulation or dopaminergic therapy, compared with healthy controls (McIntosh et al., 2015). EPD patients were impaired on all emotion recognition tasks. Neither therapy type nor therapy state (ON/OFF) altered emotion recognition performance. Finally, elderly controls were impaired on vocal emotion recognition relative to young controls, suggesting a physiological decline related to normal aging. In conclusion, emotion recognition is impaired early in PD, implicating the disruption of fronto-striatal loops mediating emotional function is an early phenomenon in PD.

Other interventional study addressed the effects of combined therapy (MAO-B inhibitors with levodopa) vs. monotherapy in PD (Krishna et al., 2014). Authors found that combined MAO-I and levodopa improves cognition compared to monotherapy. MAO-I combined with levodopa improves neuropsychiatric measures such as depression, apathy, anxiety as well as quality of life. This enhancing effect of combined therapy was more pronounced in PD patients with severe akinesia, compared to patients with severe tremor, suggesting akinetic patients particularly benefit from combined therapy.

A third interventional study, this time from France, assessed neuropsychiatric fluctuations in PD in ON and in OFF and compared them to controls (Fleury et al., 2014). The Visual Analog Mood (VAMS) and the Apathy scores improved by the acute intake of levodopa. Negative emotional Stroop task induced a lengthening of the mean reaction time during the incongruent trials compared with the congruent trials in controls and in ON patients, but not in OFF patients. OFF patients showed lower activation than Controls and ON patients within the right pregenual anterior cingulate cortex (pACC), an area specifically involved in emotional conflict resolution. Thus, pACC hypoactivation may contribute to explain neuropsychiatric fluctuations in PD. Emotional conflict processes should be understood as dopamine-dependent, therefore the practical learning point is that adjustments of dopaminergic medication might be helpful for treating these non-motor symptoms.

To summarize, this Research Topic is plenty of interesting articles from different and mutually enriching perspectives and methodologies, all around neuropsychological and neuropsychiatric aspects of neurodegenerative diseases. For us, it has been a pleasure serving as editors of these publications. We have learned both from authors and reviewers in the process of improving the original manuscripts until the final version of these papers was ready to be published. We hope readers also find this collection of articles interesting. Enjoy!

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OPINION
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Visual-motor embodiment of language: a few implications for the neuropsychological evaluation (in Alzheimer's disease)

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Keywords: vision disorders, embodied cognition, modularity of mind, enaction, enactivism, neuropsychological assessment, neuropsychological testing, sensory deficits

> *"The productive combination of adjectives, nouns, verbs, and other linguistic elements corresponds to the productive combination of perceptual symbols for properties, entities, processes, and other conceptual elements"* (Barsalou, 1999, p. 594)

Introduction

For several decades, researchers in cognitive neuroscience and cognitive psychology have developed works concerning the close relationships between "lower-level" perceptual/motor and "higher-level" conceptual/linguistic processes (Harnad, 1987; Goldstone, 1994; Barsalou, 1999; Pulvermüller and Fadiga, 2010). Some of them suggested to "reunit" perception and conception (Goldstone and Barsalou, 1998), studied the interactions between language and action (Glenberg and Kaschak, 2002; see Pulvermüller and Fadiga, 2010, for a global picture in neuroscience), or were interested in the relationships between language and other bodily (emotional) states (Glenberg et al., 2005).

On the neurophysiological and ophthalmological grounds, contemporary studies suggest that not only does Alzheimer's Disease (AD) lead to alteration in brain cortical structure and cognitive impairment, but it also conducts to deep changes in both visual system organization and vision-based performances (Tzekov and Mullan, 2013).

We recommend that both the perceptual impairment found in AD and the interactions between lower-level and higher-level cognition be taken into account by neurospychologists in order to avoid misattribution of performance deficits.

We first mention a recent research concerning language evaluation in AD and discuss main limitations of modular evaluation in that type of context. Then, we present main features of the visual "function" impairment in AD, the impacts of perceptual changes over higher-level cognition, and finally, we provide general recommendations for neuropsychological testing of higher-level cognitive "functions".

Linguistic Evaluation in AD

Drummond et al. (2015) reported an interesting research in which language production processes were evaluated in patients with AD, amnestic mild cognitive impairment (a-MCI), and controls. In contrast with many neuropsychological tests aiming at evaluating language on the basis of simple concept production (e.g., naming), the authors developed

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a "narrative test," in which participants were supposed to narrate a story from a sequence of visually presented actions. Overall, the authors found that patients with a-MCI already presented narrative deficits in comparison with the control group. Interestingly, a-MCI discursive deficits were lower than those presented by patients with AD, which may be interpreted as an intermediate level of deficiency between healthy elderly and patients with AD.

The research is interesting and allows us to examine usual practices in neuropsychology and neuropsychological research. Although the participants in this kind of research generally undergo both neuropsychological and visual (i.e., acuity) assessments, the real involvement of language "function" in the deficits found in patients with AD or a-MCI can be questioned. As we will see later, AD can lead to several visual processing impairments that influence higher-level cognitive performance so that checking for normal or corrected-to-normal visual acuity is not sufficient to control for lower-level influence on cognitive performance. Typically, whether in neuropsychology or in speech therapy, language abilities in AD are often evaluated by tests involving the visual "function". For instance, in the naming tests, patients have to orally produce the word represented by a drawing picture. Similarly, oral comprehension tests ask patients to indicate, among several pictures, which corresponds to a word or a sentence read by the examiner. In other words, patients have to visually recognize a picture (as in the naming tests) based on an oral description. The matching category tests also require patients to choose—among several visual items—the one that is semantically associated with a target item. Finally, tests that focus on graphic abilities (e.g., dictation, free writing, writing description) also rely on visual "functions".

When performances are altered in the tests such as those described above, any earlier level of information processing can be involved (Greene, 2005). Although they are mainly employed to evaluate language, these tests can also reflect visual "function" impairment. The semantic recognition of drawings or pictures implies that patients rely on good visual acuity, color vision, contrast sensitivity, and oculomotor processing. Misinterpretations of AD patient troubles may arise if visual performances are not taken into account (and controlled for in statistical analyses).

The Visual "Function" in AD

If cognitive—and especially memory—disorders are a hallmark of AD, it is less widely known but well established, that many visual processes are also altered at multiple levels of the nervous system in AD.

The lens (equatorial supranuclear cataracts), the retina (loss of ganglion cells, narrowing of venous blood column), macula (volume decrease), the retinal nerve fiber layer (reduction in thickness at the optic nerve head), the optic nerve (widespread axonal degeneration of the retinal ganglion M-cells), the lateral geniculate nucleus (demyelination, amyloid plaques especially in parvocelullar layers), the superior colliculus [amyloid plaque and neurofibrillary tangle (NFT) accumulation], the pulvinar (amyloid plaques and neuritic plaques), the visual cortex—possibly at later stages of the disease—(neuronal loss, amyloid plaques, and NFT, especially in early-onset forms) have been found to be affected in AD (see Tzekov and Mullan, 2013, for an excellent synthesis).

Psychophysical measures have also revealed noteworthy differences between patients with AD and controls. Visual acuity might be decreased in AD, as a function of disease severity, and/or under low luminance conditions. Color perception—at least in the blue-violet spectrum—is altered in AD, and visual field measures—when possible with AD patients—have revealed field constriction with deficits being more severe in the inferior part of the visual field (potentially because of the distribution of senile plaques and NFT in the visual cortex; Tzekov and Mullan, 2013). Contrast sensitivity (CS) is also reduced in both patients with AD and patients with MCI. Clearest evidence of differences in CS as a function of group (AD, MCI, Cognitive complaints without performance deficits, Controls) has been found in the upper right visual field using frequency-doubling technology, and CS has been regarded as a potential biomarker of AD (Risacher et al., 2013). Finally, both depth (Mendez et al., 1996) and motion (Gilmore et al., 1994; see Fernandez et al., 2013, for an electrophysiological approach) perception abilities are impaired in AD (see also Mandal et al., 2012, for a global picture).

Several studies demonstrated oculomotor processing impairment in AD, even at early stages of the disease or in mild cognitive impairment (Peltsch et al., 2014; Pereira et al., 2014; Molitor et al., 2015). Increased reaction time to trigger saccades, difficulty to inhibit saccadic reflex, and decreased smooth pursuit velocity, acceleration, and accuracy, have been consistently reported (Boxer et al., 2006, 2012; Garbutt et al., 2008; Crawford et al., 2013). Eye movements involved in visual exploration/search are also impaired in AD (Rösler et al., 2000, 2005; Mosimann et al., 2004; Molitor et al., 2015). For instance, when they were supposed to search for a number among 79 letters randomly distributed on a screen, patients with AD had deficits in target detection and detection time, associated with more fixations and longer fixation duration (Rösler et al., 2000). Mosimann et al. (2004) found that during a clock-reading task, patients with AD displayed fewer fixations at the end of each clock hand, a significant delay before their first fixation landed inside these regions of interest, longer fixations, and smaller saccade amplitudes.

Not only do the reviewed studies lend support for an impairment of basic visual anatomy, physiology, and behaviors, but they also suggest that higher-level cognition can be influenced by visual impairment in AD.

Interactions between Visual and Higher-level Cognitive Processes

As rightly noted by Tzekov and Mullan (2013), psychophysical evaluations in AD should be carried out with caution because of the potential influence of cognitive (and affective) variables—such as compliance with the instructions, assignment comprehension, and memorization, vigilance required during testing—over the measured performances. In the other way round, the numerous changes that affect vision and eye movements in AD should be taken into account while evaluating

later and higher-level processes. This should also be the case of language production tasks. If visual acuity (which is the simplest but most controversial psychophysical measure to identify low-level visual impairments in AD, see Tzekov and Mullan, 2013, p. 419) is generally controlled for, other visual or visuomotor variables are scarcely taken into account when testing language performances.

This state of affair, which tends to undermine the granted weight of lower-level perceptual-motor processes in cognitive neuropsychological testing, is all the more problematic that previous empirical studies reported clear interferences, not only between visual impairment and Benton's Facial Recognition, or Visual Form Discrimination tests (Kempen et al., 1994), but also between visual impairment and higher-level-cognition evaluation. For instance, Killen et al. (2013) found that visually impaired elderly individuals scored lower than controls in both the vision-dependent items of the Mini-Mental-State-Examination (MMSE) and the Clock drawing test (CDT), but not when vision-independent items of the MMSE and the CDT were proposed. Even if the visual impairments under consideration were rather severe (e.g., macular degeneration, glaucoma), the data suggest that attention should be paid to the control of visual impairment when higher-level cognition is tested. Note that the reduction in macular volume has been reported in AD and has been found to be related to cognitive performance (as indexed by the MMSE; Iseri et al., 2006).

A recent review of the literature outlined the overlap between cataract and cognitive impairment (Jefferis et al., 2011). Wood et al. (2010) reported impaired performance in older adults across three cognitive tests (the digit symbol substitution test, trail making test A and B, the Stroop color word test) when cataract conditions were simulated.

As another example, by manipulating the stimulus strength of each item of several tests through contrast sensitivity function filtering (i.e., low-degraded, medium-normal, and high-enhanced stimulus-strength conditions), Cronin-Golomb et al. (2007) demonstrated that the modification of stimulus strength altered performances in several tests in AD. They found that performances in *letter identification, word reading, picture naming*, and *face discrimination* decreased more in AD patients in comparison with healthy elderly in the low-degraded condition. Interestingly, AD patients improved their performances to a level equal to their healthy counterparts when stimulus strength was enhanced.

In this context, and given the extent of visual alteration in AD, much care should be taken when considering language performances, especially if the task requires conceptual production from visual stimulation.

Conclusion: The Need for More Systematic Evaluations of Visual Processes and More Systemic Reasoning in Neuropsychology

Taken as a whole, data concerning the effects of AD over visual processes and those demonstrating the influence of visual impairment on higher-level cognition suggest that controlling for visual impairments in patients with AD could provide critical information to attribute capacity loss to appropriate processing levels. Neuropsychologists know well the strong time constraints that often feature clinical neuropsychological testing. However, when and where possible, some measures should be performed or taken into account (when they are provided by an ophthalmologist) in order to control for any effect of lower-level process (e.g., visual or visuomotor-encoding processes) impairment over higher-level cognitive "functions" (e.g., language production). This is critical when the protocol involves *the perception and the interpretation of a visual scenario*. Disentangling the language production impairments from other disorders in this kind of settings implies to (i) more thoroughly examine potential differences in visual and eye movement performances between patients and controls through the use of appropriate visual/visuomotor tests, and (ii) consider those performances as covariates in any further group comparison concerning cognitive abilities, namely when the cognitive test requires the processing of visual information. This is needed to better understand and characterize the cascade of alterations associated with AD; especially because AD patients "*are less likely than healthy elderly individuals to report vision problems to their physicians*" and that "*sensory deficits can be hidden and may masquerade as higher order deficits*" (Gilmore et al., 2004).

Beyond the specific case of language, the proposed approach asks basic questions about our conception of cognitive processes. The discussed effects remind us how much cognition is situated, grounded, embodied in specific perceptual (Goldstone and Barsalou, 1998; Barsalou, 1999) and perceptual-motor (Laurent, 2014) systems, which allow recursive processes, conceptual elaboration, and the enaction of what is more classically regarded as modular "cognitive functions."

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Motor and cognitive changes in normal aging

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A commentary on

Effects of aging and involuntary capture of attention on event-related potentials associated with the processing of and the response to a target stimulus
by Cid-Fernandez, S., Lindin, M., and Diaz, F. (2014). Front. Hum. Neurosci. 8:745. doi: 10.3389/fnhum.2014.00745

In a recent study, Cid-Fernandez et al. (2014) tested attentional performance in 3 age groups: young (21–29 years old), middle-aged (51–64 years old), and older adults (65–84 years old). The task used in this study involved presenting both visual and auditory cues to the participants, and they were required to pay attention to the visual cues while ignoring the auditory cues (for task details, see Escera et al., 1998). Cid-Fernandez et al. (2014) found that there was an increase in distractibility and changes in motor selection in the middle-aged and older groups, compared to the young group. These findings were revealed using electroencephalography analyses which related different cognitive and motor processes to different event-related potential components. Impairment in sensory filtering (e.g., longer time to characterize stimuli in working memory), as revealed by differences in the N2b component among the groups are presumed to explain the deficits in motor processes (i.e., selection of motor responses). It is thus suggested that these cognitive working memory changes in aging lead to slowing down of motor response selection.

Other prior studies have investigated changes in motor changes in aging (Light, 1990). In one study, it was reported that abnormalities in motor processing are not related to cognitive processing (Kolev et al., 2006). For example, Falkenstein et al. (2006) also reported abnormalities in motor selection processes in healthy aging. However, unlike the Cid-Fernandez et al. (2014) study did not find a different in early stimulus processing. Further, Falkenstein et al. (2006) have only tested younger and older adults. The differences in findings could be related to different age groups in both studies.

What is important about this study is Cid-Fernandez and colleagues (a) have tested both motor and cognitive processes in (b) three age groups. Most existing studies on aging often compare younger (aged 18–30 or 50 years old) with older (aged 60 or 65 years and over) adults (Scott, 1994; Braver and Barch, 2002; Cabeza et al., 2002; Fera et al., 2005; Weiler et al., 2008; Willemssen et al., 2011; Brehmer et al., 2012; Trewartha et al., 2014), and thus do not reveal the subtle motor and cognitive changes that may occur during the aging process, for example at 40s or 50s years old. Cid-Fernandez et al. (2014) found that attentional and motor changes can occur from 50 years onwards. Since Cid-Fernandez and colleagues did not recruit participants in their 40s, it is not known whether such cognitive and motor changes can occur at a younger age than reported by the authors. Similarly, studies that recruit only younger and older adults may not reveal the exact age (or age group) at which behavioral and neural changes occur.

Importantly, there are studies that test behavioral performance in more age groups than those recruited in the Cid-Fernandez et al. (2014) study (see for example studies by Davis et al., 2003, 2013; Krishna et al., 2012; Stark et al., 2013). One such study (Davis et al., 2003) recruited 4 groups: Group 1 (30–44 years old), Group 2 (45–59 years old), Group 3 (60–74 years old), Group 4 (75–90 years old). Using the Rey Auditory Verbal Learning Test (RAVLT, which measures memory acquisition, recall, and recognition), Davis et al. (2003) found that memory acquisition was lower in the 60–74 and 75–90 age groups, compared to the other groups. They also found that forgetting after one-day delay was more common in some, but not all, participants in the 75–90 years old group, in comparison to the other age groups. These findings suggest that impairment in cognitive performance occur at different times during the aging process, depending on the cognitive process being investigated as well as age group.

Other studies have recruited participants across various age groups and correlated behavioral performance with age (Davis et al., 2013; Stark et al., 2013). For example, Stark and colleagues reported impaired performance in hippocampus-based tasks in some aging populations (Stark et al., 2013; Bennett et al., 2014). One recent study by Davis and colleagues have recruited participants from age 5 to age 90 (Davis et al., 2013), which is probably among the most inclusive studies of age groups. Davis and colleagues found that verbal learning impairments were common among children and older adults in comparison to younger and middle-aged adults. The benefit of having such a wide range of age groups is to reveal at which age acquisition (in early ages) as well as decline (in older age) of motor and cognitive processes may occur.

Cid-Fernandez et al. (2014) suggest that their behavioral and physiological findings of impaired attentional performance can be related to changes in the prefrontal cortex during aging. This is supported by an extensive body of research showing impaired prefrontal function during the aging process (West, 1996; Braver and Barch, 2002; Solbakk et al., 2008; Wang et al., 2011; Johnson et al., 2013). In addition to the prefrontal cortex, healthy aging also affects the basal ganglia and hippocampus structures as well as neurotransmitters projecting to these brain regions, including dopamine and acetylcholine (Kaasinen et al., 2000; Inoue et al., 2001; Small et al., 2011). However, it is argued that brain changes (as well as corresponding behavioral decline) do possibly occur at different degrees and at different times during the aging process (Krishna et al., 2012). In one recent study, we found that older adults (75 and above) show more impairment in hippocampal-based tasks compared to less older adults (60–70 years old), but basal ganglia-based learning was impaired in the older and less older groups (Krishna et al., 2012; also see Moustafa et al., 2012). Thus, future work should explain how alterations to certain brain regions and neurotransmitters correspond to specific motor and cognitive changes in different age groups.

The Cid-Fernandez et al. (2014) findings have implications for the understanding of motor and cognitive problems associated with age-related neurodegenerative disorders, including Parkinson's and Alzheimer's diseases. Prior studies have also reported deficits in the selection of motor responses, yet it is not known whether these deficits are due to motor and/or cognitive abnormalities in these in different age-related neurodegenerative disorders (Hocherman et al., 2004; van Deursen et al., 2009).

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Deficits in narrative discourse elicited by visual stimuli are already present in patients with mild cognitive impairment

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Language batteries used to assess the skills of elderly individuals, such as naming and semantic verbal fluency, present some limitations in differentiating healthy controls from patients with amnestic mild cognitive impairment (a-MCI). Deficits in narrative discourse occur early in dementia caused by Alzheimer's disease (AD), and the narrative discourse abilities of a-MCI patients are poorly documented. The present study sought to propose and evaluate parameters for investigating narrative discourse in these populations. After a pilot study of 30 healthy subjects who served as a preliminary investigation of macro- and micro-linguistic aspects, 77 individuals (patients with AD and a-MCI and a control group) were evaluated. The experimental task required the participants to narrate a story based on a sequence of actions visually presented. The Control and AD groups differed in all parameters except narrative time and the total number of words recalled. The a-MCI group displayed mild discursive difficulties that were characterized as an intermediate stage between the Control and AD groups' performances. The a-MCI and Control groups differed from the AD group with respect to global coherence, discourse type and referential cohesion. The a-MCI and AD groups were similar to one another but differed from the Control group with respect to the type of words recalled, the repetition of words in the same sentence, the narrative structure and the inclusion of irrelevant propositions in the narrative. The narrative parameter that best distinguished the three groups was the speech effectiveness index. The proposed task was able to reveal differences between healthy controls and groups with cognitive decline. According to our findings, patients with a-MCI already present narrative deficits that are characterized by mild discursive difficulties that are less severe than those found in patients with AD.

Keywords: narrative discourse, language, mild cognitive impairment, Alzheimer's disease, aging

Introduction

Elderly individuals may seek treatment for a wide range of cognitive deficits. Because of the lack of specificity of these complaints, clinicians can have difficulty determining the real deficit. Difficulties associated with narrative discourse deficits, such as repetitions or information gaps during the narrative, are often the main reason for referral, but patients and relatives often attribute such deficits to memory problems. Because the traditional language tests used in clinical practice, such as naming and semantic or phonemic verbal fluency tests, do not address narrative discourse, such deficits may go unnoticed during evaluations.

Discourse is a complex linguistic activity that involves different levels of the linguistic system (phonological, morphosyntactic, semantic-lexical, and semantic-pragmatic) in conjunction with other cognitive aspects, including executive functions (Mar, 2004; Cannizzaro et al., 2012). Narrative discourse can be distinguished from other types of discourse—such as spontaneous and descriptive discourse—because it requires the speaker to verbally reproduce an episode experienced in the present (perception) or in the past (memory) while respecting the temporal and causal relationships among events that unfold in particular scenarios. This cognitive structure confers an ecological advantage to such evaluations (de Lira et al., 2011; Cannizzaro and Coelho, 2013).

Studies on narrative discourse have been conducted with individuals with right hemisphere injury (Marini et al., 2005; Fonseca et al., 2008; Ferré et al., 2011; Scherer et al., 2012a) and traumatic brain injury (TBI) (Davis and Coelho, 2004; Coelho et al., 2012). Difficulties with narrative discourse are already evident in the early stages of Alzheimer's disease (AD)-related dementia (Chapman et al., 2002; Duong et al., 2005; Mansur et al., 2005; Ska and Duong, 2005; Ash et al., 2007; Ferris and Farlow, 2013; Tsantali et al., 2013). However, little is known about the discursive characteristics of patients with amnestic mild cognitive impairment (a-MCI), which is a transitional stage between full cognitive ability and AD (Petersen, 2004).

Elderly individuals maintain the microstructural (phonological, lexical, and morphosyntactic) aspects of language and are able to understand discourse. However, they display a number of difficulties related to both the complexity of the task and the speed of the interlocutor's speech (Burke and Shafto, 2007; Peelle et al., 2010). An individual's discursive profile may be related to cognitive processes that usually decline with aging, such as processing speed, memory, attention (Wright et al., 2011), executive processes (Cannizzaro and Coelho, 2013), and visual perception or auditory processing (Bidelman et al., 2014). Several authors have suggested that changes in the discursive profile may also be associated with socio-affective aspects of adaptation to aging (Brandão and Parente, 2011), such as the need to reinforce one's identity (Lin et al., 2004). Repetitive and lengthy discourse with the addition of information or memories that reflect life experiences are among the most commonly described characteristics of elderly people's spontaneous discourse (Lin et al., 2004; Scherer et al., 2012b). At around the age of 70 years, specific difficulties with propositional content (Kemper et al., 2001) and spontaneous lexical access occur, such as slow word-finding, difficulty recalling names, and the "on the tip-of-the-tongue phenomenon" (Stamatakis et al., 2011). In contrast, lexical evocation via visual confrontation is preserved. The communicative difficulties of healthy aging are subtle (Madhavan et al., 2014) and stable and thus have less impact on daily functioning compared with the memory and executive changes that are present in normal aging (Scherer et al., 2012b; Caselli et al., 2014).

Even during the early stages of AD, individuals present linguistic difficulties (Teichmann and Ferrieux, 2013). In addition to the recognized difficulties with semantic lexical evocation (Henry and Crawford, 2004) and naming in response to visual stimuli (Lin et al., 2014), difficulties with narrative discourse occurs (Ash et al., 2007). Studies investigating narratives and recounting showed that AD patients have more deficits in macrolinguistic areas (semantic-pragmatic) than in microlinguistic ones (phonological, lexical and basic syntactic structure components) (de Lira et al., 2011). The discourse of AD patients is characterized by reduced information and less effective communication. The major difficulties might include exacerbated repetitions; a smaller number of propositions (Ska and Duong, 2005); difficulty reporting events in sequence (Mansur et al., 2005); information gaps that hinder overall meaning (Chapman et al., 2002; Mar, 2004); cohesion and overall coherence (Ash et al., 2007; Brandão and Parente, 2011); and difficulty making inferences. Such features could not be exclusively explained by difficulties with lexical access (Taler and Phillips, 2008).

a-MCI is clinically characterized by episodic memory deficits, but language impairment may also occur (Petersen, 2004). The literature suggests that confrontation naming and semantic verbal fluency tasks, regardless of the suggested category (Taler and Phillips, 2008), might be capable of differentiating patients with MCI from healthy older adults. However, there are some controversial findings. Some studies showed that the visual stimulus naming test is not suitable for detecting early AD (Testa et al., 2004) or distinguishing between normal controls and patients with MCI (Beinhoff et al., 2005). A study demonstrated that semantic verbal fluency tasks (naming animals and fruits) are useful for differentiating between normal elderly and AD groups but were less able to accurately differentiate the MCI groups from normal healthy elderly adults or people with AD (Radanovic et al., 2009; Lopez-Higes et al., 2014). There are few studies of narrative discourse in patients with MCI. Typically, these studies involve assessing narrative comprehension's demands on the memory domain by requiring patients to either recount or understand a heard story. The MCI group might exhibit difficulties in the global understanding of narratives; their performance is likely to be similar to that of the AD group and worse than that of the Control group (Chapman et al., 2002). The few studies that analyzed discourse production were based on single-scene description tasks, such as "The Cookie Theft Picture" task (Forbes-McKay and Venneri, 2005; Tsantali et al., 2013). To the best of our knowledge, no study has investigated the characteristics of the a-MCI group's narrative performance on narrative tasks using visual stimuli with sequences of actions.

Narrative production might be associated with different neuropsychological processes, such as episodic memory (Chapman et al., 2002; Taler and Phillips, 2008), executive function (Mar, 2004; Troiani et al., 2008; Cannizzaro and Coelho, 2013), and the semantic-pragmatic component of language (Fonseca et al., 2008; Troiani et al., 2008). Distinguishing the limits of these different functions is a challenge for clinicians and researchers. According to Lezak et al. (2012), it is very difficult to demarcate the boundaries of different but integrated cognitive processes.

The discourse tasks used to assess the narrative productions of elderly individuals are often based on an illustrated story without a text, a recounting of a heard story or a narrative describing a single picture (from a storybook or from a sequence of actions). The examined aspects might vary depending on the objectives and sample type of the study. Such studies typically consider macrolinguistic (i.e., related to planning, overall consistency, and coherence) or microlinguistic (i.e., related to words and sentences) aspects. Local and global coherence, referential cohesion, the narrative's structure and planning, the lexical index, the number and type of sentences, the generated propositions and the contextual suitability are among the most consistently analyzed criteria (Davis and Coelho, 2004; Ska and Duong, 2005; Ash et al., 2007; Wright et al., 2011; Coelho et al., 2012).

A review of the literature demonstrated the importance of using visual stimuli when evaluating individuals with AD (Davis and Coelho, 2004; Ska and Duong, 2005; Brandão and Parente, 2011). The use of tasks that involve describing a sequence of actions has advantages, such as requiring the participant to link facts, integrate scenes and establish the relationships among the events (unlike stimuli that present a single scene) and providing increased objectivity and reproducibility (compared with the analysis of autobiographical or spontaneous discourse). In addition, such tasks decrease the demand on episodic memory (Duong et al., 2003; de Lira et al., 2011).

As stated above, some studies have investigated discourse deficits among individuals with AD, but little is known about those deficits during the a-MCI stage. This issue is of clinical interest because a-MCI may constitute a predementia stage of AD (Petersen, 2011). As previously described, tests that elicit narrative discourse with visual stimuli could offer advantages such as avoiding memory overload, which could be a confounding factor for individuals with memory deficits such as a-MCI. To the best of our knowledge, no available tests use visual stimuli to assess this population.

The "car accident" task consists of seven scenes telling the story of an accident (Ska and Duong, 2005). The scenes are portrayed on cards that are presented to the individual, who is prompted to narrate the "story" without a time limit. This task was chosen because it involves a narrative situation that targets previously consolidated knowledge, that is, a common event that occurs in daily life. The number of scenes (seven) allows the narrative to be divided into three blocks of events (i.e., the initial event, the development and the outcome), thus permitting coherence and cohesion analyses that cannot be assessed with tests that present fewer sequences.

The present study has two objectives: (1) to introduce quantitative parameters for assessing the narrative discourse of the elderly based on a visually presented sequence of actions and (2) to investigate whether these parameters are able to distinguish among healthy controls, people with a-MCI and people with AD.

Based on the literature review, we hypothesized that narrative discourse deficits may be present in MCI because such deficits are already evident in the mild stages of AD.

Materials and Methods

Our study required a brief preliminary study (pilot study) because there are no data available in the literature regarding the psychometric properties of the task. The study had two phases. The first phase was exploratory and conducted with a non-clinical sample to define the parameters with which to quantify the narrative discourse based on visual stimuli (the car accident task (Ska and Duong, 2005), not standardized for the Brazilian population). The psychometric investigation of the task was beyond the scope of our study; however, we needed preliminary findings for a representative Brazilian sample that included individuals from both genders and different educational levels and ages. We needed to investigate how individuals narrate the story in our country; that is, we needed to determine which words and sentences were most often used in Portuguese to describe each of the seven scenes and which sentences were most often used to convey the main ideas of the story. The parameters of the pilot study were then used in the analyses of the second phase of our study. The second phase was the clinical application of these parameters to a-MCI and AD clinical groups. The Research Ethics Committee of the D'Or Institute approved this project (CEP 226/11). All of the participants provided written informed consent to participate in the study.

Experiment 1: Pilot Study

Sample

Thirty individuals (males and females) ranging in age from 30 to 80 years were selected from a convenience sample. The sample was divided into two education levels (8 years or more and less than 8 years of education) to better represent Brazilian population. The individuals were recruited from the Federal University of Rio de Janeiro and the D'Or Institute of Research and Education via direct invitation. The sample comprised professors, undergraduate students, staff members and relatives of patients attending the university's outpatient unit. The participants were screened for global cognitive functioning using the Mini-Mental State Examination (MMSE). The participants with normal MMSE results based on Brazilian normative data (Brucki et al., 2003) were included. Three participants were excluded: two with more than 8 years of education and one with 2 years of education. All of the excluded volunteers had MMSE scores lower than expected for their age and education level.

Narrative Task Using Visual Stimuli

The participants were asked to narrate a story (the "car accident" task) based on seven scenes that were visually presented in the correct order. We explained that the story was a sequence

of actions and checked to ensure that the individual clearly understood this. There was no time limit, and the following standardized instruction was given: *"Look carefully at this sequence of actions and tell me what you think happened."*

All of the narratives were recorded on digital high-definition (HD) media and fully transcribed using semi-orthographic transcription. We transcribed exactly what the participant said, regardless of its grammatical correctness. This type of procedure is considered more suitable for the type of analysis we intended to perform because no interference or corrections occurred as the oral language was transposed into writing. All of the words, synonyms or equivalents were listed and compared, with the aim of identifying the keywords and central ideas (macropropositions) used to construct the narrative. All of the narratives were manually transcribed. After the transcription, we listed all of the open-class words that all of the participants used when telling the story. This procedure defined the most significant words related by group for each scene. Thus, we were able to define the keywords and central ideas used to construct the narrative. We obtained nine main ideas that were used to narrate the seven scenes (Supplementary Material).

Statistical Analysis

Exploratory factor analysis (EFA) was used to extract the most relevant recalled words (i.e., "keywords") and to identify the latent factors expressed by the scenes and keywords without the need to discriminate *a priori* the number of factors (keywords) to be identified. Next, confirmatory factor analysis (CFA) was used to define the groups of words that statistically related best to each core action (Supplementary Material). For the purposes of the pilot study and because of the limited sample size we decided to adopt a significance level (α) of 0.10, less stringent (Maas and Snijders, 2003; Gilani et al., 2013). This significance level was chosen to identify possible differences in load factor (scenes); using an (α) of 0.05 we could not be able to identify many "words" that could be important in different "scenes." For these analyses, Mplus software, version 6.0, was used (Tucker and Lewis, 1973; Muthén and Muthén, 1998–2010). Some could argue that EFA was not the best method for this analysis because of the small sample size. Therefore, other analyses were performed. The Kaiser–Meyer–Olkin test (KMO) had a low value (0.55), but according to Schwab (2006) values above 0.5 allow factor analysis to proceed. The Bartlett test had a highly significant value ($p < 0.001$, indicating correlations among variables ("words") and scenes.

Pilot Study Results

The mean age was 52.43 (± 17.7) years (range = 30–80 years), the average number of years of schooling was 10.7 (± 4.8; range = 4–16 years), and the MMSE score averaged 26 points (± 2.7; range = 21–30 points). There was a slight female predominance; the gender ratio was 18:12.

From the analysis of the 30 narrative transcripts, 101 open-class words (nouns, verbs, adjectives, and adverbs) with at least one occurrence were obtained. Through the EFA and the subsequent CFA, we identified 68 words, and their respective loadings related to nine aspects of the seven-scene story (Supplementary Material). For scenes two and six, two factors were related because they described two different, interdependent core actions. Supplementary Material presents the nine macropropositions. They were based on the nine story factors and 33 statistically significant words relative to each scene. The words (variables) showed significant correlations with their corresponding "scenes" (factors or latent variables) based on model fit (CFA).

Frequently occurring synonyms for or words and expressions related to the statistically significant words (those that appeared in the transcripts and were considered correct answers in this study) are also listed. The mean number of words used for the elaboration of each narrative was 97.4, and the mean time to complete the narratives was 51.3 s. The pilot study group averaged 7.1 (± 1.4) macropropositions (range 5–9). Regarding discourse type, there was a predominance of narrative discourse relative to descriptive discourse, with a ratio of 28:2.

Some results obtained from the pilot study (total number of words or micropropositions, time to execute the narrative or discourse type) were not used on the second phase of the study with clinical groups (Experiment 2) because the psychometric properties of the task needed to be further investigated. Furthermore, in experiment 2 we used a control group to compare all variables.

The initial experiment (pilot) was exploratory and aimed to define which words and which macropropositions were necessary for an adequate narrative telling the whole story.

Experiment 2: Clinical Study

Sample

A total of 186 participants from a research project on diagnostic tools for AD-related dementia were evaluated. Of these, 77 met the inclusion criteria for the present study: age equal to or >60 years, education equal to or >8 years, and Brazilian Portuguese as the first language. The exclusion criteria were as follows: a clinical dementia rating (CDR) score higher than 1.0, frontotemporal dementia, primary progressive aphasia, dementia with Lewy bodies, advanced cerebrovascular disease, non-amnestic MCI or a major neuropsychiatric disorder, including major depression. We used the Geriatric Depression Scale (Brief Form) to screen patients for depression.

Most of the participants were referred by physicians, such as geriatricians, neurologists, and psychiatrists, from the City of Rio de Janeiro. The subjects were informed about the procedures and signed an informed consent form. All of the participants underwent a medical evaluation followed by a neuropsychological assessment, magnetic resonance imaging (MRI) and an assessment conducted by a speech-language therapist that included the narrative task. The participants' vision was checked during the clinical/neurological evaluation to ensure normal or normal corrected vision. Diagnoses were made in meetings coordinated by a senior board-certified psychiatrist (PM) and were based on clinical, neuropsychological and MRI assessments collected by a multidisciplinary team of neurologists, neuropsychologists and speech-language therapists. The participants were then divided into three diagnostic groups: Control, a-MCI, and AD. For the a-MCI variable, the Winblad

et al. (2004) criteria were adopted. Memory impairment was objectively defined as performance below 1.5 SD for age on delayed recall tasks of the WMS-III Logical Memory and Visual Reproduction subtests. For the AD variable, we used the diagnostic criteria from the fourth revised edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000) and the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984).

Procedures

The complete language evaluation included a narrative discourse elicited with visual stimuli, confrontation naming (Boston Naming Test – Short Form) and verbal fluency (semantic and orthographic) tasks (Bertolucci et al., 2001). For the narrative discourse task, the participants were shown the same picture cards as the pilot group and were given the same instructions. The same researcher (a speech-language pathologist) assessed all of the participants and was blinded to their pathological status. The task was always administered in the same sequence. All of the sessions were videotaped, and the participants' verbal output was transcribed for further analysis. All of the narratives were then analyzed in accordance with the fixed set of parameters. These parameters were based on previous findings (Davis and Coelho, 2004; Duong et al., 2005; Ska and Duong, 2005; Coelho et al., 2012) and the results of the pilot study. We proposed the effectiveness of speech index as a new parameter. For better control of the variables, we presented the tests in the same order to each participant. The parameters are described below.

(a) *Narrative time:* The total time in seconds required to perform the narrative task, excluding the latency period between the examiner's verbal directions and the beginning of the participant's narrative.

(b) *Total number of words:* The automatic word count function of Word software was used, and repeated words were included in the counts. Open-class and closed-class words (these were of limited number and included articles, conjunctions, prepositions, and quantifiers) were counted separately. For open-class words, words that were repeated once or more per sentence were not counted. A separate index was created for this purpose.

(c) *Discourse type:* Two parameters were established: (1) predominantly narrative and (2) predominantly descriptive. The discourse was considered descriptive when there were no elements of cohesion between the sentences and the narrative comprised frame-by-frame descriptions and the use of present tense or adverbial expressions, such as "here," or demonstrative pronouns, such as "in this frame." The discourse was considered narrative when the story was treated as an event that had already occurred, with temporal ordering and causal links between facts. Because some individuals presented a "mixed" discourse with both descriptive and narrative characteristics, we defined narrative discourses as those in which at least 50% of the text presented narrative characteristics. This quantitative criterion was defined by the authors because the literature lacked relevant guidelines.

(d) *Overall coherence:* We analyzed the number of semantic propositions (macropropositions and micropropositions) following the proposal of Ska and Duong (2005) but did not examine the grammatical complexity of the discourse. Based on Kintsch and van Dijk (1978), this study considers macropropositions as the central idea of each context of action; they are the key feature that determines whether the participant understood the narrative's global meaning and could represent it with a coherent narrative production. Our analysis was based on the nine macropropositions defined in our pilot study. The total number of macropropositions per participant was counted; the maximum was nine main ideas. The number of micropropositions, relevant or irrelevant to the context, was also counted. Micropropositions were defined as information given in addition to the central ideas of the scene (details). All of the ideas that were part of the scene's context were considered relevant, regardless of whether they were essential. Only incorrect or out-of-context ideas were considered irrelevant micropropositions.

Example:
Macroproposition: "The boy set the car's parking brake" (main idea).
Relevant microproposition: "His sister, who was wearing a blue shirt, leaned her head on the seat and waved to her mother" (actual data – details).
Irrelevant microproposition: "When it rains, it is not possible to drive" (the statement has no meaning in the context of the action or to the story as a whole).

(e) *Referential cohesion:* Following principles A, B, and C of Chomsky's binding theory (Leitão, 2005), referential cohesion was defined as the use of cohesive elements between sentences during the narrative. It is indicated by the presence of transitional elements and the use of anaphoric co-reference. The "repeated-name penalty (RNP)" phenomenon (Leitão et al., 2012), which occurs when the narrator uses autonomous referents instead of co-reference, was also considered. In this case, RNP penalizes the listener and/or requires him/her to share the speaker's knowledge because the length of the speaker's sentences makes comprehension effortful. Two parameters that encompass the previously mentioned linguistic criteria and the adapted criteria of Davis and Coelho (2004) were defined: (1) appropriate, when the reference elements were used correctly, and (2) inappropriate, when transitional elements were omitted, when there were errors or ambiguities in relation to Principals A, B, or C or when RNP occurred. Only responses classified as narrative were selected for analysis because the cohesion aspects are less observable in descriptive discourse.

Example of inappropriate referential cohesion:
"The man is driving. At a certain point, she got out of the car. The passenger who was in the backseat threw himself

forward; then the boy who was in the backseat released the parking brake."
*(The use of the pronoun **she** does not make appropriate reference to the antecedent **man.** In the next sequence, the nouns **passenger** and **boy** are used as autonomous referents to signify the same subject, which denotes an RNP).*

(f) *Index of discourse effectiveness:* This index was obtained by dividing the total number of words recalled by the number of macropropositions. The use of fewer words with more macropropositions indicates a more effective discourse. We proposed this index because it is already known that patients with AD could use many words without using a coherent discourse (Ash et al., 2007). We intend to observe if there is a relationship between the numbers of macropropositions and the total number of words evoked for each group.

(g) *Narrative structure:* The ability to elaborate the content of the story from a sequence of events that form a full episode was evaluated. The criteria of Stein and Glenn (1979) adopted by Coelho et al. (2012) for evaluating discourse production in patients with TBI were used. The story was considered complete when it involved three aspects: (a) *initial event*, (b) *story development*, and (c) *outcome*. The story was considered incomplete when it involved only one or two of these aspects:

(1) *Initial event: The explanation of the characters and initial action (characterized by the presence of the first and/or second macropropositions).*
(2) *Story development: The unfolding of the episode, the intentions of the characters and the flow of facts (characterized by the presence of the third to the eighth macropropositions).*
(3) *Outcome: The direct consequence of the actions of the characters and the conclusion of the episode (characterized by the presence of the ninth macroproposition).*

Statistical Analysis

None of the variables of interest exhibited normal distribution (according to Kolmogorov–Smirnov and Shapiro–Wilk tests), with the exception of phonemic and semantic verbal fluency. Therefore, the non-parametric Kruskal–Wallis test was used for continuous variables (i.e., Items A, B, D, and F of the narrative assessment and naming test). *Post-hoc* analysis was performed for all pairwise comparisons with Mann–Whitney (U) test (with Dunn–Bonferroni corrections). ANOVA with the *post-hoc* Bonferroni test was used for the parametric variables. The statistical significance was set at 0.05. As required, we performed a complementary analysis of our test results, with level of education as a covariant. We carried out non-parametric ANCOVA – Quade's rank analysis of covariance (Quade, 1967) for non-parametric variables (**Tables 2**, **3**) and parametric ANCOVA for parametric variables (**Table 3**). For categorical variables (i.e., Items C, E, and G), the chi-square and Fisher's exact tests were used. Because the chi-square test does not indicate which cell is responsible for the significant difference, the adjusted standardized residuals were used. Residuals >1.96 indicated statistically significant association among the categories. For all statistical tests, we adopted a level of significance (α) of 0.05 (two-tailed). The Statistical Package for the Social Sciences (SPSS) software, version 20.0, was used for all of the analyses.

Results

There was no significant difference among the groups regarding gender and age. As expected, the global cognitive level of the AD group was significantly lower than that of the Control and a-MCI groups. We used the Geriatric Depression Scale (Brief-form) to perform the screening for depression (exclusion criteria – see Section Sample). The means of the three groups included in this sample did not differ ($p = 0.600$). In addition, the means of all of the groups were below the suggested cut-off for significant depression symptomatology: Control group (2.9 ± 3.09), MCI group (3.52 ± 2.29), and AD group (3.69 ± 3.37). The educational levels of the Control and a-MCI groups were significantly higher than those of the AD group (**Table 1**). Therefore, we performed an additional analysis with education (years of schooling completed) as a covariant. The significance found previously was maintained for almost all variables (**Tables 2**, **3**). It was possible to observe the effect of schooling in two linguistic variables: phonemic verbal fluency and total number of words – close class, which will be discussed later.

Narrative Evaluation Results

Table 2 summarizes the comparison between the groups regarding narrative discourse. The Control and AD groups differed significantly in nearly all narrative measures, and the a-MCI group's performance represented an intermediate stage between the Control and AD groups for most of the variables.

The index of discourse effectiveness was the only parameter that differentiated the Control, a-MCI, and AD groups. Comparatively, the AD group displayed the worst performance, using more words to generate fewer macropropositions.

For the lexical aspect, there was no significant difference among the three groups regarding the total number of recalled words. A detailed analysis of the type of recalled and repeated words revealed that the three groups did not differ in their evocation of open-class words ($p = 0.22$). The Control group recalled a lower number of closed-class words than the AD group.

TABLE 1 | Demographic and global cognitive comparisons of the Control and patient groups.

	Control Mean (SD)	a-MCI Mean (SD)	AD Mean (SD)	Comparisons
N (total)	41	22	14	
Gender (F/M)	26/15	11/11	10/4	Control ≈ MCI ≈ AD
Age	69.6 (5.8)	72.1 (4.4)	73.4 (7.3)	Control ≈ MCI ≈ AD
Years of education*	14.5 (2.6)	13.1 (2.3)	12 (3.3)	Control ≈ MCI > AD
MMSE (0–30)*	27.2 (2)	26.2 (1.9)	22.7 (3.5)	Control > MCI > AD

**Globally significant differences (Kruskal–Wallis).*

TABLE 2 | Comparison of narrative discourse among the groups.

N (total)	Control Mean (SD) 41	MCI Mean (SD) 22	AD Mean (SD) 14	Kruskal-Wallis *p*-value < 0.05*	Comparisons	ANCOVA *p*-value < 0.05**
Total words	97.7 (43.2)	117.4 (46.9)	128.0 (61.2)	0.072		0.234
Words – open class	50.2 (21.8)	60.9 (30.4)	57.93 (25.5)	0.225		0.413
Words – closed class	45.2 (21.6)	54.9 (23.3)	64.5 (40.0)	*	Control > AD	0.115
Repeated words	2.1 (1.7)	4.2 (3.6)	6.1 (5.8)	*	Control > MCI	**
Narrative time (s)	65.6 (30.1)	71.4 (34.2)	86.3 (39.2)	0.147		0.280
Total macropropositions	7.6 (1.1)	6.8 (2.0)	4.4 (2.0)	*	MCI > AD	**
Total micropropositions	1.6 (1.5)	3.1 (3.0)	5.0 (3.7)	*	Control > MCI	**
Irrelevant micropropositions	0.27 (0.7)	1.1 (1.9)	2.5 (3.8)	*	Control > MCI	**
Index of discourse effectiveness	13.0 (7.2)	19.6 (12.4)	47.4 (56.9)	*	Control > MCI > AD	**
	Control 41	**MCI 22**	**AD 14**	**Chi-square *p*-value***	**Fisher's test contrasts**	
Type of discourse: Narrative/descriptive	38/3	19/3	7/7	*	MCI > AD	
Narrative structure: Complete/incomplete	34/7	12/10	4/10	*	Control > MCI	
Referential cohesion: Adequate/inadequate	($n = 38$) 30/8	($n = 19$) 12/7	($n = 7$) 2/5	*	MCI > AD	

*Significant differences. **Quade's rank analysis of covariance with years of education (non-parametric ANCOVA).*

TABLE 3 | Comparison of language variables among the groups.

N (total)	Control Mean (SD) 41	MCI Mean (SD) 22	AD Mean (SD) 14	*p*-value < 0.05*	Comparisons	ANCOVA *p* < 0.05
Naming	14.2 (1.0)	13.4 (1.3)	11.7 (2.3)	*	Control > MCI > AD	***
Phonemic verbal fluency, F.A.S (total sum)	40.8 (18.7)	33.7 (16.4)	24 (10.7)	**	Control > AD	0.103
Semantic verbal fluency, animals (total)	18.1 (4.5)	15.2 (4.6)	9.6 (5.3)	**	MCI > AD	****

*Significant differences *Kruskal–Wallis. **ANOVA, ***Non-parametric ANCOVA – Quade's rank analysis of covariance with years of education, ****parametric ANCOVA – Covariance with years of education.*

However, the a-MCI group did not differ from the other two groups. According the posterior ANCOVA analysis, we could see the effect of education level in this specific variable. The covariance showed that the three groups did not differed in total number of closed-class word recalled (**Table 2**). The Control group used more repeated words in the same sentence than the a-MCI and AD groups did. There were no group differences regarding the time taken to tell the story.

Regarding the macrolinguistic aspect, the AD group's performance differed significantly from that of the Control and MCI groups in terms of global coherence: a smaller number of macropropositions were composed. The AD and MCI groups exhibited similar performances, using more micropropositions and irrelevant micropropositions than the Control group did ($p < 0.05$).

The discourse type was predominantly narrative for the Control (92.65%) and a-MCI (86.3%) groups, which did not differ significantly ($p = 0.413$). Both groups differed from the AD group ($p < 0.01$), in which 50% of the individuals produced predominantly descriptive discourses.

The Control group performed better on the development of the narrative structure, completing 83% of the narrative episodes. The Control group differed ($p < 0.03$) from the a-MCI and AD groups, which provided 54.5 and 28.5% completed narratives, respectively; the a-MCI and AD groups did not differ from one another ($p = 0.176$).

The Control and a-MCI groups did not differ in their use of referential cohesion elements ($p = 0.220$). A total of 79% of the individuals in the Control group and 63.1% of the individuals in the a-MCI group did not commit any errors in explicit referents and anaphoric co-reference use. None of the individuals in the Control group displayed the RNP phenomenon. The AD group performed worse than the Control and a-MCI groups ($p < 0.02$); only 28% of their narratives showed adequate cohesion. The most common errors in the AD group were omissions of the explicit referent, inadequate or ambiguous use of pronouns to characterize the antecedent and the RNP phenomenon.

Table 3 summarizes the comparison among the groups regarding their usual language variables. The Control and AD groups differed significantly in all measures, as expected. The a-MCI group only differed from the Control group on a naming test. In both semantic and phonemic verbal fluency, these two groups had a similar performance. a-MCI and AD had a significant difference in semantic verbal fluency but not in phonemic verbal fluency. ANCOVA showed the effect of education level to differentiate Control and AD groups in

phonemic verbal fluency test; previous significance (ANOVA) between these two groups is no longer seen.

Discussion

The objective of the present study was to introduce parameters for evaluating narrative discourse based on a sequence of action pictures that were visually presented to elderly people with and without cognitive decline. Because of the ceiling effect that may result from the use of simpler tasks, a more complex task, such as the one used here, might be more suitable for determining the difficulties that patients experience during the early stages of cognitive decline (Forbes-McKay and Venneri, 2005). In addition, narrative discourse in everyday's life requires the speaker to verbally reproduce an episode experienced in the past and the investigation of deficits in MCI and AD may provide more insight into the autobiographical memory deficits already described in those disorders (Urbanowitsch et al., 2013).

The proposed task offers some advantages for clinical use in language assessment batteries. The major advantage is that it decreases the influence of episodic memory on linguistic performance. Other advantages include the analysis of micro- and macro-linguistic aspects using a single ecological activity that takes advantage of individuals' familiarity with the context of the action and the relatively easy and quick administration procedures (approximately 5 min).

The second and main objective of this study was to assess the applicability of this task for differentiating among healthy controls, people with a-MCI and people with AD.

Performance Differences Between the Control and AD Groups

The results indicated that the Control group differed from the AD group in nearly all of the analyzed macro- and micro-linguistic parameters. However, the total number of words recalled and the time required to produce the narrative could not discriminate any of the three groups. Although many individuals from the Control group also used an excessive number of words, extended the narrative and provided comments about the scene, they produced an effective discourse with the expected macropropositions and a complete narrative structure. In contrast, regardless of the number of words used, the individuals in the AD group had great difficulty presenting a structured and semantically appropriate narrative discourse. These results suggest that the lexical-semantic deficit alone does not adequately explain the macrolinguistic discursive problems in AD individuals.

The AD group displayed difficulties with global coherence, produced fewer macropropositions and less complete stories and exhibited lower discourse effectiveness compared with the Control and a-MCI groups. Thus, analyzing the number of words and the time required for the narrative is less important than analyzing the relationship between the words and the number of macropropositions generated and the ability to structure the full narrative episode.

The significant differences between the Control and AD groups are corroborated by other studies that reveal a decline in narrative discourse in AD (Ska and Duong, 2005; Ash et al., 2007; Taler and Phillips, 2008; de Lira et al., 2011). In our study, the AD group consisted of individuals with CDR scores of 0.5 or 1.0, which emphasizes that this linguistic impairment is already present during the disease's initial stages (Chapman et al., 2002). The phonological and basic syntactic structuring aspects were not the focus of our study because they are not expected to decline in early AD.

Some authors argue that discursive difficulties might be related to episodic memory and executive deficits (Carlomagno et al., 2005; Cannizzaro et al., 2012). Given that the test used in this study does not overload episodic memory, our findings might reinforce the hypothesis that the coherence and cohesion difficulties observed in AD individuals are associated with the executive and semantic-pragmatic components of language. Semantic memory is required for pre-verbal planning and, along with working memory, promotes top-down processing. Working memory is also required during verbal assertion (Caselli et al., 2014).

In a study on a French population that used the same task, Ska and Duong (2005) also observed less complete discourses with decreased content in the AD group compared with a group of healthy elderly. The authors explained that these findings differ for narrative tasks elicited with visual sequences and description tasks, in which individuals can maintain the primary content even if they do not clearly describe details or complementary information. Only narrative discourse demands an integration of successive events and the relationship between such events and previous knowledge to formulate the meaning of the story to be narrated.

Although the visual stimuli remained available throughout the entire narrative time, the participants were instructed to carefully observe the depicted sequence of actions before starting the narrative. To elaborate the pre-verbal content, the individual must rely on sociocultural and discursive models previously stored in long-term memory. Subsequently, the beginning of the structuring process and the use of cohesive elements during the narrative require working memory because completely elaborating the narrative requires maintaining and updating the narrated sequence until the story ends.

In a study comparing AD patients, aphasic patients and a control group, Carlomagno et al. (2005) argued that individuals with AD had greater difficulty integrating information and mentally representing discourse formulation. The author suggested that decreased working memory in association with semantic-pragmatic difficulties could also explain the predominance of descriptive discourse and verbal productions with absent or erroneous referential cohesion. In our sample, the AD group presented similar patterns, such as the omission of transition elements, the absence of explicit referents and an excess of inappropriate or ambiguous personal pronouns in relation to the prior referent. Those issues might be caused by lexical retrieval difficulties during the discourse formulation stage and by working memory impairment. It is possible that the inadequate use of cohesion elements, which affects discourse coherence, is caused by a problem with the interface between the semantic-pragmatic component and working memory. It is important to note that is very difficult to demarcate the

boundaries between this cognitive processes and verbal language abilities, such as generating narratives.

The MCI Group's Performance

The a-MCI group's results represented an intermediate performance between the Control and AD groups for most of the studied parameters. For some tasks, their performance was similar to that of the Control group; for others, their performance was similar to that of the AD group, as described below.

The linguistic pattern of the a-MCI group resembled that of the Control group in terms of the type of discourse used, the coherence and the cohesion. The production of predominantly narrative discourse, the ability to establish the global coherence of the story with the use of more macropropositions and the production of a more effective discourse than the AD group might indicate that these macrolinguistic aspects of narrative were less impaired in a-MCI patients.

The good performance of the a-MCI group in these aspects might be related to two factors: the low use of episodic memory during the task (cognitive deficits are a characteristic of the a-MCI group) and the proper functioning of working memory (Troiani et al., 2008; Cannizzaro and Coelho, 2013). However, the a-MCI group used more irrelevant micropropositions and propositions than the Control group, and their performance was more similar to that of the AD group. We found no studies of this population and task; therefore, we had no basis with which to compare these results. Given that a-MCI is a transitional stage between senescence and AD, we hypothesized that the first manifestations of the macrolinguistic plan in the narrative discourse in this group could be intrusions of information, particularly information that is irrelevant to the story's context. This hypothesis was based on the fact that individuals in senescence provide additional information in their discourse, though without using inadequate propositions. In addition to the exacerbated presence of relevant and irrelevant micropropositions, the AD group displayed more global decline, with an absence of the macropropositions that were needed to properly construct the narrative.

Considering that there was no interference from episodic memory in the construction of discourse and in top-down planning and that adequate generation of macropropositions was present, we may hypothesize that such additions of unnecessary information and the introduction of out-of-context elements by the a-MCI group might represent problems with the semantic-pragmatic component of language. In addition, the a-MCI group had difficulties completing the story; 45% of the individuals produced incomplete narratives, most often without providing a necessary description of the story's outcome.

In the microlinguistic plan, the a-MCI and AD groups displayed similar performances in relation to the mean number of repeated words in the same sentence. This aspect differentiated these groups from the Control group, in which such repetitions rarely occurred. This finding may illustrate the need to rephrase ideas when structuring the assertion, an act that is an expected part of the discourse among the AD population (Mansur et al., 2005) but that has not been described in a-MCI.

Differences Among the Three Groups

The discourse effectiveness index is obtained by dividing the total number of words recalled by the number of macropropositions generated. It has been proposed for analyzing the efficiency of the discourse. This discursive measure was suitable to differentiate the three groups, and it involves the relationship between lexical assessment and the semantic-pragmatic domain in the context of narrative. A larger number of words does not necessarily provide more macropropositions. There was no difference between the total number of words and open class words among the three groups; however, there was a large difference in the number of macropropositions generated.

Aspects related to naming and lexical evocation could be analyzed independently of the context of the narrative form. There are controversial studies in the literature regarding the effectiveness of these tests for discriminating control and MCI groups. In our sample, the naming test differentiated the three groups; this differs from the semantic verbal fluency tests, which could only discriminate between the control and AD groups (**Table 3**), and the phonemic verbal fluency test, which could differentiate the control group from the a-MCI and AD groups. After we performed the covariance analysis with years of education this difference between control group and AD group in phonemic verbal fluency disappears, indicating the effect of education in this task. Such result is consistent with other studies that have shown the influence of sociodemographic characteristics such as age and education level (Senhorini et al., 2006). Others however (Steiner et al., 2008) have demonstrated that age but not the level of education has the largest effect on that task.

It is important to consider that analysis of covariance using the quantitative education (years of schooling completed) should be carefully analyzed. Such analysis has been criticized because it does not allow control over recency or quality of education (Miller, 2013). This aspect is particularly important for the Brazilian population where there is great heterogeneity in the quality of formal educational provided.

Limitations

This study has some limitations. First, the sample of the pilot study was relatively small. The psychometric properties of the task are presently being investigated. Although the overall objective of this study was to investigate the continuum of language deficits from healthy individuals to those with a-MCI and AD using a task that did not make episodic memory demands, our a-MCI group comprised individuals with single and multiple domain subtypes; we did not control the influence of such heterogeneity because of our small sample size. We analyzed the effect of educational level in different linguistic variables but we did not analyze the effect size of this influence for each variable because it was beyond of the scope of this study, but it should be considered in future research. It is important to ponder that narrative tasks elicited by visual stimuli may require increased visual perception, which must be evaluated during clinical evaluation of the patients. In addition, it is possible that the scenes themselves could be used as supports, which could result in a more descriptive discourse. Such tendencies must

be minimized during instruction. Visual integration and other cognitive domains, such as executive function, are interrelated, and tasks designed to evaluate one specific cognitive aspect must consider other potential contributors to performance; future studies might include a regression analysis.

Conclusions

In conclusion, narrative discourse elicitation with visual stimuli is useful for analyzing language in elderly people with cognitive decline, including a-MCI and AD. The task was suitable for differentiating the Control and AD groups, and it showed that the a-MCI group exhibited discursive deficits compared with the Control group. This task has advantages. In addition to being an ecological assessment, it allows a characterization of the subtle and complex language skills (semantic-pragmatic processing) that are initially affected during the early stages of AD, such as a-MCI. The influence of the educational level in this kind of study must be considered.

Author Contributions

CD and GC contributed equally to this study. CD, GC, PM, and RF designed the study. CD, GC, AT, and NA conducted the experiment. CD and GC analyzed the data. CD, GC, RD, RF, JM, PM, and FT contributed to the manuscript.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2015.00096/abstract

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Visuospatial characteristics of an elderly Chinese population: results from the WAIS-R block design test

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Visuospatial deficits have long been recognized as a potential predictor of dementia, with visuospatial ability decline having been found to accelerate in later stages of dementia. We, therefore, believe that the visuospatial performance of patients with mild cognitive impairment (MCI) and dementia (Dem) might change with varying visuospatial task difficulties. This study administered the Wechsler Adult Intelligence Scale-Revised (WAIS-R) Block Design Test (BDT) to determine whether visuospatial ability can help discriminate between MCI patients from Dem patients and normal controls (NC). Results showed that the BDT could contribute to the discrimination between MCI and Dem. Specifically, simple BDT task scores could best distinguish MCI from Dem patients, while difficult BDT task scores could contribute to discriminating between MCI and NC. Given the potential clinical value of the BDT in the diagnosis of Dem and MCI, normative data stratified by age and education for the Chinese elderly population are presented for use in research and clinical settings.

Keywords: WAIS-R block design test, visuospatial characteristics, mild cognitive impairment, dementia, normative data

INTRODUCTION

Dementia, one of the most common geriatric diseases, greatly affects the quality of life of older adults, bringing with it a series of related economic and public health issues (Weinberger et al., 1993; Zencir et al., 2005). Early detection of dementia and intervention to treat dementia are essential. Researchers have identified a pre-dementia syndrome, named "mild cognitive impairment" (MCI; Petersen et al., 1999). MCI is an intermediate condition lying between normal aging and progression towards Alzheimer's disease (AD), the most common type of dementia. Patients with MCI will progress to AD at a rate of 10–15% per year, while healthy control subjects who convert at a rate of only 1–2% per year (Petersen et al., 2001; Petersen, 2004).

Research has shown that patients who developed dementia experienced accelerated rates of cognitive decline before diagnosis, and considerable attention has been paid to the area of memory (Rubin et al., 1998; Grober et al., 2000), as an example of cognitive decline. Visuospatial deficits among individuals with MCI and/or Dem have been studied to a much lesser extent than memory has (Iachini et al., 2009). Although some studies found that scores on visuospatial tests did not correlate with the severity of dementia (Kurylo et al., 1996), more studies demonstrated degenerative visuospatial deficits during the progression of dementia (Herlitz et al., 1995; Kaskie and Storandt, 1995; O'Brien et al., 2001; Alegret et al., 2009). The rate of visuospatial ability decline has been found to accelerate in the later stages of dementia (Herlitz et al., 1995), and the stages of visuospatial deficits follow the typical order of memory impairments as dementia progresses. The earliest manifestation of dementia was identified as episodic memory deficit, especially episodic memory disorders (Fox et al., 1998; Wolk and Dickerson, 2011; Romero and Moscovitch, 2012). With the progression of dementia, episodic memory has been found to show a slow decline; while other cognitive functions, such as visuospatial ability, began to show an accelerated decline. Based on the association between visuospatial ability and dementia progression, we expected that visuospatial ability would be able to be used to differentiate between patients with MCI and Dem.

Visuospatial performance in patients with MCI and Dem might change with varying visuospatial task difficulties. Kaskie and Storandt (1995) adopted a simple visuospatial discrimination test to compare Dem patients with healthy controls and found visuospatial deficits in Dem patients. Marcos et al. (2006) found that as impairment toward Dem symptoms progressed, patients with MCI exhibited increased difficulty during complex or demanding visuospatial tasks. In the current study, we will investigate the visuospatial characteristics of MCI and Dem against the backdrop of increasing task difficulty. The Wechsler Adult Intelligence Scale-Revised (WAIS-R) Block Design Test (BDT; Wechsler, 1981) has adaptive difficulty, and is regarded as reflecting of visuospatial ability (Kaufman, 2001). The primary objective of the current study was to explore whether visuospatial ability could make a further contribution, aside from that of episodic memory, in distinguishing patients with MCI patients from those with Dem and from normal controls (NC). We hypothesized that simple visuospatial tasks could help differentiate patients with MCI from those with Dem, and

difficult visuospatial tasks could help in the differentiation of patients with MCI from NC.

Given the potential clinical value of measuring visuospatial abilities, the secondary objective of the present study was to collect normative data from a large sample of elderly Chinese individuals, using the BDT (Gong, 1992). The BDT has been found to be effective in diagnosing age-related decline, with performance declining as age increases (Wechsler, 1981; Kaufman et al., 1989), particularly in those aged over 60 years (Rönnlund and Nilsson, 2006). Level of education has also been found to affect BDT performance (Bolton et al., 1966; Ryan et al., 1996; Brooks et al., 2011). Due to the significant potential effects of age and education on BDT performance, and the absence of recent normative data for the BDT, we provide age-and education-adjusted normative data using a large sample of healthy Chinese older adults.

In summary, the current study aims were to (1) determine whether visuospatial ability could help discriminate patients with MCI from those with Dem and from NC; (2) collect normative data from a Chinese elderly population for use in future research and clinical settings.

METHOD

WAIS-R BLOCK DESIGN TEST (BDT)

The BDT (Wechsler, 1981) requires that a set of either four or nine, two-colored blocks be arranged so as to duplicate a maximum of 10 target patterns presented in order of ascending difficulty.

This test comprises the following three levels: (1) level 1 (two items), in which participants are asked to arrange a set of four blocks to match that of an experimenter's illustration, within 60 s of the illustration being created (score range: 0–8); (2) level 2 (four items), in which participants are asked to arrange a set of four blocks according to a presented target pattern, also within 60 s (score range: 0–16); and (3) level 3 (four items), in which participants are asked to arrange a set of nine blocks to duplicate a presented target pattern, within 120 s (score range: 0–24). The tasks in level 1 are relatively the most simple, those in level 2 are of moderate difficulty, and those in level 3 are the most difficult. The maximum aggregated score is 48, with higher scores reflecting better functioning.

DATA COLLECTION

This study was conducted in three communities from Chaoyang, Xicheng, and Changping Districts in Beijing. Residents aged 60 and above, who appeared on the census list of the three communities, were contacted for participation. Selection was based on the following inclusion criteria: (1) being aged 60 years old or over, and being registered as permanent residents in their respective residing districts in Beijing (n = 1007), and (2) having completed the three measures described below (n = 959). The study then excluded individuals (1) whose clinical diagnoses were missing (n = 25), and (2) who had received a clinical diagnosis of depression (n = 10) according the depression and anxiety subscales of the Structured Clinical Interview for DSM disorders (SCID). The final sample size was 924.

PROCEDURES AND PARTICIPANTS

All participants were first informed of the aims and procedures, and assured that their information would remain anonymous and confidential. Each participant signed a voluntary consent form that was approved by the Ethics Committee of the Institute of Psychology at the Chinese Academic of Sciences. Individual demographic information was obtained for each participant before the examination.

Each participant was invited to complete a series of examinations, including a battery of neuropsychological tests, and a clinical assessment. The clinical assessment included items to assess participants' medical history, a basic physical examination, as well as the Neuropsychiatric Inventory (NPI; Cummings et al., 1994), the Activities of Daily Life (ADL; Katz et al., 1963), the Global Deterioration Scale (GDS; Reisberg et al., 1982), the Clinical Dementia Rating (CDR; Morris, 1993), the Hachinski Ischemic Score (HIS; Hachinski et al., 1975), and the SCID (Spitzer et al., 1985). The neuropsychological battery was administered by undergraduate or graduate research assistants who majored in psychology. The Auditory Verbal Learning Test (Ryan and Geisser, 1986) was used to measure episodic memory ability, with the number of words recalled after a delay interval of 30 min was taken as an indicator of delayed recall (DR). The Mini Mental State Examination (MMSE) was administered to assess global cognition among the participants; this test was scored according to the procedures described in the original paper (Folstein et al., 1975).

All research assistants and clinicians were intensively trained. High inter-rater reliability (above 90%) was obtained with the support of a consensus diagnosis meeting at which the neuropsychological and clinical data were reviewed. The screening process was standardized with a comprehensive Case Report Form, on which each participant's results were recorded. Experienced neurologists performed all clinical diagnoses. Eighteen patients were diagnosed with dementia, based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV, 1996), including 12 with AD and six with vascular dementia. Participants that met the following criteria of Peterson (2004) were diagnosed with MCI: (1) preserved general cognitive function as confirmed by meeting an education-based criterion of the MMSE, specifically, an MMSE score of $\geq$24 for those who had received more than or equal to 7 years of education, $\geq$20 for those who had received less than 7 years of education, and $\geq$17 for those who were illiterate; (2) being $\geq$1.5 SD below the sample mean scores on tests in at least one cognitive domain within the areas of episodic memory, language, executive function, or visuospatial skills; (3) a global CDR score of 0.5; (4) receiving a result of level 2 or level 3 in the GDS; (5) intact ADL ratings; and (6) an absence of dementia. Sixty individuals were diagnosed with MCI, including 48 with amnestic MCI and 12 with non-amnestic MCI. The other 846 individuals were considered as NC.

The 846 participants in the NC group were aged 60–93 years (M = 70.12, SD = 6.99) and had education levels ranging from 0 to 24 years (M = 10.65, SD = 5.23). The mean MMSE score for this group was 26.80 (SD = 3.53). The gender ratio (males/females) was approximately 45/55 (380/466).

Table 1 | Demographic characteristics and mean scores on the neuropsychological tests ($M \pm SD$).

	NC ($N = 846$)	MCI ($N = 60$)	Dem ($N = 18$)	p-value
age	70.12 ± 6.99	71.40 ± 7.29	77.00 ± 7.08	<0.001[a]
%female	55.0%	58.3%	77.8%	0.20
years of education	10.66 ± 5.22	7.75 ± 4.53	3.33 ± 3.97	<0.001[b]
ADL	14.40 ± 2.50	15.12 ± 4.10	27.38 ± 10.15	<0.001[a]
MMSE	26.83 ± 3.47	24.45 ± 3.68	12.83 ± 5.50	<0.001[b]
DR	9.04 ± 4.32	5.57 ± 3.99	1.11 ± 2.11	<0.001[b]
BDT	26.94 ± 9.53	21.05 ± 8.38	6.78 ± 6.80	<0.001[b]
Adjusted mean ± SE[d]	26.57 ± 0.26	23.82 ± 0.99	15.04 ± 1.84	
BDT Level 1	6.87 ± 1.86	6.30 ± 2.01	1.89 ± 2.32	<0.001[a]
Adjusted mean ± SE[d]	6.83 ± 0.06	6.65 ± 0.23	2.91 ± 0.42	
BDT Level 2	13.80 ± 3.64	11.87 ± 4.03	4.89 ± 5.75	<0.001[b]
Adjusted mean ± SE[d]	13.69 ± 0.11	12.80 ± 0.42	7.69 ± 0.77	
BDT Level 3	6.29 ± 6.10	2.88 ± 4.54	0	<0.001[c]
Adjusted mean ± SE[d]	6.09 ± 0.18	4.40 ± 0.67	4.48 ± 1.24	

Notes: NC = Normal Controls; MCI = Mild Cognitive Impairment; Dem = Dementia; MMSE = Mini-mental State Examination; ADL = Ability of Daily Living; BDT = Block design test; DR = Delayed Recall.

[a]Dem < NC = MCI; [b]Dem < MCI < NC ; [c]Dem = MCI < NC; [d]p-value obtained from non-parametric analysis of covariance with adjustment for age and education.

STATISTICAL ANALYSIS

Group differences in demographic variables, and neuropsychological test results were examined by using one-way analysis of variance (ANOVA) or a chi-square analysis. Moreover, as statistically significant differences in age and education were found among the three diagnosed groups, non-parametric analysis of covariance (rank ANCOVA) was used to compare scores on the BDT among the diagnosed groups (NC/MCI/Dem), adjusting for age and level of education. *Post hoc* analyses were further performed with the significant level adjusted by the Bonferroni method.

Binary logistic regression was conducted respectively to evaluate the contribution of the BDT in differentiating individuals with MCI from those with dementia, and those with MCI from NC. In model 1, we entered age, level of education, and the DR score into the regression equation. In model 2, we added the total score of the BDT plus the variables in model 1 into the equation. In models 3, 4, and 5, we added the scores of BDT levels 1, 2, and 3, respectively, plus the variables in model 1 into the equation. To compare models 1 and 2, we were able to investigate the additional contribution of the BDT, other than DR in differentiating patients with MCI from those with Dem and NC. Through the comparison of models 3 to 5, we were able to find which level of the BDT was most effective in differentiating patients with MCI from those with Dem, and patients with MCI from NC. Receiver operating characteristic (ROC) analysis was used to assess the effectiveness of each level of BDT in differentiating MCI from NC and Dem.

The second aim of the current study was to provide normative data for the Chinese elderly population. Due to the potential significant contributions of demographic variables to BDT performance, stepwise multiple linear regression analyses were employed among the NC group to demonstrate probable correlations between the demographic variables (age, level of education, and gender) and measures of BDT. Given that BDT performance correlates with age and level of education, participants were divided into five age groups (60–64, 65–69, 70–74, 75–79, and 80–95), each with three educational levels (≤8, 9–12, and ≥13 years of education).

All statistical analyses were conducted using SPSS version 19.0 (IBM Corporation, Somers, NY).

RESULTS

DEMOGRAPHIC CHARACTERISTICS AND GROUP DIFFERENCES

The sample's demographic characteristics are summarized in **Table 1**. There were significant differences among NC, MCI and AD with respect to age ($F_{(2,920)} = 9.22$, $p < 0.001$) and level of education ($F_{(2,919)} = 25.76$, $p < 0.001$), with patients with AD being older than those in the MCI and NC groups, and NC participants more highly educated than those in the AD and MCI groups. The proportion of men and women did not differ among groups ($\chi^2 = 4.48$, $df = 2$, $p = 0.110$).

Group differences were found for the total score ($F_{(2,921)} = 49.21$, $p < 0.001$) and each level of the BDT ($ps < 0.01$). Results remained significant when further analyzed with age and level of education as covariates. *Post hoc* analyses showed that for the BDT ($F_{(2,916)} = 21.72$, $p < 0.001$), the performance of the MCI group was better than that of the Dem group ($t = 4.24$, $p < 0.001$) and worse than that of the NC group ($t = 2.68$, $p = 0.007$). Specifically, for level 1 ($F_{(2,914)} = 42.70$, $p < 0.001$), the performance of the MCI group was similar to that of the NC group ($t = 0.84$, $p = 0.404$), and significantly better than that of the Dem group ($t = 7.93$, $p < 0.001$); for level 2 ($F_{(2,914)} = 30.88$, $p < 0.001$), the performance of the MCI group was worse than that of the NC group ($t = 2.06$, $p = 0.040$), and significantly better than that of the Dem group ($t = 5.90$, $p < 0.001$); while for level 3 ($F_{(2,914)} = 3.59$, $p = 0.028$), the performance of the MCI group was significantly worse than that of the NC group ($t = 2.44$, $p = 0.015$), and similar to that of the Dem group ($t = 0.06$, $p = 0.954$).

Table 2 | Summary of logistic regression analysis in differentiating MCI from NC and Dem.

		MCI vs. NC					MCI vs. Dem				
Model	**Variables[a]**	**B[b]**	**S.E.**	**Exp (95%CI)[c]**	***p***	***R^2***	**B[b]**	**S.E.**	**Exp (95%CI)[c]**	***p***	***R^2***
1	Age	−0.019	0.020	0.981 (0.944–1.019)	0.326	0.106	0.019	0.046	1.020 (0.931–1.117)	0.674	0.463
	Education	−0.062	0.028	0.940 (0.889–0.994)	0.030		−0.190	0.089	0.827 (0.695–0.983)	0.032	
	DR	−0.150	0.033	0.860 (0.806–0.918)	0.000		−0.385	0.141	0.681 (0.516–0.898)	0.006	
2	Age	−0.022	0.020	0.978 (0.941–1.016)	0.255	0.114	0.016	0.059	1.016 (0.905–1.141)	0.790	0.631
	Education	−0.034	0.033	0.967 (0.906–1.031)	0.304		−0.115	0.113	0.892 (0.715–1.112)	0.308	
	DR	−0.141	0.034	0.869 (0.812–0.929)	0.000		−0.248	0.169	0.781 (0.561–1.087)	0.142	
	BDT	−0.029	0.018	0.971 (0.939–1.005)	0.098		−0.189	0.064	0.827 (0.730–0.938)	0.003	
3	Age	−0.019	0.020	0.981 (0.944–1.019)	0.325	0.106	0.025	0.066	1.026 (0.902–1.166)	0.699	0.656
	Education	−0.063	0.030	0.939 (0.886–0.996)	0.035		−0.081	0.110	0.923 (0.744–1.144)	0.463	
	DR	−0.151	0.034	0.860 (0.805–0.918)	0.000		−0.287	0.172	0.751 (0.536–1.052)	0.096	
	BDT level 1	0.006	0.067	1.006 (0.882–1.148)	0.924		−0.593	0.190	0.553 (0.381–0.082)	0.002	
4	Age	−0.021	0.020	0.979 (0.942–1.018)	0.287	0.108	0.019	0.054	1.019 (0.916–1.132)	0.732	0.559
	Education	−0.051	0.032	0.950 (0.893–1.011)	0.108		−0.171	0.103	0.843 (0.689–1.031)	0.096	
	DR	−0.147	0.034	0.863 (0.808–0.922)	0.000		−0.285	0.153	0.752 (0.557–1.014)	0.062	
	BDT level 2	−0.028	0.035	0.972 (0.908–1.041)	0.421		−0.187	0.074	0.829 (0.717–0.958)	0.011	
5	Age	−0.023	0.020	0.977 (0.940–1.015)	0.233	0.124	−0.028	0.051	0.972 (0.879–1.075)	0.585	0.555
	Education	−0.026	0.032	0.974 (0.915–1.037)	0.411		−0.128	0.103	0.880 (0.720–1.076)	0.212	
	DR	−0.143	0.034	0.866 (0.811–0.926)	0.000		−0.457	0.166	0.633 (0.457–0.877)	0.006	
	BDT level 3	−0.087	0.038	0.917 (0.851–0.988)	0.022		−4.664	1077.60	0.009 (0–)	0.997	

Note: [a]variables entered into models 1 to 5; [b]original coefficient for each variable obtained from the logistic regression analysis; [c]exponentiated coefficient and its 95% confidence interval for each variable obtained from the logistic regression analysis. Exponentiated coefficient less than 1 reflects negative relationships while value above 1 denotes positive relationship.

THE EFFECTIVENESS OF THE BDT IN DIFFERENTIATING INDIVIDUALS WITH MCI FROM THOSE WITH DEMENTIA AND NC

Binary logistic regression analysis was conducted to assess the contribution of the BDT in distinguishing patients with MCI from those with Dem (**Table 2**). In model 2, the BDT score was indicated as a significant predictor ($p = 0.003$) in the differentiation between MCI and Dem groups. Compare models 1 and 2, we found that the BDT would make an additional contribution ($\Delta R^2 = 0.168$, $p < 0.001$) other than DR in the discrimination between MCI and Dem groups. When we compared models 3 to 5, we found that the BDT level 1 ($p = 0.002$) and BDT level 2 ($p = 0.011$) scores were significant predictors, while the BDT level 3 score was not a significant predictor ($p = 0.997$). These results showed that the BDT could contribute to the discrimination between MCI and Dem groups, and that the score on simple BDT tasks (BDT levels 1 and 2) could significantly distinguish patients with MCI from those with Dem.

Binary logistic regression analysis was used to assess the effectiveness of the BDT in differentiating patients with MCI from NC (**Table 2**). In model 2, the score on the BDT could not significantly differentiate patients with MCI from NC ($p = 0.098$). When compared models 1 and 2, we found that the BDT could not make an additional contribution ($\Delta R^2 = 0.008$, $p = 0.097$) other than DR in the discrimination between MCI and NC groups. Compare models 3 to 5, we found that only the score on BDT level 3 was a significant predictor ($p = 0.022$) in differentiating patients with MCI from NC. These results showed that the difficult BDT tasks (BDT level 3) could contribute to discriminating between patients with MCI and NC.

ROC curves (**Figure 1**) were drawn to determine the discriminatory validity of each level of BDT for MCI vs. Dem groups, as well as MCI vs. NC groups. The area under the curve (AUC) of BDT level 3 (0.67, 95% CI: 0.61–0.74) was the largest for the discrimination between MCI and NC groups (**Figure 1A**). With regard to the discrimination between MCI and Dem groups, BDT level 1 (0.91, 95% CI: 0.83–0.98) demonstrated the largest AUC in comparison to the other levels of BDT (**Figure 1B**).

NORMATIVE DATA OF THE BDT IN NC

Stepwise multiple linear regression analyses revealed that, age and level of education significantly predicted BDT scores ($ps < 0.010$). However, there was no significant effect of gender on BDT performance ($ps > 0.050$). **Table 3** depicts normative data for each of the 15 age groups according to level of education.

DISCUSSION

The results confirmed our hypothesis that the BDT could help discriminate between individuals with MCI and those with Dem. In particular, the score on simple BDT tasks was the best for distinguishing patients with MCI from those with Dem, and the score on difficult BDT tasks could contribute to discriminating between patients with MCI and NC.

Visuospatial ability was effective in discriminating patients with MCI from those with Dem. Episodic memory seems to show an accelerated decline during the early stages of MCI (Hall et al., 2001); however, with the progression of dementia, visuospatial ability shows larger declines than does memory. It is assumed that memory impairment might have reached its limit at a certain stage of the disease. Yu et al. (2012) drew

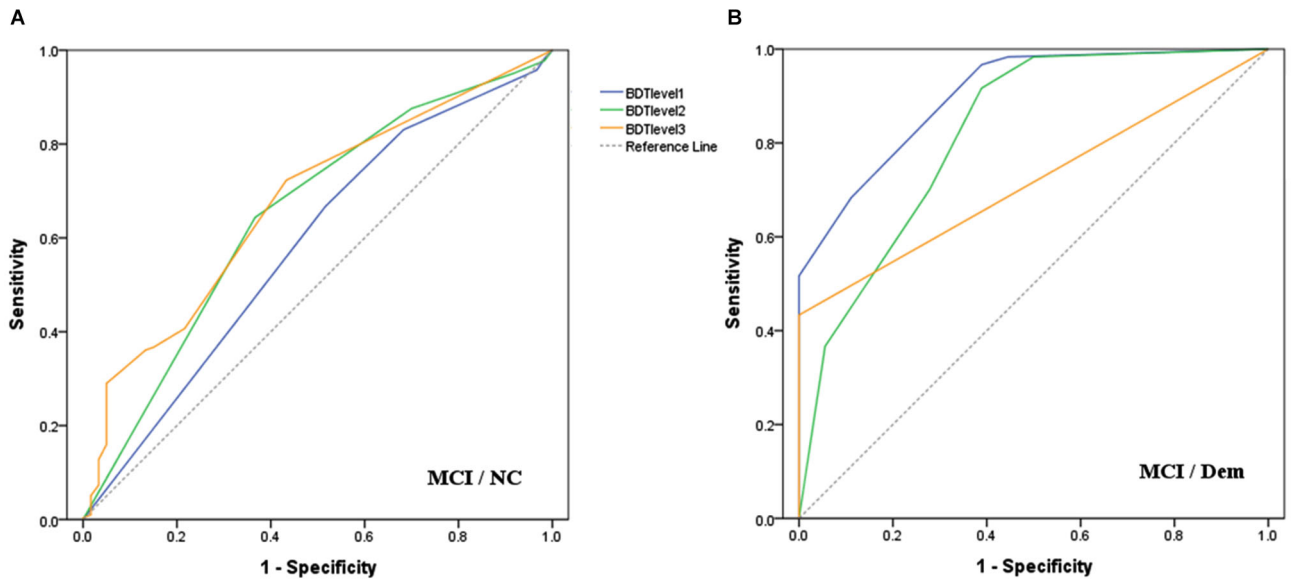

FIGURE 1 | Receiver operating characteristic curves for each level of BDT to detect: (A) MCI from NC; (B) MCI from Dem.

Table 3 | Normative data on the BDT stratified by age and education among NC (*M*, SD).

			BDT		Level 1		Level 2		Level 3	
Age	**Education**	***N***	***M***	**SD**	***M***	**SD**	***M***	**SD**	***M***	**SD**
60–64	0–8	40	20.75	8.14	5.85	2.58	11.90	3.89	3.00	3.19
	9–12	122	28.00	8.73	7.07	1.65	14.33	2.90	6.57	6.17
	13–	72	33.69	7.25	7.67	1.01	15.50	1.49	10.53	6.40
	Total	234	28.51	9.25	7.04	1.79	14.27	3.00	7.18	6.37
65–69	0–8	35	22.40	9.81	6.11	2.65	12.69	4.39	3.60	5.17
	9–12	73	26.71	8.01	7.04	1.67	14.08	3.06	5.59	5.28
	13–	69	34.04	6.77	7.68	0.81	15.65	1.14	10.71	6.34
	Total	177	28.72	9.12	7.11	1.76	14.42	3.05	7.19	6.37
70–74	0–8	35	20.89	8.77	5.89	2.05	11.77	5.22	3.23	4.04
	9–12	53	25.04	6.91	6.75	1.63	13.96	2.67	4.32	4.62
	13–	120	32.67	7.15	7.47	1.09	15.43	1.49	9.78	6.14
	Total	208	28.75	8.77	7.02	1.55	14.44	3.06	7.28	6.19
75–79	0–8	63	19.60	8.18	6.41	2.13	11.11	4.95	2.08	3.17
	9–12	36	23.86	7.70	6.72	1.67	13.11	3.53	4.03	4.78
	13–	43	29.53	7.70	7.40	1.28	15.16	1.65	7.63	5.36
	Total	142	23.69	8.93	6.79	1.83	12.89	4.20	4.25	4.92
80–	0–8	54	15.54	8.45	5.22	2.65	9.19	5.13	1.13	2.15
	9–12	18	25.00	6.47	6.56	1.65	14.00	2.47	4.44	4.44
	13–	13	28.85	7.83	6.62	2.36	15.08	1.75	7.15	4.96
	Total	85	19.58	9.61	5.72	2.49	11.11	4.99	2.29	3.96
Total	0–8	227	19.47	8.84	5.90	2.43	11.14	4.89	2.43	3.59
	9–12	302	26.50	8.11	6.93	1.69	14.04	2.97	5.51	5.52
	13–	317	32.62	7.33	7.51	1.14	15.44	1.47	9.75	6.17
	Total	846	26.91	9.57	6.87	1.86	13.79	3.64	6.27	6.10

ROC curves to determine the discriminatory validity of seven cognitive domains of the MoCA for patients with MCI vs. NC, and for patients with MCI vs. Dem. The study found that the most sensitive domains with regard to the discrimination between patients with MCI and Dem were the orientation and visuospatial/executive domains (see Nasreddine et al., 2005). These findings indicated that visuospatial abilities might be a more sensitive predictor in the discrimination between patients with MCI and those with Dem, also making a larger contribution towards such discrimination. Furthermore, scores on the BDT level 1 were significant in differentiating patients with MCI from those with Dem, with a high AUC found (0.91, 95% CI:

0.83–0.98), and this measure can be completed in a very short time. This suggests that the BDT level 1 measure could be applied as an effective tool in the diagnosis of Dem for clinical use, with the advantages of high diagnostic accuracy and time efficiency. In addition, visuospatial performance of patients with MCI and those with Dem changed with varying visuospatial task difficulties. Future studies must consider the difficulty and type of tasks when assessing visuospatial ability in patients with cognitive impairment, because increasing the difficulty of a visuospatial task could lead to an increase in the diagnosis of more subtle cognitive deficits (i.e., MCI).

Given the potential clinical value of visuospatial abilities in the diagnosis of Dem and MCI, normative data stratified by age and level of education were collected for use in future research and clinical settings. In the present study, BDT performance declined with age, which is consistent with previous research findings (Rönnlund and Nilsson, 2006). This age-related decline in BDT performance indicated that visuospatial ability degraded with normal aging. Level of education was positively correlated with BDT performance, such that less educated participants performed worse on the BDT. Higher education might be related to participation in more intellectual activities during leisure time, which might in turn protect aging individuals against dementia (Kliegel et al., 2004). Furthermore, the normative information presented in this study could serve as assessment criteria. As such, this study contributes to the development of appropriate neuropsychological test norms for the Chinese population. As age and level of education were significantly correlated with BDT performance, these two demographic factors should be taken into consideration when interpreting BDT scores.

The current study highlighted the contribution of BDT in discriminating MCI from Dem and provided a potential diagnostic tool of Dem for research and clinical use in the field of age-related cognitive impairment. The present findings also constitute a significant contribution to the expanding knowledge on age-related changes in visuospatial ability by providing normative data sourced from a large sample of older adults. These findings have potential future clinical utility in that they provide clinicians with information on normative differences across education levels and age. Nevertheless, there are several limitations that should be acknowledged. First, this was a cross-sectional study; therefore, age differences in performance on the BDT might have been exacerbated by factors that are associated with the use of cohorts. Furthermore, we could not directly analyze the predictive accuracy of the BDT score on the conversion rate of MCI to dementia. Future research could assess the value of the BDT score in the differential diagnosis, prognosis, and conversion prediction of MCI to dementia by recruiting participants from more diverse regions or countries. Second, we did not distinguish between different types of dementia. Patients with different types of dementia may demonstrate varying patterns of visuospatial performance (Heyanka et al., 2010); consequently, the ability of visuospatial ability to discriminate between MCI and dementia may vary across different types of dementia. Third, the sample sizes of the NC, MCI, and dementia groups were different. The number of dementia patients was quite small, which might have compromised the statistical potency of between-group comparisons. Lastly, the current study presented cross-sectional data with respect to BDT performance, but longitudinal normative data should be developed to investigate the cognitive trajectory of normal aging and neurodegenerative diseases, and determine the clinical and experimental significance of longitudinal changes on this measure.

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Psychometric properties of the Brazilian version of Pfeffer's Functional Activities Questionnaire

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Pfeffer's Functional Activities Questionnaire (FAQ) is one of the most commonly employed tools in studies on pathological cognitive aging. Despite the different versions of the questionnaire translated for use in clinical practice, few studies have analyzed the psychometric properties of the Brazilian version of the FAQ (P-FAQ). Thus, the aim of the present study was to analyze the P-FAQ with regard to internal consistency, factorial structure and associations with demographic factors (age, sex, and schooling), depressive symptoms, cognitive measures and other measures of functionality. One hundred sixty-one older adults were divided into four groups (91 with dementia, 46 with mild cognitive impairment, 11 with psychiatric disorders and 13 healthy controls). All participants were evaluated by cognitive, behavioral and functional tests and scales. Their caregivers answered the P-FAQ. The questionnaire showed high internal consistency ($\alpha = 0.91$). Factor analysis revealed a two-factor structure, which, accounted for 66% of the total variance. The P-FAQ was not correlated with demographic factors, was weakly correlated with depressive symptoms ($\varrho = 0.271$, $p < 0.01$, $R^2 = 7\%$) and strongly correlated with cognitive measures (Matttis Dementia Rating Scale total score: $\varrho = -0.574$, $p < 0.01$, $R^2 = 33\%$) as well as complex instrumental activities of daily living ($\varrho = -0.845$, $p < 0.01$, $R^2 = 71\%$). Cognitive performance and depression status were independent predictors of P-FAQ scores in regression models. The present findings indicate that the P-FAQ has satisfactory reliability, internal consistency, construct validity and ecological validity. Therefore, this questionnaire can be used in clinical practice and research involving the Brazilian population of older adults.

Keywords: functional assessment, older adult, instrumental activities of daily living, psychometric properties, neuropsychology, validity, reliability

INTRODUCTION

The proportion of older adults in the general population has increased in recent years due mainly to the demographic explosion in past decades as well as improvements in living conditions and quality of life (Lin et al., 2012). With the increase in life expectancy, disabling diseases associated with the aging process have become more prevalent.

Functional status is one of the most important aspects of geriatric evaluations and extremely relevant to diagnostic procedures, as atypical cognitive and behavioral manifestations often stem from normal aging. Moreover, neuropsychiatric disorders, such as dementia (Lopes and Bottino, 2002), depression and psychosis (Hoffmann et al., 2010) are characterized by persistent cognitive and functional dysfunction, resulting in limitations that worsen with the progression of the disease. The formal diagnosis of dementia requires the adequate characterization of functional impairment, which is non-existent or less impacting in conditions such as mild cognitive impairment (Petersen et al., 2001; Yassuda et al., 2010; Brown et al., 2011; de Paula and Malloy-Diniz, 2013). Thus, evidence of functional impairment constitutes an important indicator of pathological aging (Freitas and Miranda, 2011).

The use of questionnaires that evaluate basic and instrumental activities of daily living is a common method for evaluating the functional status of older adults. Basic activities include self-care, toileting, eating, dressing, bathing, hygiene, functional locomotion and sphincter control, whereas instrumental activities are those related to enjoying an independent, active life, such as household chores, managing finances, taking

medication, running errands as well as using transportation and the telephone.

Despite the importance of scales for the evaluation of functionality, few functional status measures employed in Brazil have been submitted to formal adaptation and validation procedures for use on older adults (Vasconcelos et al., 2007). Pfeffer's Functional Assessment Questionnaire (FAQ) is one of the most widely used measures of functional status in research and is often employed in epidemiological studies on dementia (Nitrini et al., 2004; Laks et al., 2005, 2010; Aprahamian et al., 2011). The interest of researchers in different centers on the questionnaire has grown in recent years, especially after its inclusion in the assessment protocol of the Alzheimer's Disease Neuroimaging Initiative (2014). This questionnaire is particularly useful due to its potential in discriminating individuals with and without cognitive impairment (Devanand et al., 2008; Steenland et al., 2008). Moreover, the FAQ exhibits greater sensitivity (0.85) in comparison to the Lawton Scale (0.57) when used to distinguish individuals with and without dementia (Pfeffer et al., 1982).

The FAQ was formally adapted to the Brazilian context in a recent study (Sanchez et al., 2011), although other versions with subtle differences have been used in clinical and research contexts. While the translated version, denominated the Pfeffer's Functional Activities Questionnaire (P-FAQ), has similar characteristics to Pfeffer's original questionnaire, a number of items have been completely changed, with the possible alteration of the original structure. However, no previous studies have evaluated the psychometric properties of the P-FAQ on a heterogeneous sample of older Brazilian adults.

Thus, the aim of the present study was to analyze the P-FAQ with regard to internal consistency, factorial structure and associations with demographic factors (age, sex, and schooling), depressive symptoms, cognitive measures and other measures of functionality.

MATERIALS AND METHODS

SUBJECTS

The participants were aged 60 years or older and recruited from the Jenny de Andrade Faria Institute of Healthcare for Older Adults and Women, which is a secondary/tertiary public health center in the city of Belo Horizonte, Brazil. This institute receives older adults referred from primary healthcare units in metropolitan Belo Horizonte as well as other municipalities in the state of Minas Gerais. The participants were sent for neuropsychological exams as part of routine evaluations or follow up and were subsequently invited to participate in the present study. A total of 161 older adults (96 women and 65 men; mean age: 75.51 $\pm$ 7.22 years; mean schooling: 4.43 $\pm$ 4.08 years) were included in the study.

This project integrates a comprehensive study which aims to evaluate the psychometric properties of a neuropsychological protocol designed to assess older adults with low formal education (de Paula et al., 2013a). The project was approved by the Research Ethics Committee of the Federal University of Minas Gerais (COEP-334/06). All participants and/or legal guardians signed a statement of informed consent. Individuals with severe sensory or motor impairment or without caregivers to provide information were excluded from the study.

PARTICIPANTS

The cognitive evaluation involved the Mini Mental State Examination (Folstein et al., 1975), the Brazilian version of the Mattis Dementia Rating Scale (MDRS) and its five subscales (Porto et al., 2003), the Clock Drawing Test (Shulman, 2000) and one of the Brazilian versions of the Frontal Assessment Battery (de Paula et al., 2013b). These measures were selected for representing different aspects of cognition (general and specific), involving language, memory, visuospatial skills, attention and executive functions, as recommended in previous studies (Salmon and Bondi, 2009; Weintraub et al., 2009). All aforementioned measures have been cross-culturally adapted and validated for use on the Brazilian population (de Paula et al., 2010, 2013a).

The participants were also evaluated with regard to psychiatric symptoms, involving the administration of the Brazilian version of the 15-item Geriatric Depression Scale (GDS-15) (Almeida and Almeida, 1999) and an interview with open-ended answers on functional status for the determination of functional complaints based on caregivers' reports focused on lost skills. The Clinical Dementia Rating (CDR) (Morris, 1993) was used to determine the stage of dementia. Only individuals with a CDR score of 1 or less were included in the study. The diagnosis was performed by consensus among a geriatrician, psychiatrist and neuropsychologist. The clinical evaluation of the geriatrician also involved an interview with the participant and caregiver to investigate symptoms, disease progression, functional loss, family history and possible confounders. Clinical and neuroimaging exams were performed when necessary.

Following the descriptive evaluations, the participants were allocated to different groups based on the clinical condition: dementia [$n = 91$; 71 with mild to moderate dementia (probable Alzheimer's disease); five with frontotemporal dementia; four with vascular dementia; and three with mixed dementia); mild cognitive impairment (46 with amnesic mild cognitive impairment); psychiatric disorder ($n = 11$; nine with a diagnosis of depression and two with late-onset psychosis); and healthy controls (13 individuals with no disorders that could affect cognition or behavior).

P-FAQ

The P-FAQ is a version of the FAQ that is frequently employed in Brazil in both clinical practice and research (Ministério da Saúde, 2007; Jacinto, 2008; Moraes, 2008; Brito, 2010; Hoffmann et al., 2010; Damin, 2011; Lino, 2011; Jacinto et al., 2012). This questionnaire allows the evaluation of the degree of independence on the performance of ten instrumental activities of daily living: managing one's own finances; shopping; heating water and shutting off the stove; making meals; keeping track of current events, watching news reports and discussing them; maintaining oneself orientated when walking outside the neighborhood; remembering commitments; managing one's own medications; and being at home alone (Moraes, 2008). The last three items on the P-FAQ differ from the original version of the FAQ: remembering appointments and taking care of one's own medication; playing

cards or performing other hobbies; and dealing with business or documents. The scoring was the same, with the total score ranging from 0 to 30 points (worst performance). Caregivers also answered the General Activities of Daily Living (GADL) Scale, which is divided into self-care activities, domestic activities and complex activities, as described elsewhere (de Paula et al., 2014).

STATISTICAL PROCEDURES

The sample size was calculated using the G*Power program, version 3.1.7. As the Kolmogorov-Smirnov test demonstrated that most data exhibited non-normal distribution, a sample of 161 individuals was considered adequate to detect large (98%), moderate (93%) and small (73%) effect sizes in the comparisons of non-parametric groups. Descriptive statistics were performed for the demographic characteristics of the participants as well as the scores on the Mini-Mental State Examination, Frontal Assessment Battery, MRDRS, Clock Drawing Test, GADL scale and Geriatric Depression Scale. Differences among the four groups (dementia, mild cognitive impairment, psychiatric disorders and control) were analyzed using the Kruskall-Wallis test, followed by the Mann-Whitney tests with the Bonferroni correction for group-by-group analyses. The chi-square test was used to determine differences among categorical variables.

The validity of the P-FAQ was evaluated using exploratory factor analysis of the ten items. Principal axis factoring and varimax rotation were selected for the procedure. Eigenvalues greater than 1 and scree plot analysis, the latter of which was performed by two independent observers (JJP and LFMD), were employed for the selection of the factors. Based on the sample size, factor loadings equal to or greater than 0.45 were considered significant (Hair et al., 2009).

Internal consistency of the P-FAQ was investigated using Cronbach's alpha coefficient. Spearman's non-parametric correlation coefficients were calculated to determine associations between the questionnaire and socio-demographic (age and schooling), cognitive (Mattis scale, Mini Mental Health Examination, Clock Drawing Test and Frontal Assessment Battery), neuropsychiatric (Geriatric Depression Scale) and functional (three components of the GADL scale) measures. Coefficients of determination were calculated for the analysis of shared variance among these variables. A forced-entry multiple regression model was used for the evaluation of the main predictors of the P-FAQ score. To minimize the collinearity of the model, only the total Mattis score, age, schooling, sex and depressive symptoms were used as predictors. All statistical procedures were conducted using the SPSS 17.0 (SPSS Inc., 2008).

RESULTS

Table 1 displays the description of the socio-demographic, functional, psychiatric and cognitive characteristics of the participants. The different groups were similar with regard to age, schooling and activities of daily living related to self-care. Significant differences were found in the proportion of men to women ($\chi^2 = 8.23$; $p = 0.041$). The psychiatric disorder group

Table 1 | Description of groups according to socio-demographic, functional, cognitive and psychiatric variables.

	Control (1)	Mild cognitive impairment (2)	Dementia (3)	Psychiatric disorder (4)	KW	*Post-hoc*
	Median (25th–75th percentile)	Median (25th–75th percentile)	Median (25th–75th percentile)	Median (25th–75th percentile)		
Age	79	74	76	77	2.00	–
Schooling	4 (4–11)	3 (2–4)	4 (1–4)	4 (0–5)	6.67	–
Female gender (*n*)	7	25	53	11	–	–
P-FAQ	0 (0–2)	4 (1–8)	14 (9–19)	12 (2–15)	51.63**	1 < 2, 1 < 3, 1 < 4, 2 < 3
GADL—self-care	8 (8–8)	8 (8–8)	8 (8–8)	8 (8–8)	2.38	–
GADL—domestic activities	8 (8–8)	8 (7–8)	6 (4–8)	6 (5–8)	36.53**	1 > 3, 1 > 4, 2 > 3, 2 > 4
GADL—complex activities	8 (7–8)	7 (6–8)	4 (2–7)	7 (2–8)	44.25**	1 > 3, 1 > 4, 2 > 3
Geriatric depression scale	2 (0–3)	3 (1–4)	4 (2–6)	8 (5–11)	20.09**	1 < 3, 1 < 4, 2 < 3, 2 < 4, 3 < 4
Mini-mental state examination	27 (23–28)	25 (20–27)	20 (17–23)	22 (19–26)	28.16**	1 > 3, 2 > 3
frontal assessment battery	15 (12–17)	12 (10–13)	8 (6–11)	8 (6–13)	40.28**	1 > 2, 1 > 3, 1 > 4, 2 > 3
Clock drawing test	5 (3–5)	2 (1–4)	2 (0–3)	3 (2–4)	19.05**	1 > 2, 1 > 3
MDRS attention	36 (35–36)	35 (33–36)	34 (32–35)	35 (34–36)	19.27**	1 > 3, 2 > 3
MDRS I/P	34 (31–37)	29 (25–31)	23 (21–28)	26 (22–29)	34.20**	1 > 2, 1 > 3, 1 > 4,2 > 3
MDRS construction	6 (6–6)	6 (4–6)	5 (2–6)	6 (3–6)	9.99*	1 > 3
MDRS conceptualization	33 (32–37)	32 (27–35)	24 (21–31)	28 (22–37)	26.50**	1 > 3, 2 > 3
MDRS memory	23 (22–24)	18 (16–21)	13 (10–17)	18 (13–20)	39.20**	1 > 2, 1 > 3, 1 > 4, 2 > 3
MDRS total	131	118	102	115	53.82**	1 > 2, 1 > 3, 1 > 4, 2 > 3

*Significant difference at p < 0.01; KW, Kruskall-Wallis test; P-FAQ, Pfeffer's Functional Activities Questionnaire; GADL, General Activities of Daily Living Scale; MDRS, Mattis Dementia Rating Scale; I/P, Initiative/Perseveration. 1 – interpreted as: 0.02–0.12 (low), 0.13–0.25 (medium), 0.26 or higher (high). *p < 0.05; **p < 0.01.*

had a larger proportion of women than the other three groups. Significant differences were also found for the other variables analyzed.

The results of the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO = 0.889) and Bartlett's Sphericity Test ($\chi^2 = 929.48$; $p < 0.001$) suggest that the sample was appropriate for factor analysis of the P-FAQ. Following the extraction of the factors and orthogonal rotation of the data, a two-factor structure was considered the most suitable for the data (**Table 2**). The first factor explained 55% of the variance (eigenvalue: 5.50) and the second factor explained approximately 11% of the overall variance (eigenvalue: 1.07). The latent structure therefore suggests bi-factor distribution.

The P-FAQ exhibited high internal consistency ($\alpha = 0.91$). The correlation analyses suggest that the P-FAQ was not correlated with age or schooling in the present sample. Significant correlations were found between the questionnaire and the three components of the GADL: a small effect size was found for the self-care component and large effect sizes were found for the domestic and complex components, with more than 70% shared variance with the latter component. A significant association, albeit with a small effect size, was found between the P-FAQ and depressive symptoms. Correlations between the questionnaire and cognitive measures ranged from weak to strong. The strongest correlations were found with general cognition (MDRS total score) and executive functions (Mattis Initiative/Perseveration). Weak correlations were found with measures of visuospatial skills (Clock Drawing Test and MDRS Construction). The other correlations between the P-FAQ and cognitive measures exhibited a moderate effect size (**Table 3**).

The multiple regression model designed for the determination of predictors of functional performance was significant [$F_{(5,\ 155)} = 17.68$; $p < 0.001$; adjusted $R^2 = 34\%$]. The significant predictors were the MDRS total score ($\beta = -0.234$; $SE = 0.03$; $p < 0.001$) and Geriatric Depression Scale ($\beta = 0.426$; $SE = 0.15$; $p = 0.007$). Marginally significant predictors were schooling ($\beta = 0.271$; $SE = 0.14$; $p = 0.054$) and the female sex ($\beta = 1.818$; $SE = 1.073$; $p = 0.092$), but not age ($\beta = 0.072$; $SE = 0.07$; $p = 0.330$). **Figure 1** displays the relationship between standardized predictors and performance on the P-FAQ.

Table 2 | Rotated factor structure (varimax) of P-FAQ.

Components	Factor loadings	
	Factor 1	**Factor 2**
Is he/she capable of walking outside the neighborhood and finding the way back home?	**0.704**	0.295
Is he/she capable of buying clothes, food and other things by himself/herself?	**0.690**	0.405
Is he/she capable of making a meal?	**0.689**	0.230
Is he/she capable of heating water for coffee and turning off the stove?	**0.647**	0.313
Does he/she manage his/her money?	**0.579**	0.361
Is he/she capable of managing his/her medications?	**0.566**	**0.448**
Is he/she capable of remembering appointments, family events and holidays?	**0.562**	**0.532**
Can he/she be left alone at home safely?	**0.512**	0.119
Is he/she capable of paying attention, understanding and discussing a radio or television program, newspaper or magazine?	0.238	**0.934**
Is he/she capable of keeping track of current events and occurrences in the community or neighborhood?	0.366	**0.771**

P-FAQ: Pfeffer's Functional Activities Questionnaire.
Significant factor loadings according to sample size.

Table 3 | Spearman's correlation coefficients and shared variance (R^2) between P-FAQ and socio-demographic, cognitive, functional and psychiatric variables.

Variable	ϱ	R^2(%)
Age	0.117	<1
Schooling	−0.109	<1
GADL self-care	−0.232**	5
GADL domestic activities	−0.687**	47
GADL complex activities	−0.845**	71
Geriatric depression scale	0.271**	7
Mini mental state examination	−0.420**	18
Frontal assessment battery	−0.440**	19
Clock drawing test	−0.260**	7
MDRS attention	−0.361**	13
MDRS I/P	−0.537**	29
MDRS construction	−0.239**	6
MDRS conceptualization	−0.388**	15
MDRS memory	−0.457**	21
MDRS total	−0.574**	33

*Significant to $p < 0.01$; P-FAQ, Pfeffer's Functional Activities Questionnaire; GADL, General Activities of Daily Living Scale; MDRS, Mattis Dementia Rating Scale; I/P, Initiative/Perseveration. **$p < 0.01$.*

DISCUSSION

The present findings demonstrate the psychometric adequacy of the P-FAQ in terms of reliability and validity. Moreover, the questionnaire demonstrated satisfactory internal consistency (Cronbach's $\alpha = 0.91$). Although this version has three items that differ from the original FAQ, the items that comprise the P-FAQ are homogeneous, maintaining the internal consistency of the questionnaire, and possibly interchangeable. These results are similar to data described by Sanchez et al. (2011), who report $\alpha = 0.95$ in the administration of the scale to a sample of older Brazilian adults.

The factorial structure of the P-FAQ exhibited two components. The first incorporated complex instrumental activities of daily living, with the largest loading factor found for "*capable of walking outside the neighborhood and finding the way back home*," followed by "*capable of buying clothes, food and other things by himself/herself*." The second factor addresses activities strongly related to planning and prospective memory, which are considered complex activities, but possibly with different

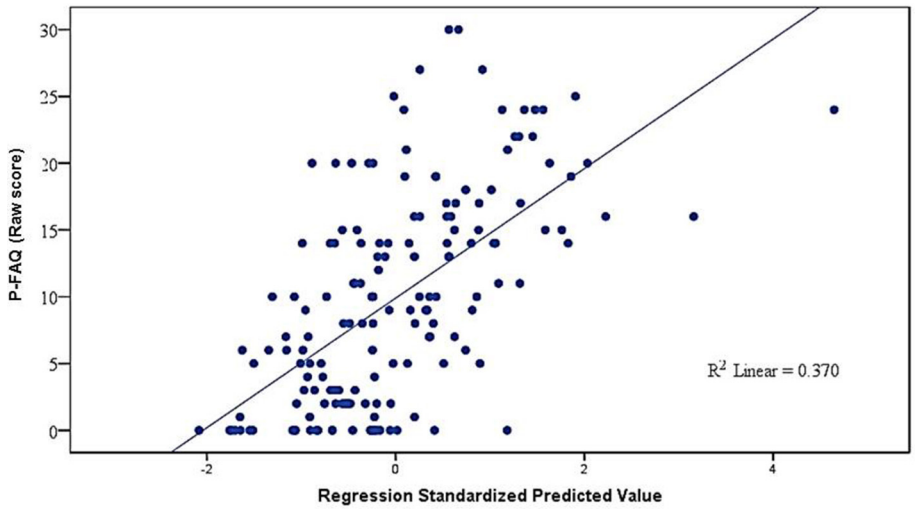

FIGURE 1 | Multiple linear regression of P-FAQ score, socio-demographic characteristics, cognition and depressive symptoms. P-FAQ: Pfeffer's Functional Activities Questionnaire.

cognitive and procedural demands. The findings demonstrate the construct validity of the questionnaire, with two factors associated with complex activities. Moreover, the correlations were stronger for complex instrumental activities involving greater cognitive involvement in comparison to basic routine activities of a domestic nature. These results are in agreement with data reported in the original study by Pfeffer et al. (1982), who considers the items on the FAQ to be more complex than those on previous scales, such as that proposed by Lawton et al. (Lawton and Brody, 1969).

A heterogeneous correlation pattern was found between the P-FAQ and the cognitive, functional and psychiatric tests selected for the present study. The strongest correlations were found for a global cognitive variable (MDRS total score) and a variable related to executive functions (Mattis Initiative/Perseveration) and moderate correlations were found for more general executive functions (Frontal Assessment Battery) and a cognitive screening test (Mini-Mental State Examination). These findings are in agreement with data described in a previous study, in which executive functions and functional performance were strongly correlated in a similar population (de Paula and Malloy-Diniz, 2013). Greenaway et al. (2012) also found the MDRS to be a predictor of functional decline in older adults. The present findings are in agreement with data described in a review of the literature conducted by Royall et al. (2007), in which measures of executive functions and general cognition were more strongly associated with performance on activities of daily living. It should be stressed that the Mattis Initiative/Perseverance subscale involves verbal fluency tasks that depend on both executive functions and processing speed (de Paula et al., 2013c), the latter of which has been associated with functional performance in studies with heterogeneous populations (Brown et al., 2013).

Moderate correlations were found between the P-FAQ and tasks related to memory (Mattis Memory), language/semantic memory (Mattis Conceptualization) and attention/work memory (Mattis Attention), suggesting that such aspects of cognition play a secondary role in the performance of complex activities of daily living. The weakest correlations found between cognitive and functional measures were related to visuospatial skills (Mattis Construction and Clock Drawing Test). However, previous studies have found significant associations between functional performance and visuospatial skills (Davies et al., 2011; Farley et al., 2011). This divergence reflects the need for components directed at the evaluation of activities strongly related to the processing of spatial information. The P-FAQ has only one item addressing this aspect ("*Is he/she capable of walking outside the neighborhood and finding the way back home?*"), which, however, is strongly influenced by other cognitive aspects, such as non-declarative memory (habits and procedural memory).

Depressive symptoms constituted another significant predictor of functional performance in the present study. These symptoms were estimated using a scale that has been validated for the Brazilian population (Almeida and Almeida, 1999). Although the association was weak, depressive symptoms were independently associated with cognitive and socio-demographic aspects. Such symptoms are important determinants of functional decline in older adults (Hoffmann et al., 2010; de Paula, 2012; de Paula et al., 2013c), but can be understood as either a cause or consequence of functional decline, which is an aspect that should be analyzed further in future studies.

Significant differences were found among the different groups evaluated using the P-FAQ, the largest of which were between the healthy controls and patients with dementia. Significant differences were also found among the healthy controls, patients with mild cognitive impairment and those with psychiatric disorders as well as between patients with mild cognitive impairment and those with dementia. Analyzing healthy older adults, those with mild cognitive impairment and those with dementia, Jacinto (2008), also found that the P-FAQ demonstrated sufficient efficacy in the diagnosis of cognitive decline. The capacity of the

FAQ to distinguish health older adults from those with dementia (Pfeffer et al., 1982) gives the questionnaire clinical importance (Alzheimer's Disease Neuroimaging Initiative, 2014). The P-FAQ also has this characteristic. Further studies should be conducted to evaluate the possible additive of effect between this version of the FAQ and cognitive measures for the differential diagnosis of pathological aging, as performed with another functional scale used as a parameter in the present investigation.

The present study has limitations that should be addressed. The participants were grouped in general categories (dementia, mild cognitive impairment and psychiatric disorders) without considering subdivisions, such as Alzheimer's disease and frontotemporal dementia in the group of patients with dementia, since the sample size is relatively small. As functional impairment may differ among these patients, the present findings cannot be directly transposed to these specific groups. The comparison of an ecological parameter for the evaluation of the FAQ, which is the gold standard for functional assessments, would allow a more accurate analysis of the ecological validity of the questionnaire (Chaytor and Schmitter-Edgecombe, 2003).

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Differences in prefrontal cortex activation and deactivation during strategic episodic verbal memory encoding in mild cognitive impairment

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In this study we examined differences in fMRI activation and deactivation patterns during episodic verbal memory encoding between individuals with MCI ($n = 18$) and healthy controls (HCs) ($n = 17$). Participants were scanned in two different sessions during the application of self-initiated or directed instructions to apply semantic strategies at encoding of word lists. MCI participants showed reduced free recall scores when using self-initiated encoding strategies that were increased to baseline controls' level after directed instructions were provided. During directed strategic encoding, greater recruitment of frontoparietal regions was observed in both MCI and control groups; group differences between sessions were observed in the ventromedial prefrontal cortex and the right superior frontal gyrus. This study provides evidence suggesting that differences of activity in these regions may be related to encoding deficits in MCI, possibly mediating executive functions during task performance.

Keywords: mild cognitive impairment, age-related memory disorders, verbal episodic memory, fMRI, semantic encoding

Introduction

Mild cognitive impairment (MCI) is a heterogeneous syndrome that in some cases is transitional between normal age-related cognitive changes and dementia (Patel and Holland, 2012). MCI with episodic memory (EM) impairment, namely amnestic MCI (aMCI), has been identified as a possible precursor of Alzheimer Disease (AD) (Petersen et al., 2001; Albert et al., 2011). Impairments in EM in aMCI and early AD patients can be identified by reduced performance in delayed free recall measures, such as word list-learning tasks (Jak et al., 2009). In AD patients, this impairment is often attributed to medial temporal lobe (MTL) neuropathology. In MCI, however, the exact nature of the verbal episodic memory deficit is unknown. It has been proposed that it can result not only from deficits in acquisition and consolidation processes characteristic of AD, but also from attentional or executive functions deficits that lead to inefficient encoding and/or retrieval of verbal material (Twamley et al., 2006).

Prior fMRI studies of memory in aMCI have produced mixed results of decreased and increased hippocampal activation that seems to result from the large variability between studies in disease classification and severity as well as in the characteristics of the memory tasks (for a review see Dickerson and Sperling, 2008). Specifically, studies examining verbal memory encoding processes have also reported inconsistent activation patterns in frontoparietal regions in MCI relative to controls (Hämäläinen et al., 2007; Dannhauser et al., 2008; Clément and Belleville, 2010). There is also evidence suggesting that these group differences may be modulated by task characteristics, since individuals with MCI and clinical AD exhibited less suppression of the so-called default mode network regions than healthy older adults in response to increases in task demands during, for example, working memory encoding (Lustig et al., 2003; Buckner et al., 2008; Kochan et al., 2010).

Despite the above contributions to the identification of the neural substrates underlying memory impairment in MCI, an aspect still not investigated is the contribution of the cognitive strategies adopted by MCI subjects to perform episodic verbal learning tasks. It has been demonstrated that older adults are not as able as young subjects in using spontaneous verbal learning strategies during word-list learning tasks in order to improve episodic memory recall (Fernandes and Grady, 2008), and that this pattern tends to deteriorate along the MCI-AD continuum (Ribeiro et al., 2007; Hutchens et al., 2012). At least in cognitively healthy older adults, the provision of semantic elaboration strategies during intentional verbal encoding was shown to be effective in improving memory performance and inducing increases in ventrolateral PFC activation, a region that was initially under-recruited (i.e., decreased fMRI activation) in comparison to young adults (Logan et al., 2002). However, it is unknown whether similar cognitive mechanisms would operate in the presence of episodic learning impairment that may be accompanied by subclinical neuropathology in memory-related regions, such as in MCI. Findings from memory strategic-training studies in MCI show that increased activation of prefrontal, temporal, and parietal regions were associated with improved memory performance for word-lists (Belleville et al., 2011) and face-name associations (Hampstead et al., 2011), suggesting a possible malleability of changes in cognitive and neural processing in this population.

In the present study we therefore investigated the neural correlates of differences in verbal learning strategy application during episodic memory encoding in MCI and age-matched healthy controls (HCs). Different levels of strategic processing were manipulated during unconstrained intentional encoding of word lists and after an explicit, direct instruction to apply a semantic encoding organizational strategy. We predicted that, relative to the unconstrained intentional encoding condition, both control and MCI groups would exhibit increases in memory and strategic performance after the explicit orientation to apply the semantic organizational strategy that would be paralleled by increased recruitment of frontoparietal network regions during episodic verbal encoding. We also investigated whether the MCI group would exhibit patterns of overactivation in frontoparietal network regions and/or impaired suppression (i.e., less deactivation) in DMN regions compared to controls during verbal episodic encoding after the explicit orientation to apply the semantic learning strategy.

Materials and Methods

Participants

A total of 18 MCI patients and 17 HCs, all right handed, were included in the study (demographic and neuropsychological profile are given in **Table 1**). The MCI patients were recruited from a specialized Alzheimer's disease clinic (CEREDIC) and in the Behavioral Neurology section at the Hospital das Clínicas, University of Sao Paulo, Sao Paulo, Brazil. Patients with MCI were diagnosed using the criteria suggested by Petersen (Petersen et al., 2001), which was operationalized in our study as the following: presence of memory complaint corroborated by an informant, performance of at least 1 SD below the mean adjusted by age on the Rey Auditory Verbal Learning Test (RAVLT) adapted to the Brazilian elderly population (Malloy-Diniz et al., 2007), normal general cognitive function assessed by Mini-Mental State Examination (adjusted for age and education) (Brucki et al., 2003) and no impairment in activities of daily living. Given our interest in including individuals at the very early stages of the MCI spectrum, the threshold for determining memory impairment was 1 SD below the age norms instead of the more conventional criteria of 1.5 SD, on the delayed recall of the RAVLT. In addition, to be included in the study, each participant had to receive a consensus diagnosis (Winblad et al., 2004) incorporating clinical history, medical records, laboratory evaluation, and neuroimaging exams by an evaluating physician and a neuropsychologist from the team. The HCs were independently functioning members of the community and did not meet MCI criteria. Exclusion criteria included presence of intracranial lesion detected in the structural MRI visually checked by a neuroradiologist (i.e., evidence of ischemic or hemorrhagic stroke or space-occupying lesions; small foci of T2 hyperintensities were not excluded, but were classified qualitatively), any type of dementia or any other type of disease that might impair cognitive function (e.g., depression), and current or past alcohol or drug abuse. Participants were excluded also on factors based on MRI contraindications such as metallic implants and claustrophobia. All subjects had normal vision or that corrected to the normal standard by the use of MRI-compatible eyeglasses. The study was approved by the local ethics committee (CAPPesq *0349/09*) and the patients signed a written informed consent form prior to their inclusion in the study.

fMRI Experimental Paradigm and Procedure

Participants were scanned in two fMRI sessions, being described here as spontaneous and directed encoding conditions, distinguished by an explicit guidance on how to apply semantic clustering during intentional verbal encoding. The fMRI word list learning paradigm consisted of alternating blocks of encoding and resting baseline conditions. The encoding blocks required subjects to read silently and intentionally memorize lists of concrete nouns visually presented on the screen for subsequent recall. Two different sets of lists were

TABLE 1 | Characteristics of the subjects with MCI and controls.

	Controls ($n = 17$)	MCI ($n = 18$)	p-values
Age	68.25 (1.54)	69.50 (1.91)	0.652
Education	11.19 (1.35)	9.20 (1.13)	0.332
Sex	8M/9F	8M/10F	0.870
Fazekas score—DWM	1.21	1.5	0.107
Fazekas score—PVWM	1.38	1.15	0.375
GDS	1.25 (0.38)	1.69 (0.41)	0.444
Pfeffer	0.27 (0.2)	2.19 (0.52)	0.002
MMSE	28.33 (0.37)	27.06 (0.53)	0.167
Paragraph immediate recall (WMS-R)	26.36 (1.60)	20.61 (2.10)	0.053
Paragraph delayed recall (WMS-R)	24.36 (1.44)	9.06 (1.59)	< 0.001
RAVLT immediate total recall	46.60 (2.89)	27.94 (2.22)	< 0.001
RAVLT immediate recall	9.80 (0.67)	5.24 (0.59)	< 0.001
RAVLT delayed recall	9.44 (0.65)	4.41 (0.46)	< 0.001
Digit span forward (WAIS)	7.17 (0.52)	5.06 (0.34)	0.002
Digit span backward (WAIS)	5.42 (0.41)	3.06 (0.14)	< 0.001
Rey figure (copy)	31.50 (1.24)	30.69 (1.22)	0.649
Rey figure (recall)	14.62 (1.78)	8.63 (1.71)	0.024
Stroop (time on third plate)	31.77 (2.28)	43.39 (5.31)	0.045
Verbal fluency (supermarket, MDRS)	24.64 (2.53)	17.79 (1.40)	0.020
Verbal fluency (FAS)	38.33 (2.61)	27.24 (3.42)	0.024
Boston naming	54.07 (1.20)	48.83 (2.18)	0.020

Results are expressed as mean (SE).
DWM, deep white matter; PVWM, peri-ventricular white matter; GDS, Geriatric Depression Scale; MDRS, Mattis Dementia Rating Scale; MMSE, Mini-Mental State Examination; WMS-R, Wechsler Memory Scale Revised; WAIS, Wechsler Adult Intelligence Scale.

used. One included lists formed by 16 words grouped into four semantic categories of four words each (SR list, ex. fruits, musical instruments, vegetables, tools). The general approach to manipulating semantic organization was modeled closely on the CVLT, which is a well-characterized clinical measure of strategic verbal memory (Delis et al., 1998). The other set was formed by 16 words that were not semantically related to one another (UR list). Word lists were balanced for word length and their validity in prompting significant differences in semantic clustering was tested in previous studies (for a more detailed description, see Savage et al., 2001; Miotto et al., 2006). During the encoding blocks, each word was presented for 2.06 s. Words from the SR list were presented so that no two words from the same semantic category occurred consecutively, thus requiring active semantic and executive processing to clustering on subsequent free recall. Encoding blocks of each list condition were alternating with a 12 s resting baseline in which participants were oriented to fix the gaze into a cross in the center of the screen. Each word list block repeated three times through one run, in each fMRI session. To perform the spontaneous fMRI session, participants were not instructed about the semantic organization of the words in the lists beforehand or given any practice with related lists. Therefore, any grouping by category observed in the subsequent free recall at the end of this fMRI acquisition was presumed to be self-initiated by the subject. At the end of the spontaneous session, participants were taken to a different room and given a period of instructions and practice to apply semantic organizational strategies to a set of five different word lists. Subjects were equally instructed to organize the words into categories and to retrieve them according to their category. The practice period had occurred during a limited time (i.e., up to 30 min) until each participant was able to apply the categorization strategy to at least three different word lists. All participants were able to learn and apply the semantic strategies. Immediately after practicing the application of the strategy, participants were scanned again using the same type of paradigm as in the first session, except for the use of new set of word lists and the explicit instruction to apply semantic clustering. The presentation order of the words in each list was randomized and block conditions were counterbalanced within fMRI sessions, across participants. Free recall (i.e., total number of words from the SR list correctly recalled) were assessed offline, immediately after each fMRI session. Semantic clustering index scores were defined as the consecutive recall of two words from the same category. They reflected the proportion of clustered responses out of the total possible clusters defined as follows: clusters/(words recalled-categories recalled). The serial clustering score was defined as follows: clusters/(words recalled–1). Recognition was also assessed outside the scanner. Stimulus presentation and response recording were performed with E-Prime 1.0 software (Psychology Software Tools). Visual stimuli presentation was projected through a magnetic shielded glass window to a screen inside the scanner room and was synchronized with image acquisition.

Scanning

The fMRI acquisition was based on T2*-weighted echo planar (EPI GRE) images for the whole brain acquired in a 3 Tesla Philips Achieva system with an eight-channel head coil. The acquisitions parameters were: TR = 3000 ms, TE = 30 ms, 40 slices, 3 mm slice thickness, 0.3 mm slice gap, FOV = 240 mm^2 and matrix 64 × 64, 3 mm^3 voxels, with 94 volumes per run. Functional acquisitions were preceded by four dummy scans to ensure steady-state magnetization. A T1-weighted structural image (voxel size: 1 mm^3) was acquired before the functional sessions for coregistration with the fMRI data and to exclude brain pathology. In particular, white matter lesions were analyzed according to the Fazekas Score (Fazekas et al., 1987).

Data Analysis

Data processing and statistical analyses were conducted using FSL (www.fmrib.ox.ac.uk/fsl/) (Smith et al., 2004). Functional volumes were processed by movement correction (MCFLIRT), spatial smoothing (FWHM = 5 mm) and spatial normalization to standard space (affine, 12 DoF). Time-series from each voxel were high-pass filtered with a cut-off period of 1/100 Hz to remove signal drift and low-frequency noise. Statistical maps of activity at the individual level were calculated using the general linear model (GLM) using FILM routines (Woolrich et al., 2004), which is based on semi-parametric estimation of residuals autocorrelation. Each block (SR and UR) was modeled using a boxcar function convolved with a gamma-derived hemodynamic response function (standard deviation of 3 s, mean lag of 6 s),

and the contrasts SR > fixation and UR > fixation were estimated for each participant. In a preliminary analysis, these contrasts were then entered into a second-level analysis to test if there were a main effect of list (SR, UR) or a list*session (spontaneous, directed) interaction, in order to identify regions whose activation increased or decreased between sessions, more for the SR contrast than the UR contrast. There were no brain regions that showed a significant interaction or a main effect of list. The remaining analyses focused only in the contrast SR > fixation, giving the sensitivity of the SR word list in prompting measurable semantic clustering scores.

Differences between the two encoding conditions were initially examined in each group separately. The effect of the explicit orientation to apply the semantic learning strategy in activation (directed > spontaneous) and deactivation (spontaneous > directed) between sessions were identified using a paired *t*-test. To answer the primary aim of this study (i.e., understanding the contribution of differences in verbal learning strategic processing between normal controls and MCI during episodic memory encoding), the interaction group (MCI, control)*session (spontaneous, directed) was examined, using free recall score as a covariate to control for the possibility that activation differences between groups could reflect only the performance differences between groups. To further explore the relation between differences in session-related changes in activation and strategic performance behavior, the change in semantic clustering index for each participant (directed—spontaneous, Δ strategy session) was regressed on the corresponding change in BOLD activation (Δ BOLD session). For this, a map was created in a second-level analysis subtracting the map associated with the encoding of the SR list at the spontaneous session from the directed session for each participant. To verify whether there was a difference in brain-behavior correlation patterns between groups, the interaction group*Δ strategy session was examined. All the statistical images were thresholded by using Gaussian random field-based cluster inference with a threshold of $Z > 2.3$ at the voxel level and a corrected cluster significance threshold of $P < 0.05$.

Results

Neuropsychological and Behavioral Data

Table 1 shows participants demographic and neuropsychological characteristics. There were no differences in age, years of formal education, socioeconomic status, and Fazekas score between groups. Subjects with MCI showed, as expected, a pattern of mild neuropsychological deficits relative to controls, particularly in the domain of memory. There were statistically significant differences in the mean scores between groups in the word list (RAVLT) and prose passages recall (WMSR), visual memory recall (Rey Figure), semantic and phonemic verbal fluency tests (Mattis), in the forward and backward digit span subtest (WAIS III), and in the Boston naming test. The groups were matched on qualitative measures of cerebrovascular integrity (e.g., white matter hyperintensities).

Behavioral performance in the word list learning paradigm related to the spontaneous and the directed fMRI sessions (**Table 2**) was analysed with two (session) × two (group) × two (list) repeated measures ANOVA (**Table 2**). A significant session effect was observed, [$F_{(1, 33)} = 20.766$, $p < 0.001$], as both groups improved their free recall from the spontaneous to the directed session, after instructions to apply the semantic encoding strategy. There was also a significant list effect [$F_{(1, 33)} = 115.447$, $p < 0.001$], showing better performance for the SR than the UR list. Controls recalled more words than MCI, independently of the list type and session [$F_{(1, 33)} = 13.923$, $p = 0.001$]. *Post-hoc* analysis indicated that MCI free recall performance after the orientation session to apply the semantic encoding strategy became similar to the controls performance at the spontaneous session [$t_{(33)} = -1.18$, $p = 0.272$].

A two (group) × two (session) repeated measures ANOVA on the semantic clustering score revealed a significant session effect [$F_{(1, 33)} = 28.859$, $p < 0.001$], indicating that both groups recalled a great number of clustered words after the explicit orientation to apply the verbal learning strategy than using self-initiated encoding strategies. The mean number of clusters generated by MCI patients was lower than the performed by controls in both sessions [$F_{(1, 33)} = 4.275$, $p = 0.047$], as expected from their lower recall performances relative to controls. Mean recognition scores indicated above chance level of performance for both groups (**Supplementary Material Table S1**).

fMRI Results

Brain Activation Related to Changes in the Application of the Semantic Encoding Strategy Across Sessions—Within-group Comparisons

We first assessed differences in verbal learning strategy application across sessions in fMRI activation and deactivation during episodic encoding of word lists separately for each group (**Table 3**, **Figure 1**). Increased activation during encoding after the explicit orientation to apply the verbal organizational learning strategy (directed > spontaneous) were observed in both the MCI and control group in clusters encompassing portions of the left middle frontal gyrus (midDLPFC), inferior frontal gyrus (VLPFC) dorsal premotor cortex, and posterior parietal cortex (PPC), in the angular gyrus and within intraparietal sulcus (IPS) borders. Decreased activation (directed < spontaneous, **Supplementary Material Figure S1**) was observed in a set of clusters within the right superior frontal gyrus, the vmPFC, left

TABLE 2 | Free recall and semantic clustering performance relative to the spontaneous and the directed sessions.

	Controls		MCI	
	Spontaneous	Directed	Spontaneous	Directed
FREE RECALL				
SR	7.53 (0.575)	9.41 (0.810)	4.77 (0.583)	6.38 (0.813)
UR	3.58 (0.496)	3.29 (0.496)	1.33 (0.489)	2.278 (0.424)
Clustering index	0.242 (0.053)	0.452 (0.052)	0.182 (0.051)	0.447 (0.050)
Number of clusters	3.23 (0.390)	4.58 (0.575)	1.55 (0.379)	3.77 (0.537)

Results are expressed as mean (SE).

TABLE 3 | Statistical information of significant clusters highlighted when comparing the BOLD response between the spontaneous and directed use of learning strategies during word list encoding compared to fixation baseline.

Contrast	Region	Side	MNI coordinates				Size	Cluster *p*-values
			x	*y*	*z*			
Directed > spontaneous								
controls	VLPFC						2131	< 0.0001
	Inferior frontal gyrus (BA 44)	L	−42	10	32	4.6		
	Inferior frontal gyrus (BA 45)	L	−46	24	24	4.25		
	midDLPFC						1021	< 0.0001
	Middle frontal gyrus (BA 46)	L	−36	0	54	4.04		
	Precentral gyrus (BA 6)	L	−42	0	32	4.17		
	Posterior parietal cortex						977	< 0.0001
	Intraparietal sulcus (BA 7)	L	−24	−70	48	4.27		
	Angular gyrus (BA 40)	L	−34	−58	40	3.58		
	Pre−SMA						545	0.0032
	Superior frontal gyrus (BA 6)	L	−6	2	58	4.66		
	Cingulate gyrus (BA 32)	R	6	20	36	3.86		
	Cerebelum	L	−40	−76	−26	4.27	412	0.0021
MCI	DLPFC						1416	< 0.0001
	Middle frontal gyrus (BA 9)	L	−42	8	50	3.95		
	Middle frontal gyrus (BA 46)	L	−44	44	12	3.82		
	Inferior frontal gyrus (BA 47)	L	−46	38	6	3.44		
	Inferior frontal gyrus (BA 47)	L	−52	18	0	3.71		
	Left temporoparietal cortex						1003	< 0.0001
	Intraparietal sulcus superior	L	−28	−62	32	4.56		
	Angular gyrus (BA 39)	L	−34	−70	38	4.45		
	Superior temporal gyrus (BA 22)	L	−42	−50	22	3.27		
	Right temporo−parietal						654	0.00015
	Supramarginal gyrus (BA 40)	R	42	−52	32	3.51		
	Middle temporal gyrus (BA 39)	R	40	−62	32	3.48		
	Precuneus(BA 7)	R	32	−62	42	3.44		
Directed < spontaneous								
controls	Precuneus/Posterior cingulate						1894	< 0.0001
	Posterior cingulate (BA 30)	L	−16	−66	12	3.97		
	Precuneus (BA 7)	L	0	−54	46	3.83		
	mPFC						1710	< 0.0001
	Superior frontal gyrus (BA 10)	R	12	54	−4	4.37		
	Anterior cingulate gyrus (BA 24)	R	0	36	8	3.77		
	Inferior temporal						896	< 0.0001
	Inferior parietal lobule (BA 39)	R	50	−48	24	4.16		
	Supramarginal gyrus (BA 40)	R	60	−50	30	3.69		
	Middle temporal gyrus (BA 21)	R	52	−56	6	3.34		
	Superior temporal gyrus (BA 22)	R	52	−44	10	3.26		
	Superior temporal						619	0.0012
	Middle temporal gyrus (BA 21)	R	64	−6	−16	3.74		
	Superior temporal gyrus (BA 22)	R	56	−26	8	3.72		
	Superior frontal						528	0.004
	Superior frontal gyrus (BA 9)	R	24	40	46	3.77		
	Middle frontal gyrus (BA 46)	R	26	46	30	3.35		
	OFC						421	0.0171
	Middle orbital gyrus (BA11)	R	18	16	−22	3.5		
	Rectus gyrus (BA 11)	L	−6	6	−22	3.1		
MCI	Parieto−occipital						710	< 0.0001
	Lyngual gyrus (BA 18)	L	−2	−100	−4	3.65		
	Cuneus (BA 17)	L	−6	−98	8	3.6		
	Cuneus (BA 17)	R	6	−92	26	3.38		

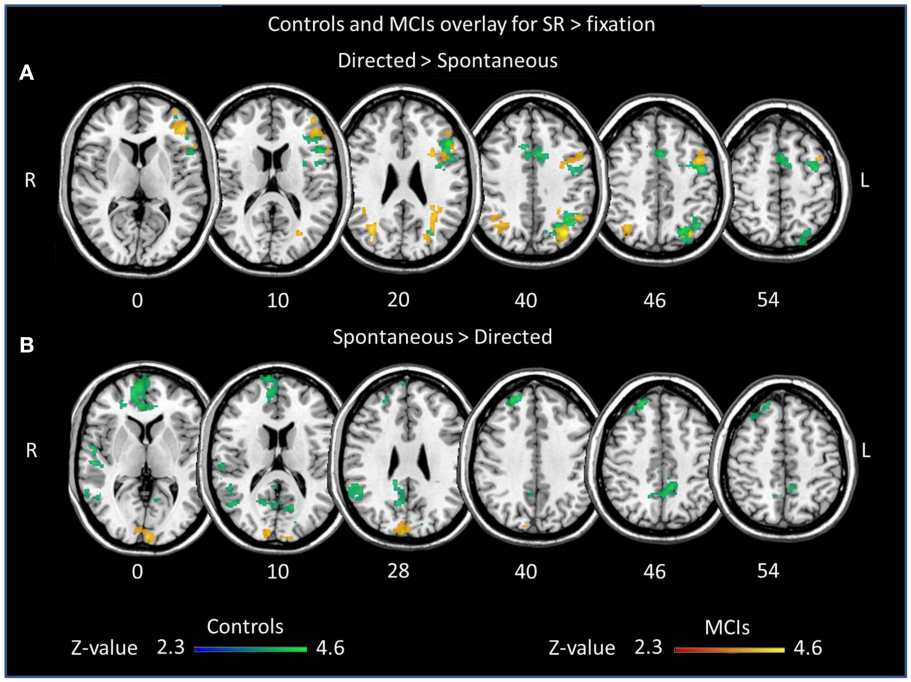

FIGURE 1 | Overlay of clusters exhibiting significant differences in activation between sessions during word list encoding for controls (blue/green) and MCIs (red/yellow). Brain regions showing greater **(A)** activation and **(B)** deactivation in the directed session, during the explicit application of the semantic strategy, in comparison to the spontaneous encoding session.

inferior parietal cortex and infero-lateral temporal cortex, and posterior cingulate/precuneous cortex only in the control group. In the MCI group, deactivation was observed only in a small cluster located in the parieto-occipital cortex.

Different Patterns of Activation/Deactivation Related to Changes in the Application of the Semantic Encoding Strategy Across Sessions Between Groups

In order to identify brain regions that exhibited different activation/deactivation patterns during encoding with explicit instructions to apply the semantic organizational strategy relative compared to unconstraint encoding session in MCI relative to controls, the interaction group × session were examined at the whole-brain level. A significant interaction was observed in two clusters: mPFC, extending to the anterior cingulate (peak voxel MNI coordinate 2 68 8, $Z = 4.13$, cluster p-value corrected = 0.016), and in the right superior frontal gyrus, extending to the middle frontal gyrus (peak voxel MNI coordinate 36 32 44, $Z = 3.46$, cluster p-value corrected = 0.003). Plots showing the mean magnitude estimates of activity in the significant clusters for each session indicate the nature of the interaction effects, showing that only controls, but not MCI patients, exhibited a significant modulation (i.e., significant deactivation) of the mMPFC function in response to the explicit orientation to apply the encoding strategy. In the right superior frontal gyrus, group level differences followed a "cross-over" pattern, such that in controls, activation decreased after the guided use of the semantic clustering, while patients showed increased activation. The interaction remained similar after controlling for behavioral differences in word recall between groups (**Figure 2**).

Relationship Between Changes in Strategy-based Verbal Learning Behavior and Brain Activation

Given the observed group-related differences associated with the directed use of the encoding strategy in brain activation and deactivation in core regions of the cognitive control and the default-mode networks during the word list encoding task, we examined if individual changes in the fMRI BOLD signal between sessions (directed—spontaneous) in these regions would be differently associated with changes in strategic performance in controls and MCIs. A significant group by strategy interaction was observed in a cluster located in the OFC, extending to the mPFC and anterior cingulate (peak voxel MNI coordinate 4 18–16 in the rectus gyrus; $z = 3.58$; cluster p-value corrected = 0.0429). Scatter plots of changes in BOLD signal between sessions against changes in strategy use for this region revealed a strong negative correlation in controls, such that higher performer participants exhibited the greatest decrease in activation ($r = -0.734$), whereas in MCIs, higher performer participants exhibited the greatest increase in activation from the spontaneous to the directed session ($r = 0.339$) (**Figure 3**).

Discussion

In the current study, we examined differences in fMRI brain activation and deactivation related to semantic strategy application during verbal memory encoding in MCI and HC

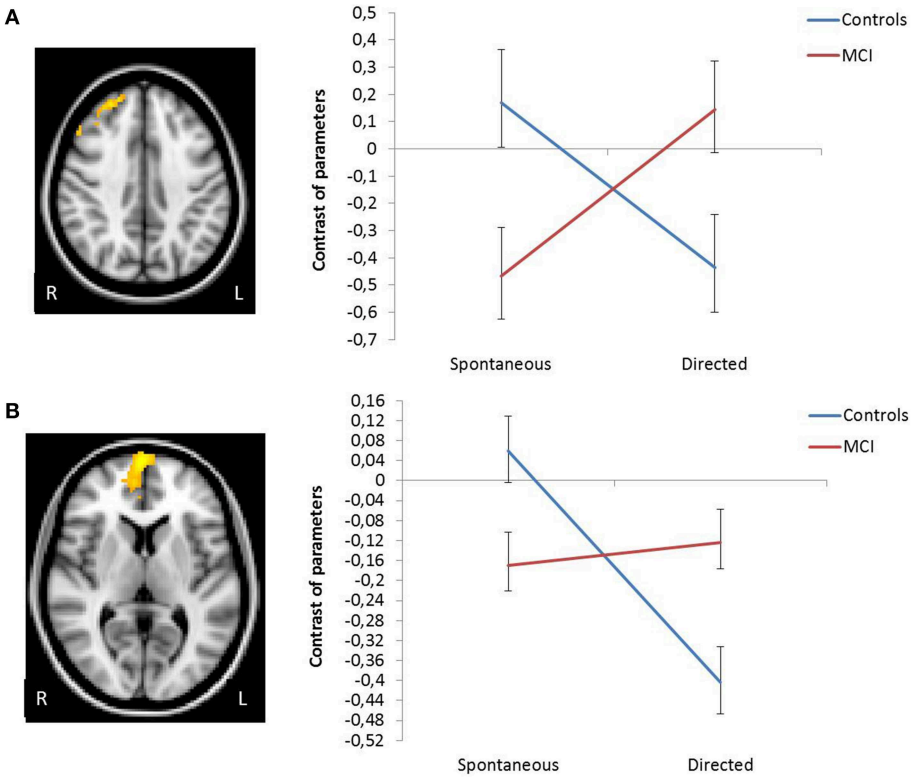

FIGURE 2 | Change in functional activation in the regions of significant group × session interaction showing that in right superior frontal gyrus (A) and in vmPFC (B) there is a decrease in activation in the controls, and an increase in MCIs.

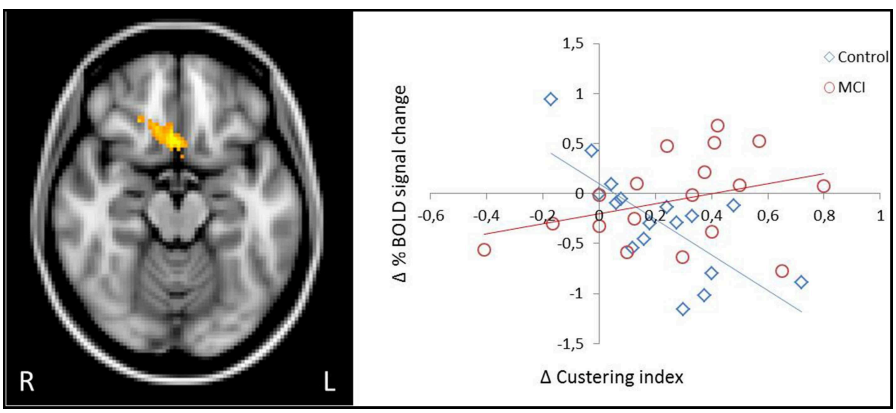

FIGURE 3 | Different patterns of association between change in semantic clustering and change in activation due to the explicit application of the encoding strategy in the OFC. In controls, greater increase in strategic behavior was predictive of greater decrease in activation in the OFC, whereas in MCI increase in behavior was predictive of increased activation.

subjects. As expected from prior reports on episodic memory performance in subjects at risk for dementia (Ribeiro et al., 2007; Hudon et al., 2011), free recall scores of the MCI group were worse than that of the control group when using self-initiated encoding strategies. However, the explicit guidance to apply the semantic strategy improved the verbal memory performance of the MCI group to the same level exhibited by control subjects when using self-initiated strategies. Improvements in verbal learning due to the application of the strategy were able to reverse MCI recall deficits to baseline controls level, which is consistent with previous findings showing that MCI can benefit from environmental support or cognitive training to reduce memory impairment (Simon et al., 2012).

Results from the fMRI analysis revealed that after the explicit orientation to apply the verbal learning strategy, greater recruitment of frontoparietal network regions, including

portions of the left DLPFC, VLPFC, were observed in both MCI and control groups in relation to the unconstrained encoding condition, as expected. The initial prediction of over-recruitment of portions of the DLPFC and within the IPS in MCI reflecting possible compensation mechanisms due to increased cognitive demand to perform the task was not confirmed. Group-differences in functional deactivations, however, were observed in the vmPFC and in the right superior frontal gyrus, related to the absence of modulation in the activity of the vmPFC, along with a lack of suppression of the right superior frontal gyrus in MCI. A different association between improvement in strategy use and session-related changes in activation of the medial OFC between groups was also confirmed. As previously stated (Savage et al., 2001), participants' increased use of semantic organization strategies due to explicit orientation allowed them to monitor, update and manipulate the studied words as they mentally regrouped related words together to subsequent recall them. The increased recruitment of regions of the frontoparietal network in response to increases in strategy use during intentional episodic encoding observed in our study is consistent with findings from previous intervention studies examining the effects of memory strategy training protocols in brain activation in MCI and controls during encoding and retrieval of word lists and face-name associations (Belleville et al., 2011; Hampstead et al., 2011).

In fMRI studies, increases in activation with the development of a new strategy acquired by learning are often thought to reflect recruitment of additional cortical units, seen as strengthening of BOLD response within brain regions (Poldrack, 2000). In this context, increased activation of the frontoparietal network can be considered as an evidence of redistribution or functional reorganization of brain activation (Kelly and Garavan, 2005; Bor and Owen, 2007). However, it has also been proposed that practice improvements in applying previously learned strategies that involve organization and "chunking" (i.e., organization of small pieces of information together) also results in increased frontoparietal activation, even when task demands decrease while using these strategies (Bor and Owen, 2007). In light of these proposals, the cognitive mechanisms underlying the observed increases in frontoparietal recruitment with increases in strategy use in our study might reflect a combination of greater skill in applying a previously learned strategy, particularly in controls, and the engagement of a new strategy, especially in MCI.

Improvements in strategy application and memory performance were represented by different activation changes in the mPFC and in the right superior frontal gyrus responses between groups. These activations did not appear to be exclusively related to performance differences between groups, as we controlled for free recall scores in our fMRI analyses.

While controls clearly suppressed the responses of mPFC and of the right superior frontal gyrus during encoding after the explicit orientation to apply the semantic strategy, MCI subjects exhibited a pattern of less deactivation in these regions. Similar results were reported by the memory training study above cited (Belleville et al., 2011), but they did not directly compared the amount of training-related deactivations between MCI and controls. In the context of task induced deactivation studies, these two regions, along with the posterior cingulate and medial parietal regions and the inferior parietal lobule, have been consistently reported as nodes of the DMN of the brain (Toro et al., 2008). Although the DMN was originally proposed as a set of regions that exhibited greater blood flow or BOLD signal during baseline rest or fixation conditions than during task performance, subsequent studies demonstrated similar patterns of results when tasks with different cognitive demands were contrasted (Raichle et al., 2001). Recent studies have shown, for example, that mPFC activity is greater during performance of a relatively easy 0-back task than a 2-back working memory task (Leech et al., 2011), and that suppressing activity in DMN regions during working memory encoding was predictive of subsequent better performance (Anticevic et al., 2010). In our study, when an increased working memory demand was imposed by the direct application of the encoding strategy, controls exhibited a suppression of these regions (i.e., less activation), but not the MCI patients. Similar findings were recently reported in MCI during a graded working memory paradigm (Papma et al., 2014). It has been suggested that DMN deactivation is progressively disrupted along the continuum from normal aging to MCI and AD, with increased impairment in subjects at risk for AD, such as *APOE4* genotype carriers (Pihlajamäki and Sperling, 2009). In this context, the pattern of results in the present study suggests that MCI may be less efficient than controls in processing external task-irrelevant information during encoding, suffering in a great extent the effects of distraction.

An alternative interpretation for the different pattern of results between our groups came from evidence suggesting that when performance of a task improves after learning or practice due to better application of strategies, task becomes less effortful and demands on executive control processes are reduced (Jonides, 2004). This reduction in executive control can lead to a reduction of activation that is correlated with better performance (Poldrack, 2000). In the present study, greater reductions in activation on a region encompassing the OFC, extending to the mPFC and the anterior cingulate were strongly predicted by greater increases in strategic performance in controls, supporting a possible automaticity hypothesis.

The results of our study should be interpreted in the context of some potential limitations. The MCI diagnosis was performed based on cross-sectional neuropsychological data rather than on longitudinal subject-by-subject objective evidence of progressive memory loss, and so it is not possible to completely rule out the possibility that patients defined on the basis of their clinical profile belong to different subgroups and different points of a severity continuum. It must also be acknowledged that the ability to discriminate changes in activity that represent differences in cognitive function from those that represent physiological change due to a MCI diagnosis is a challenge for fMRI research, especially since neurovascular coupling processes may change with age or disease risk (D'Esposito et al., 2003). We attempted to decrease the possibility of confounding compromised vascular responses with changes in cognitive processing by excluding individuals with cerebrovascular disease and matching groups on qualitative measures of cerebrovascular integrity (e.g., white matter hyperintensities). Moreover, although we observed

improvement in recall performance after the directed strategy application in MCI, it is possible that the relative limited stimulus exposure time (e.g., one word per 2 s) may have constraint the use of spontaneous mnemonic strategies by these participants (Hampstead et al., 2014). Present findings also require further replication in the context of an independent control group to ensure that the observed brain changes associated with the greater use of the encoding strategy are not solely due to repetition effects.

In sum, the results of our study extend the existing literature on differential cognitive processing and neural recruitment in MCI during performance on episodic memory encoding tasks. The novel contributions of our study involved an assessment of the neural correlates of strategic processes employed during encoding of episodic memory in this population. Examining differences in the patterns of deactivation during verbal encoding with increased strategic processing, we found that MCI patients failed to show modulation of activation in mPFC and less deactivation in the right superior frontal gyrus compared to normal controls. Such different pattern of responses may reflect changes in the set of cognitive processes, particularly executive functions, adopted during verbal memory encoding between normal aging and MCI.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2015.00147

Supplementary Material Figure S1 | Mean contrast of parameter estimates (SR > Rest) and standard errors for the comparison Directed < Spontaneous in the control group in clusters encompassing the precuneus, mPFC, and inferior temporal cortex. The MCI group exhibited significant deactivation between sessions only in the parietal-occipital cluster (indicated with an asterisk).

Supplementary Material Table S1 | Recognition performance on the fMRI paradigm. Results are expressed as mean (SE).

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Mild cognitive impairment, poor episodic memory, and late-life depression are associated with cerebral cortical thinning and increased white matter hyperintensities

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In various independent studies to date, cerebral cortical thickness and white matter hyperintensity (WMH) volume have been associated with episodic memory, depression, and mild cognitive impairment (MCI). The aim of this study was to uncover variations in cortical thickness and WMH volume in association with episodic memory, depressive state, and the presence of MCI simultaneously in a single study population. The participants were 186 individuals with MCI (clinical dementia rating [CDR] of 0.5) and 136 healthy elderly controls (HCs; CDR of 0) drawn from two community-based cohort studies in northern Japan. We computed cerebral cortical thickness and WMH volume by using MR scans and statistically analyzed differences in these indices between HCs and MCI participants. We also assessed the associations of these indices with memory performance and depressive state in participants with MCI. Compared with HCs, MCI participants exhibited thinner cortices in the temporal and inferior parietal lobes and greater WMH volumes in the corona radiata and semioval center. In MCI participants, poor episodic memory was associated with thinner cortices in the left entorhinal region and increased WMH volume in the posterior periventricular regions. Compared with non-depressed MCI participants, depressed MCI participants showed reduced cortical thickness in the anterior medial temporal lobe and gyrus adjacent to the amygdala bilaterally, as well as greater WMH volume as a percentage of the total intracranial volume (WMHr). A higher WMHr was associated with cortical thinning in the frontal, temporal, and parietal regions in MCI participants. These results demonstrate that episodic memory and depression are associated with both cortical thickness and WMH volume in MCI participants. Additional longitudinal studies are needed to clarify the dynamic associations and interactions among these indices.

Keywords: mild cognitive impairment, episodic memory, late-life depression, cortical thickness, white matter hyperintensity

INTRODUCTION

Mild cognitive impairment (MCI) is a heterogeneous clinical condition that may precede Alzheimer disease (AD) as well as vascular and other dementias (Meyer et al., 2002). In patients with MCI, memory is impaired whereas other cognitive functions are relatively spared (Petersen et al., 1999). With regard to features of the brain observed with structural MRI, compared with healthy elderly controls (HCs), MCI patients tend to show decreased cortical thickness in the temporal lobe, reduced hippocampal volume (Liu et al., 2011), and increased white matter hyperintensity (WMH) volume in the cerebrum (Smith et al., 2011; Iorio et al., 2013; Yates et al., 2014). More recently, an association between decreased cerebral cortical thickness and increased WMH volume has been reported (Seo et al., 2012). Furthermore, increased WMH volume is thought to be related to normal aging and hypertension as well as decreased cognitive functions (Smith et al., 2011; Rostrup et al., 2012; Birdsill et al., 2014).

Patients with MCI are at increased risk of progression to dementia (Richard et al., 2013), of which manifestations of depression could be regarded as early symptoms (Panza et al., 2010). In the context of associations between depressive symptoms and structural changes in the brain, reductions in amygdalar volume, hippocampal volume, or both have been reported in elderly patients with depression (Egger et al., 2008; Burke et al., 2011). Associations between WMHs and affective disorders in elderly populations have also been elucidated. Herrmann et al. (2008) reviewed the literature and reported that WMHs are also

observed more frequently in elderly patients with depression than in controls. In the study by Disabato et al. (2014), patients with late-onset late-life depression had increased WMHs and thinner cortices in the left anterior cingulate relative to those with early-onset depression. Kieseppä et al. (2014) showed that only middle-aged patients with bipolar disorder type I were at increased risk of deep WMHs, which in turn were independently associated with deficits in visual attention. Serafini et al. (2010) reported that MRI findings of deep WMHs could be a useful biological predictor of severity in patients with late-onset late-life bipolar disorder type II.

As described above, various clinical features in MCI are mutually interrelated. However, few studies have investigated the relationships among these features in a single study population. Therefore, in the present study, we explored the following questions. (1) Do MCI patients have thinner cerebral cortices (especially in the temporal lobe) and higher WMH volume than do HCs? (2) Does their deteriorated cognitive function, including problems with episodic memory, reflect thinner cortices, increased volume and spatial distribution of WMH in the brain, or both? (3) Compared with non-depressed MCI patients, do depressed participants have thinner cortices, higher WMH volumes, or both, and if so in what regions? (4) Is increased WMH volume associated with cerebral cortical atrophy and its spatial distribution? To address these issues by novel, automated, and quantitative methods, we analyzed the structural MRI scans of MCI and HC participants drawn from two community cohorts: the Osaki-Tajiri (Meguro et al., 2002) and Kurihara (Meguro et al., 2012) projects.

MATERIALS AND METHODS

THE OSAKI-TAJIRI AND KURIHARA PROJECTS

Participants were drawn from the two aforementioned community-based cohort studies. The Tajiri project was undertaken to study preventive strategies against stroke, dementia, and bed-confinement with a target population of people aged over 65 in old Tajiri, northern Japan from 1988 (Meguro et al., 2002). The project was renamed the Osaki-Tajiri project in 2005 and recruited 1654 participants. The Kurihara project, whose aims were the same as those of the Osaki-Tajiri project, recruited 590 participants aged over 75 in Kurihara, located to the north of Osaki, starting in 2008 (Meguro et al., 2012). The ethical committees of the Tajiri SKIP Center, Tohoku University Graduate School of Medicine, the Kurihara Central Hospital, and the National Center of Neurology and Psychiatry approved the data collection procedures and data analysis. Written informed consent was obtained from all participants. In the event that the participant was unable to express agreement, a family member signed the informed consent form on behalf of the participant.

PARTICIPANTS

To perform quantitative analyses on structural MRI scans of the brain to compute cerebral cortical thickness and WMH labeling, the present study required a pair of high-quality images comprising a three-dimensional (3D) T1-weighted image and a two-dimensional (2D) fluid-attenuated inversion-recovery (FLAIR) image. Although 497 participants underwent brain MRI in the Osaki-Tajiri project, only 160 were examined with both 3D T1-weighted imaging and FLAIR imaging. In the Kurihara project, 220 of the 577 participants had both a 3D T1-weighted image and a FLAIR image scanned with the same sequences and head coil as in the Osaki-Tajiri project. Then, through quality control of the imaging data, participants were excluded if they had old infarctions, except for 1 or 2 lacunar infarcts ($n = 4$); remarkable motion artifacts ($n = 3$); post-surgical operation of a brain tumor ($n = 1$); or imaging data loss ($n = 16$). Furthermore, participants with a Clinical Dementia Rating (CDR) of 1 or more were excluded ($n = 34$). Eventually, 322 participants in total (127 from the Osaki-Tajiri project and 195 from the Kurihara project) were included in this study. Clinical demographic data and a flow diagram of the current study are shown in **Table 1** and **Figure 1**, respectively.

Table 1 | Demographics of the participants ($n = 322$).

	HC (CDR of 0)	MCI (CDR of 0.5)	*p*-value
Number	136	186	
Age, y	78 (76–81)[b]	80 (76–83)[b]	<0.005
Gender, M/F	55/81	84/102	0.40
Education, y	9 (8–11)[a]	8 (8–9)[a]	0.01
MMSE	26 (24–27)[c]	23 (21–25)[c]	<0.001
WMS-R LMII	9 (5–13)[c]	4 (1–10)[c]	<0.001
GDS-15	3.5 (2–6)	4 (2–7)	0.07
WMHr, %	1.03 (0.54–1.82)[c]	1.76 (0.87–3.06)[c]	<0.001

Data are presented as numbers or medians (interquartile ranges). Abbreviations: CDR = Clinical Dementia Rating; F = female; HC = healthy elderly control; GDS-15 = 15-item Geriatric Depression Scale; M = male; MCI = mild cognitive impairment; WMHr = cerebral white matter hyperintensity volume as a percentage of the total intracranial volume; WMS-R LMII = Wechsler Memory Scale-Revised Logical Memory II. [a]$p < 0.05$, [b]$p < 0.005$, [c]$p < 0.001$.

CLINICAL AND NEUROPSYCHOLOGICAL ASSESSMENT

All participants underwent clinical and neuropsychological testing. This involved medical interviews with the participants themselves or their family members. Furthermore, we assessed each participant on the following four neuropsychological scales. (1) We used the Japanese version (Otoyama et al., 2000) of the CDR scale (Hughes et al., 1982) to rate each participant's severity of dementia symptoms with information on the six areas of memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. By integrating the assessments on the six areas, each participant was assigned to healthy (CDR of 0), very mild dementia (CDR of 0.5), mild dementia (CDR of 1), moderate dementia (CDR of 2), and severe dementia (CDR of 3). (2) The Mini-Mental State Examination (MMSE; Folstein et al., 1975) was used as a global measure of cognitive function including orientation, memory, and numeracy skills. (3) We used the Logical Memory II test of the Wechsler Memory Scale-Revised (WMS-R LMII; Wechsler, 1987) to evaluate delayed recall as a measure of episodic memory performance. (4) Finally, the 15-item Geriatric Depression Scale (GDS-15; Sheikh and Yesavage, 1986) was used to assess depressive symptoms. The cutoff point of the GDS-15 was determined as 4/5 (Almeida and Almeida, 1999). Participants

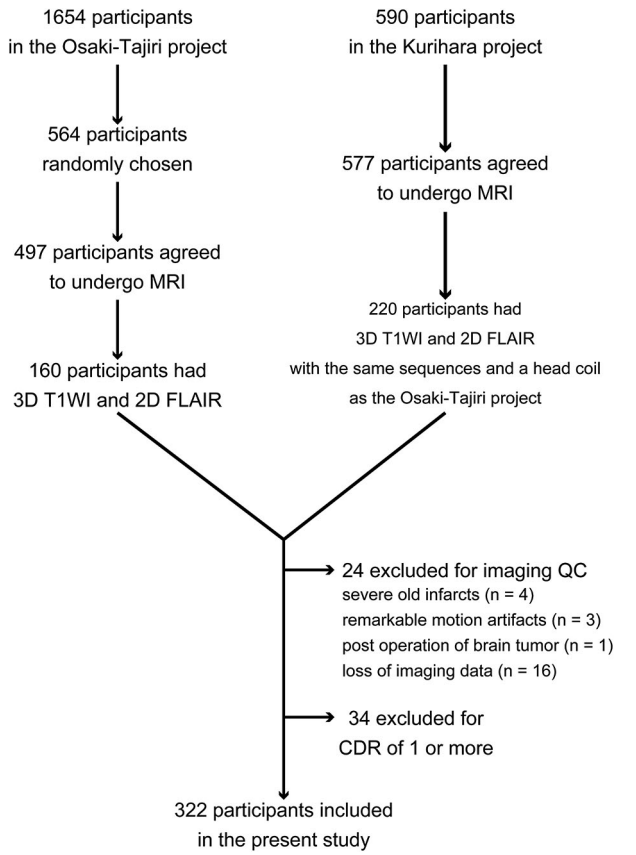

FIGURE 1 | Flow diagram for inclusion and exclusion of participants in this study. Abbreviation: QC = quality control.

with GDS-15 scores of 5 or more were judged depressive and those with scores of 4 or less were judged non-depressive. In the present study, to capture the widest possible range of MCI conditions, participants with a CDR of 0.5 were assigned to the MCI group (DeCarli et al., 2004; Celone et al., 2006; Miller et al., 2008). Those with a CDR of 0 were classified as HCs. Those with a CDR of 1 or greater were excluded from the study because participants with MCI constituted the target population. The 322 participants consisted of 186 individuals with MCI and 136 HCs.

MRI ACQUISITION

Brain scans for the Osaki-Tajiri and Kurihara projects were acquired on Achieva 1.5T MRI scanners (Philips Medical Systems, Best, Netherlands) by using a sagittal 3D T1-weighted turbo field echo (3D T1-TFE) sequence and an axial 2D FLAIR sequence with eight-channel phased array head coils. The acquisition parameters for the 3D T1-TFE sequence were as follows: repetition time (TR), 9.3 ms; echo time (TE), 4.6 ms; flip angle (FA), 8° for Osaki-Tajiri and 10° for Kurihara; field of view (FOV), 240 mm; and in-plane resolution, 256 × 256 (0.94 × 0.94 mm) with a slice thickness of 1 mm and no intersection gap. Axial FLAIR images were scanned with the following parameters: TR/TE/inversion time (TI), 11000/140/2800 ms for Osaki-Tajiri and 8000/120/2500 ms for Kurihara; FA, 90°; FOV, 230 mm; in-plane resolution, 512 × 512 (0.45 × 0.45 mm); and slice thickness/intersection gap, 5/0.5 mm for Osaki-Tajiri and 6/1 mm for Kurihara.

IMAGE PROCESSING

Measurement of cortical thickness

The cerebral cortical thickness of each participant was estimated from the 3D T1-TFE image by using antsCorticalThickness.sh (Tustison et al., 2014), a shell script in the Advanced Normalization Tools (ANTs) software package development version[1]. The image processing workflow is summarized as follows: (1) initial N4 bias correction for alleviating intensity inhomogeneity (Tustison et al., 2010); (2) skull-stripping by using a study-specific template created in advance from 30 participants (20 randomly selected participants with MCI and 10 HCs) with the Symmetric Group Normalization framework (SyGN; Avants et al., 2010) implemented in antsMultivariateTemplateConstruction2.sh; (3) six-tissue segmentation with Atropos (Avants et al., 2011); (4) cortical thickness estimation with DiReCT (Das et al., 2009); and (5) nonlinear spatial registration to the template with SyN (Avants et al., 2008). Manual corrections were added to the results of the skull-stripping as needed by using ITK-SNAP version 3.0.0[2] (Yushkevich et al., 2006) and a Cintiq 13HD tablet (Wacom Co., Ltd., Kazo, Japan). The six-tissue priors for the study-specific brain template were generated using 3D T1-weighted images from the Open Access Series of Imaging Studies (OASIS) database[3] (Marcus et al., 2007) and the corresponding expertly and manually segmented labels. These data had been used for the MICCAI 2012 Grand Challenge and the Workshop on Multi-Atlas Labeling[4], and released under the Creative Commons Attribution-NonCommercial (CC BY-NC) license. The labels were provided by Neuromorphometrics, Inc. (Somerville, MA, USA)[5] under an academic subscription. The thickness map for each individual was transformed to the template space, using Gaussian interpolation with a sigma of two voxels.

Measurement of white matter hyperintensity

White matter hyperintensity segmentation was performed by using the Lesion Segmentation Toolbox version 1.2.3[6] (Schmidt et al., 2012). The initial threshold for the lesion growth algorithm was set at 0.30 in the current study. The optimal initial threshold value of 0.30 was determined by visually comparing the resultant WMH probability lesion maps derived by using various thresholds between 0.05 and 1.0. Furthermore, gray matter and white matter were empirically chosen as lesion belief maps, whereas only gray matter was set to the lesion belief map by default. Any infratentorial WMH in each image was excluded from the resultant WMH probability map of the whole brain by applying a supratentorial

[1]http://stnava.github.io/ANTs
[2]http://www.itksnap.org
[3]http://www.oasis-brains.org
[4]https://masi.vuse.vanderbilt.edu/workshop2012
[5]http://www.neuromorphometrics.com
[6]http://www.applied-statistics.de/lst.html

parenchymal mask generated in the processing of cortical thickness (i.e., Atropos six-tissue segmentation). Each WMH probability map was visually inspected and nonlinearly transformed to the study-specific template space by applying the warp estimated in registering the 3D T1-TFE image to the template. The spatially normalized WMH probability maps were then smoothed by using a Gaussian kernel with a sigma of two voxels.

STATISTICAL ANALYSIS

Statistical analyses of demographic data and differences between the MCI and HC groups and between participants with and without depression in the MCI group were performed by using R version 3.1.0[7] (R Core Team, 2014). Continuous variables were tested for normality by using the Shapiro-Wilk test. Means for age, years of education, neuropsychological examination scores, and WMH ratio (WMHr; the cerebral WMH as a percentage of the total intracranial volume estimated with VBM8) were compared between the MCI and HC groups and between participants with and without depression in the MCI group by the Wilcoxon rank sum test owing to their non-normal distributions. Frequency distributions for gender between the MCI and HC groups and between participants with and without depression in the MCI group were compared by using chi-square tests.

Statistics for voxel-wise image analyses were performed with a voxel-wise general linear model implemented in the FMRIB Software Library (FSL), version 5.0.6[8] (Jenkinson et al., 2012). Differences in cerebral cortical thickness and WMH probability between MCI and HC participants were tested by using independent sample *t*-tests. Regional associations between cerebral cortical thickness or WMH probability and WMS-R LMII scores within MCI participants were assessed by linear regression analyses, setting cortical thickness or WMH probability as the independent variable and a WMS-R LMII score as the dependent variable. Differences in cortical thickness and WMH probability between MCI participants with GDS-15 scores of 4 or less and those with scores of 5 or more were compared by independent sample *t*-tests. Associations between cerebral cortical thickness and WMHr were also assessed by linear regression analyses, setting cortical thickness as the independent variable and WMHr as the dependent variable. All voxel-wise statistical analyses including *t*-tests and linear regression analyses used age, gender, and years of education as covariates.

Permutation-based nonparametric testing was carried out by the "randomize" function in FSL with 5000 permutations (Nichols and Holmes, 2002), using gray and white matter masks for analyses of cortical thickness and WMH probability, respectively. The gray matter and white matter masks were generated by binarizing the corresponding prior probability maps of the template with the lowest probability threshold of 0.5. By using threshold-free cluster enhancement (TFCE; Smith and Nichols, 2009), the results of all statistics were thresholded at $p < 0.05$, family-wise error (FWE)-corrected for multiple comparisons. Threshold-free cluster enhancement was used to avoid arbitrarily setting the smoothing kernel size and using an arbitrary cluster-forming threshold.

[7]http://www.r-project.org/

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

Table 2 | Demographic differences between MCI participants with GDS-15 ≤4 and ≥5.

	MCI with GDS ≤4	MCI with GDS ≥5	*p*-value
Number	105	81	
Age, y	80 (76–82)	80 (77–83)	0.13
Gender, M/F	45/60	39/42	0.57
Education, y	9 (8–10)	8 (8–9)	0.08
MMSE	23 (21–26)	23 (21–25)	0.77
WMS-R LMII	4 (1–10)	4 (1–8)	0.44
WMHr, %	1.29 (0.75–2.88)[a]	2.35 (1.26–3.19)[a]	0.006

Data are presented as numbers or medians (interquartile ranges). Abbreviations: F = female; GDS-15 = 15-item Geriatric Depression Scale; M = male; MCI = mild cognitive impairment; WMHr = cerebral white matter hyperintensity volume as a percentage of the total intracranial volume; WMS-R LMII = Wechsler Memory Scale-Revised Logical Memory II. [a]$p < 0.01$.

RESULTS

DEMOGRAPHIC AND CLINICAL DATA

A summary of the demographic characteristics and neuropsychological examination scores of the MCI and HC groups is shown in **Table 1**. The difference in GDS score between the two groups was not statistically significant, whereas the MCI group showed significantly lower MMSE and WMS-R LMII scores and higher WMHr than did the HC group (Wilcoxon rank sum test; $p < 0.05$). **Table 2** shows the differences between MCI participants with a GDS score of 4 or less (a non-depressed state) and those with a score of 5 or more (a depressed state). The WMHr of depressed MCI participants was significantly higher than that of non-depressed MCI participants. There were no other significant differences between the two groups.

DIFFERENCES IN CORTICAL THICKNESS AND WMH PROBABILITY BETWEEN MCI AND HC

As displayed in **Figure 2A**, MCI participants exhibited thinner cortices than HCs mainly in the bilateral temporal pole, fusiform gyrus, anterior parahippocampal gyrus (anterior part of the entorhinal cortex), middle and inferior temporal gyrus, inferior parietal gyrus, and left supramarginal gyrus ($p < 0.05$, FWE-corrected). In **Figure 2B**, MCI participants also showed higher WMH probabilities than HCs in bilateral cerebral white matter, especially in the corona radiata, semioval center, and regions anterior to the genu of the corpus callosum and around the trigone and anterior horn of the lateral ventricle ($p < 0.05$, FWE-corrected).

ASSOCIATION BETWEEN EPISODIC MEMORY SCORE AND CORTICAL THICKNESS OR WMH PROBABILITY IN MCI

Figure 3 displays locations in the cerebral cortex or white matter where cerebral cortical thickness (A) or WMH probability (B), respectively, was associated with the episodic memory score (i.e., WMS-R LMII score) in participants with MCI ($p < 0.05$, FWE-corrected). Only the most posterior part of the left entorhinal cortex exhibited a positive correlation between cortical thickness and the episodic memory score. In contrast, negative correlations

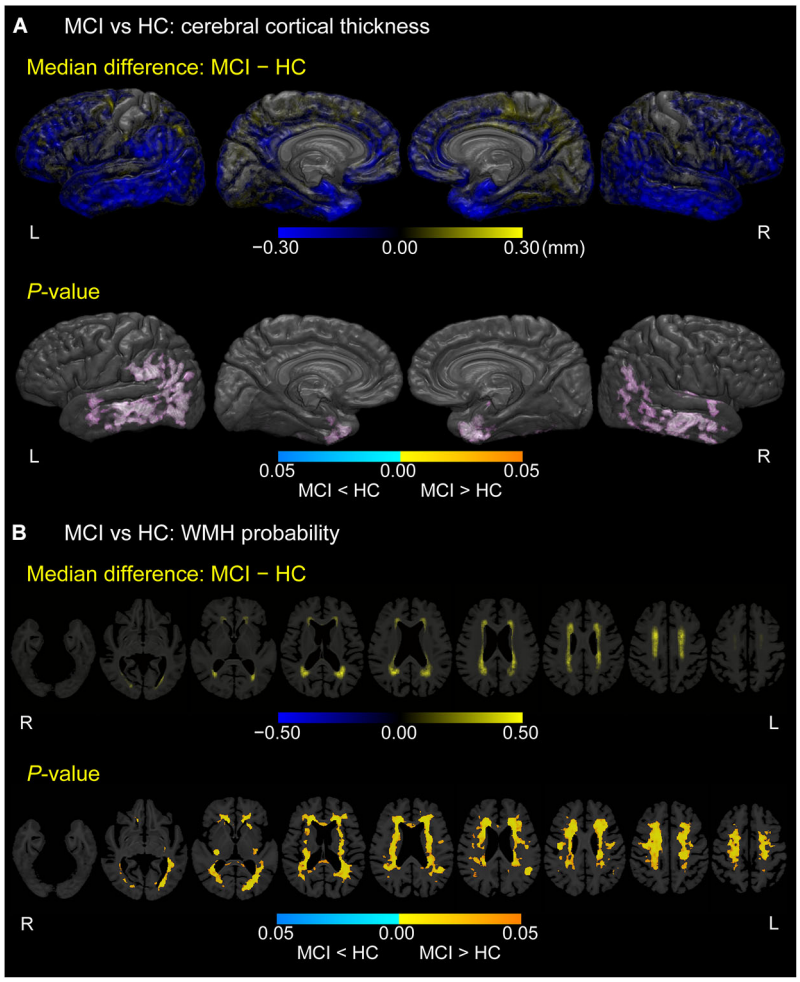

FIGURE 2 | Difference between MCI and HC groups in cerebral cortical thickness and WMH probability. (A) Cortical maps of median differences in cortical thickness (MCI − HC; in millimeters) and family-wise error (FWE)-corrected *p*-values between participants with MCI and HCs. **(B)** White matter maps of median differences in WMH probability (MCI − HC) and FWE-corrected *p*-values between participants with MCI and HCs. These results are overlaid on the skull-stripped study-specific template, adjusted for effects of age, gender, and years of education.

between WMH probability and episodic memory score were detected mainly in the bilateral posterior periventricular regions and near the right anterior horn of the lateral ventricle.

DIFFERENCES IN CORTICAL THICKNESS AND WMH PROBABILITY BETWEEN DEPRESSED AND NON-DEPRESSED MCI PARTICIPANTS

Figure 4 shows cortical or white matter regions where differences in cortical thickness (A) or WMH probability (B), respectively, were observed between depressed MCI participants (GDS score of 5 or more) and non-depressed MCI participants (GDS score of 4 or less; $p < 0.05$, FWE-corrected). Thinner cortical regions were seen in the temporal pole, left parahippocampal gyrus, and uncus of the left hemisphere in the depressed group. Moreover, coronal sections also showed the gyrus adjacent to amygdala to be thinner in depressed MCI participants bilaterally. In contrast, increased WMH probability was seen only in a small region of the right periventricular white matter in depressed MCI participants.

ASSOCIATIONS BETWEEN CORTICAL THICKNESS AND WMH RATIO IN MCI

Figure 5 displays cortical regions where a linear regression model estimated associations between cortical thickness and WMHr, adjusted for age, gender, and years of education in participants with MCI ($p < 0.05$, FWE-corrected). Negative correlations between cortical thickness and WMHr were seen broadly in the lateral, medial frontal, and temporal lobes, including the gyrus adjacent to the amygdala and entorhinal cortex in the bilateral hemispheres.

DISCUSSION

We have explored cortical thickness and WMH volume in connection with MCI, as well as associations among episodic memory, depression, cortical thickness, and WMH in the brain MR scans of participants with MCI (median age, ∼80 years old). In participants drawn from two community-based cohort studies in northern Japan, we used automated quantitative procedures

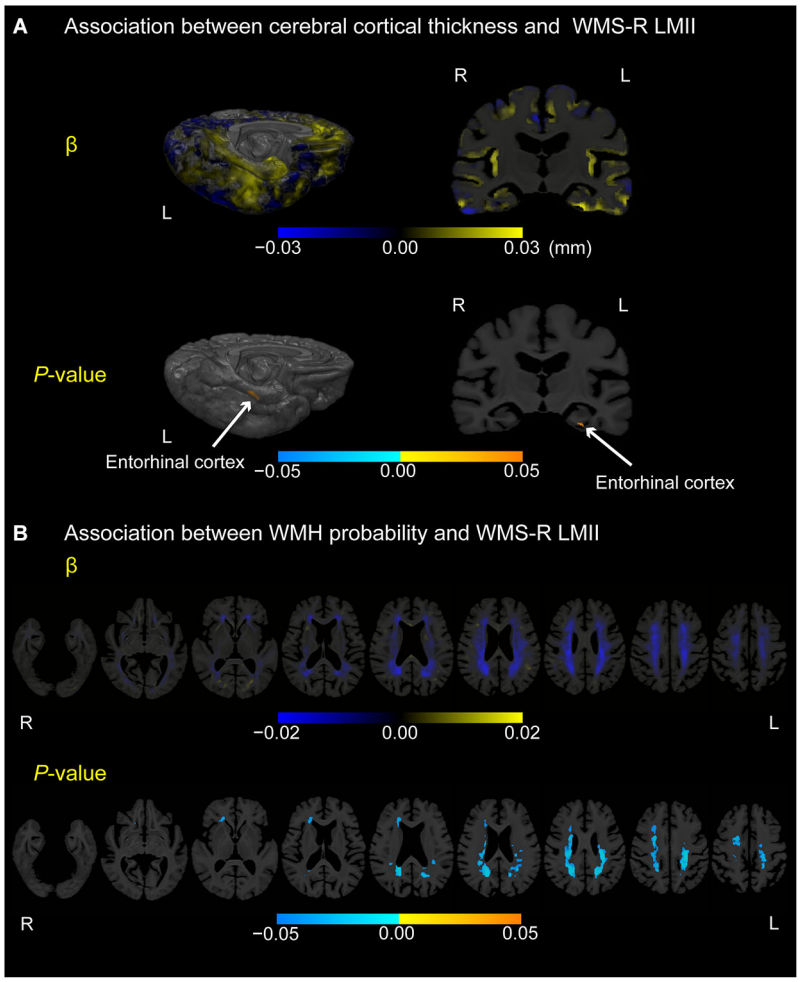

FIGURE 3 | Association between cerebral cortical thickness and WMH probability or WMS-R LMII score in participants with MCI. (A) Cortical maps of regression coefficients (unstandardized beta values) and FWE-corrected *p*-values. The regression coefficients represent the estimated contribution to cerebral cortical thickness as millimeters per unit increase in WMS-R LMII score. **(B)** White matter maps of regression coefficients (unstandardized beta values) and FWE-corrected *p*-values. The regression coefficients represent the estimated contribution to WMH probability per unit increase in the WMS-R LMII score. These results are overlaid on the skull-stripped study-specific template, adjusted for effects of age, gender, and years of education. White arrows in **(A)** point to the left entorhinal cortex.

to clarify these associations and spatial distributions of cortical thinning as well as increases in WMH volume simultaneously. We obtained four main findings. First, MCI patients have thinner cortices and more WMHs in specific regions of the cerebrum than do HCs. Second, poor episodic memory is associated with reduced cortical thickness in the left entorhinal cortex and increased WMHs in specific locations of the cerebrum. Third, a depressive state is associated with reduced cortical thickness in the left temporal pole, left entorhinal cortex, and gyrus adjacent to the amygdala bilaterally, as well as with a higher WMH volume as a percentage of the total intracranial volume. Fourth, a larger WMH volume is associated with thinner cortices in the lateral, medial frontal, and temporal lobes. These findings indicate that both the cerebral cortex and white matter are altered in MCI patients as changes occur in episodic memory and depressive state.

DIFFERENCES IN CEREBRAL CORTICAL THICKNESS AND WMH BETWEEN MCI AND HEALTHY AGING

In this study, at the stage of MCI or mild dementia (classified as CDR 0.5), thinning of the cerebral cortex occurred mainly in the medial and lateral portions of the temporal lobe and the inferior parietal lobe. This finding is in accord with the results of other studies (Singh et al., 2006; Fennema-Notestine et al., 2009). However, the middle and posterior portions of the entorhinal cortex did not exhibit significant differences between MCI participants and HCs in the present study. One possible reason is that MCI participants included not only those with amnestic MCI but also some with non-amnestic MCI, given that a global CDR score of 0.5 was sufficient to classify a participant as having MCI. Hence, the etiologies of MCI could include vascular dementia, depression, frontotemporal dementia, dementia with Lewy bodies, and other

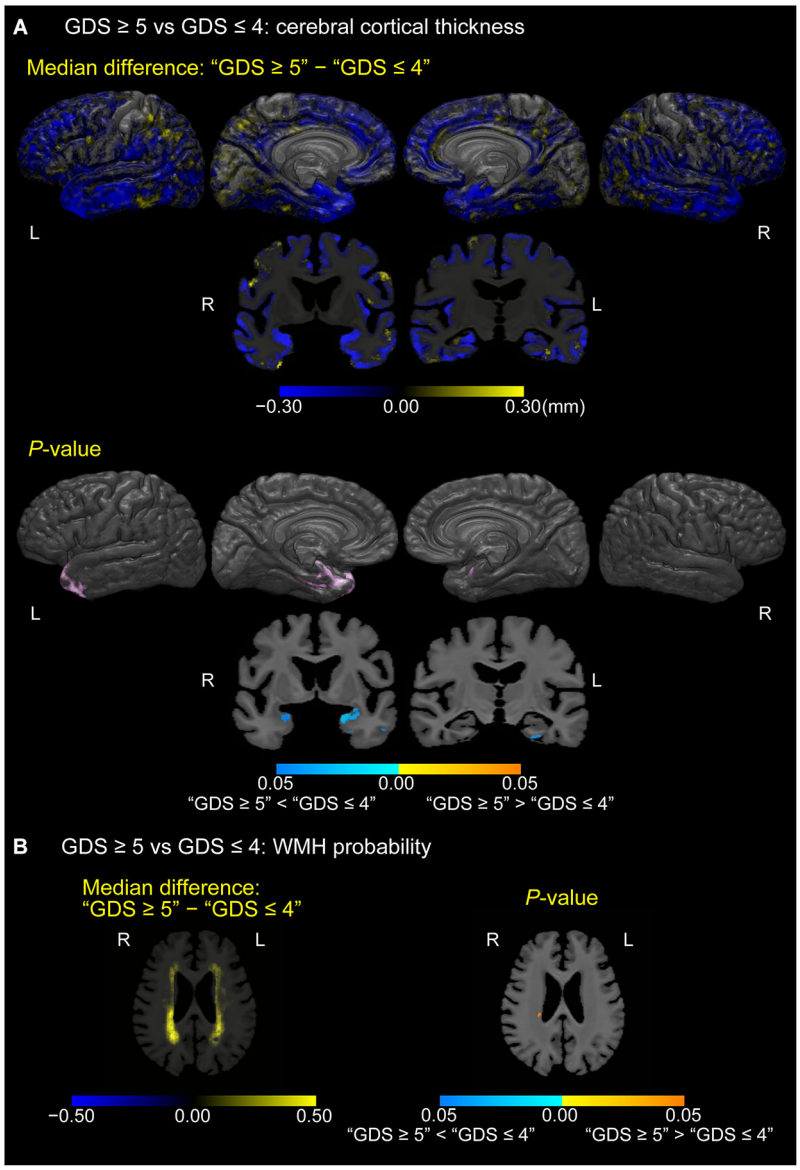

FIGURE 4 | Differences in cerebral cortical thickness and WMH probability between depressed and non-depressed MCI participants. (A) Cortical maps of median cortical thickness (MCI with GDS ≥5 − MCI with GDS ≤4; in millimeters) and family-wise error (FWE)-corrected *p*-values between MCI participants with "GDS ≥5" and "GDS ≤4." **(B)** White matter maps of median differences in WMH probability ("GDS ≥5" − "GDS ≤4") and FWE-corrected *p*-values between MCI participants with "GDS ≥5" and "GDS ≤4." These results are overlaid on the skull-stripped study-specific template, adjusted for effects of age, gender, and years of education.

dementing disorders as well as AD (Petersen and Morris, 2005).

White matter hyperintensities were more prominent for MCI participants than HCs, particularly in the corona radiata, semioval center, and near the genu of the corpus callosum and anterior horn of the lateral ventricle in the bilateral hemispheres. These regions are distributed along the lateral and medial cholinergic pathways that have been elucidated with immunohistochemical procedures in autopsied brains (Selden et al., 1998). Accordingly, our findings lend support to the previous finding that the severity of WMHs in the cholinergic pathway, as measured by a semi-quantitative visual rating scale, is correlated with cognitive status (Bocti et al., 2005). Our findings are consistent with the hypothesis of the authors that WMHs disrupt the cholinergic pathway and contribute to deterioration of cognitive function.

ASSOCIATIONS AMONG EPISODIC MEMORY, CORTICAL THICKNESS, AND WMH

We confirmed a relationship between episodic memory and cerebral cortical thickness. Our finding suggests that the left entorhinal cortex is involved in episodic memory. Similar findings

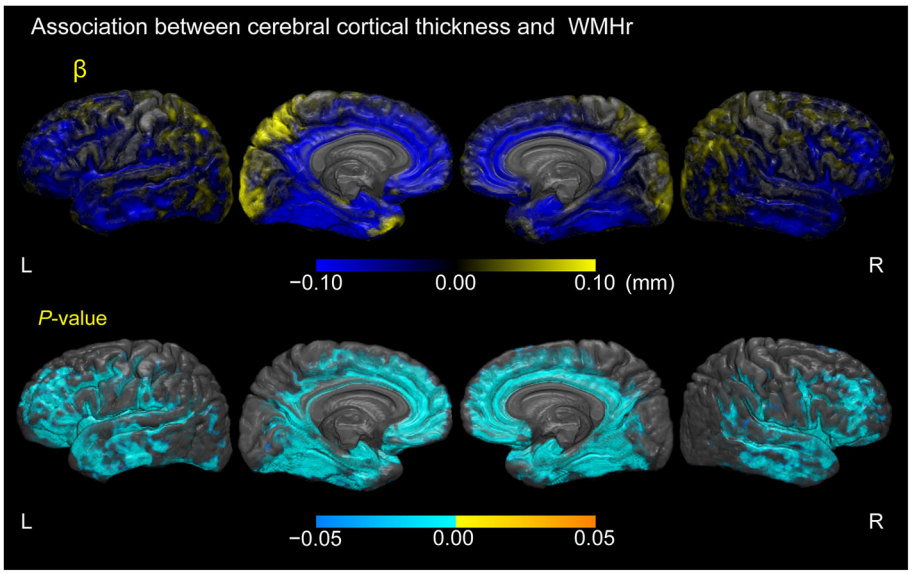

FIGURE 5 | Association between cerebral cortical thickness and white matter hyperintensity volume as a percentage of the total intracranial volume in participants with MCI. Cortical maps of regression coefficients (unstandardized beta values) and FWE-corrected *p*-values. The regression coefficients represent the estimated contribution to cerebral cortical thickness as millimeters per unit increase in WMH volume as a percentage of total intracranial volume. These results are overlaid on the skull-stripped study-specific template, adjusted for effects of age, gender, and years of education.

have been observed by others (Di Paola et al., 2007; Sarazin et al., 2010) in individuals with AD by using voxel-based morphometry (VBM). Hippocampal atrophy is also reportedly associated with poor episodic memory in studies using VBM (Chetelat et al., 2003; Leube et al., 2008; Sarazin et al., 2010) although we did not assess the hippocampal volume using VBM in the present study. A recent study has reported that, compared with VBM, the cortical thickness measure is better at detecting pathological changes in the cerebral cortex (Diaz-de-Grenu et al., 2014). Given that the entorhinal cortex is thin and located in a relatively small area, smoothing with a large kernel size—a method commonly adopted in VBM procedures—might induce contamination from voxels in adjacent areas and reduce the sensitivity to relatively slight atrophic changes of this structure in MCI patients.

We observed that episodic memory was negatively correlated with WMH volume in the region near the right anterior horn of the lateral ventricle as well as the bilateral posterior periventricular white matter, specifically in the parietal-occipital area. These regions contain the superior longitudinal fasciculus including the arcuate fasciculus, as well as the posterior and superior thalamic radiations (Mori et al., 2005). Our findings suggest that WMHs directly impair episodic memory by disrupting the thalamocortical connections as well as the connections between the parietal and temporal cortices and prefrontal cortex. However, our findings are not in line with those of Smith et al. (2011), who showed an association between episodic memory and WMH volume in the temporal-occipital and parietal periventricular white matter and internal capsule (anterior limb). The discrepancy may be partly explained by differences in subject population depending on whether the selection criteria included individuals with major vascular risk factors.

ASSOCIATIONS AMONG DEPRESSIVE STATE, CORTICAL THICKNESS, AND WMH

In the present study, the depressive state in MCI participants was associated with reduced thickness in the left temporal pole, left entorhinal cortex, and gyrus adjacent to the amygdala bilaterally. These results partially concur with the results of some who reported a relationship between late-onset depression and volume reduction in the amygdala (Egger et al., 2008; Burke et al., 2011) or entorhinal cortex (Gerritsen et al., 2011). However, others have reported that acceleration of hippocampal atrophy was associated with late-onset depression in a longitudinal study (den Heijer et al., 2011). Although the reason for these differences is unclear, they may be attributable to differences in the method of image processing. Furthermore, they may also be attributed to differences in the subject population, because only participants with MCI were analyzed to assess the relationship between brain atrophy and depressive state in the present study.

The total WMH volumes in depressed MCI individuals were higher than those in non-depressed participants, although only a small suprathreshold cluster was observed in the right periventricular white matter. Our finding is congruent with a meta-analysis that has shown that individuals with late-life depression have more frequent and severe WMHs than do HCs (Herrmann et al., 2008). Some research groups have reported that greater WMH volume in the superior longitudinal fasci-

culus, uncinate fasciculus, or cingulum bundle is relevant to late-life depression (Sheline et al., 2008; Dalby et al., 2010; Taylor et al., 2013). The disparity between the results of these groups and our findings may be related to the fact that we simply assigned MCI participants to two groups based on GDS-15 scores, instead of using operational diagnostic criteria such as the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR; American Psychiatric Association, 2000) to obtain a diagnosis for depression with higher reliability.

RELATIONSHIP BETWEEN CORTICAL THICKNESS AND WMH BURDEN

We found that larger WMH volumes were associated with thinner cortices in the lateral, medial frontal, and temporal lobes. This finding is roughly in keeping with that reported by Ye et al. (2014) despite methodological differences in measuring cortical thickness and WMH. The result may indicate that WMHs contribute to cerebral cortical thinning by disrupting specific fiber tracts additively or synergistically with pathophysiological processes in AD (Bloom, 2014). We observed that WMHs were involved in cerebral cortical thinning across broad areas of the cerebrum including the frontal cortex and posterior cingulate. According to a recent study, cortical thickness in the prefrontal cortex and that in the posterior cingulate are associated with executive function in MCI patients (Chang et al., 2010). Although we did not assess executive function in the present study, our results lead us to surmise that WMHs contribute to poor executive function by causing cerebral cortical thinning in these regions.

INFLUENCE OF CEREBRAL CORTICAL THINNING AND WMH BURDEN ON MCI AND DEPRESSION

We cannot determine whether Wallerian or retrograde degeneration is involved in the relationship between cortical thinning and increased WMH volume in MCI and late-life depression. However, Brickman et al. (2014) reported in their longitudinal study that higher baseline parietal WMH volume, increasing parietal WMH volume, smaller baseline hippocampal volume, and atrophic change in hippocampal volume independently predicted progression to AD from a non-demented state. Measures of cortical thickness did not predict disease progression. Moreover, in a longitudinal study, Duering et al. (2012) reported marked thinning of the cerebral cortex in regions that showed a high probability of connectivity with incident subcortical infarcts. Thus, cortical thinning in a specific area might occur secondarily after incident subcortical WMHs connected with the cortical area. Therefore, WMH volume may have greater influence on MCI than cortical thinning.

One possible interpretation of the associations among cortical thinning in the medial temporal lobe, increased WMH volume, and depression is that some patients have depression as an early symptom associated with a neurodegenerative process that leads to dementia, as discussed by Lebedeva et al. (2014), whereas WMHs may predispose an individual to or precipitate depression as well as perpetuate pre-existing depression (Alexopoulos et al., 1997).

LIMITATIONS OF THIS STUDY

This study has 10 principal limitations. First, we could not assess pathological microstructural alterations in cerebral white matter by diffusion tensor imaging (DTI), which has the potential to evaluate changes in white matter tracts that appear normal with T2-weighted images or FLAIR (Shimony et al., 2009; Papma et al., 2014) as well as sites of pathological changes in cerebral white matter tracts. Second, we used 2D FLAIR instead of 3D FLAIR owing to equipment limitations. The segmentation results were visually inspected with care because the automated WMH segmentation process adopted here was designed mainly for 3D FLAIR (Schmidt et al., 2012). However, 3D FLAIR images should be obtained and assessed in a future study with a newer automated WMH segmentation program that is optimized for 3D FLAIR and is now publicly available (Ithapu et al., 2014). Third, because participants with a CDR of 0.5 were classified into the MCI group as noted above, the etiologies for this group could include AD, vascular dementia, depression, frontotemporal dementia, dementia with Lewy bodies, and mixed dementia. Therefore, to test whether AD, for example, is the primary etiology for such participants given its primary role in causing dementia (Querfurth and LaFerla, 2010), participants in future studies could be additionally tested for amyloid beta peptide and tau in cerebrospinal fluid or for amyloid and fluorodeoxyglucose by positron emission tomography (Albert et al., 2011). Fourth, the method of cortical thickness estimation adopted here could not assess the volume of the amygdala or hippocampus. We found no significant differences in cortical thickness between MCI participants and HCs or between depressed and non-depressed MCI participants, unlike previous reports that have frequently reported hippocampal volume loss (Ballmaier et al., 2008; Steffens et al., 2011; den Heijer et al., 2011). Therefore, subcortical structures might be assessed more effectively in the future with surface shape analysis (Ballmaier et al., 2008; Zhao et al., 2008; Costafreda et al., 2011; Devanand et al., 2012). Fifth, we used a GDS-15 cut-off value to determine the depressive state of each participant. This could have resulted in overdiagnosis of depression. In future studies, depression should be diagnosed according to the criteria of DSM-IV-TR or the 10th revision of the International Classification of Diseases (ICD-10; World Health Organization, 1992) and assessed for severity by the Hamilton or Montgomery–Asberg Depression Rating Scale (Hamilton, 1960; Montgomery and Asberg, 1979). Additional information about patient history, for example, of attempts or current desire to commit suicide, might also yield interesting results from voxel-wise analyses of cerebral cortical thickness or WMH probability. The sixth limitation is the cross-sectional nature of this study. Clarifying the dynamic associations between increasing WMH volume or cortical thinning and changes in cognitive functions or affective states, for instance, will require intra-individual serial MRI and repeated neuropsychological assessments for a specified period. The seventh limitation is the lack of accounting for cognitive effects of psychoactive agents. For example, participants with depression who take antidepressants could have an improved GDS-15 score and might therefore be assigned to healthy elderly controls despite having some atrophy in specific brain areas due to the disease. The eighth limitation is that we did not control

for the effects of vascular risk factors (e.g, hypertension, history of cardiovascular disease, diabetes mellitus, cigarette smoking) associated with increased WMH volume (Jeerakathil et al., 2004; Tiehuis et al., 2008). The ninth limitation is the selection bias owing to the different criteria of the two projects. The Osaki-Kurihara project recruited participants aged over 65, whereas the Kurihara project recruited participants aged over 75. The prevalence of WMHs could be higher in the participants of the Kurihara than those of the Osaki-Tajiri. The tenth limitation is the heterogeneity of the slice thickness and intersection gap of 2D FLAIR sequence between the two projects. Although we did not examine any difference in WMH volume between the participants of the two projects, the heterogeneity might affect the measurement of WMH volume.

CONCLUSION

In this study, we simultaneously revealed an association between reduced cortical thickness and increased WMH volume in MCI, as well as associations among episodic memory, depressive state, cortical thickness, and WMHs and their spatial distributions in a single population. We found that MCI, poor memory performance, and depressive state involve both cortical thinning and WMH expansion in specific regions, and that increased total WMH volume is closely associated with cortical thinning. Our results verify the importance of clarifying specific pathophysiologic processes by examining combinations of cerebrovascular lesions, AD, and other dementing disorders in future studies (Kling et al., 2013). In addition, they support the view that cerebrovascular lesions could contribute to the pathogenesis of late-life depression (Alexopoulos et al., 1997). Further longitudinal studies of cortical thinning and white matter disruption in elderly individuals with MCI are needed to elucidate the pathophysiological processes of dementia and late-life depression in greater detail.

AUTHOR AND CONTRIBUTORS

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Interactive effects of vascular risk burden and advanced age on cerebral blood flow

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Vascular risk factors and cerebral blood flow (CBF) reduction have been linked to increased risk of cognitive impairment and Alzheimer's disease (AD); however the possible moderating effects of age and vascular risk burden on CBF in late life remain understudied. We examined the relationships among elevated vascular risk burden, age, CBF, and cognition. Seventy-one non-demented older adults completed an arterial spin labeling MR scan, neuropsychological assessment, and medical history interview. Relationships among vascular risk burden, age, and CBF were examined in *a priori* regions of interest (ROIs) previously implicated in aging and AD. Interaction effects indicated that, among older adults with elevated vascular risk burden (i.e., multiple vascular risk factors), advancing age was significantly associated with reduced cortical CBF whereas there was no such relationship for those with low vascular risk burden (i.e., no or one vascular risk factor). This pattern was observed in cortical ROIs including medial temporal (hippocampus, parahippocampal gyrus, uncus), inferior parietal (supramarginal gyrus, inferior parietal lobule, angular gyrus), and frontal (anterior cingulate, middle frontal gyrus, medial frontal gyrus) cortices. Furthermore, among those with elevated vascular risk, reduced CBF was associated with poorer cognitive performance. Such findings suggest that older adults with elevated vascular risk burden may be particularly vulnerable to cognitive change as a function of CBF reductions. Findings support the use of CBF as a potential biomarker in preclinical AD and suggest that vascular risk burden and regionally-specific CBF changes may contribute to differential age-related cognitive declines.

Keywords: aging, vascular risk factors, arterial spin labeling, cognition

INTRODUCTION

Vascular risk factors increase risk of cognitive impairment and Alzheimer's disease (AD) (Luchsinger et al., 2005; Gorelick et al., 2011). Although it is known that the link between vascular risk and cognitive decline is independent of clinical stroke (Gorelick et al., 2011) and vascular brain lesions imaged with magnetic resonance imaging (MRI) (Zheng et al., 2012), how vascular risk might lead to increased risk of AD has yet to be determined. According to the "two-hit vascular hypothesis" of AD (Zlokovic, 2011), vascular risk factors may lead to blood–brain barrier (BBB) dysfunction and reduced cerebral blood flow (CBF), initiating a cascade of processes that lead to dementia. In the primary (non-amyloid-β) pathway (hit one), accumulation of neurotoxic molecules and capillary hypoperfusion lead to neuronal dysfunction. Vascular dysfunction also leads to increased production and decreased clearance of amyloid-β, leading to amyloid-β accumulation. This increase in amyloid-β (hit two) leads to further neuronal dysfunction, accelerating neurodegeneration, and the development of dementia. Both amyloid-β and hypoperfusion may increase hyperphosphorylation of tau thereby leading to neurofibrillary tangle formation. Vascular-mediated neuronal dysfunction may be particularly relevant for very-old adults (i.e., those over age 80) given that arterial stiffness and vascular disorders are more common and more severe in this age group (De Leeuw et al., 2001).

Evidence from neuropathologic studies suggest that the presence of cerebrovascular disease (CVD) may lower the threshold of AD pathology accumulation necessary to clinically unmask dementia (Chui et al., 2012). AD patients with comorbid CVD show less AD pathology than AD patients without CVD (Esiri et al., 1999), even when patients with and without CVD are identical in terms of level of dementia severity (Bangen et al., in press).

The association between AD pathology and dementia is attenuated among very-old adults (Prohovnik et al., 2006; Savva et al., 2009) and neuropathologic studies show a greater prevalence of mixed pathology with both AD and vascular features along with fewer cases of "pure" AD pathology among the oldest-old (Jellinger and Attems, 2010). Taken together, these studies suggest that vascular dysfunction is an important mechanism leading to cognitive impairment (Pantoni, 2010), and may play an even more prominent role among very-old adults.

Arterial spin labeling (ASL) MRI has been employed to reliably measure CBF across the aging-MCI-AD continuum (Johnson et al., 2005; Restom et al., 2007; Bangen et al., 2009). Abnormal resting state CBF may be an early indicator of brain dysfunction in individuals at risk for developing dementia (Fleisher et al., 2009; Bangen et al., 2012; Wierenga et al., 2012). ASL studies of dementia and at-risk populations demonstrate patterns of regional hypoperfusion similar to those revealed by positron emission tomography (PET) and single photon emission computed tomography (SPECT) (Detre and Alsop, 1999; Alsop et al., 2000). Advantages of ASL over PET and SPECT include the use of an endogenous tracer (rather than an intravenously administered contrast agent), relatively brief scan times (typically 5–10 min), and the ability to provide dynamic CBF estimates (with a temporal resolution on the order of seconds) due to the rapid decay of the tracer (Johnson et al., 2005). Given these factors, ASL MRI may provide a sensitive technique for identifying at-risk individuals, monitoring changes in neural activity due to developing neuropathology, and assessing effectiveness of disease-modifying treatments.

Although associations have been consistently found between aging and hypoperfusion and between vascular risk factors and hypoperfusion (Grolimund and Seiler, 1988; Bangen et al., 2009; Muller et al., 2012), the *interaction* between advanced age and vascular risk burden on CBF remains unclear. Furthermore, most studies have focused on individual vascular risk factors, however, multiple vascular risk factors often co-exist (Genest and Cohn, 1995) and have been shown to incrementally increase risk for AD (Luchsinger et al., 2005; Whitmer et al., 2005). Studies often examine individual risk factors while adjusting for additional risk factors, but this approach may lead to over-adjustment and underestimation of effects (Szklo and Nieto, 2000; Luchsinger et al., 2005). Our previous research demonstrated that aggregate vascular risk in particular was associated with CVD in a sample of autopsy-confirmed AD patients, highlighting the potential importance of aggregate risk in the vascular contribution to cognitive impairment (Bangen et al., in press).

Therefore, the present study aimed to elucidate the relationships among elevated vascular risk burden, age, CBF, and cognition. We predicted that the presence of elevated vascular risk burden (i.e., multiple vascular risk factors) would interact with advancing age to result in reduced CBF. CBF was measured in regions of interest (ROIs) that were selected based on previous results suggesting their vulnerability to small vessel disease and association with AD and aging. We further predicted that reduced CBF in specific ROIs would correlate with poorer neuropsychological performance in associated cognitive domains [e.g., reduced CBF in medial temporal (MTL) regions would be associated with poorer memory]. Finally, we predicted that, those older adults with elevated vascular risk burden would be particularly vulnerable to cognitive change as a function of CBF reduction. Such findings may improve detection of individuals at risk for cognitive impairment and may help inform the development of treatments designed to slow or prevent cognitive decline.

MATERIALS AND METHODS

PARTICIPANTS

Seventy-one independently living, non-demented older adults were recruited from the San Diego community and ongoing studies at the University of California San Diego (UCSD) Shiley-Marcos Alzheimer's Disease Research Center (ADRC). Potential participants were excluded if they were younger than 65 years of age; had dementia identified by medical, neurological, and neuropsychological examinations; or had a history of neurologic disease, head injury with loss of consciousness, learning disability, or major psychiatric disorder. Of the 71 participants, two had three vascular risk factors (hypertension, diabetes, cardiovascular disease, atrial fibrillation, history of transient ischemic attack [TIA]/minor stroke, or current smoking), 14 had two vascular risk factors, 33 had one vascular risk factor and 22 had none. For analytical purposes, those with multiple (i.e., two or three) vascular risk factors were collapsed into one category and compared to those with no or one vascular risk factor. Thus, we compared higher and lower vascular risk burden groups. All data were collected in accordance with UCSD and VA San Diego Healthcare System institutional review board-approved procedures and within the guidelines of the Helsinki Declaration. All participants provided written informed consent prior to enrollment.

CLINICAL AND NEUROPSYCHOLOGICAL ASSESSMENT

All participants underwent a semi-structured interview regarding medical and psychiatric history; neurological examination; assessment of functional abilities; physical examination with brachial artery blood pressure measurement using an automated blood pressure cuff; neuropsychological testing; buccal swab DNA extraction for APOE genotyping; and brain MRI. The presence or absence of vascular risk factors derived from the Framingham Stroke Risk Profile (D'Agostino et al., 1994) was determined by self-report, medical chart review, and physical examination. Targeted vascular risk factors included: (1) hypertension; (2) diabetes; (3) history of cardiovascular disease (e.g., coronary artery disease (myocardial infarction, angina pectoris, coronary insufficiency), intermittent claudication, cardiac failure); (4) atrial fibrillation; (5) TIA or minor stroke; and (6) current smoking. Hypertension was defined as systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg, or use of antihypertensive medications. Each vascular risk factor was assigned a value of 0 if absent and 1 if present. Global cognition was assessed by the Dementia Rating Scale (DRS) (Mattis, 1988), episodic memory was assessed by the California Verbal Learning Test-Second Edition (CVLT-II) (Delis et al., 2000), and executive functioning was assessed by Part B of the Trail Making Test (one participant in the high vascular risk group did not complete the Trail Making

Test). APOE genotype was determined using a polymerase chain reaction-based method (Saunders et al., 1993).

MRI ACQUISITION

Participants were scanned on a GE Signa HDx 3.0 Tesla whole body MR scanner using an 8-channel receive-only head coil (General Electric Medical Systems, Milwaukee, WI, USA). A T1-weighted anatomical scan was acquired at 1 mm^3 resolution using either a 3D MPRAGE sequence (26 cm FOV, 256 × 256 matrix, $TR = 7$ ms, $TE = 3$ ms, flip angle = 8°, inversion time = 900 ms, bandwidth = 31.25 kHz, and 170 1.2 mm sagittal slices) or a 3D FSPGR sequence (identical parameters to MPRAGE except 25 cm FOV, 256 × 192 matrix, $TR = 8.1$ ms, inversion time = 600 ms, 172 1 mm sagittal slices). T2-weighted fluid attenuated inversion recovery (FLAIR) images (20 cm FOV, 256 × 256 matrix, flip angle = 90°, $TE = 142$ ms, $TR = 10000$ ms, 5 mm axial slices with no interslice gap) were acquired for a subset of 41 participants (35 with low and 6 with high vascular risk burden). A resting state pulsed ASL scan was acquired using a modified flow-sensitive alternating inversion recovery sequence (Kim, 1995) [post-saturation and inversion times of TI1 = 600 ms and TI2 = 1600 ms, $TR = 2500$ ms, $TE = 3.2$ ms FOV = 22 × 22 cm, 64 × 64 matrix, 20 5 mm axial slices, 40 volumes (20 tag+control image pairs)]. This sequence utilized presaturation pulses and PICORE QUIPSS 2 post-inversion saturating pulses and a spiral read out with four interleaves (Wong et al., 1998). A scan with the inversion pulses turned off was acquired to obtain an estimate of the magnetization of cerebrospinal fluid (CSF). The CSF signal was used to estimate the equilibrium magnetization of blood, which was used to convert the perfusion signal into calibrated CBF units (millimeters of blood per 100 g of tissue per minute) (Chalela et al., 2000). In addition, a minimum contrast scan was acquired to adjust for coil inhomogeneities during the CBF quantification step (Wang et al., 2005).

MRI DATA PROCESSING

MRI data were processed using Analysis of Functional NeuroImages (AFNI) (Cox, 1996), FMRIB Software Library (FSL) (Smith et al., 2004), and locally created MATLAB scripts.

T1-weighted anatomical images

Following N3 bias correction of field inhomogeneities, structural scans were skull-stripped using Brain Surface Extractor (Version 3.3) (Shattuck et al., 2001), an approach shown to be very effective when working with the images of older adults (Fennema-Notestine et al., 2006). Scans were manually edited when necessary to remove any residual non-brain material remaining after the automated skull stripping. Whole brain images were then segmented into gray matter (GM), white matter (WM), and CSF compartments using FSL's FMRIB's Automated Segmentation Tool (FAST) (Zhang et al., 2001). High-resolution anatomical images and partial volume segmentations were registered to ASL space and down-sampled to the resolution of the ASL image using AFNI.

T2-weighted FLAIR images

Although CBF is of primary interest, we assessed white matter lesions (WML) given evidence linking them to advanced aging and vascular risk (Raz et al., 2012). For quantification of WML volume, we applied a semiautomated volumetric approach to T2-FLAIR images using a reliable, previously published method (Delano-Wood et al., 2008). This type of semi-automated volumetric approach is a methodology shown to be the most reliable approach for the analysis of WML when compared to other image types and traditional quantitative visual rating scales (Price et al., 2005). Briefly, using AFNI, circumscribed areas of increased signal intensity within the WM were manually traced in 17–21 image slices per participant in the axial plane. WML volume was quantified as the total number of voxels (mm^3) of these hyperintense regions.

ASL images

Each ASL dataset was reconstructed using the SENSE algorithm (Pruessmann et al., 1999). To minimize effects of participant motion, the ASL time series was co-registered to the middle timepoint. A mean ASL image was formed for each participant from the average difference of control and tag images using surround subtraction. Slice timing delays were accounted for to ensure the inversion time (TI2) was slice specific (Liu and Wong, 2005). The mean ASL image was converted to absolute units of CBF (milliliters per 100 g of tissue per minute) using the CSF image as a reference signal (Chalela et al., 2000). To correct CBF data partial volume effects and minimize the effects on the CBF estimates of the lower perfusion in WM and the lack of perfusion in CSF, we used a previously published method that assumes that CSF has zero CBF and that CBF in GM is 2.5 times greater than in WM (Johnson et al., 2005). Partial-volume-corrected CBF signal intensities were calculated using the following formula: $CBF_{corr} = CBF_{uncorr}/(GM + 0.4 * WM)$. CBF_{corr} and CBF_{uncorr} are corrected and uncorrected CBF values, respectively. GM and WM are GM and WM partial volume fractions, respectively, and were computed based on the tissue content of each perfusion voxel as determined by FSL's FAST program. The CBF_{corr} data were spatially smoothed to a resolution of 4 mm full-width at half-maximum. CBF voxels with negative intensities were replaced with zero (Brown et al., 2003). In addition, we used a conservative threshold that removed CBF values outside of the expected physiological range of CBF (below 10 or greater than 150) from analyses. CBF data were normalized to the atlas of Talairach and Tournoux (1988) and re-sampled at a 4 mm^3 resolution.

STATISTICAL ANALYSES

Demographic and clinical data

Independent samples *t*-tests for continuous variables and χ^2 tests for categorical variables were used to compare the participant groups on demographic and clinical variables.

Neuroimaging data

ROI analyses for the CBF data included two subcortical and four cortical regions defined in AFNI using the Talairach atlas. The two subcortical ROIs, caudate and thalamus, were selected because of their vulnerability to small vessel disease. The four cortical ROIs were MTL (hippocampus, parahippocampal gyrus, uncus), inferior parietal (supramarginal gyrus, inferior parietal lobule, angular gyrus), posteromedial (posterior cingulate, precuneus, cuneus), and frontal (anterior cingulate, middle frontal

gyrus, medial frontal gyrus) cortices. These four cortical regions were selected because MTL, inferior parietal, and posteromedial cortices have been implicated in early AD, and frontal lobe has been associated with aging and elevated vascular risk. The average quantified CBF corrected for partial volume effects was extracted for each of the six ROIs. Hierarchical multiple regression analyses were performed to investigate the interaction between age and vascular risk burden (high and low) in each ROI. All models included sex and APOE ε4 status (ε4 carrier or non-carrier) as covariates in the first block, main effects of age and vascular risk burden in the second block, and age by vascular risk burden interaction terms in the third block.

Associations between cognitive performance and CBF in ROIs with significant interactions between age and vascular risk burden were examined for each subgroup (high and low vascular risk burden) with bivariate correlations. Performance on the CVLT-II (List A Trials 1–5 raw score) was correlated with CBF in MTL and posteromedial ROIs given the role of these regions in episodic memory. Performance on Part B of the Trail-Making Test (total time raw score) was correlated with CBF in frontal and parietal ROIs given the role of these regions in executive functioning and visual attention/spatial cognition/visuomotor integration. Significance levels of 0.05 were used for all tests. All analyses were performed using SPSS (version 18.0).

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Participants with low and high vascular risk burden did not significantly differ in terms of mean age, years of education, sex distribution, global cognitive functioning as assessed by the DRS, depressive symptomatology, or APOE genotype (all p-values > 0.05; **Table 1**). As expected, the group with high vascular risk burden had significantly greater frequencies of many of the vascular risk factors including hypertension, diabetes, cardiovascular disease, and history of TIA or clinical stroke. In addition, compared to those with low vascular risk burden, they had significantly reduced diastolic blood pressure, which may result from atherosclerosis and arterial stiffening (Qiu et al., 2005), and they were also more likely to be taking anti-hypertensive medication (see **Table 1**).

CEREBRAL BLOOD FLOW: INTERACTION OF VASCULAR RISK BURDEN AND AGE

Figure 1 displays the average quantified CBF (corrected for partial volume effects) in each of the four cortical ROIs as a function of age and vascular risk burden. Multiple regression analyses showed that, after adjusting for sex and APOE ε4 status, there were significant interactions between age and vascular risk burden for the MTL ($R^2 = 0.07$, $B = -0.86$, $p = 0.02$), inferior parietal ($R^2 = 0.07$, $B = -1.05$, $p = 0.02$), and frontal ROIs ($R^2 = 0.11$, $B = -0.66$, $p = 0.002$) and a trend toward a significant interaction for the posteromedial ROI ($R^2 = 0.03$, $B = -0.63$, $p = 0.12$). As illustrated in **Figure 1**, the interaction effects were characterized by a negative relationship between age and CBF in the high vascular risk burden group but no such relationship in the low vascular risk burden group. Follow up bivariate correlational analyses were conducted for those with low and high vascular risk burden separately. These follow up analyses demonstrated that, among those with high vascular risk burden, aging was associated with significantly reduced CBF or trends toward significantly reduced CBF across all four ROIs (MTL CBF: $r = -0.49$, $p = 0.06$; Posteromedial CBF: $r = -0.46$, $p = 0.08$; Inferior parietal

Table 1 | Demographic and clinical characteristics of participants.

	Low vascular risk Mean (*SD*)	High vascular risk Mean (*SD*)	*t* or χ^2	*P*
N	55	16		
Age (years)	74.73 (7.92)	75.94 (7.35)	0.55	0.59
Education (years)	16.31 (2.41)	15.13 (2.03)	1.79	0.08
Women/men (% Women)	33/22 (60.0%)	8/8 (50.0%)	0.51	0.48
APOE ε4 carrier/non-carrier (% ε4 carrier)*	16/37 (29.1%)	9/7 (56.3%)	3.61	0.06
DRS total score	139.82 (3.94)	139.56 (4.50)	0.22	0.83
CVLT-II trials 1–5 total (*T*-score)	46.43 (11.87)	46.13 (11.74)	0.43	0.67
Trails B (s)	80.57 (29.81)	78.67 (34.19)	0.21	0.83
Geriatric depression scale	3.40 (3.82)	4.27 (3.54)	0.79	0.43
Systolic blood pressure	129.16 (16.21)	130.06 (12.45)	0.21	0.84
Diastolic blood pressure	77.05 (9.30)	71.31 (8.26)	2.23	0.03
Use of antihypertensive medications, *n* (%)	23 (41.8%)	14 (87.5%)	10.39	0.006
Hypertension, *n* (%)	27 (49.1%)	15 (93.8%)	10.23	0.001
Diabetes, *n* (%)	1 (1.8%)	4 (25%)	10.18	0.001
Cardiovascular disease, *n* (%)	0	7 (43.8%)	26.69	<0.001
History of atrial fibrillation, *n* (%)	3 (5.5%)	3 (18.8%)	2.83	0.09
Current smoker, *n* (%)	2 (3.6%)	0	0.60	0.44
History of TIA or stroke, *n* (%)	0	5 (31.3%)	18.49	<0.001

SD, standard deviation; APOE, apolipoprotein E; DRS, Mattis Dementia Rating Scale; CVLT-II, California Verbal Learning Test-Second Edition.

**Two participants with low vascular risk factors burden were missing APOE genotype data.*

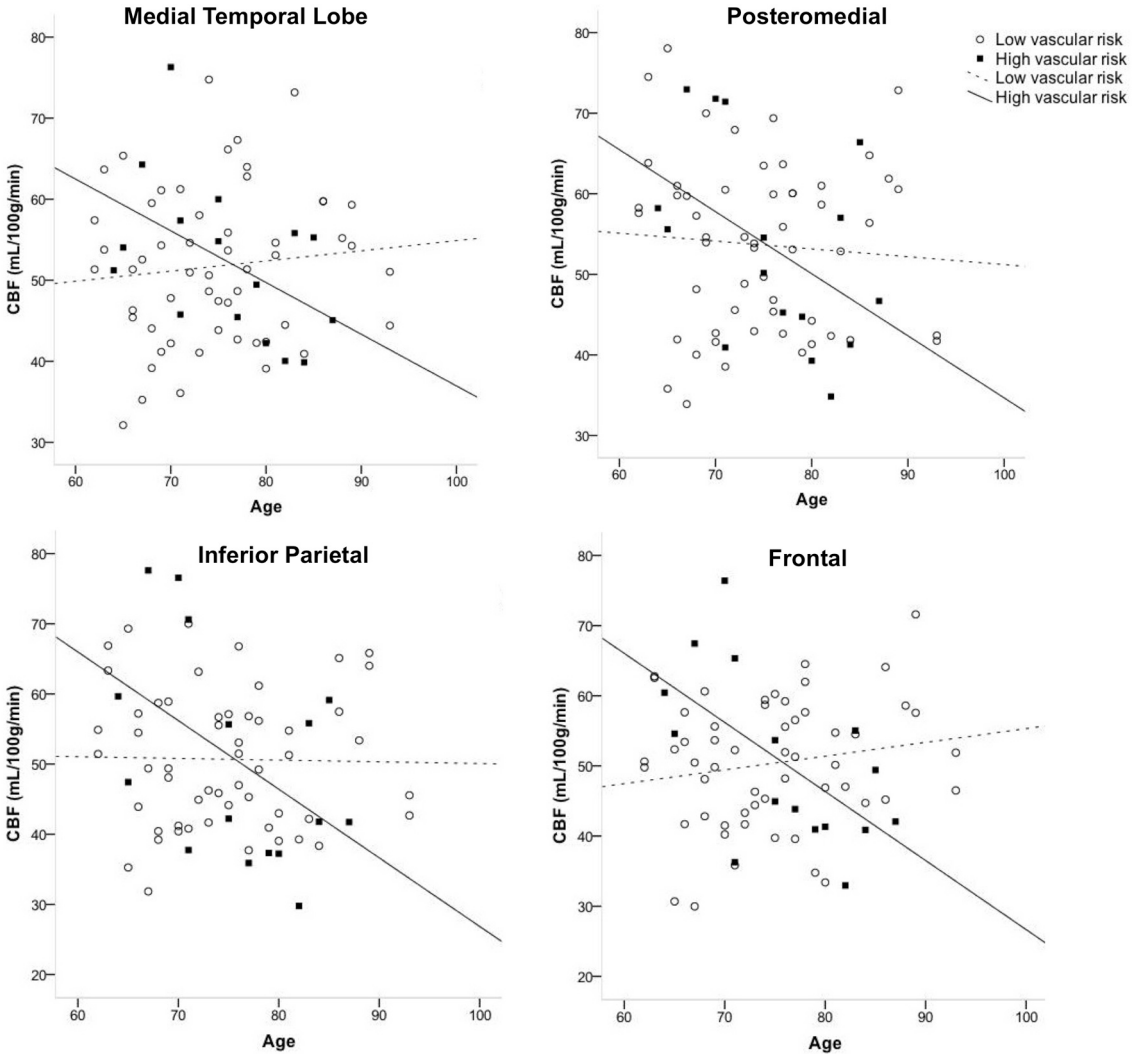

FIGURE 1 | Interaction of age and vascular risk burden on cerebral blood flow (CBF) for four *a priori* cortical regions of interest. Interactions were statistically significant for the medial temporal, inferior parietal, and frontal regions of interest (p-values < 0.05) and there was a trend toward an interaction for posteromedial CBF ($p = 0.12$). High vascular risk indicates the presence of two or more vascular risk factors whereas low vascular risk indicates the presence of no or one vascular risk factor.

CBF: $r = -0.48$, $p = 0.06$; Frontal CBF: $r = -0.59$, $p = 0.01$). In contrast, among those with low vascular risk burden, there were no significant associations or trends toward significant associations between age and CBF (MTL CBF: $r = 0.10$, $p = 0.45$; Posteromedial CBF: $r = -0.07$, $p = 0.60$; Inferior parietal CBF: $r = -0.02$, $p = 0.89$; Frontal CBF: $r = 0.17$, $p = 0.21$). There were no significant interactions between age group and vascular risk status for the two subcortical regions: thalamus ($R^2 = 0.02$, $B = -0.75$, $p = 0.18$) and caudate ($R^2 = 0.008$, $B = -0.28$, $p = 0.42$). There were no main effects of age or vascular risk status on CBF across any of the six ROIs (p-values > 0.05 see **Table 2**).

In the subset of participants with T2-FLAIR imaging, hierarchical regression models adjusting for sex and APOE ε4 status showed that there was no interaction between age and vascular risk burden on total WML volume ($R^2 = 0.005$, $B = -139.19$, $p = 0.54$). Furthermore, there was no main effect of vascular risk burden on total WML volume ($B = -1211.40$, $p = 0.48$). There was, however, a main effect of age, with advancing age associated with greater total WML volume ($\beta = 0.32$, $p = 0.02$). WML volume was not significantly correlated with CBF in any ROI (p-values > 0.05).

ASSOCIATION BETWEEN CBF AND COGNITION

As shown in **Figure 2**, among those with high vascular risk burden, reduced CBF in inferior parietal ($r = -0.77$, $p < 0.001$) and frontal ($r = -0.72$, $p = 0.001$) ROIs was associated with poorer performance on an executive functioning measure requiring visual attention, spatial perception, and visuomotor integration. Also, among this group, there was a trend toward reduced CBF in MTL being associated with poorer memory performance ($r = 0.31$, $p = 0.13$). Among those with low vascular risk

Table 2 | Main and interaction effects of age and vascular risk burden on CBF.

Block	Variable	Medial temporal CBF		Inferior parietal CBF		Posteromedial CBF		Frontal CBF		Thalamus CBF		Caudate CBF	
		ΔR^2	*B (SE)*	ΔR^2	*B (SE)*	ΔR^2	*B (SE)*	ΔR^2	*B (SE)*	ΔR^2	*B (SE)*	ΔR^2	*B (SE)*
1	Sex	0.06	7.41 (3.80)	0.10*	12.30** (4.68)	0.16**	14.53*** (4.04)	0.18***	8.44*** (2.22)	0.19**	22.68*** (5.74)	0.19***	12.68 (3.5)***
	APOE ε4 status		2.00 (3.96)		4.54 (4.88)		0.86 (4.2)		−0.59 (2.3)		5.68 (5.99)		6.42 (3.65)
2	Age	0.01	0.16 (0.25)	<0.001	−0.05 (0.31)	0.003	−0.12 (0.27)	0.003	0.03 (0.15)	0.03	−0.57 (0.37)	0.04	−0.22 (0.27)
	Vascular risk		2.34 (4.68)		0.46 (5.79)		0.67 (4.50)		1.16 (2.74)		4.78 (6.97)		6.83 (4.23)
3	Age × vascular risk	0.07*	−0.86 (0.37)	0.07*	−1.05* (0.45)	0.03	−0.63 (0.40)	0.11**	−0.66 (0.21)**	0.02	−0.75 (0.56)	0.008	−0.28 (0.34)

*$p < 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.
B, unstandardized coefficient estimate.

burden, although there was a trend toward a significant association between inferior parietal CBF and Trails B performance ($r = 0.19$, $p = 0.09$), there were no significant relationships between cognitive performance and CBF in this group (frontal CBF and Trails B: $r = 0.10$, $p = 0.24$; MTL CBF and CVLT: $r = -0.13$, $p = 0.17$).

DISCUSSION

The present study extends previous cerebral perfusion studies of dementia risk by demonstrating an interaction between advancing age and vascular risk burden on CBF. We found that, among those with elevated vascular risk burden, advancing age was associated with reduced CBF, whereas for the low vascular risk burden group, there was no such relationship.

This pattern was observed for cortical ROI that have been implicated in aging and AD, namely, MTL, inferior parietal, and frontal cortices. In addition, reduced CBF was associated with poorer cognitive performance in participants with elevated vascular risk burden, and this relationship was not seen in participants with low vascular risk burden, suggesting that older adults with multiple vascular risk factors may be particularly vulnerable to cognitive change as a function of CBF reduction.

Regional decreases in CBF have often been interpreted as a reflection of decreased brain function whereas increases in perfusion have frequently been interpreted as a cellular and vascular compensatory response to pathologic changes (Dai et al., 2009; Bangen et al., 2012; Wierenga et al., 2012). It has been suggested that advancing age increases risk for dementia via its tendency to reduce CBF (De La Torre, 2012a,b). Studies have reported that aging may account for an approximately 0.45–0.50% reduction in CBF per year (Leenders et al., 1990; Parkes et al., 2004). Therefore, the presence of vascular risk factors may add to the already diminished CBF that results from aging and these two burdens on CBF could decrease neuronal energy thereby leading to cognitive decline (De La Torre, 2012a). The findings of the present study suggest that the presence of multiple vascular risk factors and advanced age interact to impart more detrimental effects on CBF than either one alone.

Among those with high vascular risk burden, younger age was associated with higher CBF. In addition, greater CBF in frontal and posterior regions was associated with better executive function among those with elevated vascular risk. Taken together, these findings raise the possibility that elevated CBF may represent a compensatory mechanism in this group. It is possible that older adults with multiple vascular risk factors are able to invoke compensatory mechanisms at younger ages. However, as they age, they may have less capacity for compensation given that their brain perfusion may be already reduced due to advanced age. Burgeoning evidence suggests that cerebral autoregulation is unlikely to reverse brain hypoperfusion if cardiac output is compromised (De La Torre, 2012a). Therefore, the presence of cardiovascular pathology in older adults may have long-term effects on CBF because it may limit neuronal responses from being able to maintain normal regulatory and/or compensatory capabilities.

The present findings are consistent with our previous reports suggesting that multiple dementia risk factors interact to reduce

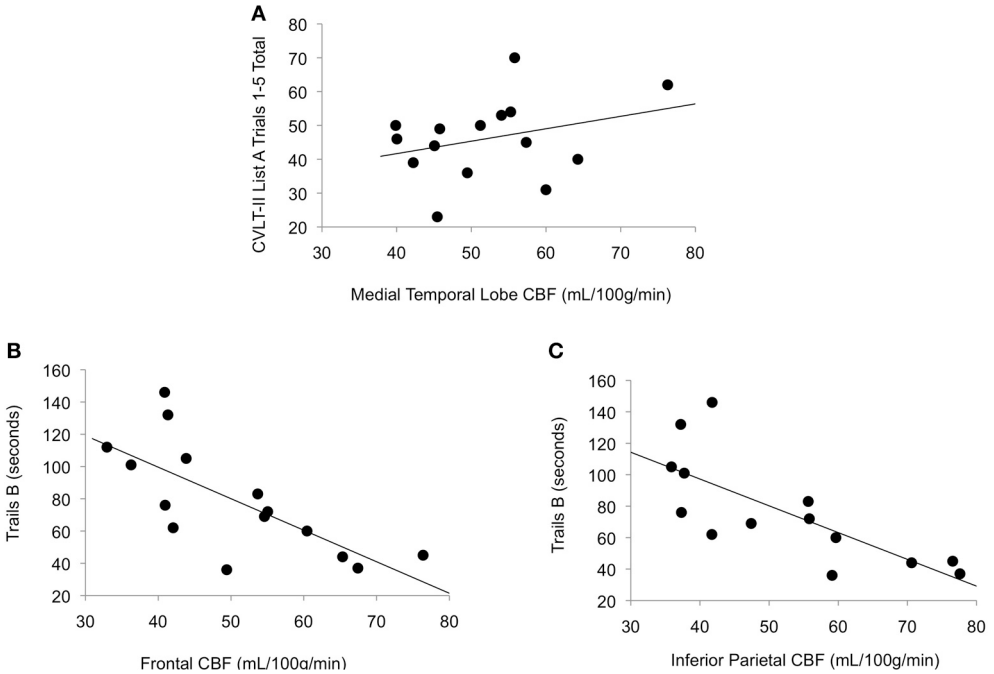

FIGURE 2 | Scatterplots of correlations between cerebral blood flow and cognitive performance for older adults with elevated vascular risk burden. (A) Correlation between memory performance and medial temporal lobe cerebral blood flow ($p = 0.13$), **(B)** Correlation between executive function performance and frontal cerebral blood flow ($p = 0.001$), and **(C)** Correlation between executive function performance and inferior parietal cerebral blood flow ($p < 0.001$). Higher scores on Trails B represent poorer (slower) performance.

CBF in regions vulnerable to early AD and aging (Wierenga et al., 2012) and may be valuable for characterizing changes in structure-function relationships in individuals at risk. We observed interactions between age and vascular risk burden on CBF in cortical regions implicated in early AD or aging (Braak and Braak, 1991, 1996; Raz et al., 1997; McDonald et al., 2009), but no interactions in subcortical regions. Notably, amyloid burden has been shown to increase over time in non-demented older adults in the posterior cingulate, frontal, parietal, and temporal cortical regions in which we observed interactions with CBF (Rowe et al., 2007; Aizenstein et al., 2008; Villemagne et al., 2008). Although conflicting reports exist regarding whether vascular risk factors directly increase AD pathology (Chui et al., 2012), some evidence links vascular dysfunction to the development of AD pathology (Altman and Rutledge, 2010) and to the accumulation of amyloid in particular (Craft, 2009; Zlokovic, 2011; Reed et al., 2012). Indeed, the "two-hit vascular hypothesis" of AD (Zlokovic, 2011) suggests that the presence of vascular risk factors initiates a cascade of events involving BBB dysfunction, hypoperfusion, and impaired clearance and accumulation of amyloid, thereby leading to dementia. Given that we did not collect markers of amyloid deposition or BBB integrity as part of this study, we cannot directly examine these mechanisms. However, regardless of the precise mechanism, the present findings suggest that, as non-demented older adults age, the presence of multiple vascular risk factors may influence neuronal function within areas that are vulnerable to early AD (Braskie et al., 2010; Bangen et al., 2012; Beason-Held et al., 2012).

In contrast to some previously published studies (Beason-Held et al., 2007; Bangen et al., 2009), we did not observe main effects of age or vascular risk factors on CBF (cf., Glodzik et al., 2011). However, these previous studies often focused on longitudinal change rather than cross-sectional group differences in CBF (Beason-Held et al., 2007, 2012), and compared young and old adults (Bangen et al., 2009; Wierenga et al., 2013) rather than the two groups of older adults included in the present study. WML volume was not significantly correlated with CBF in the current sample even though it is thought that hypoperfusion may lead to the development of WML (Zlokovic, 2011). Furthermore, there was no interaction between age and vascular risk on total WML volume. Given that hypoperfusion and the development of WML may be on a continuum, it is possible that older adults with multiple vascular risk factors who are demonstrating reduced CBF have not yet developed increased WML volume but may do so over time as chronic hypoperfusion worsens.

Several limitations should be considered when interpreting the present findings and should be addressed in future studies. As is commonly the case in neuroimaging studies, our sample size was relatively small which may have attenuated our ability to detect some group differences. It should be noted, however, that significant interactions between age and vascular risk burden on CBF were found even in this small sample with relatively low vascular risk burden. In addition, our sample was relatively well educated and medically healthy which may reduce the generalizability of the present results. Vascular risk factors in this study were categorized dichotomously as being either present or absent and

vascular risk burden was characterized as high or low, rather than considering vascular risk factors as continuous variables based on risk factor severity. Further, given the cross-sectional nature of the study, causality of the relationships among aging, vascular risk, and CBF cannot be inferred. We identified hypertension based in part on the use of anti-hypertensive medications, however, it is unclear whether use of anti-hypertensive medications imparts risk or is protective (Beishon et al., 2014). Limitations of ASL MRI techniques more generally include relatively low signal-to-noise ratios and reliance on assumptions in the perfusion quantification (e.g., transit delay). The use of partial volume corrected quantitative ASL data was a strength of the study. Despite these limitations, the present study is one of the only attempts to assess the interaction between vascular risk burden and advancing age on ASL measures of CBF and cognition, making these findings a potentially useful step toward elucidating the influence of vascular risk factors on CBF abnormalities among non-demented older adults.

The present results add to a growing body of evidence demonstrating the possible influence of vascular risk burden on functional brain changes (CBF abnormalities) that may increase risk of cognitive impairment and dementia (Tzourio et al., 2001; Bangen et al., 2012). Among older adults with multiple vascular risk factors, we found association between advancing age and reduction in CBF in cortical regions implicated in early AD. Evidence suggests that cognitive and brain changes observed in very-old AD patients are less salient than those seen in younger AD patients (Stricker et al., 2011). Thus, the inclusion of additional markers may improve our ability to predict progression to dementia in very-old adults (Stricker et al., 2011). The present findings provide support for ASL MRI as a candidate biomarker for detecting changes in the central nervous system associated with vascular risk factors, particularly in older adults with elevated vascular risk burden. Finally, the present results highlight the potential utility of interventions designed to treat reduced CBF in older adults who present with vascular risk factors. Given that many vascular risk factors such as diabetes and smoking can be treated, interventions designed to target vascular risk factors in order to maintain CBF may represent important opportunities for preventing or delaying the onset of cognitive impairment and dementia (Gorelick et al., 2011; Qiu, 2012).

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Bone mineral density, adiposity, and cognitive functions

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Cognitive decline and dementia due to Alzheimer's disease (AD) have been associated with genetic, lifestyle, and environmental factors. A number of potentially modifiable risk factors should be taken into account when preventive or ameliorative interventions targeting dementia and its preclinical stages are investigated. Bone mineral density (BMD) and body composition are two such potentially modifiable risk factors, and their association with cognitive decline was investigated in this study. 164 participants, aged 34–87 years old (62.78 ± 9.27), were recruited for this longitudinal study and underwent cognitive and clinical examinations at baseline and after 3 years. Blood samples were collected for apolipoprotein E (APOE) genotyping and dual energy x-ray absorptiometry (DXA) was conducted at the same day as cognitive assessment. Using hierarchical regression analysis, we found that BMD and lean body mass, as measured using DXA were significant predictors of episodic memory. Age, gender, APOE status, and premorbid IQ were controlled for. Specifically, the List A learning from California Verbal Learning Test was significantly associated with BMD and lean mass both at baseline and at follow up assessment. Our findings indicate that there is a significant association between BMD and lean body mass and episodic verbal learning. While the involvement of modifiable lifestyle factors in human cognitive function has been examined in different studies, there is a need for further research to understand the potential underlying mechanisms.

Keywords: dual energy x-ray absorptiometry, cognition, apolipoprotein E, bone mineral density, episodic verbal memory, executive function, aging

INTRODUCTION

Dementia is a major debilitating disorder and a cause of significant concern for the currently aging population. In 2010, more than 35.6 million individuals were diagnosed with dementia worldwide (Prince et al., 2013). Prevalence projections indicate that dementia cases will dramatically increase worldwide by 2050 (Norton et al., 2014). In particular, a report from the Australian Institute of Health and Welfare estimates that the number of dementia patients in Australia will increase from 175,000 to 465,000 by the year 2031 (Australian Institute of Health and Welfare, 2007). Preventative research to reduce dementia-related burden is essential to tackle the financial as well as social consequences of this condition. Based on recent modeling reported by Alzheimer's Australia, if the onset of dementia was delayed by 2 years, a reduction of 13% or 398,000 cumulative new cases by 2050 would be achieved. Further, a delay of 5 years would reduce the number of cumulative new cases by 30%, or 935,000 individuals by 2050. Dementia-prevention programs would have a significant economic impact and improve the quality of life for affected individuals and their family (Vickland et al., 2012). Identification of potentially modifiable risk factors, including lifestyle factors,

is a promising avenue for facilitating reductions in dementia incidence.

Dementia due to Alzheimer's disease (AD) is the most common form of dementia worldwide (Di Carlo et al., 2012). While the underlying causes of the late-onset form of the disease remain poorly understood, a complex mix of genetic, lifestyle, and hormonal factors is thought to contribute to the cerebral accumulation of a small peptide, beta amyloid (Aβ) (Butterfield et al., 2002; Isacson et al., 2002; Verdile et al., 2004; Wirths et al., 2004), resulting in the formation of extracellular amyloid deposits (Glenner and Wong, 1984; Masters et al., 1985). Research suggests that one third of AD cases are preventable (Norton et al., 2014). Because lifestyle and hormonal factors are potentially modifiable risk factors for AD, they remain a focus of intense research scrutiny. One such hormone-related risk-factor is osteoporosis, which is defined as bone mineral density (BMD) more than 2.5 standard deviations below the mean for healthy adults aged between 20 and 40 years (W.H.O, 1994). The prevalence of osteoporosis increases with age. According to Osteoporosis Australia, 1 in 2 women and 1 in 3 men over the age of 60 will experience an osteoporotic fracture. In addition to age, female sex and menopause-related changes, previous fragility, previous fragility fractures, family history of hip fracture, and the use of oral corticosteroids are also significant risk factors for low BMD (Kanis, 2002; Finkelstein et al., 2008).

Osteoporosis and low BMD (osteopenia) have been associated with cognitive impairment and dementia (Lui et al., 2003; Rothman et al., 2007). BMD is regulated through the brain (Haberland et al., 2001; Karsenty and Oury, 2010), and this may partially explain the underlying relationship between BMD, cognitive dysfunction, and dementia. The brain regions involved in adiposity, (specifically the hypothalamus), also regulate bone remodeling through complicated and slow processes involving hormones including leptin (Haberland et al., 2001; Crockett et al., 2011). Leptin is thought to mediate BMD via binding to relevant receptors in the ventromedial hypothalamus (Haberland et al., 2001; Yang and Barouch, 2007) suggesting that osteoporosis may represent a neuro-skeletal condition (Takeda, 2009). Of note, plasma leptin level has been negatively associated with dementia and AD risk (Lieb et al., 2009). Additionally, the relationship between lower BMD and dementia may be modulated through cumulative exposure to estrogen as it was found in the Framingham Study, that lower femoral neck BMD was associated with a two-fold increase in risk of AD in women, potentially due to estrogen exposure (Tan et al., 2005).

Adiposity or body fat is another potentially modifiable risk factor associated with cognitive decline and dementia; however, research has produced somewhat conflicting results in this area. While most studies have supported a significant association between adiposity and cognitive decline (Luchsinger et al., 2007; Kerwin et al., 2011) other studies have failed to identify a significant relationship between these two on some of the cognitive functions associated with AD, including verbal memory (Wolf et al., 2007). In some studies, adiposity has been associated with cognitive decline only in men (Kanaya et al., 2009), in individuals above 70 years old (Levine and Crimmins, 2012), or in participants below age 70 (Yoon et al., 2012). Adiposity is a risk factor for diabetes, hypertension, and cardiovascular changes; conditions which themselves contribute to significantly increased risk of AD (for a review see: Gustafson and Luchsinger, 2013) and cognitive decline due to vascular pathologies (Gustafson, 2012). For example, it has been suggested that adiposity, as a risk factor for insulin resistance and hyperinsulinemia may increase amyloid deposits in the brain resulting in AD (Luchsinger and Mayeux, 2007). In sum, the available evidence suggests that midlife central obesity plays a significant role in age-related cognitive decline and significantly increases the risk of dementia (Whitmer et al., 2008).

It is important to note that both increased adiposity and osteoporosis have been associated with cardiovascular disease (CVD) (Banks et al., 1994), which is associated with AD-plasma amyloid-β protein (Bates et al., 2009) and has been shown to increase the risk of cognitive decline and dementia (Qiu et al., 2010; Norton et al., 2014). Interestingly, subclinical CVD increases the risk of bone loss and fracture (den Uyl et al., 2011) and BMD has been inversely associated with CVD (Farhat et al., 2007). Further, cardiovascular problems are associated with osteoporosis; moreover, lipid-related problems may play a role in increasing osteoporosis risk (Brown and Sharpless, 2004). Animal models support the association between osteoporosis and atherosclerosis (Parhami et al., 2000; Price et al., 2001). Observational studies have shown that higher atherogenic lipid profile and lipoproteins are inversely associated with bone density (Dimic et al., 2012; Sarkis et al., 2012) but the exact mechanisms underlying this relationship are unclear (Farhat and Cauley, 2008).

Cholesterol metabolism has been linked to apolipoprotein E epsilon 4 allele (APOE ε4), a major genetic risk factor for late-onset AD (Corder et al., 1993; Saunders et al., 1993; Roses, 1997). The ApoE protein is the major cholesterol transport protein in the brain, with allelic polymorphism in the APOE gene resulting in isoform-specific functional effects (e.g., higher risk of AD for ε4 carriers and more resistance to AD in ε2 carriers) (Weisgraber, 1994; Mahley et al., 1996). Some studies have indicated that, in addition to increased risk of AD, APOE can also be involved in osteoporosis through mediating vitamin K transportation and/or inhibition of osteoblast differentiation (Kohlmeier et al., 1996; Parhami et al., 1997). However, a more recent study did not support the involvement of APOE genotype in BMD, increased bone loss, or higher risk of osteoporotic fractures (Schoofs et al., 2004).

The current study evaluated the relationship between adiposity, BMD and subsequent cognitive decline with respect to both screening of functional capacity and, more specifically, verbal episodic memory. We assessed BMD, adiposity and cognition, controlling for the potential effects of age, gender, and possession of the APOE ε4 allele. Specific hypotheses included: (i) higher BMD would be significantly associated with better current and future cognitive functioning, particularly verbal memory; (ii) higher adiposity and lower lean body mass would be related to current cognitive function and predict subsequent cognitive function.

MATERIALS AND METHODS

STUDY DESIGN AND COHORT SELECTION

One hundred and sixty four participants aged 34–87 years old (62.78 ± 9.27) were recruited from a larger, longitudinal,

community-based study (the Western Australia Memory Study), investigating molecular and neuropsychological predictors of cognitive decline within younger and older adults (Clarnette et al., 2001; Sohrabi et al., 2009). The results were analyzed at baseline and after a 3-year follow-up. Participants completed annual blood and cognitive testing using standardized, validated screening and diagnostic measures. Exclusion criteria at the recruitment included: Mini Mental State Examination (MMSE) score $\leq$ 24 (Folstein et al., 1975); clinically diagnosed dementia; untreated depression [Geriatric Depression Scale (GDS) score $\geq$ 11]; history of neurological or psychiatric disorders affecting cognitive functions (e.g., stroke, Parkinson's disease, epilepsy, schizophrenia) and difficulty understanding or speaking English.

All participants provided written, informed consent to the study procedures including a body composition/BMD scan, using dual energy x-ray absorptiometry (DXA). Cognitive and clinical assessments, along with venous blood sampling occurred on the same day as DXA was performed. The study was approved by the Human Ethics Committees of Edith Cowan University, University of Western Australia, and Hollywood Private Hospital, Western Australia.

DXA ANALYSIS

The DXA technique quantifies bone mineral content by comparing the attenuation that occurs as a result of absorption of photons at two different energy levels, thereby creating a two dimensional BMD (aBMD) map (Van Loan and Mayclin, 1992). This analysis allows for the separation of body mass into bone, lean, and fat components.

In this study, DXA bone density and body composition scans were conducted on a central, whole body, Norland XR-46, pencil beam scanner using software version 4.1.1. The instrument was calibrated daily using a 77-step calibration standard QC phantom. The mean coefficient of variation (over 5 days) was 0.40 for BMD, 0.20 for lean mass and 0.14 for fat mass. Bone density measures were taken at the spine (L2-L4) and at the hip (femoral neck and total hip). The software generated: (i) *t*-scores, which compared each individual against a group, defined as possessing peak bone mass (i.e., normative data from healthy adults aged 20–40 years); and (ii) *z*-scores which compared an individual with data from their own age group.

Whole body scans were divided into regions of interest including head, chest, midriff, pelvis, and limbs. The measures taken included fat and lean mass (in kg), total body fat percentage, Siri formula, and Brozek formula, for underwater weight equivalents (UWE) (Guerra et al., 2010). To estimate body fat %, body mass density is calculated and converted to body fat %, using the Siri or Brozek equations. These are the most commonly equations available (Guerra et al., 2010).

Most of the scores produced by DXA are highly intercorrelated. In order to address potential multicollinearity, we created composite, sample-based *z* scores derived from the sum of all the *z* scores calculated for lean mass and BMD raw scores, divided by the number of scores. The following raw scores were converted to z scores and divided by six (number of scores) to compute the composite lean mass *z* score: Midriff lean mass + Pelvis lean mass + Left leg lean mass + Right leg lean mass + Left arm lean mass + Right arm lean mass. The bone density composite *z* score was calculated by summing the DXA Spine L2-L4, Femoral Neck, and trochanter computed *z* scores divided by three. We did not use a Fat mass composite *z* score, but instead used the Siri UWE as this score is strongly associated with fat % and other fat mass-related scores derived from DXA.

CLINICAL AND COGNITIVE MEASURES

Participants completed a comprehensive set of clinical and neuropsychological assessments lasting between 1.5 and 2.5 h and were offered breaks as needed. Depression at baseline was measured using the GDS (Yesavage et al., 1982). Premorbid cognitive ability was assessed using the Cambridge Contextual Reading Test (CCRT) (Beardsall, 1998). General cognitive functioning was assessed using the CAMCOG-R (Roth et al., 1998). Verbal episodic memory was assessed using the California Verbal Learning Test (CVLT) (Delis et al., 1988). Baseline and 3-year follow-up scores for the CVLT were calculated as follows: List Learning (List A; trials 1–5 total score), short delay free recall (SDFR), short delay cued recall (SDCR), long delay free recall (LDFR), long delay cued recall (LDCR), and recognition discriminability (RecDisc).

BIOCHEMICAL AND GENETIC ANALYSIS

On the same day as the DXA scan and cognitive/clinical assessment, a fasted venous blood sample was collected into serum, EDTA (containing prostaglandin E to prevent platelet activation) and heparin blood collection tubes (Interpath Services, Australia). The whole blood was then separated into different components using standard centrifugation techniques. DNA was isolated from leukocytes, and APOE genotype was determined via polymerase chain reaction (PCR) amplification and restriction enzyme digestion using the method originally described by Hixson and Vernier (1990), and outlined in Laws et al. (2002).

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel and statistical analyses conducted using IBM SPSS Version 19 (IBM SPSS Statistics, 2010 New York, IBM Corp). After testing for normality, descriptive sample characteristics were analyzed. Next, a series of two-step hierarchical linear regressions were conducted in order to explore the relationships between biological variables, general cognitive function (as assessed by the CAMCOG-R) and episodic verbal memory (as measured by the above-mentioned CVLT sub scores). In step one, potential covariates including age at scan time, gender, APOE ε4 allele status, and premorbid IQ (CCRT) were entered into the analysis. In step 2, variables derived from DXA were entered into the model. These variables included (1) Siri UWE Fat percentage, (2) Lean mass composite score, and (3) BMD composite score.

RESULTS

In the current study, participants included 69% women and 38% of participants were *APOE* ε4 carriers. Descriptive data for males/females, and *APOE* ε4 +/*APOE* ε4 – groups are presented in **Tables 1**, **2**. The percentage of participants who were *APOE* ε4 + did not differ by gender, $\chi^2(1; N = 162) = 0.010$, $p = 0.919$. There were significant differences between men and

Table 1 | Descriptive findings of dual energy x-ray absorptiometry (DXA) including the *p*-values corresponding to independent *t*†.

	Sex			APOE ε4[e] Status		
	Male (*N* = 51) Mean (±*SD*)	Female (*N* = 113) Mean (±*SD*)	*P*	Non-Carrier (*N* = 102) Mean (±*SD*)	Carrier (*N* = 61) Mean (±*SD*)	*P*
Age at scan	63.65 (± 7.88)	62.39 (± 9.85)	0.423	63.31 (± 9.36)	61.97 (± 9.21)	0.372
Total fat %	25.65 (± 6.05)	40.27 (± 8.07)	**0.000***	36.63 (± 10.36)	34.30 (± 9.61)	0.155
Siri UWE fat %	21.14 (± 5.67)	32.71 (± 7.35)	**0.000***	29.89 (± 8.75)	27.87 (± 8.62)	0.153
Lean mass composite score[a]	5.13 (± 3.08)	−2.31 (± 3.10)	**0.000***	0.100 (± 4.68)	−0.307 (± 4.49)	0.586
BMD composite score[b]	0.0007 (± 0.944)	−0.0003 (± 0.855)	0.999	0.018 (± 0.922)	−0.051 (± 0.805)	0.626
HDL[c]	1.21 (± 0.364)	1.59 (± 0.419)	**0.000***	1.46 (± 0.431)	1.50 (± 0.458)	0.570
LDL[d]	2.81 (± 0.989)	3.13 (± 0.851)	**0.032***	3.01 (± 0.878)	3.08 (± 0.959)	0.626
Cholesterol	4.93 (± 0.992)	5.44 (± 0.885)	**0.001***	5.26 (± 0.931)	5.34 (± 0.979)	0.584
Triglycerides	1.98 (± 1.40)	1.54 (± 1.03)	**0.027***	1.69 (± 1.17)	1.64 (± 1.19)	0.798

† *Equal variances not assumed; *p < 0.05;*

[a] *Lean mass composite score included the DXA Lean mass Z scores for Midriff, Pelvis, Left leg, Right leg, Left arm, and Right arm divided by six;*

[b] *Bone Mineral Density Composite Score included the Z scores for DXA Spine L2-L4, Femoral Neck, and trochanter divided by three;*

[c] *High-density lipoprotein;*

[d] *Low-density lipoprotein;*

[e] *Apolipoprotein E ε4.*

Table 2 | Descriptive cognitive data including *p*-values corresponding to independent *t*†.

	Sex			APOE ε4[i] Status		
	Male (*N* = 51) Mean (±*SD*)	Female (*N* = 113) Mean (±*SD*)	*P*	Non-Carrier (*N* = 102) Mean (±*SD*)	Carrier (*N* = 61) Mean (±*SD*)	*P*
CAMCOG[a]-baseline	99.90 (± 2.76)	97.79 (± 4.40)	**0.000***	98.72 (± 3.50)	97.98(± 4.91)	0.270
CVLT List A[b]-baseline	53.71 (± 10.37)	54.61 (± 11.20)	0.625	55.05 (± 10.83)	52.98 (± 11.07)	0.244
CVLT SDFR[c]-baseline	10.75 (± 2.86)	11.20 (± 2.91)	0.350	10.99 (± 2.98)	11.10 (± 2.73)	0.817
CVLT SDCR[d]-baseline	11.73 (± 2.50)	11.68 (± 2.95)	0.926	11.70 (± 2.95)	11.62 (± 2.54)	0.872
CVLT LDFR[e]-baseline	11.22 (± 2.77)	11.17 (± 3.04)	0.924	11.32 (± 2.92)	10.87 (± 2.96)	0.340
CVLT LDCR[f]-baseline	11.61 (± 2.81)	11.42 (± 3.23)	0.727	11.59 (± 3.06)	11.23 (± 3.15)	0.475
CVLT RecD[g]-baseline	94.77 (± 5.96)	93.26 (± 10.76)	0.354	93.70 (± 11.11)	93.66 (± 6.36)	0.981
CAMCOG-F/U[h]	98.36 (± 4.67)	98.12 (± 4.23)	0.763	98.30 (± 3.96)	97.98 (± 5.02)	0.682
CVLT List A-F/U	56.16 (± 9.77)	62.09 (± 10.41)	**0.002***	60.41 (± 10.39)	59.98 (± 11.01)	0.818
CVLT SDFR-F/U	11.30 (± 2.89)	12.43 (± 2.78)	**0.028***	11.98 (± 3.01)	12.25 (± 2.61)	0.588
CVLT SDCR-F/U	12.00 (± 2.68)	13.25 (± 2.19)	**0.004***	12.82 (± 2.44)	12.92 (± 2.41)	0.805
CVLT LDFR-F/U	11.66 (± 3.26)	12.87 (± 2.52)	**0.018***	12.58 (± 2.57)	12.31 (± 3.24)	0.584
CVLT LDCR-F/U	12.07 (± 2.98)	13.35 (± 2.30)	**0.006***	13.00 (± 2.38)	12.85 (± 2.95)	0.736
CVLT RecD-F/U	95.02 (± 4.83)	96.04 (± 6.95)	0.380	95.83 (± 4.86)	95.50 (± 8.40)	0.766

† *Equal variances not assumed; *p < 0.05;*

[a] *The Cambridge Cognitive Examination-Revised total score;*

[b] *The California List A Learning trials 1–5 total score;*

[c] *The California Verbal Learning Test (CVLT) Short Delay Free Recall;*

[d] *The CVLT Short Delay Cued Recall;*

[e] *The CVLT Long Delay Free Recall;*

[f] *The CVLT Long Delay Cued Recall;*

[g] *CVLT discriminability;*

[h] *Follow up;*

[i] *Apolipoprotein E ε4.*

women on most of the DXA measures. The differences are presented in **Table 2** with respect to gender and *APOE* ε4 status. *APOE* ε4 status and GDS (depression level) were not significantly associated with any of the DXA or cognitive variables.

CORRELATIONS

Table 3 presents the correlations between DXA, adiposity, and cognitive measures at baseline and follow up. Premorbid IQ (as measured using the CCRT) was not associated with any of

Table 3 | Associations between Dual-energy X-ray absorptiometry (DXA), baseline, and follow up cognitive measures (2-tailed).

	Scan age	CCRT[a]	HDL[b]	LDL[c]	Cholest[d]	Trigl[e]	Siri UWE[f] fat %	Lean mass comp[g]	BMD comp[h]	DXA *t*-score	DXA *z*-score
Scan age		−0.001	−0.083	−0.121	−0.061	**0.160***	0.077	−0.092	0.067	**−0.275****	0.009
GDS[i]-baseline	−0.065	−0.074	−0.110	0.061	−0.014	−0.046	0.006	0.094	−0.02	−0.009	−0.003
CAMCOG[j]-baseline	**−0.222****	**0.174***	−0.055	−0.008	0.011	0.079	**−0.204****	**0.214****	−0.061	−0.028	−0.077
CVLT List A[k]-baseline	**−0.206****	0.026	0.136	−0.052	0.026	0.035	0.035	−0.028	**0.157***	**0.240****	**0.155***
CVLT-SDFR[l]-baseline	**−0.169***	0.064	**0.195***	−0.038	0.03	−0.03	0.055	−0.095	0.049	0.158*	0.049
CVLT–SDCR[m]-baseline	−0.057	0.074	**0.160***	−0.062	0.024	0.017	0.006	−0.054	**0.166***	**0.205****	**0.160***
CVLT-LDFR[n]-baseline	−0.124	0.149	0.147	0.007	0.047	−0.039	−0.016	−0.007	0.078	0.12	0.063
CVLT-LDCR[o]-baseline	**−0.164***	0.078	**0.162***	0.043	0.067	−0.076	−0.024	−0.02	0.083	0.14	0.071
CVLT-RecD[p]-baseline	−0.146	−0.058	**0.167***	−0.015	−0.037	**−0.176***	−0.029	−0.051	0.066	**0.157***	0.091
CAMCOG-F/U[q]	**−0.218****	**0.379****	0.004	−0.006	0.048	0.094	−0.016	0.054	0.017	0.116	0.041
CVLT List A-F/U	**−0.293****	0.132	0.155	0.119	0.152	−0.043	0.149	**−0.189***	**0.187***	**0.319****	**0.190***
CVLT SDFR-F/U	**−0.350****	0.152	0.166	0.11	0.137	−0.056	0.064	−0.098	0.083	**0.233****	0.092
CVLT SDCR-F/U	**−0.337****	**0.283****	**0.184****	0.117	0.136	−0.081	0.106	−0.134	0.085	**0.186***	0.116
CVLT LDFR-F/U	**−0.332****	0.132	0.087	0.078	0.116	0.013	0.079	−0.098	0.143	**0.280****	0.159
CVLT LDCR-F/U	**−0.343****	0.166	0.105	0.026	0.06	−0.009	0.099	−0.12	0.104	**0.286****	0.152
CVLT RecD-F/U	**−0.311****	**0.266****	−0.021	0.013	0.023	0.041	0.092	0.002	0.068	**0.169***	0.083

*$p < 0.05$; **$p < 0.011$;

[a] *Cambridge Contextual Reading Test;*

[b] *High-density lipoprotein;*

[c] *Low-density lipoprotein;*

[d] *Cholesterol;*

[e] *Triglyceride;*

[f] *Underwater weight equivalents;*

[g] *Lean mass composite score included the DXA Lean mass Z scores for Midriff, Pelvis, Left leg, Right leg, Left arm, and Right arm divided by six;*

[h] *Bone Mineral Density Composite Score included the Z scores for DXA Spine L2-L4, Femoral Neck, and trochanter divided by three;*

[i] *Geriatric Depression Scale;*

[j] *The Cambridge Cognitive Examination-Revised total score;*

[k] *The California Verbal Learning Test (CVLT) List A Learning trials 1–5 total score;*

[l] *The CVLT Short Delay Free Recall;*

[m] *The CVLT Short Delay Cued Recall;*

[n] *The CVLT Long Delay Free Recall;*

[o] *The CVLT Long Delay Cued Recall;*

[p] *The CVLT discriminability;*

[q] *Follow up.*

the DXA measures, but was a significant correlate of CAMCOG Baseline and FU scores, and CVLT SDCR and RecD follow up scores. GDS score was also not significantly associated with any of the DXA or other biomarkers and therefore was not included as a covariate in regression analyses. However, we found significant associations between overall cognitive status, as measured by CAMCOG-R, and various DXA measures. A significant, negative association was found between baseline CAMCOG-R and Siri UWE ($r = -0.204$, $p < 0.01$). Lean mass and BMD composite scores were positively associated with verbal learning (CVLT scores) and general cognitive functioning (CAMCOG-R) (**Table 3**) and therefore included in the subsequent regression analyses. HDL was significantly associated with baseline CVLT subscales including, SDFR ($r = 0.195$, $p < 0.05$), SDCR ($r = 0.160$, $p < 0.05$), and LDCR ($r = 0.162$, $p < 0.05$) but not with the CVLT follow up results except for SDCR ($r = 0.184$, $p < 0.05$). Interestingly, HDL was negatively associated with the total perseveration score for List A learning trials 1–5 ($r = -0.160$, $p < 0.05$), indicating that greater perseveration was associated with lower HDL.

CLINICAL AND COGNITIVE DATA

Clinical and cognitive data were available for all participants. The mean clinical and cognitive scores for all participants are outlined in **Tables 1**, **2**. The means for all neuropsychological measures were within expected age-related norms (Yesavage et al., 1982; Delis et al., 2000). We did not find any significant differences between *APOE* ε4 carriers and non-carriers on any of the DXA or neuropsychological measures. Depression was not significantly associated with any of the DXA measures (**Table 3**) or with cognitive measures at baseline or follow up.

REGRESSION ANALYSIS

A series of hierarchical multiple regressions was conducted to examine the associations between DXA and adiposity measures

and cognitive test results whilst controlling for the effects of relevant covariates at both baseline and follow up assessments. As discussed, demographic factors including age, gender, APOE ε4 carriage, and premorbid IQ (as measured using the CCRT) were entered in Step 1. In Step 2, variables derived from DXA were entered into the model including the Siri UWE Fat percentage, Lean mass composite score, and the BMD composite score.

COGNITIVE RESULTS

The DXA scores in our two-step hierarchical regression model were not significantly associated with general cognitive functioning at baseline (R^2-change = 0.007; F change = 0.359, $p = 0.782$) or follow up (R^2-change = 0.002; F change = 0.110, $p = 0.954$). In predicting baseline CVLT List A trials 1–5, the first model containing covariates only was not significant [$F_{(4,\ 141)} = 2.014$, $p = 0.096$, $R^2 = 0.054$]. However, the second model was significant [$F_{(7,\ 138)} = 2.359$, $p < 0.05$, $R^2 = 0.107$]. Specifically, as seen in **Table 4**, the addition of the DXA variables provided unique predictive variance (R^2-change = 0.53). Higher BMD was significantly associated with higher learning scores, and there was a trend for lower Lean mass composite score ($B = -0.644$; $t = -1.926$; $p = 0.056$).

Baseline CVLT discriminability (recognition) performance was not significantly predicted by the first model containing covariates only [$F_{(4,\ 140)} = 1.115$, $p = 0.352$, $R^2 = 0.031$, but the second model was significant, $F_{(7,\ 137)} = 2.422$, $p < 0.05$, $R^2 = 0.110$]. Both lean mass composite score and BMD were significant predictors of RecDisc in the final model, with higher BMD and lower lean mass being associated with better performance (**Table 5**). Adding DXA variables contributed an additional 8% of predictive variance.

CVLT List A learning at follow-up was also significantly predicted by DXA variables. The first model containing covariates only was significant [$F_{(4,\ 124)} = 6.005$, $p < 0.001$]. The second model was also significant [$F_{(7,\ 121)} = 5.375$, $p < 0.001$, $R^2 = 0.237$]. Specifically, the Lean mass composite score ($t = -2.297$, $p < 0.05$), and BMD composite score ($t = 3.351$, $p < 0.001$) were significant predictors (**Table 6**), accounting for an additional 8% of variance.

Due to significant differences between men and women on DXA measures, we examined the interaction of gender and DXA measures (i.e., Siri UWE Fat %, lean mass composite score, and BMD composite score) in predicting cognitive functions. The cognitive functions examined here included baseline and follow up CAMCOG-R, List A learning trials 1–5, SDFR, SDCR, LDCR, LDFR, and Recognition Disc results. Interestingly, we did not find any significant results for these interactions except for Gender *X* BMD on the follow up LDFR [R^2-change = 0.052; F change = 2.707, $p = 0.048$; $F_{(10,\ 118)} = 3.944$, $p < 0.001$; B = -1.302, $SE = 0.606$, Beta = −0.698; $t = -2.150$, $p < 0.034$]. Further analysis indicated that higher BMD was significantly associated with better follow up LDFR in men, but not in women ($r = 0.459$, $p < 0.002$ and $r = -0.039$, $p = 0.707$, respectively).

Table 4 | Hierarchical linear regression predicting baseline CVLT List A trials 1–5 total score from lean mass and bone density composite scores.

	B	Std. error	β	Adj. R^2	ΔR^2
Step 1				0.027	0.054
Age	−0.247	0.097	−0.209*		
Gender	0.558	1.931	0.024		
APOE ε4 status	−2.413	1.849	−0.107		
CCRT[a]	0.062	0.185	0.028		
Step 2				0.062	0.053*
Age	−0.322	0.101	−0.272**		
Gender	−5.201	3.629	−0.221		
APOE ε4 Status	−2.457	1.837	−0.109		
CCRT	0.073	0.183	0.032		
Siri UWE[b] fat %	0.074	0.132	0.059		
Lean mass comp score[c]	−0.644	0.334	−0.274#		
BMD comp score[d]	3.123	1.138	0.252**		

*$p < 0.05$; **$p < 0.01$; #$p < 0.10$ (trend);

[a] *Cambridge Contextual Reading Test;*

[b] *Underwater weight equivalents;*

cLean mass composite score included the DXA Lean mass Z scores for Midriff, Pelvis, Left leg, Right leg, Left arm, and Right arm divided by six;

[d] *Bone Mineral Density Composite Score included the Z scores for DXA Spine L2-L4, Femoral Neck, and trochanter divided by three.*

Table 5 | Hierarchical linear regression predicting baseline CVLT discriminability score from lean mass and bone density composite scores.

	B	Std. error	β	Adj. R^2	ΔR^2
Step 1				0.003	0.031
Age	−0.155	0.086	−0.150		
Gender	−1.685	1.715	−0.082		
APOE ε4 status	−0.238	1.641	−0.012		
CCRT[a]	−0.113	0.165	−0.057		
Step 2				0.065	0.079**
Age	−0.251	0.088	−0.243**		
Gender	−10.253	3.178	−0.499**		
APOE ε4 status	−0.460	1.609	−0.023		
CCRT	−0.089	0.160	−0.045		
Siri UWE[b] fat %	0.104	0.115	0.095		
Lean mass comp score[c]	−0.970	0.293	−0.471***		
BMD comp score[d]	2.409	0.996	0.222*		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$;

[a] *Cambridge Contextual Reading Test;*

[b] *Underwater weight equivalents;*

[c] *Lean mass composite score included the DXA Lean mass Z scores for Midriff, Pelvis, Left leg, Right leg, Left arm, and Right arm divided by six;*

[d] *Bone Mineral Density Composite Score included the Z scores for DXA Spine L2-L4, Femoral Neck, and trochanter divided by three.*

Table 6 | Hierarchical linear regression predicting follow up CVLT List A trials 1–5 total score from lean mass and bone density composite scores.

	B	Std. error	β	Adj. R^2	ΔR^2
Step 1				0.135	0.162***
Age	−0.318	0.094	−0.279***		
Gender	5.476	1.872	0.241**		
APOE ε4 Status	−0.885	1.792	−0.041		
CCRT[a]	0.279	0.180	0.128		
Step 2				0.193	0.075**
Age	−0.400	0.096	−0.351***		
Gender	−0.665	3.459	−0.029		
APOE ε4 status	−0.993	1.751	−0.046		
CCRT	0.294	0.174	0.135		
Siri UWE[b] fat %	0.050	0.125	0.041		
Lean mass comp score[c]	−0.732	0.319	−0.322*		
BMD comp score[d]	3.633	1.084	0.304***		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$;

[a] *Cambridge Contextual Reading Test;*

[b] *Underwater weight equivalents;*

[c] *Lean mass composite score included the DXA Lean mass Z scores for Midriff, Pelvis, Left leg, Right leg, Left arm, and Right arm divided by six;*

[d] *Bone Mineral Density Composite Score included the Z scores for DXA Spine L2-L4, Femoral Neck, and trochanter divided by three.*

DISCUSSION

The results of this longitudinal study lend further support to the mounting body of evidence suggesting that lifestyle factors may be involved in cognitive functions and, in turn, may modulate the risk of future cognitive decline. We report a significant link between potentially modifiable indicators of systemic health, namely BMD and Lean body mass on cognitive performance in a group of community-dwelling, healthy adults. These findings have a number of potential implications for understanding the underlying, modifiable mechanisms involved in pathological cognitive decline and for developing preventive and potentially ameliorative clinical trials targeting AD, specifically with respect to the possible influence of metabolic, cardiovascular, and general health factors.

BMD was a predictor for cognitive performance with respect to CVLT measures (**Tables 4–6**) in a cross-sectional as well as longitudinal context. Interestingly, the findings from the present study indicate that the effect of *APOE* ε4 was limited, i.e., we did not observe any significant differences between the *APOE* ε4 carriers and non-carriers in the study on DXA or cognitive assessment results (**Table 1**).

In this study, we found significant differences between men and women on BMD and body composition (as expected from previous studies), but we did not find significant differences on cognitive measures at baseline. However, after 3 years of follow up, women outperformed men on most of the CVLT measures (**Table 2**) but their performance was similar on follow up global cognitive function. This may be consistent with findings implying differential cognitive decline rate in women vs. men (Maylor et al., 2007; Holland et al., 2013).

Low BMD and osteoporosis restrict morbidity amongst the elderly and have been associated to the risk of future cognitive decline (Yaffe et al., 1999; Lui et al., 2003) and dementia due to AD (Tan et al., 2005). Low BMD has been associated with lower cognitive function in cross-sectional study of post-menopausal women (Brownbill and Ilich, 2004) and with verbal memory performance in both men and women (Zhang et al., 2001). It has been suggested that BMD may be reflective of cumulative estrogen exposure, thus providing a molecular mechanism for the link between BMD and cognition as longer estrogen exposure has been associated with lower dementia risk (Fox et al., 2013). However, the evidence supporting a role for estrogen replacement in protecting against AD is currently uncertain due to a number of methodological factors and possible confounding variables including education, general health and physical activity (as reviewed in: Yaffe et al., 1998). As such, the debate surrounding the efficacy of estrogen replacement as a therapeutic target for AD continues.

We found that BMD and lean mass were significantly associated with memory abilities as measured using the CVLT (**Tables 4–6**). This is consistent with a previous report that BMD and verbal memory impairment were significantly associated (Zhang et al., 2001). Zhang et al. (2001) has postulated that this relationship may be potentially a function of cumulative exposure to estrogen. In fact, our findings, indirectly, support the notion of BMD as a marker of cumulative estrogen exposure, particularly because we observed a significant association between sex and CVLT Learning abilities at follow-up in the present study (**Table 2**). It should be noted that we did not measure estrogen exposure in this study. However, it has previously been shown that body fat serves as a source of endogenous estrogen in post-menopausal women (Bagger et al., 2004).

The relationship between memory, BMD, and body composition were more significantly pronounced on performance in the List A learning trials. In the current study, BMD and lean mass measures of DXA were significant predictors of CVLT scores representing learning capabilities. This finding is important, as encoding deficits in episodic verbal memory i.e., mental representation of new information–are the primary memory problems seen in preclinical dementia and AD (Golby et al., 2005; Twamley et al., 2006; Beck et al., 2012), and such encoding problems have been shown to correlate with cholinergic deficits that are commonly seen in AD (White and Ruske, 2002). Interestingly, the cholinergic system plays a pivotal role in BMD regulation, as has recently been reviewed (Eimar et al., 2013). These considerations are consistent with our findings discussed earlier concerning the association between learning ability and BMD.

It has been reported that executive functions are associated with episodic memory (Duff et al., 2005). More specifically, the attention component of executive function plays a significant role in verbal learning and memory (Brooks et al., 2006) and has been associated with difficulties in CVLT List A learning (Hill et al., 2012). Our findings show a significant association between List A learning and BMD (as discussed earlier). This may indirectly suggest an underlying mechanism involved in both BMD and executive functioning, although this has to be carefully examined in future studies.

While the current study furthers our understanding of the relationship between age-related memory capacities and BMD, the cohort size and the very wide age range represent the main limitations. In addition, it would be advantageous to have baseline and follow up DXA results, and general morbidity and physical activity to examine the potential differences with negative changes in the DXA results in terms of memory and other cognitive functions. Additionally, examining the sex hormones will add value to a longitudinal study investigating the relationship between DXA scores and cognitive functions.

Further research is warranted investigating the relationship between BMD and cognitive dysfunctions in various dementia patient groups and in preclinical stages, and the potential mechanisms underlying these relationships. Such research could significantly improve our knowledge of the association between bone mineral content and higher cortical capabilities, and the relevance of these factors in aging and dementia. Future research may investigate the relationship between BMD and cognitive decline in aging while controlling for the effects of general mobility and current/previous physical activity as contributing factors.

The non-significant relationship between *APOE* alleles carriage, BMD and cognition in this study may indicate differential contributing pathways for genetic vs. lifestyle factors. Of course, we requires further research in a larger cohort where various genes identified as contributing to dementia risk can be examined in relation to BMD and cognitive decline measures.

AUTHOR CONTRIBUTIONS

Study design: HRS, KAB, JKF, SML, and RNM. Study conduct: HRS, KAB, MAR, MH, KT, GM, AP, JKF. Data collection: HRS, KAB, MAR, AP, GM. Data analysis: HRS, KAB, SSD, MW. Data interpretation: HRS, KAB, TS, RSB. Drafting manuscript: HRS, KAB. Revising manuscript content: MW, RSB, SRRS, JB, AC, MW, BMB, SMB, KT, TS, PDM, JKF, IJM, NLL, FM, SML. Approving final version of manuscript: HRS, KAB, MW, SSD, FM, NLL, and RNM. HRS, SSD, MW, and RNM take responsibility for the integrity of the data analysis.

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Specific cognitive functions and depressive symptoms as predictors of activities of daily living in older adults with heterogeneous cognitive backgrounds

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Cognitive functioning influences activities of daily living (ADL). However, studies reporting the association between ADL and neuropsychological performance show inconsistent results regarding what specific cognitive domains are related to each specific functional domains. Additionally, whether depressive symptoms are associated with a worse functional performance in older adults is still under explored. We investigated if specific cognitive domains and depressive symptoms would affect different aspects of ADL. Participants were 274 older adults (96 normal aging participants, 85 patients with mild cognitive impairment, and 93 patients probable with mild Alzheimer's disease dementia) with low formal education (~4 years). Measures of ADL included three complexity levels: Self-care, Instrumental-Domestic, and Instrumental-Complex. The specific cognitive functions were evaluated through a factorial strategy resulting in four cognitive domains: Executive Functions, Language/Semantic Memory, Episodic Memory, and Visuospatial Abilities. The Geriatric Depression Scale measured depressive symptoms. Multiple linear regression analysis showed executive functions and episodic memory as significant predictors of Instrumental-Domestic ADL, and executive functions, episodic memory and language/semantic memory as predictors of Instrumental-Complex ADL (22 and 28% of explained variance, respectively). Ordinal regression analysis showed the influence of specific cognitive functions and depressive symptoms on each one of the instrumental ADL. We observed a heterogeneous pattern of association with explained variance ranging from 22 to 38%. Different instrumental ADL had specific cognitive predictors and depressive symptoms were predictive of ADL involving social contact. Our results suggest a specific pattern of influence depending on the specific instrumental daily living activity.

Keywords: activities of daily living, functional performance, neuropsychological assessment, depression, dementia, mild cognitive impairment, executive functions

Introduction

Cognitive and functional impairments are hallmarks of cognitive disorders and defining features of mild cognitive impairment (MCI) and dementia. In MCI, the cognitive deficits do not impair the capacity to live independently, in contrast to individuals with dementia that present pronounced functional deficits, such as the ones observed in Alzheimer's disease (AD; (Pereira et al., 2010; Brown et al., 2011; Seelye et al., 2013). The most usual form to assess functional performance in older adults is the investigation of activities of daily living (ADL), common activities performed by the majority of older adults in a specific cultural setting (Lawton and Brody, 1969).

Prior studies have investigated the relationship between cognitive and functional performance in older adults with MCI or AD. Longitudinal changes in cognition are related to longitudinal changes in ADL (Farias et al., 2009). In a comprehensive review, Royall et al. (2007) showed a weak to moderate association between global cognitive measures and functional impairment. However, their results are heterogeneous with cognitive features responding for 0 to 80% of the variance in functional performance (mean of 21% with a SD of 20%; Gold, 2012). Methodological differences and sample characteristics might explain part of this excessive variability.

Gold (2012) discuss some of the methodological issues. The definition and the type of ADL investigated varies between studies. Some studies focuses on a unitary construct of ADL, in contrast with several evidences from the literature of a multidimensional construct involving activities of different levels of complexity (Thomas et al., 1998; Niti et al., 2007; Gold, 2012; de Paula et al., 2014). Beyond the usual distinction between Basic (BADL) and Instrumental (IADL) activities, some studies found different latent structures in ADL questionnaires and scales. Thomas et al. (1998) reported a multidimensional structure for BADL and IADL combined, proposing its interpretation based on levels of complexity (basic, intermediate, and complex). Niti et al. (2007) found a bifactorial structure for IADL ("physical" and "cognitive") in a sample of Asian older adults with a significant influence of specific cultural aspects on participants' responses. In a sample of low educated older adults from Brazil, de Paula et al. (2014) observed a different factorial solution with a "Domestic" IADL component and a "Complex" IADL component, in addition to a third component classified as BADL. Therefore, ADL may be a multidimensional construct with different components varying according to sample characteristics (Bootsma-van der Wiel et al., 2001; Cabrero-García and López-Pina, 2008; Fieo et al., 2011). Such differences might account for some of the high heterogeneity between studies reported in Royall et al. (2007). This emphasizes the importance of investigating specificities in ADL structure (Gold, 2012).

Methodological difficulties include instrument bias. Sikkes et al. (2009) reviewed the literature concerning the different measures of IADL focusing on its psychometric properties. Their results suggest that most of the scales adopted in clinical and research settings still lack of psychometric studies, reducing its validity and reliability for the functional assessment, although great effort is being dispend on this matter (Fieo et al., 2011). The source of information (e.g., patient vs. caregiver) is also not consensual in studies including cases of dementia, which is of extreme importance to the use and interpretation of scales measurement (Farias et al., 2005). Bootsma-van der Wiel et al. (2001) also highlight the conceptual difference between a "can do" or an "actually do" score in specific activities. Additionally, there is no consensus in the literature indicating scales and questionnaires as adequate methods to functional level measurement. There are more ecological measures of ADL, which involve the observation of the patient's behavior on real life or simulated settings (a more precise estimation of functional performance; Chaytor and Schmitter-Edgecombe, 2003). However, these procedures also have limitations such as the higher costs of execution and the inherent complexity of the assessment, and might not be well suited for most of the clinical and research settings. The suitability of these ecological measures for studies involving cognitive performance is controversial. There are studies showing a stronger association of cognitive measures with ecological measures of ADL (Burton et al., 2006; Tam et al., 2008). However, the opposite pattern was also demonstrated with the relation between cognitive performance with ADL being stronger for ADL measured by scales/questionnaires (Cahn-Weiner et al., 2002; Mariani et al., 2008; de Paula et al., 2014). The cognitive processes assessed by the neuropsychological tests used in these studies might be associated with ADL in different and more specific ways (Gold, 2012).

Taking specific cognitive domains as predictors of functional performance most of the studies report associations with executive functions (Royall et al., 2007; Pereira et al., 2008; de Paula and Malloy-Diniz, 2013). This complex cognitive construct usually shows the strongest correlations with functional performance. Executive functions involve planning, initiation, monitoring, inhibition, and flexibility of goal-oriented behavior (Diamond, 2013). These specific aspects of executive functions may contribute differently to the ADL (Jefferson et al., 2006). However, other cognitive functions may contribute to specific aspects of ADL. Cognitive measures of spatial processing predicted participants' performance in an ecological measure of visuospatial abilities in which the subject had to estimate distances, positions, and directions in a "real-life" setting developed by Farley et al. (2011). Activities demanding communicative skills were related to semantic process and language (Razani et al., 2011). Schmitter-Edgecombe et al. (2009) investigated different aspects of episodic memory and its association with ADL reporting significant associations of specific memory components with specific functional components. In this sense, although most of the studies have focused on executive functions or global cognitive measures, different kinds of ADL may depend on different cognitive abilities.

Depressive symptoms can also impair functional performance in older adults, usually in more complex activities (Bombin et al., 2012; Tomita and Burns, 2013; Zahodne et al., 2013; Park et al., 2014). Our group reported a weak association between depressive symptoms and functional performance in older adults with low formal education diagnosed with MCI or AD (de Paula and Malloy-Diniz, 2013). A stronger association was recently reported

in a similar sample (Assis et al., 2014). Cahn-Weiner et al. (2002) found an association of depressive symptoms with ADL independent from cognitive functioning. Remission of depressive symptoms is associated with improvement of ADL (Nyunt et al., 2012). Nonetheless, there is evidence for the contrary, suggesting that depressive symptoms are not associated with IADL after controlling for cognitive symptoms (Reppermund et al., 2011; Wadsworth et al., 2012). Then, whether depressive symptoms affect functional performance by behavioral symptoms (depressed mood, lack of pleasure, apathy, and vegetative symptoms) or through cognitive impairment associated with depression remains unclear.

Functional deficit is one of the hallmarks of MCI and AD in older adults. Improving cognitive functions and mood symptoms may result in gains in functional performance. Therefore, a better understanding of how specific cognitive abilities and depressive symptoms contribute to the performance of specific ADL could be important to the development of tailored rehabilitation programs to improve daily functioning in individuals with cognitive disorders in a personalized way. The objective of the present study is to assess how specific cognitive abilities and symptoms of depression are associated to different aspects of ADL.

Materials and Methods

Participants

We evaluated 274 older adults from a public outpatient clinic specialized in cognitive disorders and frailty. The center usually receives elderly patients referred from primary-care physicians when they suspect of cognitive impairment, mental disorders, or multiple chronic diseases. Patients usually have a very low socioeconomic status and less than 4 years of formal education. Participants' sociodemographic characteristics are shown in **Table 1**. A more detailed description of the typical profile of patients assessed in this center was published elsewhere (Bicalho et al., 2013; de Paula et al., 2013a).

The participants underwent a detailed clinical, cognitive, and behavioral assessment for diagnostic purposes as described below. During the geriatrician examination and the clinical neuropsychological assessment, the patients underwent cognitive, functional and behavioral assessment to determine their cognitive status. Ninety-three participants were diagnosed with mild Alzheimer's disease dementia (AD), 85 patients were diagnosed with amnestic MCI (MCI) and 96 older adults were normal aging participants without clinical history, cognitive, or functional status suggestive of dementia or AD. AD was diagnosed by the NINCDS-ADRDA criteria for probable dementia (McKhann et al., 1984). Only patients with mild dementia, according to Clinical Dementia Rating (CDR; Morris, 1993), were invited for participation in this study. MCI diagnosis was based on a modified version of the Mayo Clinic diagnostic criteria (Petersen et al., 2001). Criteria for MCI was as follow:

(1) Subjective cognitive complaint, preferably corroborated by an informant/caregiver.

TABLE 1 | Participants' demographic profile.

Cognitive status	Normal aging	35%
	Mild cognitive impairment	31%
	Alzheimer's disease dementia	34%
Gender	Male	39%
	Female	61%
Depression[1]	Present	30%
	Absent	70%
Age	60–69 years	34%
	70–79 years	43%
	80+ years	23%
Formal education	Illiterate	12%
	1–4 years	57%
	5–8 years	13%
	9+ years	12%
	12+ years	6%
Occupations[2]	Craft and related trades workers	13%
	Elementary occupations	34%
	Service and sale workers	22%
	Others	31%
Retired?	No	13%
	Yes	87%
Marital status	Married	53%
	Divorced	12%
	Single	9%
	Widow	36%

[1]*According to the Geriatric Depression Scale 15 cut-off (5/6).*
[2]*According to the International Labour Office (ILO, 2012).*

(2) Objective impairment on specific cognitive measures of the assessment battery for diagnosis according to Brazilian norms and cut-off scores (Porto et al., 2003; Nitrini et al., 2004). The cognitive battery includes the Verbal Learning Test of the CERAD Neuropsychological Battery (Morris et al., 1989), the memory test from the Brief Cognitive Battery (Nitrini et al., 2007), and subscales of the Mattis Dementia Rating Scale (Mattis, 1988).
(3) Normal global cognitive functioning (MMSE above the cut-off for dementia and CDR < 1).
(4) Preserved or minimal impairments in ADL assessed by a clinical interview and the CDR.
(5) Not demented based on the DSM-IV-TR criteria (American Psychiatric Association [APA], 2000).

All groups (i.e., AD, MCI, and control) were combined in a unique heterogeneous sample. This strategy was adopted to increase statistical power and to avoid detection loss of cognitive processes that are likely to underlie functional performance (Farias et al., 2009; Gold, 2012).

Cognitive, Functional, and Mood Assessment

We adopted an unstructured protocol of neuropsychological tests designed for the assessment of older adults with low formal

TABLE 2 | Neuropsychological measures used to extract the four cognitive factors according to de Paula et al. (2013a).

Cognitive domain	Test	Test measures	Reference
Executive functions	Frontal assessment battery	Total score	Dubois et al. (2000)
	Verbal fluency	Animals	Lezak et al. (2004)
		Fruits	
		Letter "S"	
	Digit span forward	Correct trials × Span	Kessels et al. (2008)
	Digit span backward	Correct Trials × Span	Kessels et al. (2008)
Language semantic memory	TN-LIN (naming test)	Nouns	Malloy-Diniz et al. (2007a)
		Actions	
		Professions	
Episodic memory	RAVLT	Short term memory (A1)	Malloy-Diniz et al. (2007b)
		Immediate recall (A6)	
		Delayed recall (A7)	
		Sum of words	
		Recognition memory	
Visuospatial abilities	Stick design test	Total score	Baiyewu et al. (2004)
	Clock drawing test	Total score	Shulman (2000)
	Token test (short version)	Visual attention	De Renzi and Faglioni (1978)
		Complex comprehension	

TN-LIN, Naming test of the laboratory of neuropsychological investigations; RAVLT, Rey auditory-verbal learning test.

TABLE 3 | Participants' description and group comparisons.

Measures	NA ($N = 96$) M (SD)	MCI ($N = 85$) M (SD)	AD ($N = 93$) M (SD)	F/χ^2	Comparisons[2]
Age	72.61 (7.76)	73.18 (8.46)	73.18 (8.46)	1.80	–
Education	5.22 (4.29)	4.71 (4.00)	4.82 (3.46)	0.48	–
GDS-15	4.33 (3.95)	2.94 (2.84)	3.82 (3.22)	3.81	–
Sex (% Female)	67%	60%	55%	2.59[1]	–
MMSE	25.75 (3.85)	23.52 (3.62)	20.59 (3.98)	42.58**	NA > MCI > AD
Language/Semantic memory	−0.34 (1.20)	−0.76 (0.96)	−1.69 (1.16)	27.28**	NA = MCI > AD
Episodic memory	−0.28 (0.80)	−1.22 (0.72)	−1.76 (0.65)	93.47**	NA > MCI > AD
Visuospatial abilities	−0.31 (1.08)	−0.84 (1.04)	−1.47 (1.05)	30.38**	NA > MCI > AD
Executive functions	−0.58 (1.34)	−1.21 (1.05)	−2.25 (1.09)	46.91**	NA > MCI > AD
GADL self-care	9.94 (0.32)	9.99 (0.11)	9.78 (0.87)	4.01*	NA = MCI > AD
GADL domestic	7.68 (0.86)	7.41 (1.20)	5.74 (2.19)	19.61**	NA = MCI > AD
GADL complex	7.55 (1.27)	6.91 (1.47)	4.35 (2.57)	59.61**	NA = MCI > AD
GADL global score	25.16 (2.06)	24.03 (2.34)	19.88 (3.92)	75.03**	NA = MCI > AD

**0.05, **<0.001. NA, Normal aging; MCI, Mild cognitive impairment; AD, Alzheimer's disease; M, Mean; SD, Standard-deviation; GDS-15, Geriatric Depression Scale 15 items; GADL, General Activities of Daily Living Scale; [1]Chi-Square test; [2]One-Way ANOVA and Sidak's post hoc test ($p < 0.05$).*

education. The tests were not included in patients' diagnosis. Cognitive composite factors were obtained through factor analysis from our sample (statistical procedures detailed below). This approach allows the assessment of different aspects of cognitive functioning (here, the core domains recommended for the MCI and AD diagnosis) with greater specificity. The protocol comprised tests of executive functions (Frontal Assessment Battery, Verbal Fluency tests, and Digit Span); language and semantic memory (Laboratory of Neuropsychological Investigations Naming Test – Nouns, Verbs and Professions, Token Test verbal comprehension component); episodic memory (components of learning, recognition, immediate, and delayed recall of the Rey Auditory-Verbal Learning Test); and visuospatial abilities (Clock Drawing Test, Stick Design Test, and Token Test visual attention components). These tests are valid and reliable for the assessment of older adults with a low educational background (de Paula et al., 2013a). The test measures are shown in **Table 2**.

The assessment of ADL occurred during the clinical and neuropsychological assessment by an interview with participants' caregivers. For this study, we used the 'General Activities of Daily Living Scale' (GADL) (de Paula et al., 2014), a multidimensional functional measure of BADL/IADL based on the Lawton and Brody (1969) and Katz et al. (1970) indexes of ADL. The GADL shows a hierarchical structure with a general score and three components of more specific activities: a measure of

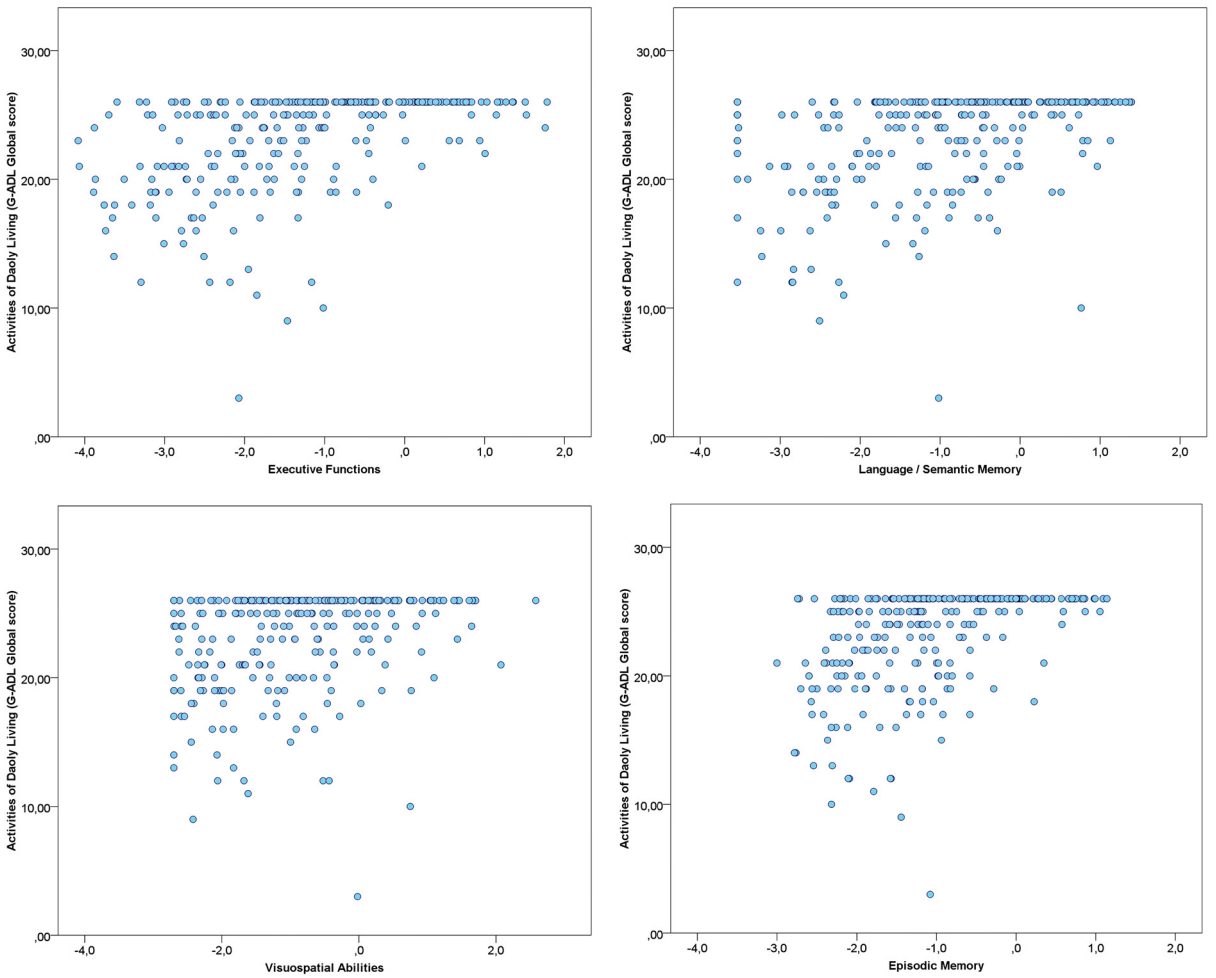

FIGURE 1 | Association between Cognitive Factors and General Activities of Daily Living Scale (GADL). The correlations of cognitive factors with functional performance were all significant ($p < 0.001$). Executive functions showed the strongest correlation with GADL total score ($r = 0.478$), followed by episodic memory ($r = 0.449$), language/semantic memory ($r = 0.410$), and visuospatial abilities ($r = 0.302$). The number of dots in the scatterplot differs from the sample size due to superposed values.

BADL (Self-care: ability to change clothes, use the toilet, use the shower, transference from bed or chair, and feed itself) and two components of IADL. Instrumental Domestic ADL include ability to perform domestic chores, use the telephone, prepare meals, and do the laundry. Instrumental Complex ADL include ability to manage financial matters, shopping, adequate use of medication, and go out alone using transportation. This structure was determined by factor analysis on the same sample of the present study and showed evidence of reliability and validity (de Paula et al., 2014). We scored each activity using a 3-point Lickert scale (dependent, partially dependent, or independent of assistance to perform the activity). The GADL subscores of Self-care (0–10), Instrumental-Domestic (0–8), Instrumental-Complex (0–8), and the Global score (0–26) represented the general ADL measures in our study. To investigate the association of different cognitive functions with specific measures of functional performance, we also used each item of the scale (13 different ADL) independently.

We assessed the depressive symptoms with the Brazilian version of the Geriatric Depression Scale-15 (GDS-15; Sheikh and Yeasavage, 1986). A validation study conducted in Brazil attested its sensitivity and specificity for the detection of depression (Almeida and Almeida, 1999). However, since our focus was not to identify patients with major depressive disorder, but to use a dimensional measure of its symptoms, we used the GDS-15 total score in this research. The GDS use for depression diagnosis in dementia is controversial. To reduce biases we selected only patients with mild dementia for the AD group (CDR ≤ 1). Due to participants' low formal education, the examiner read the GDS questions aloud to ensure the comprehension and validity of patients' report.

Statistical Procedures

Our four cognitive domains were extracted by factor analysis (principal axis factoring with an oblique rotation of the neuropsychological tests described in Cognitive Assessment)

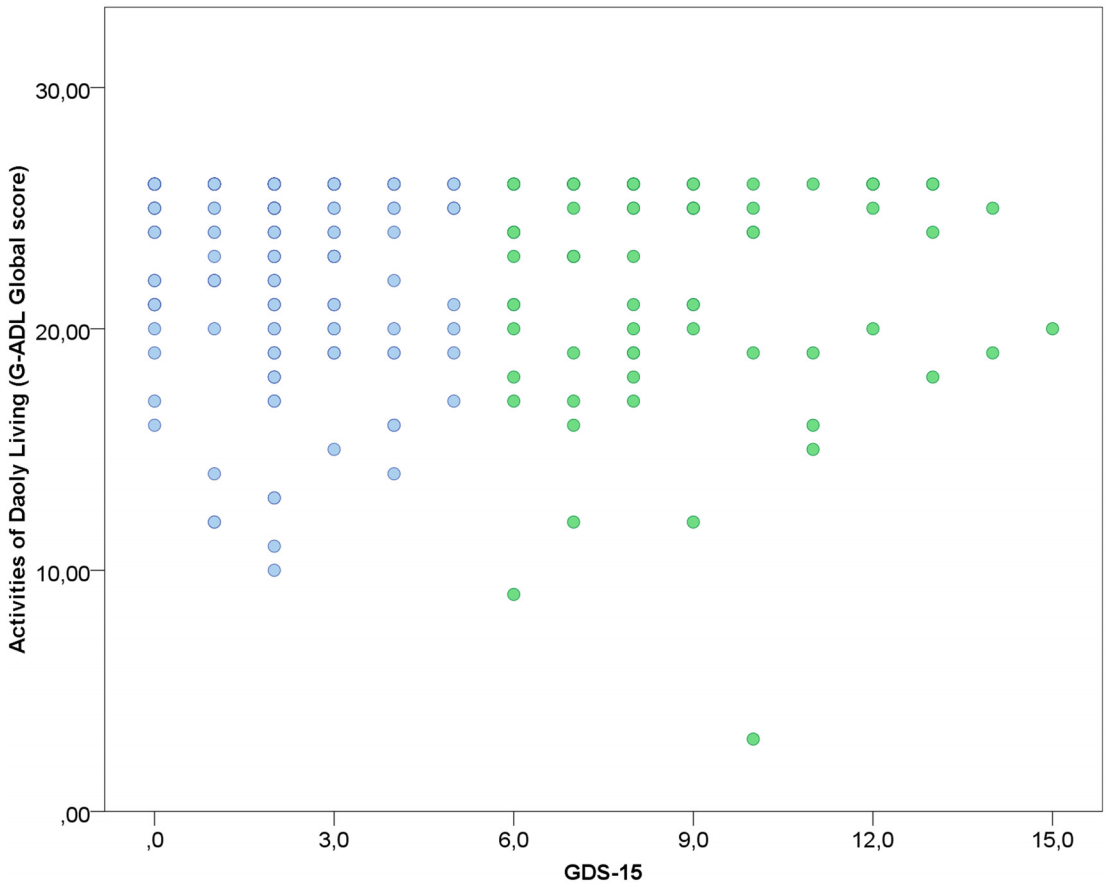

FIGURE 2 | Association between depression and GADL scores. A weak correlation was observed between depressive status and GADL total score ($r = -0.151$, $p = 0.013$). In the scatterplot, blue dots represent the participants under the cut-off for depression according to the Geriatric Depression Scale and green dots the participants above the cut-off for depression. The number of dots in the scatterplot differs from the sample size due to superposed values.

from our sample. The procedures were described in detail elsewhere (de Paula et al., 2013a). Briefly, the cognitive factors were saved by a regression method and standardized (*Z*-Score) based on the performance of our cognitively normal non-depressed participants. The four factors were *executive functions, episodic memory, language/semantic memory,* and *visuospatial Abilities*. These factors showed high internal consistency and reliability (Cronbach's alpha > 0.800 for all factors).

We carried out univariate analysis of variance (continuous variables) or chi-square tests (categorical variables) to evaluate baseline differences in sociodemographic, clinical, cognitive, and ADL measures between the AD, MCI, and control groups. Part of this data was previously published (de Paula et al., 2013a). For a preliminary assessment of the relationship between cognitive functioning, depressive level and ADL, we correlated each measure with the GADL global score. The influence of age, education, and gender in ADL was investigated by linear regression models (forced entry) containing each ADL measure as dependent variables. We also explored the pattern of association between cognitive performance and depressive symptoms through Pearson correlations to evaluate if the contribution of cognitive abilities and symptoms of depression to ADL performance is independent.

We used multiple linear regressions with a forced entry model to test whether the performance on specific cognitive domains and the intensity of depressive symptoms could predict the scores of the GADL components. *Z*-scores of executive functions, episodic memory, language/semantic memory, and visuospatial abilities, along with depressive symptoms (GDS-15 total score), were entered as independent variables in the models. In addition, we carried out ordinal regression analysis to assess whether the cognitive factors and depressive level predict the performance on each specific item of the GADL. Effect sizes were estimated by the adjusted R^2 (linear regression) or Nagelkerke Pseudo-R^2 (ordinal regression).

Results

Table 3 shows the sociodemographic, clinical data, ADL, and *Z*-scores for individual cognitive domains, according to diagnosis. Group comparisons indicate no differences in sociodemographic measures ($p > 0.05$), but significant

TABLE 4 | Linear regression models of cognitive function and depressive symptoms as predictors of different ADL.

F	df	*p*	R^2	Predictors	Standard β	*p*
GADL: Global score						
22.90	(5,268)	<0.001	29%	Executive functions	0.30	<0.001
				Episodic memory	0.25	<0.001
				Language/Semantic memory	0.18	0.011
				Visuospatial abilities	−0.13	0.084
				Depressive Symptoms	−0.08	0.140
GADL: Self-care score						
1.37	(5,268)	0.234	<1%	Executive functions	0.20	0.039
				Episodic memory	−0.02	0.780
				Language/Semantic memory	−0.01	0.931
				Visuospatial abilities	−0.14	0.116
				Depressive symptoms	−0.05	0.299
GADL: Instrumental-domestic score						
14.39	(5,268)	<0.001	19%	Executive functions	0.33	<0.001
				Episodic memory	0.18	0.009
				Language/Semantic memory	0.09	0.211
				Visuospatial abilities	−0.10	0.237
				Depressive symptoms	−0.05	0.402
GADL: Instrumental-complex score						
24.66	(5,268)	<0.001	30%	Executive functions	0.22	0.007
				Episodic memory	0.28	<0.001
				Language/Semantic memory	0.23	0.001
				Visuospatial abilities	−0.12	0.120
				Depressive symptoms	−0.08	0.108

ADL, Activities of daily living; GADL, General Activities of Daily Living Scale; df, Degrees of freedom.

differences in cognitive ($p < 0.01$), and ADL ($p < 0.05$) measures. The normal aging group outperformed both MCI and AD groups in cognitive measures, except for language/semantic memory compared with MCI patients. MCI patients showed higher scores than AD patients in all cognitive measures. Differences in ADL occurred only between AD and the other groups.

Correlations between each cognitive factor, depressive symptoms and the global score of the GADL are show in **Figures 1** and **2**. All correlations between cognitive measures and ADL were significant ($p < 0.001$) and moderate, but we found only a weak correlation between depressive symptoms and the functional measure ($r = -0.151$, $p = 0.013$). The correlations between depressive symptoms and cognitive performance were significant for executive functions ($r = -0.192$, $p = 0.001$), but we found no association with episodic memory ($r = -0.019$, $p = 0.753$), language/semantic memory ($r = -0.085$, $p = 0.162$) and visuospatial abilities ($r = -0.039$, $p = 0.525$). The influence of sociodemographic factors (age, education and gender) on ADL performance was not significant: GADL Complex ($F = 1.31$, $p = 0.272$), GADL Domestic ($F = 1.13$, $p = 0.336$), GADL Self-care ($F = 1.56$, $p = 0.200$), and the global score ($F = 2.40$, $p = 0.068$).

Table 4 shows the predictors of GADL subscales scores. For the global score of the GADL the model was significant ($F = 22.90$, $p < 0.001$, $R^2 = 0.29$) and contained as predictors executive functions ($p < 0.001$), episodic memory ($p < 0.001$), and language/semantic memory ($p = 0.011$). The Self-Care model was not significant ($F = 1.37$, $p = 0.234$, $R^2 < 0.01$). The model for Instrumental-Complex ADL was significant ($F = 24.66$, $p < 0.001$, $R^2 = 0.30$) and contained as predictors executive functions ($p = 0.007$), episodic memory ($p < 0.001$), and language/semantic memory ($p = 0.001$). The model for Instrumental-Domestic was also significant ($F = 14.39$, $p < 0.001$, $R^2 = 0.19$) and involved executive functions ($p < 0.001$) and episodic memory (p-0.009) as significant predictors.

Tables 5 and **6** show the role of specific cognitive factors and depressive symptoms as predictors of specific IADL. Since the cognitive factors and depressive symptoms were unrelated to GADL Self-Care scores, the analyses were carried out for Instrumental-Domestic and Instrumental-Complex activities only.

All Instrumental-Domestic activities were associated with executive functions ($p < 0.05$) and, except for the independence in doing personal laundry, with episodic memory ($p < 0.05$). Language/semantic memory was related only to the correct use of the telephone ($p = 0.014$). Visuospatial abilities were not significantly related to any Domestic ADL (all $p > 0.05$). Depressive symptoms were predictors of doing personal laundry ($p = 0.025$) and difficulties using the telephone ($p = 0.003$). The effect sizes for the comparisons were moderate-large, ranging from 22 to 28% of explained variance.

Episodic memory was a significant predictor of all Instrumental-Complex ADL ($p < 0.05$). Executive Functions

TABLE 5 | Ordinal regression analysis of cognitive functions and depressive symptoms as predictors of Instrumental-Domestic activities of daily living.

χ^2	df	*p*	R^2	Predictors	Est.	SE	*p*
Do simple domestic chores							
48.70	5	<0.001	26%	Executive functions	−0.74	0.22	0.001
				Episodic memory	−0.74	0.28	0.008
				Language/Semantic memory	−0.24	0.19	0.207
				Visuospatial abilities	0.49	0.25	0.065
				Depressive symptoms	0.06	0.05	0.224
Do personal laundry							
45.91	5	<0.001	24%	Executive functions	−0.72	0.20	<0.001
				Episodic memory	−0.61	0.25	0.015
				Language/Semantic memory	−0.08	0.18	0.649
				Visuospatial abilities	0.29	0.23	0.209
				Depressive symptoms	0.11	0.05	0.025
Use the telephone							
52.97	5	<0.001	28%	Executive functions	−0.57	0.20	0.005
				Episodic memory	−0.37	0.26	0.147
				Language/Semantic memory	−0.45	0.18	0.014
				Visuospatial abilities	0.14	0.24	0.558
				Depressive symptoms	0.15	0.05	0.003
Prepare meals							
42.53	5	<0.001	22%	Executive functions	−0.64	0.19	0.001
				Episodic memory	−0.47	0.24	0.046
				Language/Semantic memory	−0.01	0.17	0.978
				Visuospatial abilities	0.01	0.22	0.950
				Depressive symptoms	0.02	0.05	0.668

χ^2, Chi-Square test; df, Degrees of freedom; R^2, Nagelkerke pseudo R-Square; Est., Ordinal logistic regression model estimate; SE, Standard error.

followed a similar pattern ($p < 0.05$), but was not predictive of the individual's ability to manage finances ($p = 0.089$). Language/Semantic Memory was a significant predictor of independence in performing simple shopping ($p < 0.001$) and managing finances ($p = 0.008$). The Visuospatial abilities were a significant predictor of the ability to go out alone and use transportation ($p = 0.012$). Depressive symptoms predicted the ability to shop ($p = 0.030$), handle financial matters ($p = 0.016$), and go out alone using transportation ($p < 0.001$), but not the medication management ($p = 0.162$). The effect sizes of these models were large, ranging from 30 to 38% of explained variance.

Discussion

In the present study, we showed distinct cognitive domains having a significant impact on ADL in older adults with a wide range of cognitive deficits. Executive functioning and Episodic Memory showed the strongest significant association with functional performance. Language/Semantic Memory contributed to complex aspects of ADL and visuospatial abilities contributed only to a specific instrumental activity. Depressive symptoms had a significant influence on more complex ADL such as handling finances. Self-care ADL were not related to cognitive performance. Executive functions showed only a weak correlation with depressive symptoms. The results are in agreement with previous studies and highlight the close relationship between deficits in specific cognitive domains and functional loss.

Episodic memory and executive functions were the most important predictors of domestic ADL performance. The execution of these activities requires skills related to the identification and ordering of different steps necessary to achieve the final goal (e.g., different steps to prepare a meal) or recalling information after a period of time or in face of distractors (e.g., remembering what of house cleaning was already done and what was not). These behaviors are intrinsically related to different aspects of executive functioning and episodic memory.

Similar to our findings, previous studies showed executive functions and episodic memory tests as significant predictors of the ability to cook and to do household chores (Farias et al., 2003; Matsuda and Saito, 2005; Mariani et al., 2008). Jefferson et al. (2006) found association between verbal fluency and food preparing, cognitive flexibility, and selective attention with doing laundry, but no correlations between executive functions and house cleaning. An interesting research using extrapyramidal signs and structural brain imaging found that, controlling these previous factors and sociodemographic aspects, the performance in tests of memory and executive functions was still associated with cooking, and specific measures of executive functions with the ability to perform simple domestic chores (Bennett et al., 2006). We found an association between depressive symptoms with doing laundry. We hypothesize that this might be a sample bias. Most of the older adults assessed

in our study had a very low socioeconomic level and usually do not have laundry machines. Since they do the laundry manually, the association with depressive symptoms may be due to lack of energy or apathy since this activity is very physically demanding.

We found significant associations between executive functions, language/semantic memory, depressive symptoms, and telephone use. The engagement of these specific cognitive functions may reflect the necessity of communication to perform this activity. The neuropsychological battery used in this study included instruments related to expressive language, comprehension, and access to semantic and phonological lexicons. Therefore, we expected that ADL related to communicative skills would be influenced by the performance on these cognitive domains (Taler and Phillips, 2008; Razani et al., 2011). Farias et al. (2003), however, found motor praxis as the only predictor of telephone use. Depressive symptoms also influenced telephone use in our study. Social isolation, a common characteristic of elderly persons with depression (Corcoran et al., 2013), may reduce the individual willingness to actively pursue contact with other people, leading to impairments in this specific activity.

Two of the Complex-ADL activities involve management of finances. Episodic memory and language/semantic memory predicted financial management, while executive functions, episodic memory, and language/semantic memory predicted shopping ability. These are complex activities and involve several cognitive processes (Marson et al., 2009). Sherod et al. (2009) decomposed financial managing it in basic financial skills, financial conceptual knowledge, financial transactions, checkbook control, banking control, and financial judgment. The authors identified the cognitive predictors of financial capacity in the spectrum of normal aging, MCI, and AD using a specific questionnaire for financial management and a comprehensive battery of neuropsychological tests. Their findings suggest that arithmetic skills (which relies on working memory) are the main predictor of financial capacity. Jefferson et al. (2006) found an association between selective attention and the ability to shop and to control finances. Tasks related to episodic memory, basic math skills, and a test related to language/semantic memory predicted financial control in Matsuda and Saito (2005) study. Razani et al. (2011) evaluated skills related to the management of money and found similar predictors to the present study: how to write out checks was associated with language, control the checkbook was related to executive functions, and shopping ability was associated with memory and executive functions. Motor praxis also might be related to financial management (Farias et al., 2003). Additionally, we observed depressive symptoms predicting worse performance in the management of finances. This finding is in contrast to Farias et al. (2003) who found no significant association between depressive symptoms and management of finances.

TABLE 6 | Ordinal regression analysis of cognitive functions and depressive symptoms as predictors of Instrumental-Complex activities of daily living.

χ^2	df	*p*	R^2	Predictors	Est.	SE	*p*
Manage finances							
67.43	5	<0.001	30%	Executive functions	−0.30	0.18	0.089
				Episodic memory	−0.81	0.23	<0.001
				Language/Semantic memory	−0.43	0.16	0.008
				Visuospatial abilities	0.05	0.20	0.801
				Depressive symptoms	0.11	0.05	0.016
Shopping							
91.15	5	<0.001	38%	Executive functions	−0.42	0.18	0.018
				Episodic memory	−0.95	0.24	<0.001
				Language/Semantic memory	−0.60	0.16	<0.001
				Visuospatial abilities	0.31	0.21	0.139
				Depressive symptoms	0.10	0.05	0.030
Use of medication							
96.07	5	<0.001	38%	Executive functions	−0.71	0.18	<0.001
				Episodic memory	−0.99	0.22	<0.001
				Language/Semantic memory	−0.27	0.15	0.077
				Visuospatial abilities	0.36	0.20	0.065
				Depressive symptoms	0.06	0.04	0.162
Go out alone and use transports							
75.93	5	<0.001	33%	Executive functions	−0.51	0.18	0.004
				Episodic memory	−0.72	0.22	0.001
				Language/Semantic memory	0.23	0.20	0.252
				Visuospatial abilities	−0.40	0.16	0.012
				Depressive symptoms	0.14	0.04	0.001

χ^2, Chi-Square test; df, Degrees of freedom; R^2, Nagelkerke pseudo R-Square; Est., Ordinal logistic regression model estimate; SE, Standard error.

The correct use of medications was associated with executive functions and episodic memory in the present research in accordance with previous studies (Maddigan et al., 2003; Sino et al., 2014). A very common complaint by patients with memory impairment is to forget when to take medications or difficult to remember if he/she has already take it or not. This emphasizes the importance of different aspects of the episodic memory for the correct maintenance of medical care routine (Matsuda and Saito, 2005). Complex medication routines may demand more executive control (Maddigan et al., 2003). A study reported that performance in executive functions tests (including working memory) was a significant predictor of medication use in older adults (Insel et al., 2006). However, Jefferson et al. (2006) did not find any significant associations in this direction. Compensatory strategies might explain these discrepancies. Carlson et al. (2005) tested two objective measures of medication use capacity (schedule and pillbox) and found a significant association between memory performance and the schedule strategy and between executive functions and the pillbox.

We found executive functions, episodic memory, visuospatial skills, and depressive symptoms as predictors of ADL related to going out of home alone to distant locations using transportation. Different aspects of executive functions such as planning, cognitive flexibility, and selective attention were associated with transportation in the study of Jeferson et al. (2006). Deficits in these functions were associated with impairment in "visually" dependent activities such as driving, orientation, and transport use (Silva et al., 2009; Farley et al., 2011). However, despite the expected relationship between visuospatial abilities and the ability to travel long distances, few studies have found a significant direct association between the neuropsychological performance and its functional counterpart. Our findings provide a model in which visuospatial and navigation abilities depend on executive functions, episodic memory, and visuospatial skills. Matsuda and Saito (2005) found similar results in the Japanese population. Depressive symptoms also predicted performance in the ability to travel. As in telephone use, social isolation might mediate the association between depressive symptoms and functional performance on this task.

In our view, the strengths of the current study are the relatively large sample size, the heterogeneity of the participants and the use of fine-grained cognitive and functional measures. The use of cognitive factors validated for this population instead of tests' raw scores allow the construction of more precise conceptual models and is easier to be generalized for other settings, since the data is analyzed in the cognitive construct level and can be represented by different cognitive tests. However, the present results should be viewed in light of its limitations. Although the neuropsychological measures used comprise four specific cognitive domains, the protocol had no specific measure of processing speed, a cognitive domain related to functional performance and a potential mediator of depression influence on daily functioning (Brown et al., 2013). The executive functions factor adopted in this study may be related to processing speed in our population since processing speed and executive functions influence verbal fluency in an independent way in older adults with low formal education (de Paula et al., 2013b). Therefore, the relationship between the executive function domain and functional performance could be secondary to its processing speed component. Additional studies including specific measures of processing speed should evaluate its impact on ADL. Additionally, the present study has a cross-sectional design, which limits the interpretation of our findings. Future studies with a longitudinal design are necessary to assess the impact of changes in specific cognitive functions on the performance of specific ADL. Another aspect is the lack of an ecological functional measure since our work relies on scales of caregiver report, which may result in different results.

Conclusion

We found executive function and episodic memory as the cognitive domains most frequently related to impairment in general constructs of ADL. Nonetheless, language, semantic memory, and visuospatial abilities may influence specific functional aspects of ADL. The development of better predictive models can aid to the development of tailored and personalized rehabilitation programs to improve functional performance of subjects with neurocognitive disorders.

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REVIEW
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On the central role of brain connectivity in neurodegenerative disease progression

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Increased brain connectivity, in all its variants, is often considered an evolutionary advantage by mediating complex sensorimotor function and higher cognitive faculties. Interaction among components at all spatial scales, including genes, proteins, neurons, local neuronal circuits and macroscopic brain regions, are indispensable for such vital functions. However, a growing body of evidence suggests that, from the microscopic to the macroscopic levels, such connections might also be a conduit for in intra-brain disease spreading. For instance, cell-to-cell misfolded proteins (MP) transmission and neuronal toxicity are prominent connectivity-mediated factors in aging and neurodegeneration. This article offers an overview of connectivity dysfunctions associated with neurodegeneration, with a specific focus on how these may be central to both normal aging and the neuropathologic degenerative progression.

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Introduction

Intra-brain connectivity is indispensable for the attainment and maintenance of animal life. Genes, proteins, neurons, cell assemblies and gross brain regions all interact constantly to orchestrate the brain functions that underly sensorimotor and cognitive processing. Homeostatic mechanisms establish a basal level of functional organization upon which subtle variations are overlaid that subserve the changes in mood, attention, performance and response to external stimuli that we discern at the behavioral level. However, this delicate equilibrium may break down, particularly in the presence of neurological and psychiatric disorders where aberrant pathologic factors provoke massive alterations in connectivity at all brain levels (Konrad and Eickhoff, 2010; Bicchi et al., 2013; Iturria-Medina, 2013; Reynolds and Stewart, 2013; Gomez-Ramirez and Wu, 2014; He and Evans, 2014; Pievani et al., 2014). Recent advances in brain mapping tools, including genetics, electrophysiology and imaging techniques, with the support of new bioinformatic analysis, have extended to unprecedented levels our understanding of segregation and integration processes in the normal brain (Stam and van Dijk, 2002; Bota et al., 2003; Hagmann et al., 2007; Iturria-Medina et al., 2007; Karlebach and Shamir, 2008; Axer et al., 2011; Friston, 2011; Sporns, 2011; Evans, 2013). Additionally, they have revealed the connectional alterations associated with a wide range of psychiatric and neurological disorders (Buckholtz and Meyer-Lindenberg, 2012; Meyer-Lindenberg and Tost, 2012). Both brain disconnections and hyperconnections are commonly observed for different neurodegenerative diseases (for detailed review see Pievani et al., 2014). How disconnections and hyperconnections arise and coexist during disease progression is still not well understood. Often, disconnections

are considered a direct consequence of neurodegeneration, while hyperconnections are assumed to reflect compensatory mechanisms or spatiotemporal correlation in pathology. But such views may be a simplistic interpretation of more complex phenomena, in which brain connectivity could be playing a more causal role. In this article, we provide a brief overview of the biological mechanisms implicated in connectional dysfunctions and consequent neurodegeneration. The article is organized in four primary subsections. The first offers a brief overview of gene regulatory network alterations and their role in neurodegeneration. The second reviews the demonstrated role of the brain's structural architecture on prion-like propagation, as a main factor mediating neurodegenerative progression. The third presents and discusses the evidence supporting the neuronal activity dependent neurodegeneration hypothesis, in which functional connectivity presents an active role. The fourth integrates previous and recent findings, emphasizing the role of multimodal connections on disease spreading and progression. Finally, we highlight some outstanding questions and the challenges in building an operational model of dynamic brain organization that can account for both normal brain aging and neurodegenerative disease.

Alterations in Normal Brain Connectivity and Neurodegeneration

Deregulated Gene and Protein Networks

Gene regulatory networks control the expression levels of mRNA and proteins. Normal cellular activity depends upon the proper functioning of these networks. This makes the analysis of regulatory network dynamics a crucial step towards understanding the biological processes of health and disease (for reviews, see Bota et al., 2003; Karlebach and Shamir, 2008; Bernot et al., 2013). Aging and neurodegeneration are thought to have strong upstream genetic causes. For instance, Apoε4 and BCHE genes are considered important risk factors for the development of Alzheimer's disease (AD; Genin et al., 2011; Cramer et al., 2012; Ramanan et al., 2014). Meanwhile, increasing evidence supports the important modulatory impact of many other AD-related genes (Lambert et al., 2013). Similarly, amyotrophic lateral sclerosis (ALS) is associated with different genetic risk factors, e.g., TDP-43 and SOD, which act in combination with aging and environmental conditions (for reviews see Al-Chalabi and Hardiman, 2013; Robberecht and Philips, 2013). Such multi-factorial causes during the neuropathologic progression are common for the most prevalent neurodegenerative diseases of AD, ALS, Frontotemporal dementia (FTD), Parkinson's disease (PD) and Huntington's disease (HD). It strongly supports that "aberrant" genes do not act alone on pathologic progression but by their interaction with other collaborator genes under the influence of environmental/experience conditions (e.g., life style, epigenetic effects). Nutrition conditions have an important modulatory role on gene activities and aging disorders (Joseph et al., 2009; Bouchard-Mercier et al., 2013; Nicolia et al., 2014). For instance, caloric restriction and diet rich in anti-inflammatory and antioxidant properties have been found associated to increased longevity and preserved cognitive functioning (Roth et al., 2002; Colman et al., 2009; Joseph et al., 2009; Stice et al., 2013; Crichton et al., 2013; Sezgin and Dincer, 2014). *Nutri-epigenomics* science focus on the influence of nutrition on epigenetic modifications and its consequences on health (Gallou-Kabani et al., 2007), which ideally should contribute to develop effective nutrition-based therapeutic interventions. Modern gene expression profiling techniques allow us to quantify gene-specific activity across different tissues and time points (O'Driscoll, 2011). As a result, genetic interaction occurring between regions of interest can be characterized by means of sophisticated statistical concepts and tools (Bota et al., 2003; Karlebach and Shamir, 2008; Bernot et al., 2013), thereby contributing to our understanding of how regulatory networks are involved in disease progression (Crespo et al., 2012; Narayanan et al., 2014; Leiserson et al., 2015). In the context of brain degeneration, Zhang et al., 2013, reported a remarkable example of causal gene-gene pathologic interaction. These authors used gene expression profiles of the prefrontal cortex to identify regulatory networks causally associated with late onset AD. They identified an immune and microglia-specific gene module that is strongly regulated by the gene TYROBP, directly associated to Amyloid-β (Aβ) turnover and neuronal damage. This TYROBP causal network (**Figure 1A**), characterized in detail by means of Bayesian network analysis, showed a direct modulatory effect on late onset AD gene networks, which was verified not only in human brain but also in an experimental animal model. A salient finding was that the differential gene expression observed for late onset AD presented a distance-dependent relationship with TYROBP (**Figure 1B**). Those genes with a higher functional association with TYROBP are more likely to be altered during the disease process, as well as to propagate the pathologic effects to their connected neighbors. Although this characteristic gene network regulatory effect (Zhang et al., 2013) needs further exploration and validation in other neurodegenerative diseases, it illustrates how connectional links at the molecular level can mediate disease propagation.

Inter-Cellular Misfolded Proteins Propagation Across Structural Pathways

Proteins that fail to configure properly are called misfolded proteins (MP). Historically, they have been causally associated with aging and several human neurodegenerative diseases (Braak and Braak, 1991; Dobson, 2002, 2003; Braak et al., 2004). The prion-like hypothesis proposes that cell-to-cell transmission of toxic MP is a principal cause of neurodegeneration (Frost et al., 2009; Brundin et al., 2010; Hallbeck et al., 2013). Increasing neuropathologic evidence supports the spread of MPs from initial host regions to anatomically connected areas, spreading and simultaneously re-seeding the toxic effects (Frost et al., 2009; Waters, 2010; Nath et al., 2012; Jucker and Walker, 2013; Song et al., 2014). This fact, combined with recent evidence supporting the notion that each neurodegenerative disorder is associated with a characteristic group of MPs (Brundin et al., 2010), motivated in part the network degeneration hypothesis (NDH; Seeley et al., 2009). This hypothesis proposes that each disorder should present disease-specific anatomic,

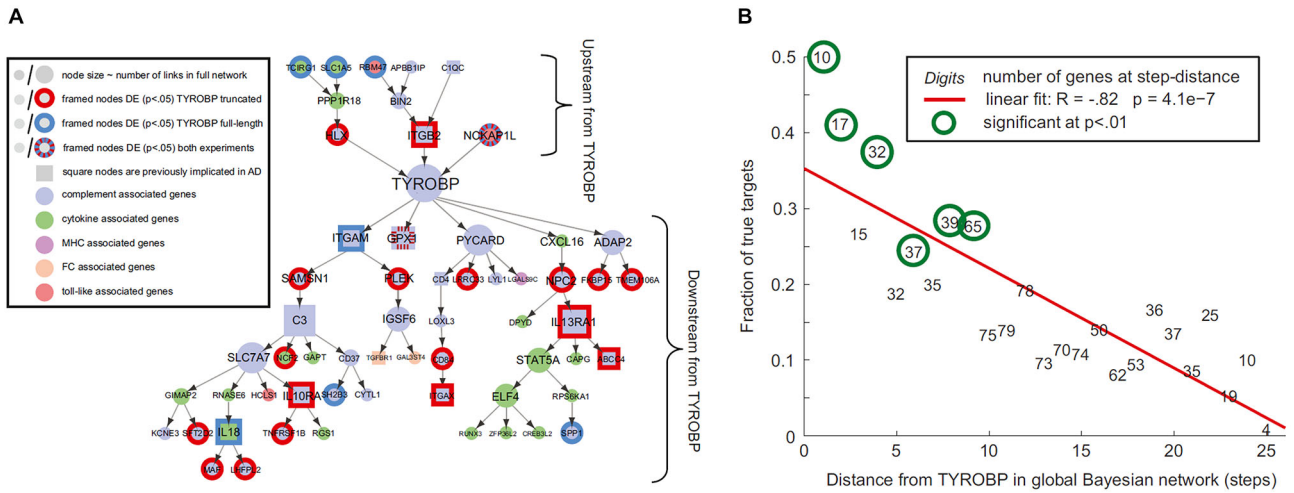

FIGURE 1 | Connectivity distances to pathologic epicenters predict diseases effects. **(A)** TYROBP causal network in late onset Alzheimer's disease (AD), **(B)** differential expression levels of deregulated genes associated with TYROBP at various functional distances from it. Note the negative association ($R = -0.82$; $P < 10^{-3}$) implying increasing impact with proximity to TYROBP. Figure adapted from Zhang et al. (2013), with permission from Elsevier.

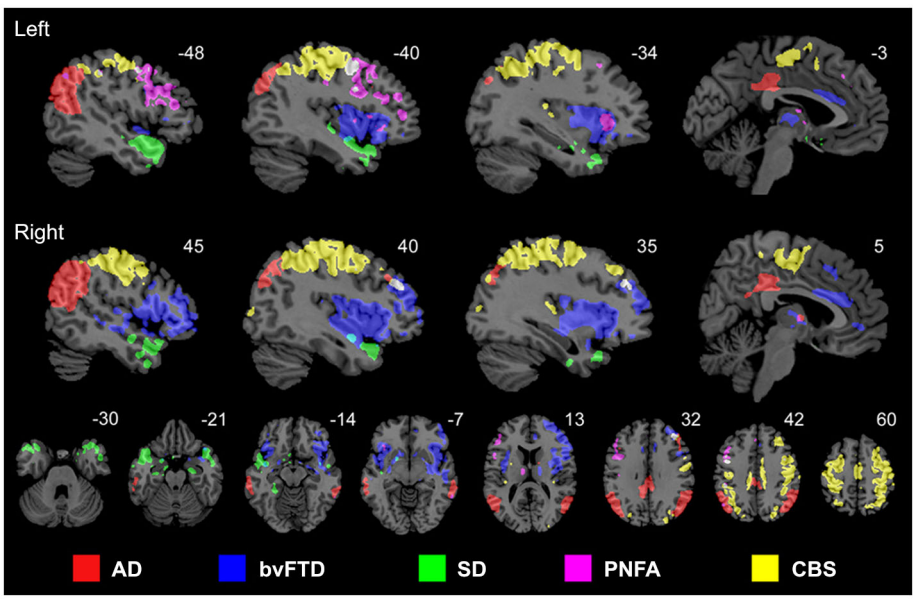

FIGURE 2 | Anatomically dissociable networks targeted by five different neurodegenerative disorders: AD, behavioral variant frontotemporal dementia (bvFTD), semantic dementia (SD), progressive nonfluent aphasia (PNFA), and corticobasal syndrome (CBS). Figure adapted from Seeley et al. (2009), with permission from Elsevier.

functional and metabolic pathways. Seeley and colleagues used MRI to demonstrate that different neurodegenerative disorders are associated with spatially dissociable atrophy patterns, each pattern corresponding to a consistent structural covariance and functional sub-network (**Figure 2**; Seeley et al., 2009). In a complementary study (Zhou et al., 2012), the same group showed that regions with higher connectivity with, and shorter functional distances to, disease-specific epicenters presented greater structural atrophy. Raj et al., 2012, introduced a diffusion network model of intra-brain MP propagation, according to which the increase over time of the number of diseased afferents from a given brain region to any other region depends upon the disease concentration factor in both regions and upon the anatomical connection strength between them (Raj et al., 2012). From this model, an analytical expression for structural atrophy dynamics was obtained. After a mathematical

decomposition of a healthy brain anatomical connectome, the authors found a significant correspondence between specific dissociable connectivity modules and the characteristic atrophy patterns of different neurodegenerative diseases (AD, behavioral variant FTD [bvFTD]). Each connectivity module's weight in the initial connectome was inversely proportional to the population prevalence of a specific disorder (AD, bvFTD or HD). This suggested that the final structural atrophy pattern in adulthood could be the weighted combination of characteristic atrophy patterns from prevalent neurodegenerative diseases, in which each disease-characteristic pattern is weighted by the individual predisposition to express such disease. In general, these three seminal studies (Seeley et al., 2009; Raj et al., 2012; Zhou et al., 2012) supported the NDH, as well as the structural and functional connectivity-mediated spread of neuropathologic effects. However, neurodegenerative gray matter atrophy patterns may not be uniquely provoked by MP toxicity. Other pathologic factors, such as neuronal activity toxicity, and metabolic and vascular deregulations (see below) may contribute to cell death.

In order to obtain straight evidence of MP spread as a function of anatomical proximity to a disease propagation epicenter, Iturria-Medina et al. (2014), analyzed PET Aβ deposition patterns in 733 healthy and diseased brains. Motivated by the remarkable similarity between intra-brain pathology propagation and the spread of human infectious diseases in social networks, we hypothesized that MP dynamics can be mathematically described and characterized by the epidemic-like interactions between infection agents (the aberrant proteins) and the brain's defense response, mediated by the brain's anatomical architecture (Iturria-Medina et al., 2014). The proposed epidemic spreading model (ESM) reproduced Aβ patterns from healthy to advanced diseases states, allowing the reconstruction of individual lifetime histories of intra-brain Aβ propagation, and the subsequent analysis of the biological factors that promote such propagation/deposition (e.g., the relationship of clinical state with MP production and/or clearance). When exploring the relation between regional Aβ deposition pattern and the connectional proximity to the Aβ outbreak regions, as identified by the ESM (anterior and posterior cingulate cortices), a significant negative linear trend was observed (**Figure 3A**), with more advanced disease states corresponding to higher deposition. Also, a significant negative relation between regional anatomical connectivity degree and Aβ arrival time (measures

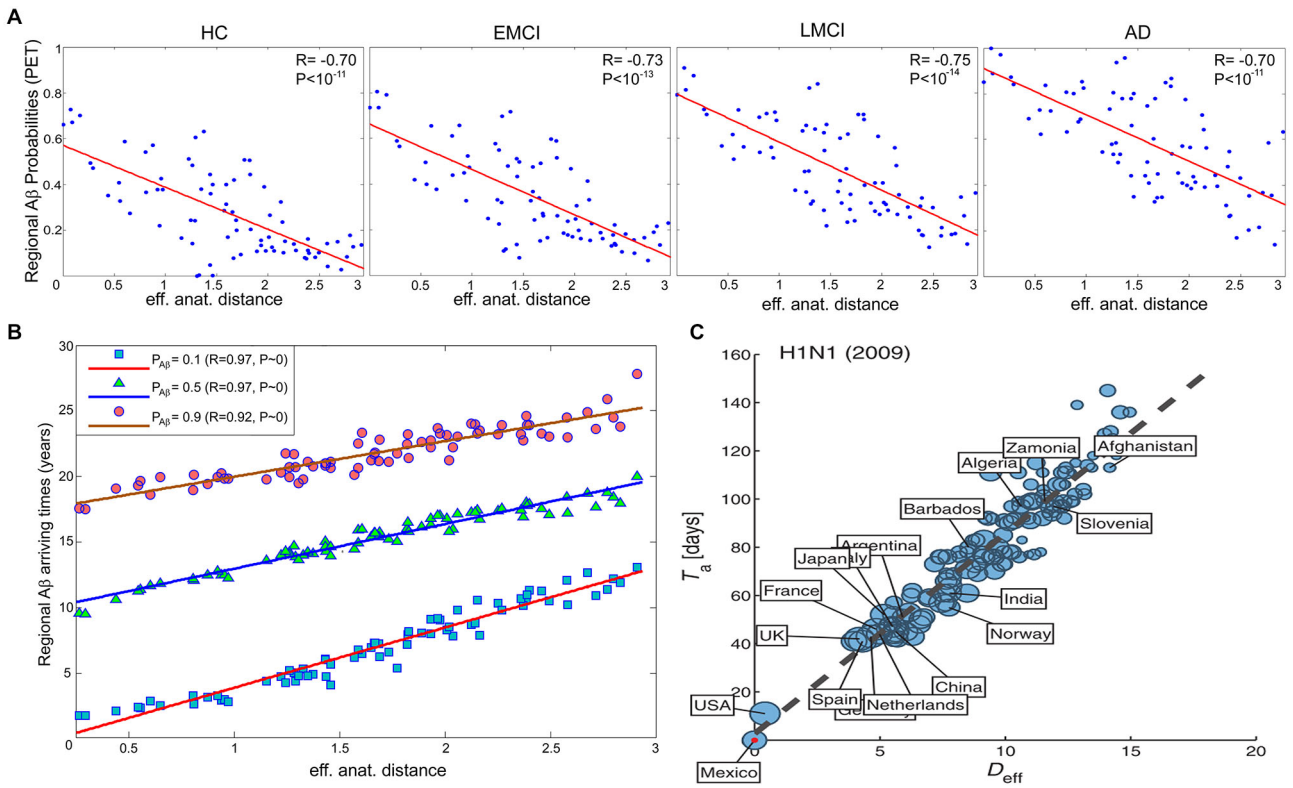

FIGURE 3 | In brain and social networks, effective proximity to an epicenter modulates the propagation of aberrant factors. **(A)** PET-based regional Aβ deposition probabilities for different clinical groups (healthy control (HC), early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI) and AD) vs. effective anatomical distances to the identified Aβ outbreak region (anterior and posterior cingulate cortices). **(B)** Regional Aβ arriving times vs. effective anatomical distances, for different Aβ probability thresholds (i.e., 0.1, 0.5 and 0.9). **(C)** N1H1 pandemic arrival time vs. effective distance (D_{eff}) to outbreak country (i.e., Mexico). In **(C)**, the effective distance was computed from the projected global mobility network between countries. Panels **(A,B)** and **(C)** were adapted with permission from Iturria-Medina et al. (2014), and Brockmann and Helbing (2013) respectively.

of hubness and disease vulnerability) was observed (**Figure 3B**). This relation was independent of the selection of different Aβ deposition thresholds, indicating that regions with a higher degree of anatomical connectivity experience earlier Aβ arrival and, consequently, larger periods of exposure to the toxic effect of the aberrant protein. Interestingly, and supporting the hypothesis of an epidemic spreading behavior for MP propagation, a similar linear predictive relationship has been reported for effective distance in human social networks and disease arrival times for real epidemic propagation of infectious disease (Brockmann and Helbing, 2013) (e.g., 2009 H1N1 pandemic; see **Figure 3C**).

Also in line with the prion-like hypothesis, the phosphorylated 43 kDa TAR DNA-binding protein (pTDP-43) has been identified as a major neuropathologic factor in ALS and frontotemporal lobar degeneration (Neumann et al., 2006; Geser et al., 2009). Recently, Brettschneider et al. (2013), identified four characteristic stages of pTDP-43 neuropathology in ALS, which suggested a sequential pTDP-43 intra-brain dissemination pattern (Brettschneider et al., 2013). Schmidt et al. (2015), found a dense level of anatomical connectivity between the regions of these four pTDP-43 stages. These authors also used a computational random walker spread model to simulate axonal spread of the pTDP-43 factor as a walking particle along the white matter pathways (Schmidt et al., 2015). Consistent with the hypothesis that pTDP-43 pathology is propagated along axonal pathways, they observed a significant overlap between the simulated pTDP-43 patterns and the sequential distribution found previously in ALS autopsy cases.

Neuronal Activity-Dependent Neurodegeneration

The upstream causal role of MP on neurodegenerative disorders is currently under scientific controversy (Soto and Castilla, 2004; Hilker et al., 2011). MP presence do not always correlate well with structural atrophy and/or cognitive decline levels, whereas therapeutic drugs created to reduce MP levels have demonstrated poor modulatory effects on disease progression (Holmes et al., 2008). Different alternative hypotheses have been proposed in order to fit the inconsistencies of the MP prion-like assumptions. For example, the Caspase-6 neurodegeneration hypothesis of AD (LeBlanc et al., 1999; Albrecht et al., 2007; LeBlanc, 2013), explains cell inflammation and death by the stress-associated action of the Caspase-6 enzyme. Caspase-6 activation modulates also Aβ and phosphorylated tau concentrations, which are strongly associated to the stress in neurons and cell lines. Moreover, consistent evidence suggests that abnormal neuronal and synaptic activity may modulate brain MP levels (Kamenetz et al., 2003; Cirrito et al., 2005, 2008; Buckner et al., 2009; Bero et al., 2011). For instance, exogenous increases in neuronal and synaptic activity in the hippocampus, induced by electric stimulation, increase the extracellular Aβ concentrations in that region (Cirrito et al., 2005). Also, endogenous neuronal activity changes have an equivalent impact on Aβ concentrations (Bero et al., 2011), suggesting that regional differences in basal neuronal activity levels could explain regional vulnerabilities to Aβ presence and toxicity. From these facts arise some relevant questions: can aberrant neuronal/synaptic activity have an upstream role in neurodegenerative progression, and, importantly, is functional connectivity a mediator of neuronal/synaptic toxicity spreading? Motivated by these questions, de Haan et al. (2012), used neural mass modeling to explore local neuronal activity in relation to large-scale connectivity in normal and abnormal conditions. For this, the authors simulated neural dynamics using a real structural brain connectome, and induced progressive damage to the regions based on their level of activity. The results suggested that, in no-task conditions, hubs should be the most active regions (due to the convergence of heteromodal activity), and also that excessive connectivity-dependent neuronal activity can have a significant role in the neurodegenerative progression, thus explaining the associated hub vulnerability (de Haan et al., 2012). Previously, a robust relationship between regional hubness (in terms of functional connectivity) and Aβ depositions had been reported (Buckner et al., 2009), whereas functional hyperconnectivity, mainly between cingulate and medio-temporal regions, had been associated with semantic memory deficits (Gardini et al., 2015). Similarly, in AD, regional metabolic alterations had been found to follow Aβ presence in many brain regions (Förster et al., 2012), whereas the spatial mismatch between these two pathologic components can be explained by functional connection to Aβ binding areas (Klupp et al., 2014). This means that non-Aβ areas can also be metabolically deregulated during disease progression if those areas are functionally linked to Aβ and/or functionally-impaired zones. Moreover, derangement of metabolic connectivity patterns have been associated with elevated Aβ burden (Carbonell et al., 2014a,b), while a significant modulatory impact of the Apoε4 genotype on hypometabolism had been observed (Jagust and Landau, 2012; Carbonell et al., 2014a). All together, these results support the contention that neuronal/synaptic toxicity spreading in neurodegeneration, and associated activity-dependent deregulation of local MP and metabolic levels, strongly depend on anatomic, functional and metabolic brain connectional patterns.

Towards a Multi-Factorial Disease Spreading Perspective

Although generally associated with specific hypotheses, previously proposed pathologic mechanisms are not unrelated. In addition to have a modulatory impact on protein expression, gene activity is markedly associated with structural connectivity patterns (French and Pavlidis, 2011; Wolf et al., 2011a; Ji et al., 2014; Fakhry and Ji, 2015) and synaptic density dynamics (Goyal and Raichle, 2013). This suggests that alterations in gene regulatory networks or aberrant signal spreading across them may induce important changes in structural, functional and metabolic brain patterns, even as an additional downstream effect of a main genetic pathologic factor. Similarly, strong associations persist among different forms of brain connectivity, under normal or abnormal conditions. The vascular and metabolic/functional systems represent a remarkable example. Among other relevant functions, the vascular system supplies oxygen, glucose and other nutrients, and clears away deoxygenated blood and metabolic products (Scremin, 2012). These functions are essential to satisfy daily

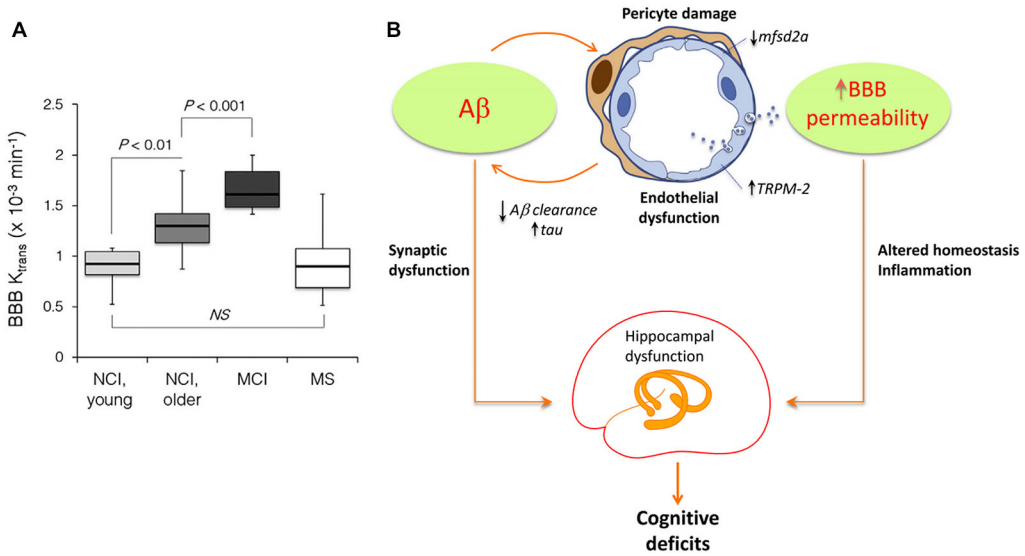

FIGURE 4 | Age-dependent blood-brain barrier (BBB) permeability breakdown may be causally associated with neurodegenerative spreading (Panels (A) and (B) were adapted from Montagne et al. (2015), Iadecola (2015), respectively, with permission from Elsevier). **(A)** Significant increases in BBB permeability in older compared young group of individuals with no cognitive impairment (NCI), and MCI compared to older NCI group in the entire hippocampus. Multiple sclerosis (MS) patients with no cognitive impairment were comparable with the age-matched young NCI group (Montagne et al., 2015). **(B)** Hypothetical pathologic mechanisms by which Aβ may induce BBB permeability alterations and hippocampal/cognitive dysfunction (Iadecola, 2015). Aβ affects endothelial cells, damaging pericytes, vesicular transport and Ca^{+} balance. This contributes to BBB disruption, homeostasis alterations, reduction on misfolded proteins (MP) clearance and tentatively to hippocampal dysfunction, cognitive deficits and intra-brain pathology spreading.

neuronal/glial energy and maintenance demands. However, this close association dates from initial neurodevelopmental processes: axon-guidance cues mediate the navigation of blood vessels along predestined tracks during development (Carmeliet and Tessier-Lavigne, 2005; Zacchigna et al., 2008), whereas angiogenic vascular endothelial growth factor regulates the migration of various neuron types to their final destination (Schwarz et al., 2004; Zacchigna et al., 2008). Recently, Lacoste et al. (2014), combined genetics, imaging and computational tools to verify that neural activity changes can modulate vascular networks. They found that decrease or enhancement of neural activity (by deafferentation, (de)stimulation or genetic impairment of neurotransmitter release) leads to equivalent effects in vascular density and branching (Lacoste et al., 2014). Together, these facts explain the anatomical positioning and behavioral similarities that have also been uncovered among the vascular and the functional/metabolic pathways (Melie-García et al., 2013; Jann et al., 2015). Moreover, the vascular system plays a major role in aging and associated neurodegenerative processes (Zacchigna et al., 2008; Quaegebeur et al., 2011; Iadecola, 2013). Capillary density loss and other vascular abnormalities have been consistently observed in healthy aging, AD, leukoaraiosis (LA) and HD (Brown and Thore, 2011; Wolf et al., 2011b). Damage to vascular network integrity leads to MP clearance deficits and resultant deposition. For instance, the efflux across the blood-brain barrier (BBB) contributes Aβ clearance (Deane et al., 2009; Qosa et al., 2014). Qosa et al. (2014), reported that around a 60% of soluble $A\beta^{40}$ is cleared across BBB while the remaining is cleared by brain degradation. Consistent with this thesis, a significant age-dependent BBB permeability breakdown, that correlates with cognitive dysfunction, has been observed in human hippocampus (Montagne et al., 2015; see **Figure 4A**). Such aging effects have a crucial impact on BBB-mediated MP clearance and deposition (Iadecola, 2015; see **Figure 4B**), contributing to structural, functional and metabolic connectional deregulation in a continuous degenerative cycle. In addition, brain neuroinflammation is characteristic feature during neurodegeneration (Streit et al., 2004; Block et al., 2007; Lull and Block, 2010). It is particularly associated to microglia cells activity, which reacts defensively in respond to different events, such as infection, brain injury or associated autoimmune processes (Gendelman, 2002). Under certain pathologic conditions (ex. presence of environmental toxins or neuronal damage), microglias can enter to a hyperactivation state and release excessive reactive oxygen species (ROS), which cause neurotoxicity and cell death (Block et al., 2007; Lull and Block, 2010). Then, local pathologic effects associated to microglia-mediated neuroinflammatory processes may impact other connected areas. Similarly that with the region-region transmission of previously mentioned aberrant factors (ex. MP, toxic neuronal/synaptic signals, metabolic deregulation, BBB damage), functional/metabolic impairment and neuronal death in a given brain region, due to neuroinflammation and ROS, may alter its vascular, functional, metabolic and anatomical links, and gradually the multi-factorial subnetworks associated to the connected regions, extending the negative neuroinflammatory effects across the interconnected brain.

Discussion and Conclusions

Converging evidence supports the central role of brain connectivity in neurodegenerative progression. Abnormal connectivity might not only be involved in the propagation of downstream effects, it might also support upstream pathologic causes (Pievani et al., 2014). This supports the strategic importance of understanding the role of brain connectivity in disease evolution. Notably, the finding of patterns of pathology that reflect known structural connectivity, suggests the active role of specific epicenter nodes during the disease processes (e.g., deregulated genes, cell assemblies, and/or gross regions). In social networks, the presence of individuals with a disproportionately large number of contacts (social hubs) accelerates considerably the spread of infectious disease (Newman, 2002; Lloyd-Smith et al., 2005; Leventhal et al., 2015). This hub-centric behavior could be also be a feature of intra-brain pathologic propagations, not only limited to neurodegeneration but also present in other disorders (e.g., schizophrenia, epilepsy, Asperger's syndrome). A recent meta-analysis study of 26 different brain diseases showed that disease-specific structural lesions were mainly located on connectivity hub regions (see **Figure 5**). This hub vulnerability could be a consequence of the high topological centrality and biological cost of the hubs, that make them more sensitive to a diverse range of pathogenic processes (Crossley et al., 2014). In addition, we showed that brain regions with a higher degree of anatomical connectivity experience early Aβ arrival and larger periods of Aβ exposition (**Figure 3B**; Iturria-Medina et al., 2014), which, in addition to excessive connectivity-dependent neuronal activity (de Haan et al., 2012), explains the higher Aβ deposition levels found on functional hubs (Buckner et al., 2009).

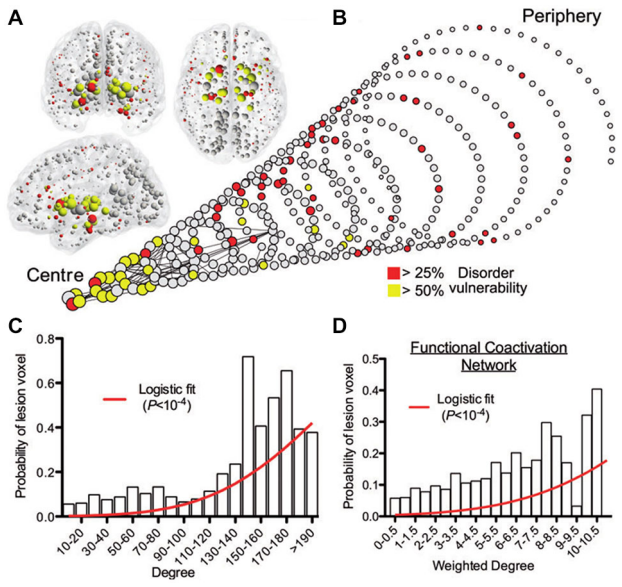

FIGURE 5 | Gray matter lesions identified on 26 clinical brain disorders impact mainly on the structural and functional hub regions. **(A)** Nodes of the normative structural connectome, represented in anatomical space, with nodes size reflecting connectivity degrees. **(B)** Spiral representation of the region vulnerability vs. hubness relationship. Nodes of similar degree are arranged in the same circle, and the different circumferences arranged so that the tip of the spiral has the highest degree hub nodes, while the base the most peripheral nodes. Nodes sizes are proportional to their connectional degree, with colors reflecting each region's lesioned percentage. The strongest 0.1% of edges between nodes, which highlight pairs of nodes with consistently high number of streamlines interconnecting them, are shown for illustrative purposes. **(C)** Plot of the probability of lesion voxels (*y*-axis) vs. connectivity degree for structural connectome nodes (*x*-axis). The red line is a fitted logistic regression model. **(D)** Plot of the probability of lesion voxels (*y*-axis) vs. the degree of the functional co-activation network nodes (*x*-axis). Figure adapted from Crossley et al. (2014), with permission.

In spite of its biological relevance, it is not totally clear yet how to quantify the role of dynamic connectivity in disease evolution. For instance, although MP propagation have been modeled and studied by means of diffusion networks (Raj et al., 2012) and epidemic-like spreading (Iturria-Medina et al., 2014), the predictive power of these models still needs further validation. Similarly, there are promising advances in the modeling and understanding of neuronal/synaptic spreading across structural networks (Sotero et al., 2007; Valdes-Sosa et al., 2009; Sanz-Leon et al., 2013, 2015; Messé et al., 2015), but these methodologies also require additional predictive validation. Increasing evidence supports that gender have a substantial impact on structural and functional brain connectivity (Gong et al., 2011), which have been suggested to explain specific gender-related cognitive differences (Ingalhalikar et al., 2014). Gender is also associated to the risk of develop specific neurodegenerative diseases. For example, women are more likely to develop AD than men (Farrer et al., 1997; Damoiseaux et al., 2012), whereas men present a significant higher risk to develop PD (Wooten et al., 2004). Thus, in order to reach a realistic operational model of dynamic brain organization during aging and degeneration, it is essential to clarify how gender-related differences, from genetic, molecular, structural and/or functional levels, might modulate the role of brain connectivity in disease development and progression. I addition, it is still unclear if a given connectivity change should be interpreted as the result of a pathologic induced alteration or as the outcome of a compensatory change. The current lack of multi-factorial trajectory analysis and particularly the absence of robust causal models of disease progression, make still unfeasible to discriminate between connectivity associated upstream and downstream effects. A decreased connection could be reflecting either a pathologic alteration or a counteracting compensation mechanism, whereas an increased connection could be responding either to a compensatory mechanism or to a pathologic spreading effect. Importantly, it is also unclear how our understanding of the role of connectivity in disease progression could be translated into the development of effective therapeutic strategies. As Zhang et al. (2013) point out, targeting highly-connected genes in deregulated gene networks may be effective in disrupting disease-related networks for the purpose of therapy, but that could be at the cost of unknown adverse effects. How to diminish the outcome of negative effects after possible therapeutic interventions is still the subject of much scientific debate, Computer simulation modeling could help considerably the exploration of the effects of intervention strategies. For example, Proctor et al. (2013) modeled DNA

damage, p53/GSK3 regulation, Aβ and tau dynamics to predict the intervention effects of Aβ immunization. However, in order to extend the simulation analyses, we will need a deeper understanding of the genetic, protean, metabolic, vascular, functional and structural aberrant interactions associated with aging and neurodegeneration.

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Differences in functional brain connectivity alterations associated with cerebral amyloid deposition in amnestic mild cognitive impairment

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Despite potential implications for the early detection of impending Alzheimer's disease (AD), very little is known about the differences of large-scale brain networks between amnestic mild cognitive impairment (aMCI) with high cerebral amyloid-beta protein (Aβ) deposition (i.e., aMCI+) and aMCI with no or very little Aβ deposition (i.e., aMCI−). We first aimed to extend the current literature on altering intrinsic functional connectivity (FC) of the default mode network (DMN) and salience network (SN) from cognitively normal (CN) to AD dementia. Second, we further examined the differences of the DMN and the SN between aMCI−, aMCI+, and CN. Forty-three older adult (12 CN, 10 aMCI+, 10 aMCI−, and 11 AD dementia) subjects were included. All participants received comprehensive clinical and neuropsychological assessment, resting-state functional magnetic resonance imaging, structural MRI, and Pittsburgh compound-B-PET scans. FC data were preprocessed using multivariate exploratory linear optimized decomposition into independent components of FMRIB's Software Library. Group comparisons were carried out using the "dual-regression" approach. In addition, to verify presence of gray matter volume changes with intrinsic functional network alterations, voxel-based morphometry was performed on the acquired T1-weighted data. As expected, AD dementia participants exhibited decreased FC in the DMN compared to CN (particularly in the precuneus and cingulate gyrus). The degree of alteration in the DMN in aMCI+ compared to CN was intermediate to that of AD. In contrast, aMCI− exhibited increased FC in the DMN compared to CN (primarily in the precuneus) as well as aMCI+. In terms of the SN, aMCI− exhibited decreased FC compared to both CN and aMCI+ particularly in the inferior frontal gyrus. FC within the SN in aMCI+ and AD did not differ from CN. Compared to CN, aMCI− showed atrophy in bilateral superior temporal gyri whereas aMCI+ showed atrophy in right precuneus. The results indicate that despite the similarity in cross-sectional cognitive features, aMCI− has quite different functional brain connectivity compared to aMCI+.

Keywords: amnestic mild cognitive impairment, amyloid-beta deposition, brain functional connectivity, default mode network, salience network

INTRODUCTION

Mild cognitive impairment (MCI) refers to the clinical state of cognitive decline that is greater than expected for a given age and educational attainment but does not interfere with the activities of daily living. In general, MCI is considered as a transitional stage or an intermediate state between normal aging and dementia. Particularly, amnestic MCI (aMCI) has been considered as a prodromal stage of Alzheimer's disease (AD) dementia (Morris, 2006). Among aMCI individuals, however, nearly half does not show abnormal levels of cerebral amyloid-beta (Aβ) accumulation, which is considered as the hallmark of AD (Price et al., 2005; Rowe et al., 2007; Wolk et al., 2009; Nordberg et al., 2013).

Given that Aβ cerebral deposition is considered as a necessary pathological process of AD (Hardy and Selkoe, 2002; Villemagne et al., 2008), aMCI with high levels of Aβ deposition (aMCI+) may more specifically be the prodromal state of AD dementia compared to aMCI with low levels of Aβ deposition (aMCI−), which may be associated with pathophysiological processes other than AD. However, only a few studies investigated the differences in the clinical or neuroimaging characteristics of aMCI+ and aMCI−. Characterizing the differences between aMCI+ and aMCI− is anticipated to shed light on the underlying mechanisms of each state more specifically.

In the past several years, converging pieces of evidence from structural and functional magnetic resonance imaging (MRI)

studies suggested that AD affects specific large-scale brain networks. Particularly, the studies using resting-state functional magnetic resonance imaging (rs-fMRI) – an imaging method that measures functional connectivity (FC, i.e., synchronous ongoing brain activity) between spatially distinct brain regions – have shown that individuals with an early stage of AD dementia or aMCI have disruptions in FC between the structures that are parts of the network referred to as the default mode network (DMN) (Biswal et al., 1995; Raichle et al., 2001; De Luca et al., 2006; Wang et al., 2006; Fox and Raichle, 2007; Sorg et al., 2007; He et al., 2009; Smith et al., 2009; Scholvinck et al., 2010). The DMN, which is comprised of a set of brain regions that are active during rest and deactivated when engaged in cognitively demanding tasks, also shows a striking overlap with the brain regions with high Aβ deposition in AD (Buckner et al., 2005; Hedden et al., 2009; Sperling et al., 2009; Sheline et al., 2010).

In addition to the DMN, abnormal activity within the salience network (SN) – comprised of paralimbic structures such as insula and anterior cingulate cortex – is implicated as another indicator of different neurodegenerative diseases, such as frontotemporal dementia (FTD) (Zhou et al., 2010; Farb et al., 2013). Furthermore, the interaction between the DMN and the SN is thought to be important in generating controlled behavior, particularly for the role of the SN in disengaging the DMN when on a task (Rilling et al., 2008; Sridharan et al., 2008; Sharp et al., 2010; Bonnelle et al., 2012).

Despite a growing literature on disruptions of the DMN and the SN in AD and other neurodegenerative conditions, little is known about differential alterations of the DMN or the SN between aMCI+ and aMCI−. In this context, we first aimed to expand current literature on changes in connectivity strengths of the DMN and the SN from cognitively normal (CN) to AD process by specifically including the analysis of aMCI+ rather than overall MCI. Second, we examined the FC differences in the DMN and the SN between aMCI+ and aMCI− by comparing them to CN as well as to each other.

MATERIALS AND METHODS

PARTICIPANTS

Forty-three older adults (12 CN, 20 aMCI, and 11 AD) were recruited from a dementia clinic of the Seoul National University Hospital. CN subjects did not have subjective or reported cognitive complaints, had Clinical Dementia Rating (CDR) score of 0 (Morris, 1993) and the Mini-Mental State Examination (MMSE) score greater than or equal to 26 (Lee et al., 2002), and performed within the normal range on comprehensive neuropsychological assessment. Individuals with aMCI met Petersen's criteria (Petersen, 2004): (a) memory complaint corroborated by an informant; (b) objective memory impairment for age, education, and gender; (c) essentially preserved general cognitive function; (d) largely intact functional activities; and (e) not demented. All aMCI individuals had an overall CDR (Morris, 1993) of 0.5. In terms of the criterion (b), a performance score for at least one of the four episodic memory tests included in the Consortium to Establish a Registry for Alzheimer's disease (CERAD) neuropsychological battery [namely, Word List Memory (WLM), Word List Recall (WLR), Word List Recognition (WLRc), and Constructional Recall (CR) test] (Morris et al., 1989; Lee et al., 2002) was at least 1.5 SD below the respective age-, education-, and gender-specific normative mean (Lee et al., 2004). Patients diagnosed with AD dementia met the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for the probable AD (McKhann et al., 1984).

The exclusion criteria for all subjects were: (a) any present serious medical, psychiatric, and neurological disorders that could affect the mental function; (b) evidence of focal brain lesions on MRI; (c) the presence of severe behavioral or communication problems that would make a clinical examination or brain scans difficult; (d) left-handedness; (e) absence of a reliable informant; and (f) illiteracy. Individuals with minor physical abnormalities (e.g., diabetes with no serious complications, essential hypertension, and mild hearing loss) were included. The Institutional Review Board of the Seoul National University Hospital, South Korea, approved the study, and subjects or their legal representatives gave written informed consent.

CLINICAL AND NEUROPSYCHOLOGICAL ASSESSMENT

All participants were administered a standardized clinical assessment according to the protocol of the Korean version of the CERAD Assessment Packet (Lee et al., 2002). The CERAD neuropsychological battery and the Stroop Test (Seo et al., 2008) were administered by experienced clinical neuropsychologists. Reliable informants provided information regarding participants' cognitive, emotional, and functional changes as well as medical history. A panel of psychiatrists and a clinical neuropsychologist with expertise in dementia research made clinical decisions on the diagnoses.

PiB-PET IMAGE ACQUISITION AND PREPROCESSING

Participants underwent Carbon-11-labeled PiB (^{11}C-PiB) PET imaging using the ECAT EXACT 47 scanner (Siemens-CTI, Knoxville, TN, USA), which has an intrinsic resolution of 5.2-mm full width at half maximum (FWHM) and the images of 47 contiguous transverse planes with a 3.4-mm thickness for a longitudinal field of view of 16.2 cm. Before administering ^{11}C-PiB, a 10-min transmission scanning was performed using rotating three germanium-68 rod sources to correct the attenuation. Sixty minutes after the intravenous injection of 370 MBq ^{11}C-PiB, three 10-min frames of data acquisition were started and later summed into a single frame (60–90 min). All the data were reconstructed in $123 \times 128 \times 47$ matrix with a pixel size of 2.57 mm × 2.57 mm × 3.75 mm using the filtered back projection method with Shepp–Logan filter (cutoff = 0.35 cycle/pixel), and reconstructed images were corrected for attenuation and rearranged onto transaxial, sagittal, and coronal images.

The details of PiB quantification image analyses were described previously (Choo et al., 2011). Briefly, image preprocessing for statistical analyses was performed using SPM2 implemented in MatLab. ^{11}C-PiB-PET data of each subject were co-registered to individual volumetric magnetic resonance image and then automatically spatially normalized into the standard MNI template in SPM2 using transformation parameters derived from the normalization of individual magnetic resonance image to the

template. All normalized images were reformatted with a voxel size of 2 mm × 2 mm × 2 mm. For quantitative normalization of cerebral ^{11}C-PiB uptake values, the cerebellum was used as a reference region (Lopresti et al., 2005) and ^{11}C-PiB retention maps as region-to-cerebellar ratio were generated by dividing regional uptake values by the individual mean cerebellar uptake values in the same images. The automatic anatomic labeling algorithm (Tzourio-Mazoyer et al., 2002) and a region combining method (Reiman et al., 2009) were applied to set regions of interest (ROIs) to characterize ^{11}C-PiB retention level in frontal, lateral parietal, posterior cingulate–precuneus (PC–PRC), lateral temporal, and basal ganglia (BG) regions. A global cortical ROI consisting of frontal, lateral parietal, PC–PRC, lateral temporal, and BG ROIs was also defined. For each ROI, mean value was calculated by averaging ^{11}C-PiB retention values for all voxels within the ROI. Each aMCI participant was classified as PiB-positive (i.e., aMCI+) if ^{11}C-PiB retention value of the image was over 1.4 in one of the five ROIs (i.e., frontal, lateral temporal, lateral parietal, PC–PRC, and BG) and PiB-negative (i.e., aMCI−) if ^{11}C-PiB retention values of all of the ROIs were equal to or less than 1.4 (Reiman et al., 2009).

MRI ACQUISITION

Imaging was performed on a 3.0-T GE whole body imaging system (GE VH/I; General Electric, Milwaukee, WI, USA). The rs-fMRI BOLD data for each participant consisted of 100 T2*-weighted single-shot gradient echo EPI sequence with the following parameters: TR/TE/FA = 3000 ms/30 ms/90°; voxel size, 1.87 mm × 1.87 mm × 5.0 mm; 26 anterior commissure–posterior commissure aligned axial slices in interleaved order; matrix 128 × 128; scan time ~9 min. During the whole functional scanning, all participants were asked to keep their eyes closed, to stay awake during the entire session, and not to focus their minds on anything in particular. Cushions and headphones were used to reduce subject motion and scanner noise. For structural imaging, we obtained a three-dimensional T1-weighted spoiled gradient recalled echo (SPGR) sequence (TR = 22.0 ms, TE = 4.0 ms, slice thickness/gap = 1.40 mm, matrix = 256 × 192, FOV = 240 mm, Flip angle = 40°).

DATA PROCESSING AND ANALYSES

Data analyses were carried out using multivariate exploratory linear optimized decomposition into independent components (MELODIC) of FMRIB's Software Library (FSL version 4.0.4)[1] to identify large-scale patterns of temporal signal-intensity coherence, interpreted as FC, in the population of subjects (Beckmann et al., 2005). Preprocessing included discarding the first five volumes to let the scanner reach equilibrium due to progressive saturation, motion correction, removal of non-brain structures, spatial smoothing (Gaussian kernel of 5-mm FWHM), slice timing correction, and high-pass temporal filtering (100 s). The rs-fMRI volumes were registered to Montreal Neurological Institute-152 standard space (MNI-152). Then, each subject's preprocessed functional data were run through single-session independent component analysis (ICA) of MELODIC to identify artifacts to be

[1] www.fmrib.ox.ac.uk/fsl

Table 1 | Demographic, neuropsychological, gray matter volumetric, and cerebral amyloid burden characteristics.

	CN	aMCI−	aMCI+	AD	*p*-value
Demographics					
n (total *N* = 43)	12	10	10	11	
Age, years (SEM)	71.75(1.21)	70.70(1.71)	71.20(2.50)	64.18(2.41)*	0.032
Sex (% female)	75	80	80	91	0.874
Education, years (SEM)	10.33(1.23)	9.00(1.50)	10.50(1.32)	9.09(1.32)	0.790
MMSE, raw (SEM)	27.40(0.45)	23.70(0.87)*	23.40(0.79)*	18.09(1.18)*,§,I	<0.001
Neuropsychological tests [T-scores (SD)]					
Category fluency[a]	58.80(13.42)	42.15(9.8)*	47.17(9.33)	37.07(12.86)*	0.001
Boston naming test[a]	54.95(13.01)	45.22(10.44)	46.04(9.90)	49.29(7.79)	0.117
Immediate word recall[a]	58.51(11.97)	39.49(9.85)*	36.39(9.80)*	31.23(9.46)*	<0.001
Visual construction[a]	55.14(6.89)	46.81(8.62)	50.69(11.91)	43.26(15.90)	0.134
Delayed word recall	53.03(8.55)	37.26(15.11)*	38.26(7.06)*	26.57(6.26)*	<0.001
Word recognition[a]	51.05(6.77)	40.52(10.31)	43.10(13.06)	25.95(15.42)*,I	<0.001
Delayed visual memory[a]	56.88(13.17)	36.83(10.59)*	37.97(10.65)*	34.75(9.53)*	<0.001
Stroop: color–word[a]	42.60(12.39)	43.07(14.12)	40.99(9.35)	24.46(7.63)*,§,I	0.001
PiB cortical retention [Mean (SD)]	1.051(0.077)	1.070(0.074)	1.542(0.241)*,§	1.692(0.228)*,§	<0.001

Data are presented as means (standard error of the mean, SEM; standard deviation, SD). If analysis of variance was $p < 0.05$, a post hoc Bonferroni test was performed. CN, cognitively normal healthy controls; aMCI−, amnestic-type mild cognitive impairment PiB-negative; aMCI+, amnestic-type mild cognitive impairment PiB-positive; AD, Alzheimer's disease: MMSE, Mini-Mental Status Examination.

[a] Missing data of one to three subjects.

** $p < 0.05$ (Bonferroni-corrected) compared with CN.*

§ $p < 0.05$ (Bonferroni-corrected) compared with aMCI− patients.

I $p < 0.05$ (Bonferroni-corrected) compared with aMCI+ patients.

denoised. Finally, the denoised functional data were temporally concatenated across all subjects (covering each comparisons) to create a single 4D group ICA (gICA) data set for the following analysis. The sample-specific DMN and SN were identified from the gICA results.

The between-subject analyses were carried out using the "dual-regression" approach, allowing for voxel-wise comparisons of resting FC (Filippini et al., 2009). In summary, dual-regression included the following steps: (1) obtaining matrices describing the temporal dynamics for each component and subject resulting from using the gICA spatial maps in a linear model fit against the separate rs-fMRI data set, which results in single-subject time-courses corresponding to each of spatial components generated by the gICA over all subjects; (2) normalizing the temporal modes to unit variance; (3) using the set of normalized individual temporal modes as regressors in a first-level GLM to derive individual subject spatial maps corresponding to each of the grand spatial maps. In the final stage of dual-regression analysis, we tested voxel-wise for statistically significant difference between the groups using non-parametric permutation testing (10000 permutations). This results in statistical maps characterizing the group differences. These maps were thresholded at $p < 0.005$ (uncorrected) using "threshold-free cluster enhancement (TFCE)" as implemented in FSL (Smith and Nichols, 2009). The effects of age, gender, and education were statistically accounted for by including these variables as subject-wise covariates in all of the statistical models. Group comparisons were performed for (a) CN and aMCI+, (b) CN and aMCI−, (c) CN and AD, and (d) aMCI− and aMCI+.

GRAY MATTER MORPHOLOGY

To verify whether altered FC in the current study might be explained by MRI-detectable loss of gray matter (GM), a customized voxel-based morphometry (VBM) approach was implemented following the combination of the VBM toolbox version 8 (VBM8 version 435)[2] and the Diffeomorphic Anatomical Registration through Exponentiated Lie algebra toolbox (DARTEL) (Ashburner, 2007) using SPM8 software package (Wellcome Trust Center for Neuroimaging, London)[3] using default parameters. The native structural T1 volumes were segmented into GM, white matter (WM), and cerebrospinal fluid tissue classifications, which were then used for partial volume estimation to facilitate accurate segmentation (Tohka et al., 2004). Two denoising methods were applied: (1) a spatially adaptive non-local means denoising filter which removes noise while preserving edges and (2) a Markov Random Field denoising filter which removes isolated voxels that are unlikely to be a member of a certain tissue class (Manjon et al., 2010). The filtered segmented data were affine registered to the tissue probability maps provided by the VBM8 toolbox and then were used in DARTEL to create a study-specific customized template. The warped GM and WM segments were modulated by multiplying voxel values in the segmented images by the Jacobian determinants derived from the spatial normalization step to correct for local differences in shape. The resulting normalized modulated non-linear GM images were smoothed with an 8-mm FWHM and were used for between-group comparisons. Based on our hypothesis, we restricted the VBM analysis to only differences in GM. Total intracranial volume was not used as a covariate as the non-linear images represent volume of GM corrected for individual brain sizes. Age, gender, and education were included in the statistical model as nuisance covariates.

The resulting set of T values constituted the SPM(T) map. The voxel-wise results were initially displayed at p less than 0.005 (uncorrected) to illustrate patterns. Then, we applied p less than 0.001 (two-tailed, uncorrected for multiple comparisons) as a significance height threshold at a voxel-level across the GM. Given the relatively small sample size, the significance threshold was set at p less than 0.001 (uncorrected) in order to avoid the unintended overlook of novel findings by too conservative a threshold. The MNI coordinates were automatically calculated in SPM8 and transformed into Talairach and Tournoux (Talairach and Tournoux, 1988) by the mni2tal program[4].

NON-IMAGING STATISTICS

All other statistical analyses were performed using Statistical Package for the Social Sciences 18.0 (SPSS, SPSS Inc., Chicago, IL,

[2]http://dbm.neuro.uni-jena.de/vbm/

[3]http://www.fil.ion.ucl.ac.uk/spm/software/spm8/

[4]http://imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/

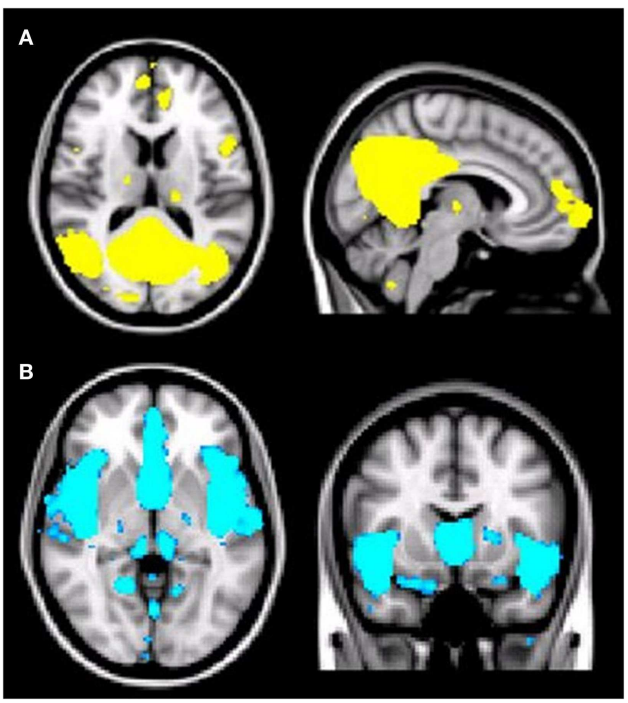

FIGURE 1 | (A) Illustration of the default mode network (DMN) regions derived from group independent component analysis (ICA). The DMN component identified by meta-ICA analysis included the posterior cingulate cortex, precuneus, medical prefrontal cortex, lateral parietal regions, lateral temporal regions, and bilateral medial temporal regions ($p < 0.001$). **(B)** Illustration of the salience network (SN) regions derived from group independent component analysis (ICA). The SN component identified by meta-ICA analysis included the anterior cingulate cortex, presupplementary motor area, and anterior insula ($p < 0.001$).

USA). For continuous measures, differences between groups were assessed using one-way ANOVA with *post hoc* Bonferroni tests to correct for multiple comparisons. Fisher's exact test was used to compare frequency distributions of gender.

RESULTS

DEMOGRAPHICS, COGNITIVE PERFORMANCE, AND AMYLOID BURDEN

Twelve CN, 10 aMCI−, 10 aMCI+, and 11 AD patients were compared on their demographic characteristics and neuropsychological performance scores (**Table 1**). Mean age of all subjects was 69.4 (SEM = 1.07) and their mean years of education was 9.7 (SEM = 0.65), of which 81% of the subjects were female. CN, aMCI−, and aMCI+ groups did not differ in their age. However, CN was older than AD. All groups did not differ in their years of education or gender distribution. As expected, CN performed significantly better on the MMSE compared to aMCI−, aMCI+, and AD groups. In addition, aMCI− and aMCI+ groups performed significantly better than AD on the MMSE.

In terms of other neuropsychological performances, four groups did not differ on the Boston Naming Test and a task of visual construction. Performances of aMCI−, aMCI+, and AD on word recall tasks (both immediate and delayed) as well as delayed visual memory task did not significantly differ from each other; however, they performed significantly worse than CN. On a recognition task, CN, aMCI−, and aMCI+ did not differ from each other; however, AD performed significantly worse than CN and aMCI+. On a task of response inhibition, CN, aMCI−, and aMCI+ performed similarly except for AD who performed significantly worse compared to the other groups. In addition, AD and aMCI− performed worse than CN on a category fluency test. Mean cortical retention of PiB of aMCI+ and AD was significantly higher than CN and aMCI− [$F(3, 39) = 39.41$, $p < 0.001$].

GROUP DIFFERENCES IN THE DMN

As predicted, ICA analysis revealed a sample-specific DMN with both the anterior and posterior regions present (**Figure 1A**). The results of voxel-wise between-group comparison for FC within the DMN at the threshold of $p < 0.005$ (uncorrected, TFCE) are shown in **Figure 2** and **Table 2**. Compared to CN, aMCI+ showed decreased FC at the left lingual gyrus. There were no regions within the DMN demonstrating increased FC in aMCI+ compared to CN. Similarly, AD showed weaker DMN connectivity in the right precuneus and left posterior cingulate cortex (PCC) than CN. There were no regions showing increased DMN connectivity in AD compared to CN. In contrast, when compared to CN, aMCI− demonstrated increased DMN connectivity in the left precuneus, right superior parietal lobule, right superior temporal gyrus, left middle temporal gyrus, and left culmen. There were no regions where aMCI− showed weaker DMN connectivity compared to CN.

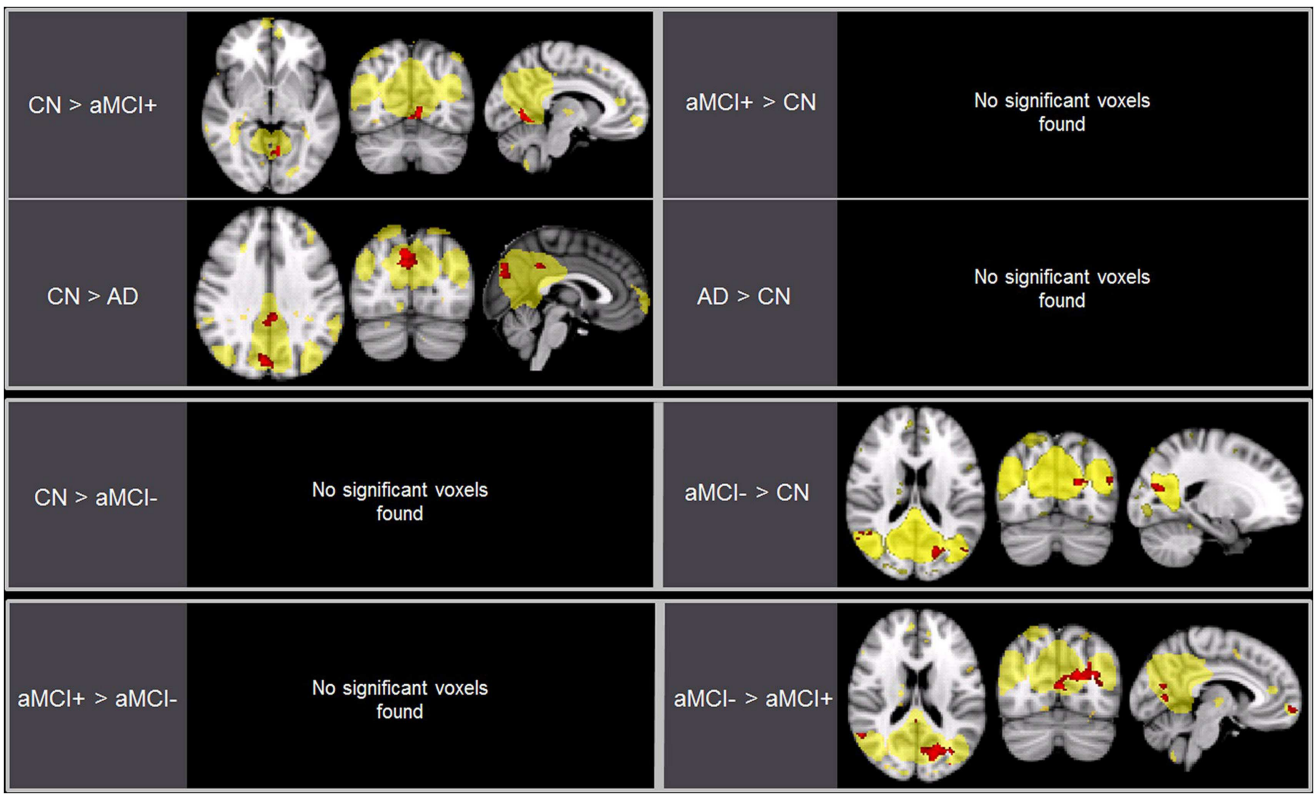

FIGURE 2 | Brain regions displaying significant differences (in red) in the default mode network (DMN) between the groups using voxel-wise comparisons ($p < 0.005$, uncorrected for multiple comparisons) masked by the overall average DMN in yellow ($p < 0.001$, uncorrected). Results are superimposed on the MNI-152 T1 1-mm brain template.

Table 2 | Regions of significant difference in functional connectivity within the default mode network between CN, aMCI−, aMCI+, and AD.

Contrast	Brain region	BA	MNI coordinates			Extent voxels (mm^3)	Peak *T* value
			X	*Y*	*Z*		
CN > aMCI+	L. lingual gyrus	18	−8	−64	2	81	4.77
CN < aMCI+	None						
CN > AD	R. precuneus	7	6	−76	36	228	5.21
	L. posterior cingulate	31	−2	−40	38	112	4.49
CN < AD	None						
CN > aMCI−	None						
CN < aMCI−	L. precuneus	31	−20	−70	16	76	4.77
	R. superior parietal lobule	7	22	−68	58	69	4.82
	R. superior temporal gyrus	22	56	−50	14	28	3.53
	L. middle temporal gyrus	39	−48	−72	18	21	4.02
	L. culmen		0	−50	−2	18	3.79
aMCI+ < aMCI−	L. cuneus	18	−16	−74	20	383	6.32
	R. superior temporal gyrus	22	54	−48	14	51	5.75
	L. superior frontal gyrus	10	−6	64	−6	47	3.67
	R. superior parietal lobule	7	28	−66	58	30	3.10
	R. inferior temporal gyrus	21	58	−8	−18	23	4.56
	L. medial frontal gyrus	10	0	64	2	17	3.27
	Culmen		0	−62	−6	11	4.16
	R. posterior cingulate	29	4	−42	16	10	3.99
aMCI+ > aMCI−	None						

CN, cognitively normal healthy control; aMCI−, amnestic-type mild cognitive impairment PiB-negative; aMCI+, amnestic-type mild cognitive impairment PiB-positive; AD, Alzheimer's disease; BA, Brodmann area.

Between aMCI+ and aMCI−, aMCI− showed stronger DMN connectivity in the left cuneus, right PCC, right superior parietal lobule, right superior temporal gyrus, right inferior temporal gyrus, left superior frontal gyrus, left medial frontal gyrus, and left culmen compared to aMCI+. There were no regions demonstrating decreased DMN connectivity in aMCI− compared to aMCI+.

GROUP DIFFERENCES IN THE SN

Independent component analysis revealed a sample-specific SN with the anterior cingulate cortex and anterior insula (**Figure 1B**). The results of voxel-wise between-group comparison for FC within the SN at the threshold of $p < 0.005$ (uncorrected, TFCE) are shown in **Figure 3** and **Table 3**. There were no differences in SN connectivity between CN, aMCI+, and AD. Compared to CN, aMCI− demonstrated decreased SN connectivity in the left inferior frontal gyrus (iFG); however, there were no regions where aMCI− showed increased SN connectivity compared to CN.

Between aMCI+ and aMCI−, aMCI− demonstrated decreased SN connectivity in the left iFG compared to aMCI+. There were no regions where aMCI− demonstrated stronger SN connectivity than aMCI+.

GROUP DIFFERENCES IN REGIONAL BRAIN VOLUME

For illustration, the results of VBM between-group comparisons are displayed at the threshold of $p < 0.005$ (uncorrected) as shown in **Figure 4**. Compared to CN, aMCI− showed atrophy in the bilateral superior temporal gyri (BA 38 and 13) and left uncus whereas aMCI+ showed atrophy in the right precuneus and left lingual gyrus (**Figure 4**; **Table 4**). The peak voxels of clusters meeting the $p < 0.001$ (uncorrected) cluster-wise criterion for the contrasts are listed in **Table 4**.

DISCUSSION

The present study showed that DMN FC differences between CN, aMCI+, and AD occur in the expected direction consistent with the results from previous studies on the differences between CN, overall aMCI, and AD. Both AD and aMCI+ showed decreased DMN FC compared to CN. The novel aspect of the present report was the comparisons of aMCI− with CN and aMCI+. aMCI− demonstrated increased FC strength in several DMN regions, mainly involving the precuneus, posterior cingulate, superior parietal, and superior temporal regions compared to aMCI+ as well as CN. Furthermore, aMCI− showed decreased FC strength of the SN in the left iFG compared to both CN and aMCI+. The present findings show that aMCI− has quite a different functional brain connectivity compared to aMCI+ despite overall similarity in cross-sectional cognitive features. To the best of our knowledge, this is the first study to directly examine FC alterations in both DMN and SN between aMCI+ and aMCI−.

Functional connectivity alterations in the posterior regions (i.e., the precuneus and PCC) of the DMN in AD and aMCI have been repeatedly found in previous task-related as well as task-free fMRI studies (Lustig et al., 2003; Greicius et al., 2004; Celone et al., 2006). The current study extends our knowledge about the DMN FC alterations in aMCI by specifying that previously

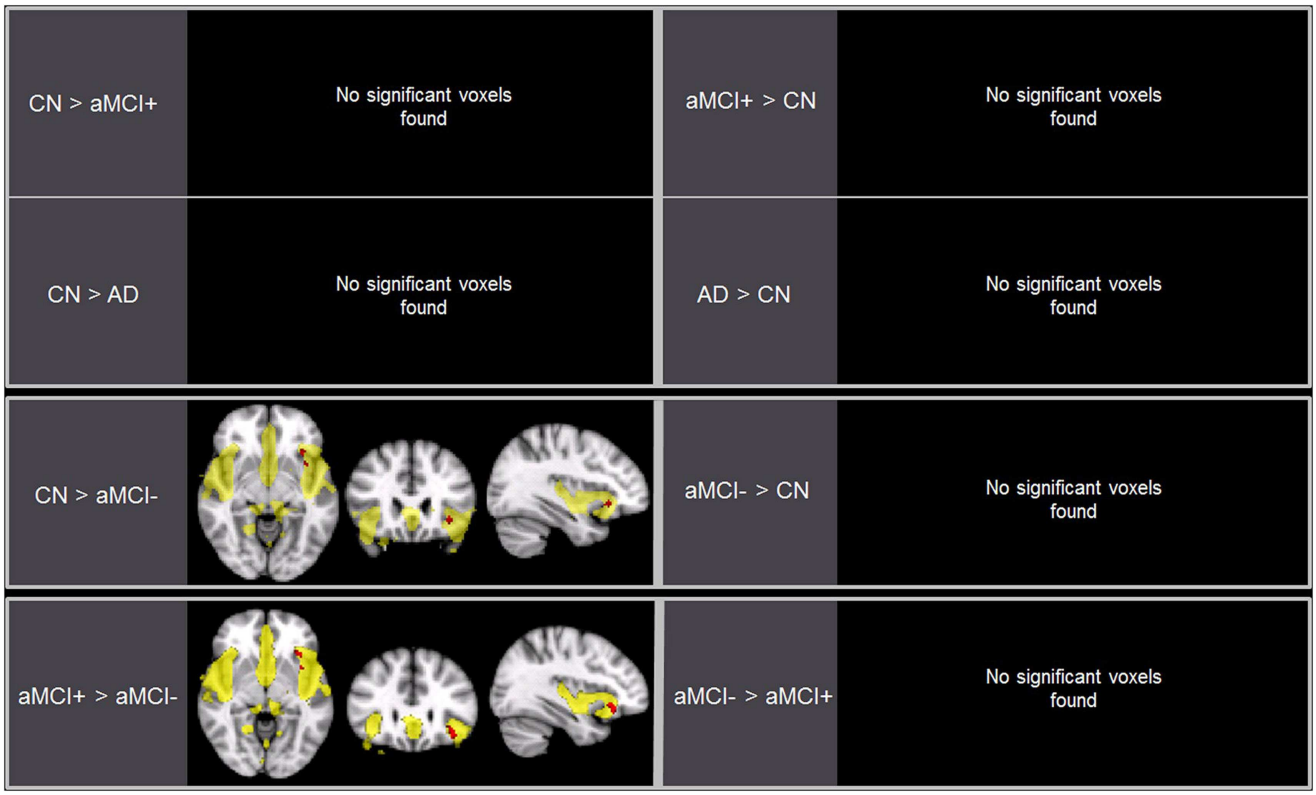

FIGURE 3 | Brain regions displaying significant differences (in red) in the salience network (SN) between the groups using voxel-wise comparisons ($p < 0.005$, uncorrected for multiple comparisons) marked by the overall average SN in yellow ($p < 0.001$, uncorrected). Results are superimposed on the MNI-152 T1 1-mm brain template.

Table 3 | Regions of significant difference in functional connectivity within the salience network between CN, aMCI−, aMCI+, and AD.

Contrast	Brain region	BA	MNI coordinates			Extent voxels (mm^3)	Peak *T* value
			X	*Y*	*Z*		
CN > aMCI+	None						
CN < aMCI+	None						
CN > AD	None						
CN < AD	None						
CN > aMCI−	L. inferior frontal gyrus	47	−36	24	−6	16	3.99
CN < aMCI−	None						
aMCI + < aMCI−	None						
aMCI + > aMCI−	L. inferior frontal gyrus	47	−34	26	−8	55	4.95

CN, cognitively normal healthy control; aMCI−, amnestic-type mild cognitive impairment PiB-negative; aMCI+, amnestic-type mild cognitive impairment PiB-positive; AD, Alzheimer's disease; BA, Brodmann area.

reported FC alterations are distinctive of aMCI with high Aβ burden. The most widely held hypothesis to account for such finding is that the decreased FC in the posterior association cortices is not only secondary to local AD-related neuropathological abnormalities in the very posterior DMN regions but also reflects distant effects of neuronal damage in the remote brain regions, such as the medial temporal lobe including hippocampus, based on its massive connectivity with widespread brain regions (Arnold et al., 1991; Jack et al., 2008; Bourgeat et al., 2010).

Unlike aMCI+ whose SN FC was comparable to CN, aMCI− exhibited decreased FC strength of the SN in the left iFG compared to CN and aMCI+. Furthermore, FC strength in the posterior regions of the DMN increased in aMCI− compared to CN and aMCI+. The present study does not demonstrate the

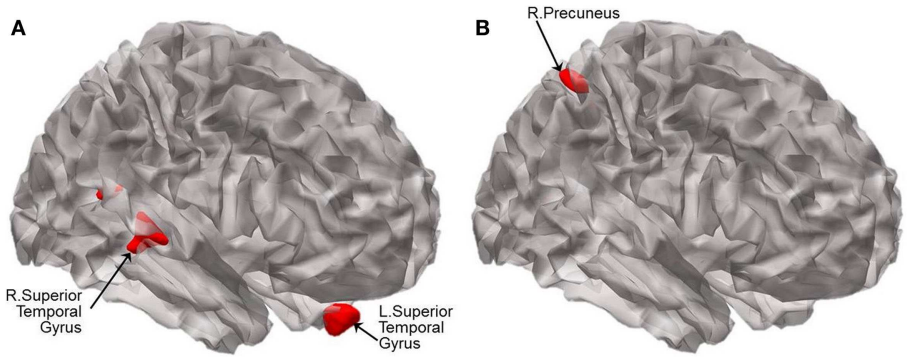

FIGURE 4 | Statistical parametric maps showing the results of analysis of covariance of VBM at $p < 0.005$ (uncorrected). Brain regions displaying significant differences in brain volume are shown in red between **(A)** CN and aMCI−, and between **(B)** CN and aMCI+. Results are superimposed using the cortex_20484.surf template in SPM8. The peak voxels of clusters meeting the $p < 0.001$ (uncorrected) cluster-wise criterion for the contrasts are listed in **Table 4**.

Table 4 | Regions of significant difference in brain volume between CN, aMCI−, and aMCI+.

Contrast	Brain region	BA	MNI coordinates			Extent voxels (mm^3)	Peak *T* value
			X	*Y*	*Z*		
CN > aMCI+	R. precuneus	7	29	−56	56	71	5.07
	L. lingual gyrus	18	−11	−89	−20	21	4.12
CN > aMCI−	L. uncus	20	−35	−12	−35	20	4.50
	L. superior temporal gyrus	38	−33	14	−38	74	4.24
	R. superior temporal gyrus	13	47	−48	15	21	4.05

CN, cognitively normal healthy control; aMCI−, amnestic-type mild cognitive impairment PiB-negative; aMCI+, amnestic-type mild cognitive impairment PiB-positive; BA, Brodmann area.

causal relationship between memory difficulties and FC disruptions. However, given the role of the SN in disengaging the DMN when on a cognitive task (Rilling et al., 2008; Sridharan et al., 2008; Sharp et al., 2010; Bonnelle et al., 2012), the ability of aMCI− to disengage the DMN may be compromised resulting in manifestation of reduced memory performance from inefficient processing of information. Alternatively, increased FC of the DMN may indicate greater recruitment of the regions in order to compensate for atrophied temporal pole given that the posterior DMN has been implicated in memory retrieval (Shapira-Lichter et al., 2013).

The differential network changes of aMCI− and aMCI+ are also noteworthy when explicated in conjunction with the differential patterns of atrophy in aMCI− versus aMCI+ compared to CN. In aMCI+, atrophy was observed in the same region where decreased FC was found in AD compared to CN (i.e., right precuneus of the DMN), which is also one of the regions where Aβ aggregates very early in the AD process. In contrast to aMCI+, the regions of FC alterations and atrophy do not necessarily overlap in aMCI−. The current findings suggest that decreased FC of the SN in aMCI− may be associated, at least in part, to atrophied superior temporal gyri given that the regions share dense connection with the regions of the SN (Augustine, 1996).

The results of the present study point to the DMN as an important intrinsic network differentiating aMCI− and aMCI+ as well as from CN. Challenges remain, however, in conceptualization of the etiology behind aberrant FC in the PCC and iFG in aMCI−. The FC alterations in aMCI− likely reflect non-AD pathology, although the exact nature of the pathology is still not clearly elucidated. Potential candidate pathologies underlying the impairment include cerebrovascular disease, hippocampal sclerosis, or Lewy body disease (Bennett et al., 2005; Jicha et al., 2006; Schneider et al., 2007). Alternatively, FC alterations in aMCI− may reflect underlying dysregulated lipid metabolism. A recent study from our research group suggested that cognitive deficits and brain atrophy in aMCI− are associated with decreased serum apolipoprotein A-1 (APOA1), the major component of HDL cholesterol independently of Aβ and vascular burden; the authors posited that antioxidant and/or anti-inflammatory properties of APOA1 as well as its critical role in reverse cholesterol transport may account for the contribution of the lipoprotein to non-AD brain damage (Choi et al., 2013). Additional etiological considerations for aberrant FC of the SN and the DMN may include mood disorders. A recent study on major depressive disorder found decreased FC within the SN as well as aberrant inter-network FC between subsystems of the DMN and the central executive network in patients

compared to healthy adults (Manoliu et al., 2013). Future studies are needed to examine and verify the relationship between non-AD pathological contributions and altered FC of the DMN and SN in aMCI− compared to aMCI+ and CN in order to elucidate the etiology.

This study has some limitations. First, the sample size in each group is relatively small. We tried to overcome statistical challenges due to small sample size by utilizing dual-regression with permutations; also, by combining all subjects in our gICA instead of using just CN, we ended up with more conservative results when describing alterations in FC in participants. Second, all presented data are cross-sectional in nature. Longitudinal changes in the networks may enable in-depth investigation of properties of alterations in these networks.

In conclusion, our results indicate that despite very similar cross-sectional profile of cognitive deficits, aMCI individuals with very low Aβ burden have quite different connectivity alteration pattern in the DMN and SN, compared to those with high Aβ burden. From a practical point of view, this discrepancy in the patterns of FC changes between the two aMCI groups may be utilized as a cost-effective biomarker for differentiating those who are at higher risk for AD dementia from those who are related to non-AD pathological processes among clinically defined overall aMCI individuals.

AUTHOR CONTRIBUTIONS

DY made contribution to the design of the work and performed the data analyses and prepared the manuscript. YC, MB, BS, ES, JH, and JP made substantial contribution to the acquisition of the data and provided critical intellectual reviews. DL provided a substantial contribution to the design of the work as well as to the analyses and interpretation of data; and, he critically revised the paper and approved the final version. JW made contribution to the conception of the work and acquisition of the data. All authors approved the final version to be published.

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Widespread increase of functional connectivity in Parkinson's disease with tremor: a resting-state fMRI study

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Parkinson's disease (PD) is a clinically heterogeneous disease in the symptomatology dominated by tremor, akinesia, or rigidity. Focusing on PD patients with tremor, this study investigated their discoordination patterns of spontaneous brain activity by combining voxel-wise centrality, seed-based functional connectivity, and network efficiency methods. Sixteen patients and 20 matched healthy controls (HCs) were recruited and underwent structural and resting-state functional MRI scan. Compared with the HCs, the patients exhibited increased centrality in the frontal, parietal, and occipital regions while decreased centrality in the cerebellum anterior lobe and thalamus. Seeded at these regions, a distributed network was further identified that encompassed cortical (default mode network, sensorimotor cortex, prefrontal and occipital areas) and subcortical (thalamus and basal ganglia) regions and the cerebellum and brainstem. Graph-based analyses of this network revealed increased information transformation efficiency in the patients. Moreover, the identified network correlated with clinical manifestations in the patients and could distinguish the patients from HCs. Morphometric analyses revealed decreased gray matter volume in multiple regions that largely accounted for the observed functional abnormalities. Together, these findings provide a comprehensive view of network disorganization in PD with tremor and have important implications for understanding neural substrates underlying this specific type of PD.

Keywords: Parkinson's disease, tremor, connectome, centrality, resting functional connectivity

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder typically characterized by motor symptoms, but distinct symptoms also include tremor, akinesia, and rigidity (Deuschl et al., 2000; Lees et al., 2009). Such a symptomatic heterogeneity is a key confounding factor in disclosing the pathophysiology of PD (Jankovic et al., 1990). Uncovering neural substrates accounting for this heterogeneity is critically important to advance our knowledge of PD and discover efficient therapies.

Pathophysiologically, PD is commonly thought to be attributed to the dysfunction of the basal ganglia circuit (i.e., the striatal-thalamic-corticoloop) triggered by deficits in dopaminergic nigrostriatal neurons (Hacker et al., 2012). However, despite success in accounting for the parkinsonian symptoms of akinesia and rigidity, this theory fails to explain tremor (Helmich et al., 2011). In clinical studies, the level of tremor severity is independent of the amount of dopamine deficiency (Toth et al., 2004; Lees, 2007; Helmich et al., 2012) and is often insensitive to dopamine treatment (Fishman, 2008; Rodriguez-Oroz et al., 2009). This is consistent with the findings from post-mortem studies, which show that PD patients with tremor have less dopaminergic dysfunction than non-tremor PD patients (Paulus and Jellinger, 1991; Jellinger, 1999). These findings suggest that PD with tremor is a unique and characteristic disease state in PD. However, brain mechanism underlying such specific state of PD is not well-established.

Recently, neuroimaging techniques have been used to reveal structural and functional brain alterations in PD patients with tremor. Compared with healthy controls (HCs), PD patients with tremor are found to show increased gray matter (GM) concentration (Kassubek et al., 2002) and hypermetabolism (Kassubek et al., 2001) in the thalamus, increased neural activity in the dorsolateral prefrontal cortex (Prodoehl et al., 2013), and elevated functional connectivity between the basal ganglia and the cerebello-thalamo-cortical circuit (Helmich et al., 2012). These studies suggest a wide involvement of multiple sites ranging from cortical to subcortical regions in PD with tremor, therefore triggering a hypothesis that there may exist a distributed network associated with PD with tremor. Given the interconnected nature of the brain to integrate various information inputs across segregated sensory systems (Bullmore and Sporns, 2009; He and Evans, 2010; Van Dijk et al., 2010), such a network hypothesis is reasonable but still lacks direct support from empirical evidence.

To fully depict abnormal brain networks in PD patients with tremor, the current study combined several approaches and applied them to resting-state functional MRI (R-fMRI) data collected from 16 PD patients with tremor and 20 matched HCs. R-fMRI measures spontaneous brain activity (Biswal et al., 1995) and

is proposed as a promising tool to map intrinsic brain networks (Van Dijk et al., 2010). We first constructed individual whole-brain, voxel-level functional connectivity networks. A graph-based measure, degree centrality, was then employed to locate the brain sites exhibiting abnormal functional connectivities in the patients. The measure is independent of the prior definition of regions of interest (ROIs), therefore providing an unbiased approach to test functional discoordination by searching over the entire brain. The identified sites were subsequently used as seed regions to trace the brain regions to which the abnormal functional connectivities were linked, therefore outlining the whole discoordination landscape in PD with tremor. Furthermore, we performed a network efficiency analysis to characterize the topological organization of the abnormal connectivity network identified in the patients. Finally, we correlated the disorganized network to clinical variables of the patients and tested their potential to serve as biomarkers in discriminating the disease. In addition, we also examined regional GM volume changes in the patients and tested the extent to which structural alterations contributed to functional abnormalities.

MATERIALS AND METHODS

PARTICIPANTS

Thirty-six right-handed participants, comprising 16 PD patients with tremor (9 men and 7 women) and 20 age-, gender-, and education-matched HCs (11 men and 9 women) were recruited from the Second Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine (Guangdong Province's Traditional Chinese Medical Hospital) in the present study. All the patients underwent a detailed clinical assessment of history of family genetic and traumatic brain injuries, neurological examinations, including the Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn and Yahr Scale (H–Y stage), and a conventional MRI scan. All the clinical assessments and MRI scans were performed when the patients were in their off-medicine condition (i.e., at least 8–12 h after the last dose of dopaminergic medication) to avoid a medication effect as much as possible. The patients were diagnosed by an experienced neurologist (XL) according to the UK PD Brain Bank Criteria (Gibb and Lees, 1988). Inclusion criterion was the presence of the resting tremor in the unilateral or bilateral upper or lower extremity. All the selected PD patients had classical parkinsonian resting tremor with ($n = 14$) or without ($n = 2$) action or postural tremor. There were no cognitive impairments for each individual PD patient, as measured by Mini-Mental State Examination (>28, mean = 29.8 ± 0.05). The participants were excluded for advanced PD stages (H–Y ≥ 4), secondary parkinsonism, atypical parkinsonian disease, and a history of any substance dependence, head trauma, or claustrophobia. All the participants gave written informed consent for the present study. This study was approved by the Institutional Review Board of the Guangzhou University of Traditional Chinese Medicine. **Table 1** lists detailed demographic and clinical information for all the participants.

Table 1 | Demographics and clinical characteristics of the participants.

	HC ($n = 20$)	PD ($n = 16$)	p value
Age (yrs)	42–78 (59.2 ± 8.7)	37–81 (60.5 ± 11.8)	0.37[b]
Gender (M/F)	11/9	9/7	0.90[a]
Education (yrs)	0–22 (11.4 ± 5.0)	0–20 (9.8 ± 4.2)	0.14[b]
Illness duration (yrs)	–	0.42–6 (2.5 ± 1.7)	–
MMSE	–	29.0–30 (29.8 ± 0.05)	–
UPDRS	–	4–49 (27.3 ± 14.3)	–
H–Y	–	1–3 (2.25 ± 0.91)	–
Tremor level	–	1–4 (2 ± 0.85)	–

Data are presented as minimum–maximum (Mean ± SD). PD, Parkinson's disease; HC, healthy control; MMSE, Mini-Mental State Examination; UPDRS, Unified Parkinson's Disease Rating Scale; H–Y, Hoehn and Yahr Scale.

[a]The p value was obtained using a two-tail Pearson chi-square test.

[b]The p values were obtained using two-sample two-tail t-tests.

IMAGE ACQUISITION

All the participants were scanned using a 1.5 T MR scanner (Siemens Magnetom Avanto, Erlangen, Germany) at the department of radiology of the Second Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine. R-fMRI data were collected using an echo-planar imaging sequence: 30 axial slices; repetition time (TR) = 2000 ms; echo time (TE) = 39 ms; slice thickness = 4 mm; gap = 1 mm; flip angle (FA) = 90°; matrix = 64 × 64; field of view (FOV) = 240 mm × 240 mm. During the data acquisition, the participants were asked to lie quietly in the scanner with their eyes closed. After scanning, a total of 180 volumes were obtained for each participant. Individual high-resolution 3D structural images were also acquired using a T1-weighted MP-RAGE sequence: 192 axial slices; TR = 1160 ms; TE = 4.21 ms; inversion time = 600 ms; slice thickness = 0.9 mm; no gap; FA = 15°; matrix = 512 × 512; FOV = 256 mm × 256 mm.

DATA PREPROCESSING

Resting-state functional MRI data preprocessing was performed with the GRETNA toolbox[1] based on SPM8[2]. After removal of the first five volumes, the functional images were corrected for time offsets between slices and geometrical displacements due to head movement. None of the participants were excluded based on the criterion of a displacement of >3 mm or an angular rotation of >3° in any direction. The summary scalars of both gross (maximum and root mean square) and micro (mean frame-wise displacement) head motions were matched between the PD patients and HCs (all $Ps > 0.15$). All the corrected functional data were then normalized to the Montreal Neurological Institute space using an optimum 12-parameter affine transformation and non-linear deformations and then resampled to a 3-mm isotropic resolution. The resulting images were further temporally band-pass filtered (0.01–0.1 Hz) to reduce the effects of low-frequency drift and high-frequency physiological noise, and linear trend was also removed. Finally, several nuisance signals were regressed out from each voxel's time course, including 24-parameter head-motion profiles (Friston et al., 1996; Yan et al., 2013), mean white matter (WM), and cerebrospinal fluid (CSF) time series within the respective brain masks derived from prior probability maps in SPM8 (threshold = 0.8). Of note, spatial smoothing was not

[1]http://www.nitrc.org/projects/gretna/

[2]http://www.fil.ion.ucl.ac.uk/spm/software/spm8/

included in the data preprocessing as in previous studies (Zuo et al., 2012) since smoothing could induce spurious local correlations for subsequent centrality analysis.

For structural images, we performed a voxel-based morphometry (VBM) analysis to determine GM volume alterations in the patients and to examine the potential effect of structural changes on functional abnormalities. Briefly, individual GM volume maps were obtained through the following steps: (i) segmentation of individual structural images into GM, WM, and CSF based on an adaptive Maximum A Posterior technique; (ii) normalization of the resulting GM maps into the Montreal Neurological Institute space using a high-dimensional DARTEL approach; (iii) non-linear modulation of GM maps to compensate for spatial normalization effects; and (iv) spatial smoothing of GM maps using a 6-mm full width at the half-maximum Gaussian kernel. Notably, the non-linear modulation essentially corrected for individual brain sizes. Structural data preprocessing was performed with the VBM8 toolbox for SPM8[3].

VOXEL-WISE WEIGHTED DEGREE CENTRALITY

Weighted degree centrality (WDC) is a measure from graph theory that quantifies the importance/centrality of a node in a network in terms of its connectivity strength to all the other nodes. For the voxel-wise centrality analysis, a node represents a voxel and the inter-node connectivity strength represents inter-voxel functional connectivity in their BOLD signals. Formally, the WDC for a given voxel i is calculated as follows:

$$S(i) = \sum_{j=1}^{N} r_{ij} - 1 \quad (1)$$

where r_{ij} is the Pearson correlation coefficient between voxel i and voxel j in their BOLD signal time series and N is the number of GM voxels according to the GM probability map in SPM8 (threshold = 0.2). To avoid the contamination of spurious weak correlations, a threshold is necessary before the summation. In contrast to an arbitrary choice, we employed a thresholding procedure based on the statistical significance level of the correlation analyses. Specifically, correlations surviving at a threshold of $P < 0.05$ (Bonferroni corrected) were screened and retained or were reset to 0. Of note, during the calculation, negative correlations were excluded given their ambiguous interpretation and detrimental effects on test–retest reliability (Fox et al., 2009; Murphy et al., 2009; Weissenbacher et al., 2009; Wang et al., 2011). After calculating WDC for each voxel, a whole-brain map was obtained for each participant, with the value at a given voxel indicating its functional integration over the entire brain. Degree centrality has been widely used in brain network studies (Buckner et al., 2009; Tomasi and Volkow, 2010; Zuo et al., 2012; Di Martino et al., 2013) due to its simplicity in understanding and implementation and high test–retest reliability (Wang et al., 2011; Cao et al., 2014).

SEED-BASED FUNCTIONAL CONNECTIVITY

Although whole-brain voxel-wise WDC analysis allows us to identify the brain sites that exhibit abnormal functional connectivity between PD patients and HCs (seven regions were identified in this study), we did not obtain any insight regarding the locations to which these abnormal connections were linked. To trace these locations, we further performed a seed-based functional connectivity analysis. Specifically, a sphere ROI was generated for each of the seven regions, with the centroid at the corresponding peak voxel (radius = 6 mm); the mean time series was then extracted and correlated to all the other voxels in the brain. This resulted in seven correlation maps for each participant, which further underwent Fisher's r-to-z transformation to improve the normality.

NETWORK EFFICIENCY

The voxel-wise WDC and subsequent seed-based functional connectivity analyses identified a total of 57 regions that exhibited abnormal functional connectivity in PD patients with tremor. To uncover the organization among these regions, we further constructed their pairwise connectivity matrix individually and fed them into graph-based analyses. Briefly, a sphere ROI was generated for each of the 57 regions, with the centroid at the corresponding peak voxel (radius = 6 mm). Seven ROIs were excluded to avoid spatial overlapping. For each of the remaining 50 ROIs, a mean time series was extracted for each participant and then correlated with all the other ROIs, therefore outputting a 50×50 correlation matrix. To exclude possible effects of spurious correlations on network topology, a sparsity threshold (i.e., the ratio of the number of existing edges divided by the maximum possible number of edges in a network) was applied to individual correlation matrices employed to convert individual correlation matrices such that only those high correlations are remained. The sparsity approach normalized all resultant networks to have the same number of nodes and edges and minimized the effects of discrepancies in the overall correlation strength between groups. However, because there is currently no definitive way to select a single threshold, we therefore empirically thresholded each correlation matrix repeatedly over a wide range of $0.08 \leq \text{sparsity} \leq 0.6$ (interval = 0.02) to obtain sparse and weighted networks. For the resultant networks at each sparsity, we calculated global and local efficiency to characterize parallel information flow within them (Latora and Marchiori, 2003; Achard and Bullmore, 2007). Similar to previous studies (He et al., 2009; Zhang et al., 2011a), we also calculated the area under the curve (AUC) for each network metric (global and local efficiency) to provide a summarized scalar independent of single threshold selection. Mathematically, the global efficiency for a network G is defined as:

$$E_{glob}(G) = \frac{1}{N(N-1)} \sum_{i \neq j \in G} \frac{1}{d_{ij}} \quad (2)$$

where d_{ij} is the shortest path length between node i and node j in G and is calculated as the smallest sum of edge lengths throughout all of the possible paths from node i and node j. The length of an edge was designated as the reciprocal of the edge weight (i.e., correlation coefficient), which can be interpreted as a functional distance that a high correlation coefficient corresponds to a short functional distance. Global efficiency measures the ability

[3]http://dbm.neuro.uni-jena.de/software/

of parallel information transmission over the network. The local efficiency of G is measured as:

$$E_{loc}(G) = \frac{1}{N}\sum_{i\in G} E_{glob}(G_i) \quad (3)$$

where $E_{glob}(G_i)$ is the global efficiency of G_i, the subgraph comprised the neighbors of the node i (i.e., nodes linked directly to node i). Local efficiency measures the fault tolerance of the network, indicating the capability of information exchange for each subgraph when the index node is eliminated.

To determine whether the constructed brain networks were topologically organized into small-world architectures, the global and local efficiency were normalized by the corresponding mean derived from 100 random networks that preserved the same number of nodes, edges, and degree distributions as the real brain networks (Maslov and Sneppen, 2002; Milo et al., 2002). Typically, a network is thought to be small-world if it has a normalized local efficiency larger than 1 and a normalized global efficiency approximately equal to 1.

STATISTICAL ANALYSIS

Between-group comparison

Two-sample t-tests were used to determine the between-group differences in GM volume, WDC, and seed-based functional connectivity. Non-parametric permutation tests (10,000 permutations) were used to test between-group differences in network measure (global and local efficiency). Gender and age were treated as unconcerned covariates for all these comparisons. Summary head-motion variables (maximum, root mean square, and mean frame-wise displacement) were treated as extra covariates for all functional comparisons (Fair et al., 2012). Additionally, voxel-specific GM volumes were added as covariates to test the structural contribution to functional abnormalities in WDC. For volume-based comparisons (GM volume, WDC, and seed-based functional connectivity), the results are presented at a statistical threshold of $P < 0.05$ (corrected) by combining a height threshold and an extent threshold determined by Monte Carlo simulations (Ledberg et al., 1998). For graph-based metrics, a false discovery rate (FDR) procedure was used to correct for multiple comparisons across different sparsities.

Brain–behavior correlation

Multiple partial Spearman correlation analyses were performed to examine the relationship between the neuroimaging results (WDC, functional connectivity, network efficiency, and GM volume) and clinical variables (duration, tremor, H–Y scale, and UPDRS score) for the patients after controlling for the corresponding confounding mentioned above.

CLASSIFICATION

To determine whether the observed between-group differences could be used to discriminate the patients from HCs, we implemented a receiver operating characteristic curve (ROC) analysis with the public code[4] (Giuseppe Cardillo, Naples, Italy).

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Table 1 summarizes the detailed demographic and clinical characteristics of all the participants. There were no significant between-group differences in age, gender, or years of education (all $Ps > 0.37$). The tremor score (an item in the UPDRS), UPDRS, H–Y, and disease duration of the patients were 2.0 ± 0.85, 27.3 ± 14.3, 2.25 ± 0.91, and 2.5 ± 1.7 years, respectively.

ALTERED DEGREE CENTRALITY IN PD WITH TREMOR

We utilized an unbiased voxel-wise WDC approach to explore abnormal functional connectivity networks in PD patients with tremor. The mean WDC distributed heterogeneously across the entire brain, with the most highly connected regions predominantly in the posterior parietal and occipital, lateral temporal, and medial prefrontal cortices and the cerebellum, a common pattern in both the HC (**Figure 1A**) and PD (**Figure 1B**) groups. Nevertheless, between-group comparison revealed widely altered WDC in the PD group (**Figure 1C**; Table S1 in Supplementary Material). Specifically, six clusters were observed to show increased WDC in the PD group, predominantly involving in the prefrontal (superior/middle/inferior frontal gyri and precentral gyrus), parietal (postcentral gyrus, precuneus, and superior parietal lobule), and occipital (calcarine and cuneus) regions. Notably, portions of the putamen and caudate also exhibited increased WDC and are thought to play critical roles in PD pathology. With regard

[4]http://www.mathworks.com/matlabcentral/fileexchange/19950

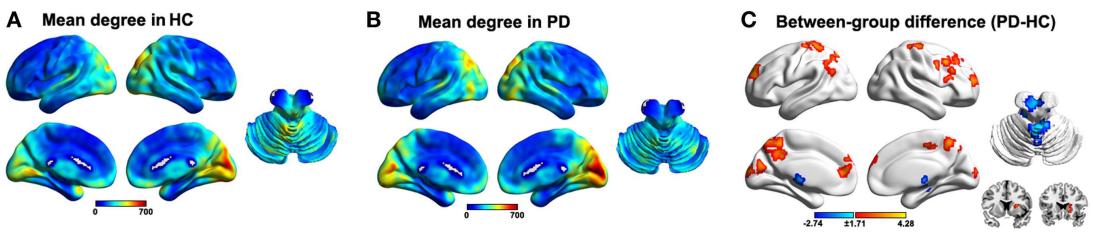

FIGURE 1 | Results of the within/between-group analysis on weighted degree centrality (WDC). **(A)**, Mean WDC pattern for the HC group. **(B)**, Mean WDC pattern for the PD group. **(C)**, Between-group differences in the WDC. The results were mapped onto the brain surface using the BrainNet viewer (http://www.nitrc.org/projects/bnv/).

to decreased WDC, only one cluster was detected and primarily encompassed the left cerebellum anterior lobe and bilateral thalamus.

ALTERED FUNCTIONAL CONNECTIVITY IN PD WITH TREMOR

To locate sites to which the above-mentioned regions were abnormally linked, we further performed a seed-based functional connectivity analysis. In total, 50 brain regions were identified as showing abnormal functional connectivity to the seven above-mentioned clusters in the PD patients (**Figure 2**). These regions distributed predominantly within the DMN (e.g., left precuneus, inferior parietal lobule, and middle/inferior temporal gyri), sensorimotor (e.g., left postcentral gyrus, supplementary motor area, paracentral lobule, and right precentral gyrus), and prefrontal [e.g., right inferior/middle frontal gyri and left superior frontal gyrus (SFG)] cortices. Notably, all the altered functional connectivities were increased in the PD group compared with the HC group, except for four connections. Table S1 in Supplementary Material lists the detailed information of these abnormal connectivities.

ALTERED NETWORK EFFICIENCY IN PD WITH TREMOR

Figure 3A represents spatial locations of the 50 sphere ROIs (radius = 6 mm) that exhibited discoordination in PD patients in the brain surface and **Figure 3B** shows the mean connectivity patterns among these ROIs for each group (sparsity = 0.3). This tremor-related network exhibited typical features of small-world topology for both the PD and HC groups, that is, compared with matched random networks, they had larger local efficiency and almost identical global efficiency (Figure S1 in Supplementary Material). Further between-group comparison revealed significantly ($P < 0.05$, FDR corrected) increased local and global efficiency over a wide range of sparsity threshold in the patients compared with HCs (**Figure 3**). As for the AUC, the patients also showed higher local ($P = 0.022$) and global ($P = 0.013$) efficiency than the HCs.

BRAIN–BEHAVIOR CORRELATION

Multiple functional connectivities were observed to be correlated with the patients' clinical variables (Table S1 in Supplementary Material) after controlling for age, gender, and head motion ($P < 0.05$, FDR corrected). **Figure 4** shows the most significant correlations for the duration, tremor, and H–Y score. No correlations were observed for the UPDRS scores. Significant correlations were also observed between local and global efficiency and tremor score for the patients (**Figure 5**).

SENSITIVITY AND SPECIFICITY OF ABNORMAL CONNECTIVITY IN DIFFERENTIATING THE PATIENTS FROM HCs

We found that most of the above-mentioned alterations in the PD group (WDC and functional connectivity) showed fair to good discriminative performances in distinguishing the patients from HCs (Table S1 in Supplementary Material). **Figure 6** presents the ROC of functional connectivity between the bilateral superior frontal gyri, which exhibited the highest power (AUC = 0.899, $P < 10^{-12}$, 95% CI area = 0.788–1.000), with a maximum sensitivity of 93.8% and a specificity of 85.0%. As such, 15 of the 16 patients with PD and 17 of the 20 HCs were classified correctly.

ALTERED GM VOLUME IN PD WITH TREMOR

Compared with the HCs, the PD patients exhibited significant GM changes in multiple regions (Table S2 in Supplementary Material). Specifically, decreased GM volumes were found in the parietal (right angular gyrus, superior and inferior parietal lobule, and bilateral precuneus), temporal (right superior and middle temporal gyri, parahippocampal gyrus, hippocampus, amygdale, and fusiform gyrus), frontal (right inferior frontal gyrus and rectal gyrus), occipital (left middle occipital gyrus and right lingual gyrus) regions, and the bilateral insula. In contrast, increased GM volume was observed predominantly in the right cerebellum.

To test the effects of altered GM volume on between-group differences in functional WDC, individual GM volume maps were treated as extra covariates in a voxel-wise manner during the between-group WDC comparison. The results demonstrated that the structural GM volume showed significant correlations with functional WDC in multiple regions (**Figure 7A**), largely accounting for the between-group differences in WDC (**Figure 7B**). Only two clusters, the left SFG and precuneus, survived after controlling the GM volume that increased WDC in the patients.

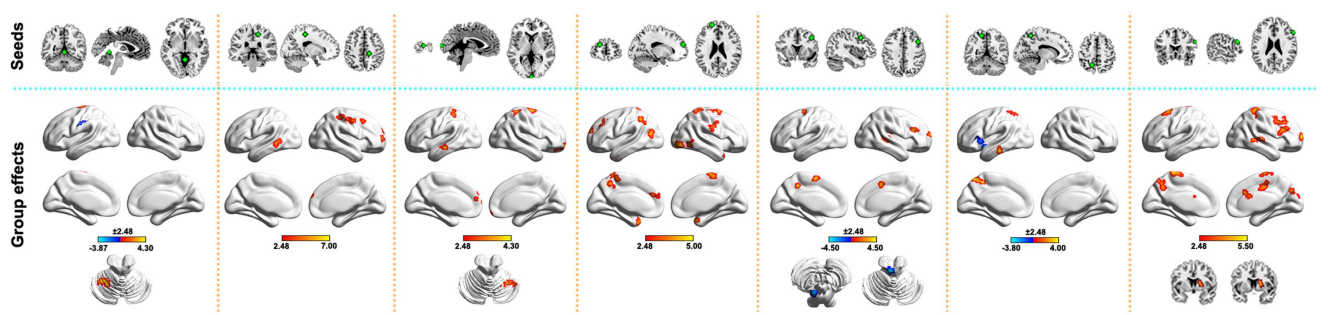

FIGURE 2 | Between-group differences in seed-based functional connectivity. The seeds (top row) were defined as spherical ROIs (radius = 6 mm) centered at the peak voxels, with the strongest group effects in WDC for clusters in **Figure 1C**. Regions showing abnormal functional connectivity in the PD (bottom row) were mapped onto the brain surface using the BrainNet viewer (http://www.nitrc.org/projects/bnv/). See Table S1 in Supplementary Material for detailed information.

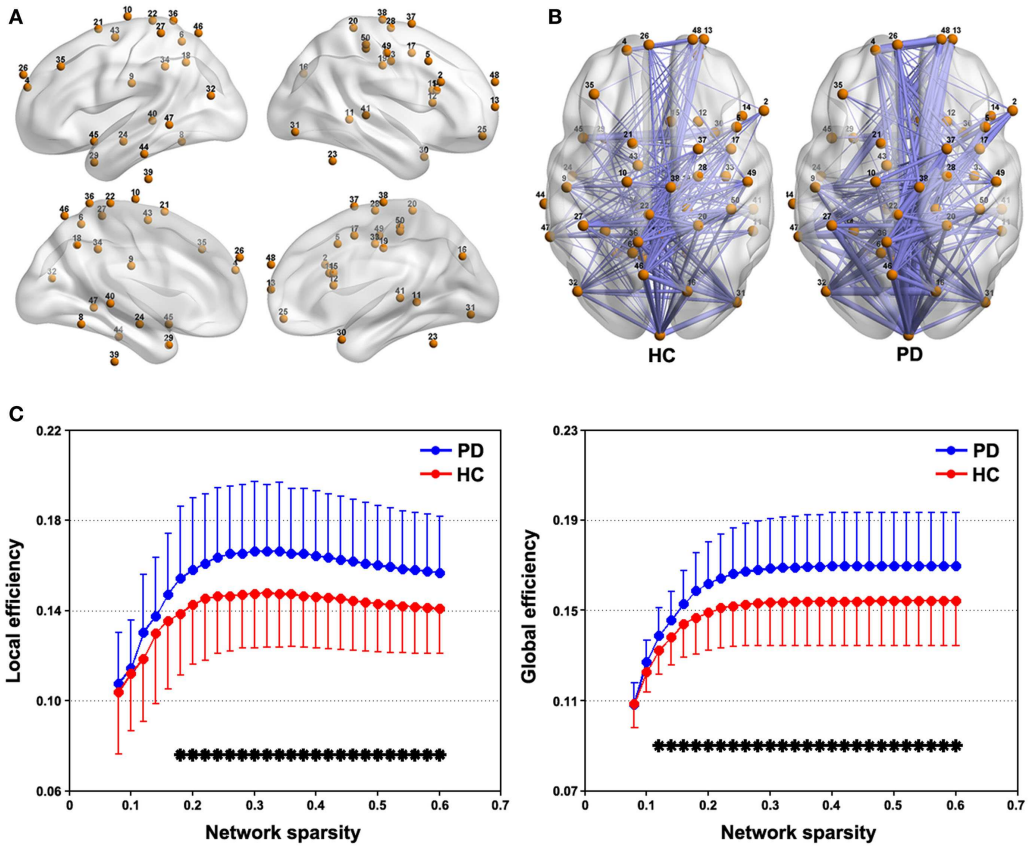

FIGURE 3 | Between-group differences in network efficiency. **(A)** Brain surface representation of the 50 ROIs showing abnormal connectivity in PD. **(B)** mean connectivity patterns among the 50 ROIs for the PD and NC groups that were thresholded at a sparsity = 0.3. **(C)** Local and global efficiency the 50-ROI network in the PD and HC groups as a function of sparsity. $*P < 0.05$, FDR corrected.

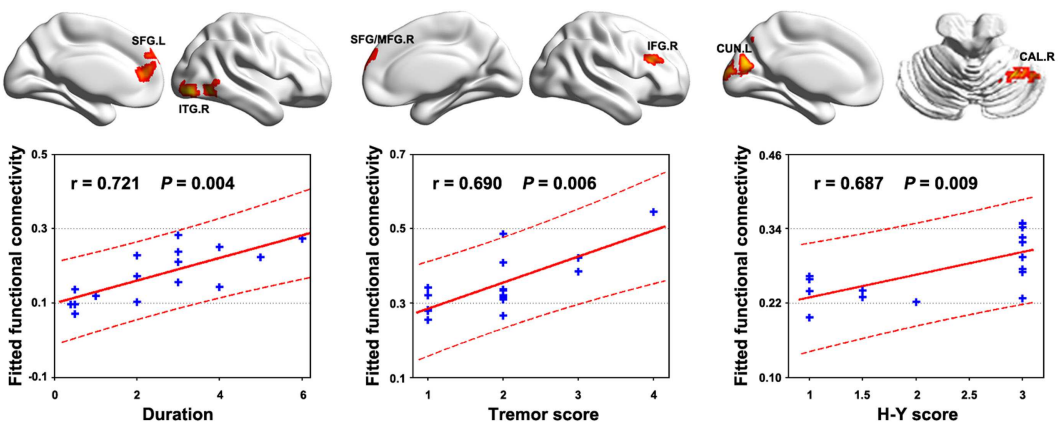

FIGURE 4 | Relationship between functional connectivity and clinical variables in PD patients. Significant correlations ($P < 0.05$, FDR corrected) of multiple functional connectivities were observed with the behavior performance of the patients. The figure shows the most significant correlations for the duration, tremor, and H–Y score. All the detected correlations are listed in Table S1 in Supplementary Material.

DISCUSSION

The current study investigated abnormal functional connectivity networks in PD patients with tremor via a novel combination of voxel-wise centrality, seed-based functional connectivity, and network efficiency analyses. The main results can be summarized as follows: (i) a spatially distributed discoordination network was

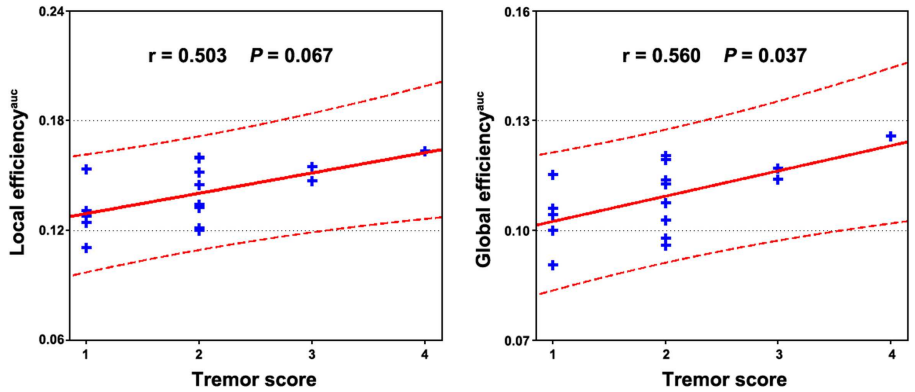

FIGURE 5 | Relationship between network efficiency and clinical variables in PD patients. Significantly positive correlations were observed between the AUC of local and global efficiency and tremor score of the patients.

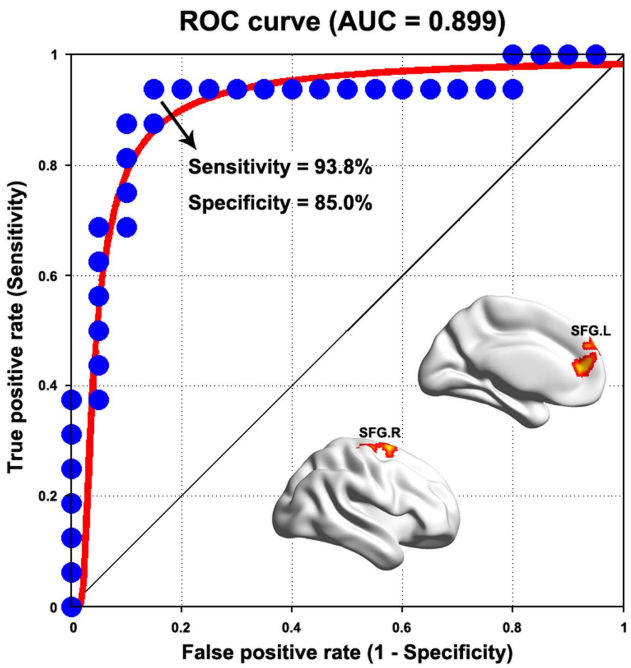

FIGURE 6 | Receiver operating characteristic curve for distinguishing PD patients from healthy controls as a function of varying functional connectivity between the bilateral superior frontal gyrus (SFG). See Table S1 in Supplementary Material for detailed classification information for weighted degree centrality and other functional connectivities.

outlined in PD with tremor that encompassed the cortical (sensorimotor, DMN, prefrontal, and occipital cortices) and subcortical (basal ganglia and thalamus) regions, cerebellum, and brainstem; (ii) the altered connectivity network was highly relevant to the patients' clinical expressions and exhibited a clinically relevant power in distinguishing the patients from HCs; and (iii) multiple brain regions showed structural changes of GM volume in the patients and the morphological changes largely accounted for the functional connectivity abnormalities.

DISTRIBUTED NETWORK ABNORMALITIES IN PD WITH TREMOR

Perhaps, the most important advancement of the current work relative to previous studies is that we provided a full delineation of abnormal functional connectivity networks in a specific PD state (i.e., PD with tremor) by searching the entire brain connectome at a refined voxel level. Focusing on focal brain regions or single neural circuit, previous studies documented structural and/or functional changes in PD with tremor (Kassubek et al., 2002; Helmich et al., 2012; Prodoehl et al., 2013). Indeed, increasing evidence has manifested that the human brain is, as a whole, an interconnected complex network. Consistent with this notion, experimental and simulation studies have collectively demonstrated that focal brain lesions not only influence local brain architecture but also spread their effects to other, even distant, brain regions (Alstott et al., 2009; Nomura et al., 2010; Gratton et al., 2012). Inspired by these findings, we speculate that, in addition to these already identified brain sites/circuits, there may exist a spatially more extensive network associated with PD patients with tremor. This was clearly demonstrated in the present study.

The identified abnormal network was involved in several subcortical regions (e.g., basal ganglia and thalamus), the cerebellum, and the brainstem (midbrain and pons). It is not surprising to observe that these sites show altered functional connectivities due to their high relevance in tremor generation in PD, as proposed by previous models (Deuschl et al., 2000; Helmich et al., 2011, 2012; Wu and Hallett, 2013). In addition to these well-established sites, many cortical areas were identified within the network that was predominantly located in sensorimotor cortices (e.g., postcentral gyrus, supplementary motor area, and paracentral lobule) and DMN (e.g., left precuneus, inferior parietal lobule, and middle temporal gyrus). Previous studies have demonstrated that rhythmic oscillations within motor- (e.g., primary motor cortex and cingulate/supplementary motor area) and sensory- (e.g., secondary somatosensory cortex and posterior parietal cortex) related regions are associated with parkinsonian tremor (Volkmann et al., 1996; Timmermann et al., 2003). Indeed, the cerebral motor cortex (e.g., primary motor cortex) has been demonstrated to be a convergence of distinct subcortical–cortical neural circuits (Helmich et al., 2012) that play important roles in tremor.

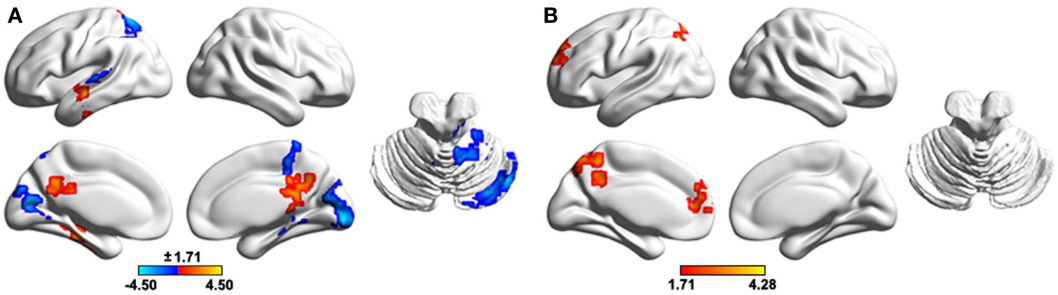

FIGURE 7 | The effects of regional gray matter volume on the functional results of weighted degree centrality. **(A)**, Spatial correlation between regional gray matter volume and weighted degree centrality. **(B)**, Between-group differences in weighted degree centrality after controlling for regional gray matter volume. The results were obtained in one general linear model, with weighted degree centrality as the dependent variable, group status (0 or 1) and voxel-specific gray matter volume as independent variables, and gender, age, and head motion as unconcerned covariates. The results were mapped into the brain surface using the BrainNet viewer (http://www.nitrc.org/projects/bnv/).

Therefore, it is reasonable to observe abnormal functional connectivity associated with sensorimotor cortices in PD patients with tremor. As for the DMN, previous studies have shown that the DMN regions are structurally interconnected (Greicius et al., 2009; Teipel et al., 2010) and functionally coherent in their brain activity to promote multiple cognitive processes (Greicius et al., 2003; Mason et al., 2007). In PD patients, disturbances of the DMN have been reported to be accompanied by various cognitive deficits, such as recognition memory (Ibarretxe-Bilbao et al., 2011), motor working memory (Rottschy et al., 2013), and motor learning (Argyelan et al., 2008). In our study, we observed that the DMN exhibited abnormal functional connectivity in PD patients. Presumably, these abnormalities may also relate to cognitive deficits, such as impaired working memory in PD (Moustafa et al., 2013). Notably, the patients in the current study were cognitively intact in terms of the MMSE score. This implies that altered network organization might be an early predictive sign of cognitive dysfunction with the progress of the disease, which could be examined in future longitudinal studies. We also detected several prefrontal (e.g., superior, middle, and inferior frontal gyri) and occipital (e.g., calcarine and cuneus) areas showing abnormal functional connectivity in PD. To our knowledge, few studies have examined the roles of these regions in PD, although their deficiencies have been reported in PD (Niethammer et al., 2012). Thus, it is difficult to speculate further on these findings, which should be studied more deeply in the future. Overall, we demonstrated a widely distributed network associated with PD patients with tremor.

HYPER CONNECTIVITY IN PD WITH TREMOR

We identified a disorganized network in which almost all the connections exhibited increased connectivity strength in PD patients with tremor compared with HCs. Previous studies have documented regional hypermetabolism in the basal ganglia, cerebellum, dorsal pons, and primary motor cortex (Mure et al., 2011) and functional connectivity increases within the cerebello-thalamic circuit (Helmich et al., 2011) in tremor PD. These results suggest that overheated functional activities may be common in PD with tremor, consistent with our findings. Moreover, this study expanded previous findings by demonstrating that the hyper connectivity spread over the entire brain in PD with tremor. We further investigated the global properties of these hot-wiring connectivities and found increased network efficiency in the patients relative to HCs.

Generally, the pathophysiology of PD is attributed to the dysfunction of the basal ganglia circuit triggered by deficits in dopaminergic nigrostriatal neurons. Several studies have demonstrated that the dopamine depletion impairs interregional functional connectivity (Nagano-Saito et al., 2008; Wu et al., 2009) and information processing efficiency of resting-state brain networks (Achard and Bullmore, 2007; Carbonell et al., 2014). In patients with PD, decreased interregional connectivity (Luo et al., 2014) and network efficiency do have been observed (Woerner et al., 2009). However, the present study found that there were a large number of connections that exhibited increased functional connectivity in PD patients with tremor compared with HCs and the efficiency among these connections was increased in the patients. The major reason for these discrepancies may be that the current study focused on a unique and characteristic disease state in PD, which was clinically characterized by resting tremor. Although there is also dopaminergic nigrostriatal neurons loss in PD patients with tremor, many clinical studies have shown that only dopamine depletion fails in completely accounting for the symptoms of resting tremor in patients with PD (Helmich et al., 2011). Particularly, clinical studies have found that the level of tremor severity is independent of the amount of dopamine deficiency and is often insensitive to dopamine treatment. Moreover, post-mortem studies also showed that PD patients with tremor have less dopaminergic dysfunction than non-tremor PD patients (Helmich et al., 2012). These findings indicate the existence of other mechanisms that underline tremor PD. Indeed, there are many previous studies that showed elevated GM concentration (Kassubek et al., 2002), regional hypermetabolism (Kassubek et al., 2001) and increased functional connectivity (Helmich et al., 2012) in tremor PD compared with HCs. These findings together with the current results are in correspondence with the notion that there exists a cerebral compensation for pathophysiological alterations in PD patients with tremor (Hallett and Khoshbin, 1980; Rivlin-Etzion et al., 2006). It should

be noted that there are few studies that have directly examined the relationship between resting-state networks and neuotransmitters reduction in PD patients with tremor, which will be an important direction for future studies to deepen our understanding of the origin of tremor in PD.

Intriguingly, we found that the increased functional connectivities and network efficiency were positively correlated with the clinical expressions (e.g., tremor level) of the patients. In other words, the more serious the tremor, the higher the strength of these functional connectivities and network efficiency. These correlations indicate the effectiveness of the detected alterations in capturing clinical expressions in PD with tremor and further support the compensatory interpretation. However, it should be pointed out that the compensatory notion in PD with tremor is still speculative currently, thereby, more studies are required on this issue. Finally, the classification analysis showed that the abnormal functional connectivities could distinguish the patients from HCs with clinically relevant discriminative power, indicating the potential of these abnormalities to serve as biomarkers for the disease. Of note, we found that the abnormal functional connectivities exhibiting high relevance with clinical performance and discriminative power were primarily related to cortical, rather than to well-recognized subcortical/cerebellum regions. This finding highlights the important roles of cortical regions in understanding tremor PD and suggests that more attention should be shifted in this direction in future.

In addition to these increased functional connectivities, we also found that there were several decreased functional connectivities in the patients, which may reflect a genuine consequence of the pathological damage of PD to brain function. Neurochemically, PD is generally characterized by an important neurotransmitter depletion, a feature that will lead to a natural assumption that PD is associated with reduced connectivity of numerous connections from a neurochemical perspective. In contrast, we found that there were numerous connections that exhibited increased connectivity strengths in the patients compared with HCs. Although the neural mechanism underlying so many increased connections in PD patients with tremor is unclear currently, as we have discussed above, our findings are consistent with numerous previous studies that showed elevated GM concentration (Kassubek et al., 2002) regional hypermetabolism (Kassubek et al., 2001) and functional connectivity increase (Helmich et al., 2012) in tremor PD. Presumably, these increases may reflect the cerebral compensation for pathophysiological changes of PD (Rivlin-Etzion et al., 2006), which is dominant (versus neurotransmitter depletion) at this specific stage of PD. Of note, there are other possibilities. For example, the current pilot study only included a relative small sample size, which may limit the power to detect more subtle decreases of functional connectivity. Future studies are thus required to provide deeper insights into this issue by recruiting a large cohort of patients. Additionally, the current findings were derived from R-fMRI, which measures spontaneous brain activity. Whether similar findings hold when the patients are engaging in cognitive tasks is an interesting topic in future although the brain's functional network architecture during task performance is strongly shaped by an intrinsic network architecture during rest (Cole et al., 2014).

GM ATROPHY IN PD WITH TREMOR

Beyond functional abnormalities, we also studied structural GM volume changes in these PD patients. There are many studies investigating GM volume in PD patients (Brenneis et al., 2007; Jubault et al., 2011; Pereira et al., 2012); however, the findings are far from a consensus (Lin et al., 2013). One possible factor accounting for such inconsistency is the mixture of different PD symptom dimensions that demonstrate distinct patterns of GM volume alterations (Benninger et al., 2009). Focusing on single-domain PD patients, Kassubek et al. studied a specific region of interest, the posterior ventral lateral thalamus in PD patients with tremor, and found increased GM volume compared with HCs (Kassubek et al., 2002). Using an unbiased VBM method, here we detailed GM alterations over the entire cortical mantle in PD with tremor. We found that PD patients with tremor were associated with widespread GM volume reductions in multiple regions of cortical (e.g., right inferior frontal gyrus, bilateral middle temporal gyrus, and left middle occipital gyrus) and limbic/subcortical (e.g., right hippocampus, parahippocampus, amygdale, and bilateral insula) sites. The volumetric reductions might reflect neural degeneration owing to the pathological damage of the disease (Benninger et al., 2009). More importantly, we found that the volumetric reductions had significant correlations with the patients' clinical expressions, suggesting a neuroanatomical significance of these abnormalities in monitoring the progression of the disease. In addition, we also found that the cerebellum exhibited increased GM volume in the PD patients. Generally, GM volume increase may be due to neuronal hypertrophy or higher neuron density (Kassubek et al., 2002), which reflect brain neuroplasticity (Brosh and Barkai, 2004) to respond external environmental cues, experience, behavior, injury, or disease (Ludlow et al., 2008). Thus, we speculate that the increased cerebellar GM volume observed in the PD patients with tremor may relate to the neuroplasticity due to long-term pathological and/or behavior changes in the patients. Of note, we did not detect GM changes in the thalamus in this dataset, which may be due to the small sample size.

Previous studies have shown that regional GM loss significantly contributes to functional abnormalities in patients with Alzheimer's disease (He et al., 2007). Analogously, we also observed that functional abnormalities can be largely accounted for by GM volume changes in PD patients with tremor. This implies a structural basis of the observed functional abnormalities in this specific PD state and suggests that more attention should be given to the impacts of regional morphological changes on functional results in future studies of neurodegenerative diseases (He et al., 2007). Notably, the functional abnormalities of two clusters (i.e., left SFG and precuneus) were independent of GM volume alterations, suggesting their important roles in understanding how the disease exerts influence on brain function.

LIMITATIONS

Several issues need to be addressed in future research. First, the small sample size limited the conclusions that we can draw; thus, a large number of participants should be included in future studies. Second, in this study, only *post hoc* motion correction methods were used to correct for motion artifacts by combining individual- and group-level strategies. However, despite of our

efforts to attenuate head-motion effects as much as possible, we cannot exclude the possibility of residual head-motion effects on our results. Further studies are required to reproduce our findings using new methods with the development of this field. Third, although this study identified the abnormally organized network related to PD with tremor, it did not answer how this abnormal component reshapes whole-brain network topology, such as large-scale small-world organization and intermediate-scale community structure (Gottlich et al., 2013; Olde Dubbelink et al., 2014). Fourth, the present study focused on functional brain networks in PD with tremor. Recent studies document tight relationships in the connectivity patterns between structural and functional brain networks (Honey et al., 2009; van den Heuvel et al., 2009; Hermundstad et al., 2013) and the structure–function coupling was found to be disrupted under the pathological condition (Zhang et al., 2011b). Therefore, combining multimodal neuroimaging datasets (e.g., task fMRI, positron emission tomography, arterial spin labeling, and diffusion tensor imaging) will provide more informative insight into neural response to task demands, neurotransmitters disruption, physiological basis, structure basis, and the influences of the disease on the collective behavior of the brain. Fifth, the present study outlined whole-brain functional network abnormalities in PD patients with tremor. In the future, it will be important to determine the similarities and differences in the abnormal network patterns among PD patients dominated by different clinical symptoms. Sixth, the current dataset was obtained on a 1.5 T MRI scanner, which is less sensitive in the detection of resting-state brain networks. The findings observed here should be further validated using high field magnet scanner. Finally, the patients recruited in the current study were under an off-medicine condition. Whether the observed changes are normalized during the drug effect should be further considered.

CONCLUSION

Combining network centrality, seed-based functional connectivity, and network efficiency analyses, the present study provides a full map of abnormal connectivity networks in PD with tremor that is distributed over cortical, subcortical, cerebellum, and brainstem sites. These altered functional connectivities correlated with the clinical performance of the patients and exhibited clinically relevant discriminative power in distinguishing the patients from HCs. Moreover, the functional changes were explained to a great extent by abnormal GM volume, indicating a structural basis of functional alterations. These findings have important implications in understanding the neural substrates underlying the specific type of PD with tremor patient.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fnagi.2015.00006/abstract

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Elevated levels of cerebrospinal fluid α-synuclein oligomers in healthy asymptomatic *LRRK2* mutation carriers

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Mutations in the leucine-rich repeat kinase 2 gene are the most common cause of autosomal dominant Parkinson's disease (PD). To assess the cerebrospinal fluid (CSF) levels of α-synuclein oligomers in symptomatic and asymptomatic leucine-rich repeat kinase 2 mutation carriers, we used enzyme-linked immunosorbent assays (ELISA) to investigate total and oligomeric forms of α-synuclein in CSF samples. The CSF samples were collected from 33 Norwegian individuals with leucine-rich repeat kinase 2 mutations: 13 patients were clinically diagnosed with PD and 20 patients were healthy, asymptomatic leucine-rich repeat kinase 2 mutation carriers. We also included 35 patients with sporadic PD (sPD) and 42 age-matched healthy controls. Levels of CSF α-synuclein oligomers were significantly elevated in healthy asymptomatic individuals carrying leucine-rich repeat kinase 2 mutations (n = 20; $P < 0.0079$) and in sPD group (n = 35; $P < 0.003$) relative to healthy controls. Increased α-synuclein oligomers in asymptomatic leucine-rich repeat kinase 2 mutation carriers showed a sensitivity of 63.0% and a specificity of 74.0%, with an area under the curve of 0.66, and a sensitivity of 65.0% and a specificity of 83.0%, with an area under the curve of 0.74 for sPD cases. An inverse correlation between CSF levels of α- synuclein oligomers and disease severity and duration was observed. Our study suggests that quantification of α-synuclein oligomers in CSF has potential value as a tool for PD diagnosis and presymptomatic screening of high-risk individuals.

Keywords: Parkinson's disease, LRRK2 mutation carriers, CSF, biomarkers, alpha-synuclien

INTRODUCTION

Parkinson's disease (PD) is the most common age-related movement disorder and the second most common neurodegenerative disorder after Alzheimer's disease. The earliest clinical features of PD are typically retrospective and not specific, and they may include depression, hyposmia, constipation, and sleep disorders. At least 70% of the neurons in the substantia nigra (SN) are lost prior to the appearance of any major motor symptoms (Schapira, 1999). The main motor symptoms, including resting tremor, rigidity, bradykinesia, and postural instability, are collectively known as parkinsonism. Currently, PD is clinically diagnosed and clinical trials of disease-modifying drugs are initiated only after most of the vulnerable dopaminergic neurons in the SN have already been lost. Individuals at risk for PD with less complete loss of dopaminergic neurons may be more responsive to and benefit most from neuroprotective therapies. Therefore, identifying biomarkers for early diagnosis may facilitate the development of novel treatments designed to slow disease progression. Furthermore, such findings may help to elucidate the pathophysiology of PD. Most PD cases are sporadic (sPD) (i.e., idiopathic; attributed to unknown causes). However, some atypical cases involve genetic susceptibility (Singleton et al., 2013). Over the last two decades, several genetic causes of PD have been identified. At present, 5–10% of all PD cases can be traced to a known genetic cause that is either monogenic or related to a combination of susceptibility factors (Singleton et al., 2013). Mutations in the gene encoding α-synuclein (α-syn) (*SNCA*) were the first genetic factors to be linked to familial PD (Polymeropoulos et al., 1997; Krüger et al., 1998; Singleton et al., 2003). Although *SNCA* mutations rarely cause late-onset familial PD, *SNCA* is still of great importance to PD etiology, as abnormal aggregation of α-syn in the brain is also found in neuropathological lesions (Lewy bodies (LBs); Spillantini et al., 1997). However, it has been previously shown that α-syn is normally released by neuronal cells and present in the cerebrospinal fluid (CSF) and peripheral plasma (El-Agnaf et al., 2003) Recent studies have demonstrated that oligomeric forms of α-syn are neurotoxic species *in vitro* and *in vivo*, whereas amyloid fibrils may not be directly toxic (Winner et al., 2011). Gene

mutations in leucine-rich repeat kinase 2 (LRRK2) are the second most common cause of autosomal dominant PD and cause 2–5% of familial PD. The most common point mutation, G2019S, has been shown to be involved in 5–6% of autosomal dominant PD cases (Di Fonzo et al., 2005; Nichols et al., 2005; Dächsel and Farrer, 2010) and 1–2% of sPD cases (Gilks et al., 2005). Patients with late-onset monogenic forms of PD may demonstrate subtle signs or symptoms several years before they suffer from any motor symptoms (Sossi et al., 2010; Johansen et al., 2011; Ruiz-Martínez et al., 2011). Similarly, recent positron emission tomography (PET) studies have confirmed dopaminergic dysfunction in asymptomatic LRRK2 mutation carriers (Nandhagopal et al., 2008). Therefore, these carriers are an ideal population for identifying novel biomarkers for the early diagnosis of PD. We and other groups recently reported elevated levels of α-syn oligomers (o-α-syn) and an increased o-α-syn/total-α-syn (t-α-syn) ratio in CSF from PD patients relative to controls (Tokuda et al., 2010; Park et al., 2011; Sierks et al., 2011; Parnetti et al., 2014a,b). These findings suggest that CSF α-syn oligomers could be a potentially useful biomarker for diagnosis and possible early detection of PD. We therefore explored the potential use of o-α-syn as an early biomarker for PD in CSF from asymptomatic LRRK2 mutation carriers and symptomatic LRRK2 PD patients relative to sPD patients and healthy age-matched controls.

MATERIALS AND METHODS

PATIENT POPULATION AND CLINICAL METHODS

In total, 33 Norwegian individuals from 12 different families with mutant LRRK2 were assessed in this study. Thirteen patients were clinically diagnosed with PD and 20 patients were healthy, asymptomatic LRRK2 mutation carriers. These families have been extensively described in previous report (Aasly et al., 2005, 2010; Johansen et al., 2010). In addition, 35 patients with sPD and 42 age-matched healthy controls were also recruited for this study from St. Olav's Hospital at the University Hospital of Trondheim in Norway. Parkinson's disease was diagnosed according to established diagnostic criteria (Gelb vs. UK Parkinson's Disease Society). Disease severity was defined according to the Hoehn and Yahr scale (H&Y). All patients with sPD were screened and tested negative for known *LRRK2* mutations. Patients with age at onset ≤50 years also tested negative for known pathogenic mutations in *Parkin* and *PINK1*. All family members were screened for clinical signs of PD and found to be asymptomatic, although a few had mild pre-motor signs with an increased Unified Parkinson's Disease Rating Scale (UPDRS) score (Johansen et al., 2011). The LRRK2-mutant PD patients were on levodopa, and some were taking other dopamine agonists and monoamine oxidase-B (MAO-B) inhibitors. The mean levodopa-equivalent dose in the LRRK2-mutant PD group varied between 300 and 1800 mg, with a mean of 580 ± 422 mg, and the mean levodopa-equivalent dose in the sPD group was 300 to 1500 mg, with a mean of 628 ± 387 mg.

All individuals underwent lumbar puncture between 08:00 am and 10:00 am following overnight fasting. A small sample of CSF was sent for routine analysis, and then 18 to 22 ml was sampled and frozen in 15 aliquots of 1.2–1.5 ml each within 15 min of completion of the lumbar puncture. The aliquots were stored at −80°C until further analysis. All patients gave signed, informed consent, and the study was approved by the Regional Committee for Medical and Health Research Ethics.

SIZE EXCLUSION CHROMATOGRAPHY (SEC) FOR SEPARATING α-syn MONOMERS AND OLIGOMERS

Size Exclusion Chromatography was carried out using an AKTA FPLC system (GE Healthcare-Sweden) and a superdex 200 column at 4°C. Concentrated 0.5 ml of CSF were loaded onto the column and eluted with PBS (pH 7.4) at a flow rate of 0.1 ml/min (0.5 ml/fraction). The elution of α-syn was monitored at absorbance wavelengths of 215 nm. Fractions of 1 ml were collected, concentrated to 100 μl using a speed vac, and analyzed by the western blotting for the presence of α-syn. To determine the elution time of monomeric and oligomeric α-syn, molecular weight standards (Thyroglobulin 669 kDa, ferritin 440 kDa, aldolase 171 kDa, abmumin 68 kDa and chymotrypsinogenA 25 kDa), fresh α-syn solution and aged α-syn solution were co-injected into the column and eluted at the same conditions mentioned above.

SODIUM DODECYL SULFATE–POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS–NuPAGE) AND IMMUNOBLOTTING

The CSF fractions were separated on NuPAGE Bis–Tris 4–12%, 1 mm gels (Invitrogen Ltd., Paisley, UK) and then transferred to nitrocellulose membranes (0.45 μm) at 30 V, 125 mA for 45 min (Invitrogen Ltd., Paisley, UK). Membranes were boiled for 5 min in PBS then blocked for 1 h with 5% marvel dried skimmed milk and dissolved in PBS–Tween 20 (0.05%) (PBST). The membranes were probed overnight at 4°C anti-α-syn (211) mouse monoclonal antibody to α-syn (aa 121–125). The membranes were washed several times with PBST followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse (Dako Ltd., Ely, UK), for 60 min. The membranes were again washed several times with PBST. The protein bands were visualized using ECL reagents (Pierce, USA) as described by the manufacturer.

IMMUNOASSAYS FOR TOTAL AND OLIGOMERIC α-SYNUCLEIN IN CSF

A sandwich enzyme-linked immunosorbent assay (ELISA) methods were employed to measure total or oligomeric α-syn levels in CSF samples as described previously (Tokuda et al., 2010).

STATISTICAL ANALYSIS

Differences between groups were compared using a Mann-Whitney U test. Significance was defined as $P < 0.05$. Correlational analysis was conducted by Pearson simple correlation. The receiver operating characteristic (ROC) was analyzed to assess the most appropriate cut-off values for the level of CSF α-syn oligomer and the oligomers/total-α-syn ratio in the CSF to distinguish between groups. All analyses were conducted using GraphPad Prism software (GraphPad Prism Version 4.0, GraphPad software, San Diego, CA).

RESULTS

PATIENT POPULATION AND DEMOGRAPHICS

In total, 33 Norwegian individuals from 12 different *LRRK2* families were investigated in the present study. Thirteen individuals

with *LRRK2* point mutations had developed symptomatic PD, including 11 males who were carrying the most common *LRRK2* point mutation, G2019S, and two females who were carrying a different *LRRK2* point mutation, N1437H. The 13 individuals had a mean age of 64.0 years ± 13.3 years. In contrast, 20 individuals were healthy asymptomatic *LRRK2* mutation carriers [G2019S (n = 16) and N1437H (n = 4)]. These 20 individuals had a mean age of 55.4 years ± 15 years. None of the healthy asymptomatic *LRRK2* mutation carriers (LRRK2-H) had any complaints of a movement disorder. Some were receiving medication for diabetes mellitus, mild hypertension, and other minor health problems. In addition, 35 patients with sPD and 42 age-matched healthy controls were also included in this study. No significant difference was noticed in disease duration between symptomatic PD patients with *LRRK2* mutations (LRRK2-PD) and sPD patients. Moreover, there was no difference between the groups with regard to CSF levels of leukocytes or total protein, albumin, and glucose levels, including plasma glucose levels. Controling for age and gender did not significantly alter the results in any case. A summary of the patient population employed in the present study and the respective demographic details are shown in **Table 1**.

LEVELS OF TOTAL α syn (t-α syn) IN CSF SAMPLES

To measure the total α-syn (t-α-syn) in CSF samples, we recently optimized our original α-syn ELISA protocol using a chemiluminescence-based read-out arm for HRP-labeled antibody detection (Tokuda et al., 2010). We demonstrated that our optimized protocol yielded excellent performance with regard to both specificity and sensitivity. Using this system, an increase of approximately 100-fold in the detection of recombinant α-syn was recorded, ranging from 0.010 to >500 ng/ml (Tokuda et al., 2010).

As illustrated in **Figure 1**, the concentration of t-α-syn varied considerably among the four studied groups, although the difference was not statistically significant. Lower mean concentrations of CSF t-α-syn were observed in patients with sPD (mean ± SEM = 22.81 ± 4.198 ng/ml, n = 35), LRRK2-PD (mean ± SEM = 20.54 ± 3.139 ng/ml, n = 13), and LRRK2-H (mean ± SEM = 17.84 ± 2.569 ng/ml, n = 20) than in age-matched controls (mean ± SEM = 24.74 ± 4.470 ng/ml, n = 42) (P = 0.7001, Mann-Whitney U-test).

CSF α syn OLIGOMERS (o-α syn) LEVELS

After measuring the total α-syn in CSF samples from our cohort, we then measured the levels of CSF α-syn oligomers (o-α-syn) in the same samples. For such measurements, we used an ELISA system for α-syn oligomers that specifically detects soluble oligomers without detecting monomeric forms of α-syn (El-Agnaf et al., 2006). Because α-syn oligomers represent a small fraction of the total α-syn in CSF, for this assay we also used a chemiluminescence-based read-out arm to allow detection of low levels of CSF α-syn oligomers. The ELISA protocol was based on a conventional sandwich system in which mAb 211 was used to capture α-syn, followed by detection with a biotinylated form of 211. Subsequently, the biotinylated mAb was detected with ExtrAvidin-HRP, followed by a chemiluminescent substrate. In this assay, no signal is detected for monomeric α-syn, as the capture mAb occupies the only available antibody binding site on the protein. In contrast, multiple mAb binding sites are available in the case of oligomeric forms of α-synuclein, thus permitting both capture and detection (El-Agnaf et al., 2006). This assay has been extensively characterized and yields excellent performance in both specificity and sensitivity (Tokuda et al., 2010). In order to investigate the size of α-syn oligomers detected by our ELISA in CSF, recently we used SEC to fractionate fresh CSF samples from those PD patients that gave a robust signal in our ELISA. The western blot revealed immunoreactive material with an elution peak in SEC fractions corresponding to MW of 70 and 170 kDa which belong to α-syn monomers and dimmers respectively. However, much of the immunereactive material was eluted at the void volume, which indicated a MW of >670 kDa (**Figure 2A**). These SEC fractionation results support the notion that the CSF from PD patients mainly contain HMW α-syn oligomers, since most of the protein material detected by the ELISA has a MW >760 kDa. Experiments are in progress in our laboratory to further characterize and analyze the structure and nature of the oligomeric species of α-syn and to discern any modifications of α-syn detected by the ELISA (i.e., nitrated, phosphorylated, etc.). This information will be useful in order to improve both the sensitivity and selectivity for α-syn protein species in our future ELISA variants.

A scatter plot representing the CSF levels of o-α-syn for each patient in the four groups is shown in **Figure 2B**; the level of o-α-syn is given as the chemiluminescence signal intensity

Table 1 | Details of patient population employed in the present study and the demographics.

Groups	Symptomatic PD due to LRRK2 mutations	Asymptomatic LRRK2 mutation carriers	Sporadic PD	Healthy controls
Number of individuals	13	20	35	42
Gender (M/F)	11/2	11/9	23/2	14/21
Age range (y)	43–87	26–76	38–71	37–74
Mean Age (y)	64 ± 13.3	55.4 ± 15	54 ± 15	59 ± 10
Levodopa equivalents	580 ± 422 mg	NA	628 ± 387 mg	NA
H-Y grade	2.7 ± 0.7	NA	2.28 ± 0.6	NA
Disease duration (months)	24–330	NA	12–300	NA

PD = Parkinson's disease; y = years; M = male; F = female; H-Y grade = Hoehn-Yahr grade.

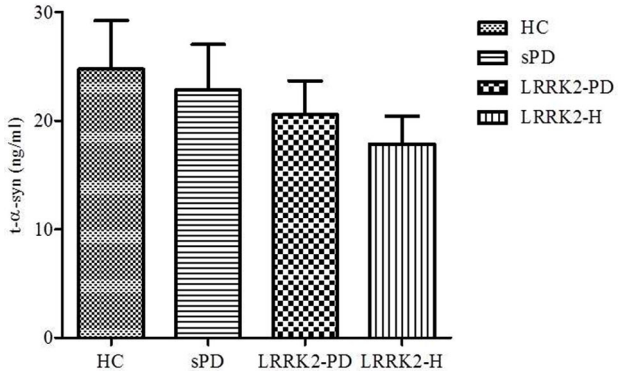

FIGURE 1 | Levels of t-α-syn (in ng/ml) in CSF from healthy controls (HC), sporadic PD patients (sPD), symptomatic PD patients with LRRK2 mutations (LRRK2-PD), and healthy asymptomatic LRRK2 mutation carriers (LRRK2-H). Values are expressed as the mean ± standard deviation.

(relative luminescence units/second [RLU/s]). The level of CSF o-α-syn was significantly higher by 4-fold in the sPD group (mean ± SEM = 80,186 ± 23,861, n = 35) relative to the healthy age-matched control group (mean ± SEM = 17,117 ± 2943, n = 42) ($P < 0.01$, Mann-Whitney U test). Significantly higher levels of CSF o-α-syn (approximately 2-fold higher) were also detected in LRRK2-H (mean ± SEM = 38,754 ± 12,514, n = 20) relative to the control group (mean ± SEM = 17,117 ± 2,943, n = 42) ($P < 0.01$, Mann-Whitney U test). Unexpectedly, LRRK2-PD patients (mean ± SEM = 24,510 ± 7,161, n = 13) did not show any significant difference in the level of o-α-syn in the CSF relative to the healthy control group (P = 0.1910, Mann-Whitney U test), possibly because of the low number of individuals in this group (n = 13 vs. n = 42). Furthermore, we observed a significant inverse correlation (Spearman $r = -0.49$, $P < 0.05$) between the severity of the disease and the level of o-α-syn in the CSF of sPD patients using H&Y grading (**Table 2**). However, no significant correlation (Spearman $r = -0.5391$, P = 0.09) between H&Y grading and the levels of o-α-syn in the CSF of LRRK2-PD patients was observed (**Table 2**). Interestingly, when sPD and LRRK2-PD cases were combined together, more significant and stronger inverse correlation was noted (Spearman $r = -0.6$, P = 0.0004, **Table 2**). Whereas, CSF t-α-syn levels did not correlate with H&Y levels. In parallel, statistically significant inverse correlation between CSF o-α-syn and disease duration was also observed in sPD group (Spearman $r = -0.45$, $P < 0.05$), but CSF o-α-syn did not correlate with disease duration in LRRK2-PD group. Whereas, we observed significant negative correlation between CSF o-α-syn levels and disease duration when the sPD and LRRK2-PD groups were combined together (Spearman $r = -0.5$, P = 0.002, testable 2). In contrast, CSF o-α-syn levels did not correlate with UPDRS scores within any of the groups (data not shown). In addition, no correlation was observed between the level of o-α-syn in the CSF and the age of the patients (data not shown).

The measurement of both total and oligomeric α-syn in RLU/s allowed us to calculate the ratio of o-α-syn to t-α-syn (o-α-syn/t-α-syn ratio [%]) in the CSF of each patient, as shown in **Figure 3**. This ratio was found to be significantly higher in the sPD group (mean ± SEM = 71.59 ± 12.76,

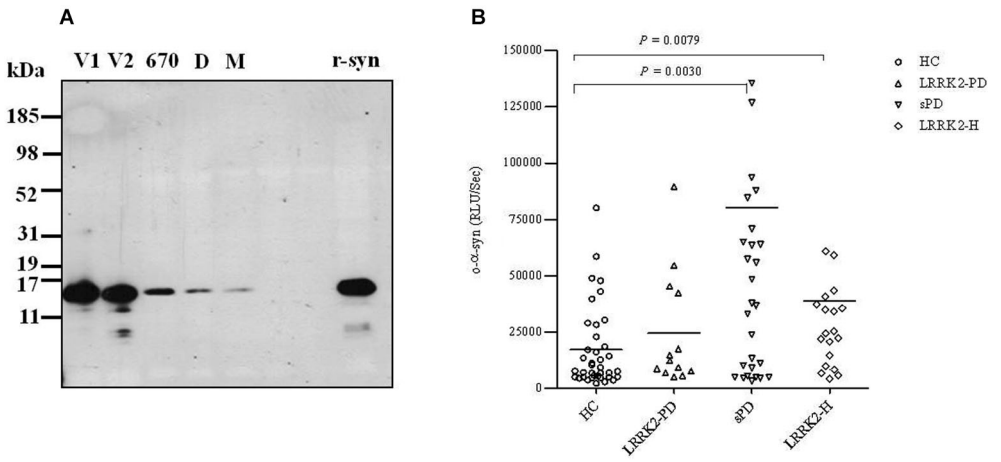

FIGURE 2 | (A) Size exclusion chromatographic analysis of α-syn oligomers in CSF. Five mls of CSF from a PD patient concentrated to 0.5 ml, was fractionated on a superdex 200 SE column. The elution volume for monomeric (M) α-syn was determined by fresh recombinant α-syn solution and molecular weight standard, and was eluted in a peak corresponding to column volume of 13–14 ml (kDa ∼68), while dimeric (D) α-syn was eluted in a peak corresponding to column volume of approximately 12 ml (kDa ∼170). High molecular weight oligomeric α-syn was determined by aged recombinant α-syn solution and molecular weight standard, and was eluted in a peak corresponding to column volume of approximately 9 ml (670 kDa). The void volume (V) corresponds to MW >670 kDa. The fractions of 1 ml which correspond to the oligomeric and monomeric α-syn peaks were separately collected, concentrated to 100 μl using a speed vac, and then analyzed by western blotting for the presence of α-syn using anti-α-syn (211) antibody (1:1000). **(B)** Scatter plot representing individual values of the levels of o-α-syn (in relative luminescence units/second [RLU/s]) in CSF from healthy controls (HC; open circles), symptomatic PD patients with LRRK2 point mutations (LRRK2-PD; open triangles), patients with (sPD; inverted open triangles), and healthy asymptomatic LRRK2 mutation carriers (LRRK2-H; open squares). Each bar represents the mean value.

Table 2 | Spearman correlations between CSF α-syn species, disease duration (months), and Hoehn and Yahr stage in sPD and LRRK2-PD.

	Disease duration (months)			Hoehn and Yahr stage		
Case	**sPD**	**LRRK2-PD**	**sPD+LRRK2-PD**	**sPD**	**LRRK2-PD**	**sPD+LRRK2-PD**
t-α-syn	NS	NS	NS	NS	NS	NS
o-α-syn	−0.45*	NS	−0.5**	−0.5*	NS	−0.6***

** P < 0.05, ** P < 0.01, *** P < 0.001, NS = Not-Significant.*

n = 35) (P < 0.001, Mann-Whitney U test) and LRRK2-H group (mean ± SEM = 103.1 ± 47.91, n = 20) (P < 0.05, Mann-Whitney U test) than in the healthy control group (mean ± SEM = 40.97 ± 21.62, n = 42). No significant difference in the ratio of o-α-syn to t-α-syn was found in the LRRK2-PD relative to controls (mean ± SEM = 24.08 ± 7.359, n = 13) (P = 0.5297, Mann-Whitney U test), possibly because of the small number of individuals in this group (n = 13 vs. n = 42).

Figure 4 shows the ROC curve for CSF o-α-syn and the ratio of o-α-syn to t-α-syn in the discrimination of PD patients from controls. The ROC curve indicates that cutoff values of 31,671.9 RLU/s for CSF o-α-syn and 24.1% for the CSF o-α-syn to t-α-syn ratio were the most reliable measures to distinguish sPD from controls. Detection of CSF α-syn oligomers yielded a sensitivity of 65% (95% CI, 53–77%) and a specificity of 83% (95% CI, 73–93%), with an area under the curve (AUC) of 0.74 (95% CI, 0.60–0.88). The o-α-syn to t-α-syn ratio yielded a sensitivity of 73% (95% CI, 62 to 84%) and a specificity of 77% (95% CI, 0.66–0.88), with an AUC of 0.79 (95% CI, 0.67–0.91). Furthermore, the ROC analysis demonstrated that cutoff values of 20,566 RLU/s for CSF o-α-syn and 9.4% for the CSF o-α-syn to t-α-syn ratio provided the most reliable measure to differentiate LRRK2-H from healthy controls. The sensitivity and specificity of CSF o-α-syn to predict LRRK2-H were 63% (95% CI, 0.50–0.76) and 74% (95% CI, 0.62–0.86), respectively, with an AUC of 0.66 (95% CI, 0.50 to 0.82). The sensitivity of the CSF oligomer to t-α-syn ratio was 53% (95% CI, 39–67%), and the specificity was 81% (95% CI, 0.70–0.92) with an AUC of 0.64 (95% CI, 0.47–0.80).

DISCUSSION

Currently the diagnosis of PD is based mainly on clinical symptoms. However, differential diagnosis from other parkinsonisms can be difficult and can lead to misdiagnosis. To date, no simple laboratory biomarker is available to detect individuals at risk for PD before most of their dopaminergic neurons have been lost.

Numerous studies have suggested that neuronal cell death may result from the formation of oligomeric species of α-syn in the brain (Irvine et al., 2008). It has been previously shown that levels of soluble o-α-syn are elevated in brain homogenates from patients with PD and DLB relative to normal brains (Paleologou et al., 2009). Recently, it has been reported by us and others significant higher levels of CSF o-α-syn in PD patients compared to age-matched controls (Tokuda et al., 2010; Park et al., 2011; Sierks et al., 2011; Parnetti et al., 2014a,b). Levels of o-α-syn and the o-α-syn to t-α-syn ratio have also been shown to be higher in patients with mild PD (H&Y grades 1 and 2) and patients with early PD (within 24 months after onset) relative to a control group (Tokuda et al., 2010). The aim of this study was to determine whether o-α-syn is suitable biomarker for detecting PD at the early stages of the disease. Recently, abnormal PET changes and olfactory dysfunction were reported in LRRK2-H (Nandhagopal et al., 2008; Ruiz-Martínez et al., 2011; Saunders-Pullman et al., 2011). Therefore, healthy family members with *LRRK2* mutations are an excellent population for validating surrogate biomarkers for early stages of PD. A recent study reported a lack of a statistically significant relationship between PET scan evidence of lost striatal dopaminergic function and the levels of DJ-1 and t-α-syn in CSF from LRRK-H or LRRK-PD cases (Shi et al., 2012). In the present study, we assessed the levels of CSF o-α-syn in symptomatic and asymptomatic *LRRK2* mutation carriers and in sPD cases. We observed significantly elevated levels of CSF α-syn oligomers in LRRK-H relative to healthy controls (**Figure 2B**), which suggests that the formation of o-α-syn in the brain commences several years before *LRRK2* mutation carriers experience any motor symptoms of PD. Furthermore, we also observed significantly higher o-α-syn levels in the CSF of sPD cases relative to healthy age-matched controls (**Figure 2B**), which confirms previous findings (Tokuda et al., 2010; Park et al., 2011; Sierks et al., 2011). Unexpectedly, we did not observe any significant difference in the CSF levels of o-α-syn between LRRK-PD patients and healthy controls, possibly because of the low number of individuals in this group (n = 13 vs. n = 42). However, we noticed in this group that patients with mild PD (H&Y grade ≤2) showed high levels of CSF o-α-syn similar to those of sPD patients. Conversely, patients with higher H&Y grades (>2) had lower levels of α-syn oligomers, which supports the hypothesis that at the early stages of the disease, high levels of toxic o-α-syn accumulate in the brain. In support of this notion, we also found that levels of CSF o-α-syn were inversely correlated with disease duration and H&Y grade in LRRK2-PD and sPD cases, which confirms that CSF levels of o-α-syn decrease with increasing PD severity (**Table 2**). Thus, we speculate that o-α-syn is formed during the early stages of the disease prior to any major clinical manifestation.

Unified Parkinson's Disease Rating Scale is the most commonly used clinical scale to provide an efficient and flexible assessment of motor performance in PD patients and to monitor the degree of resultant disability. However, thus far, no strong linear relationship has been established between UPDRS scores and the progressive nigrostriatal degeneration in PD, which may underlie the absence of a correlation between CSF α-syn levels and UPDRS scores. Cerebrospinal fluid biomarkers mirror changes within the brain as an entire unit, whereas UPDRS scores primarily reflect

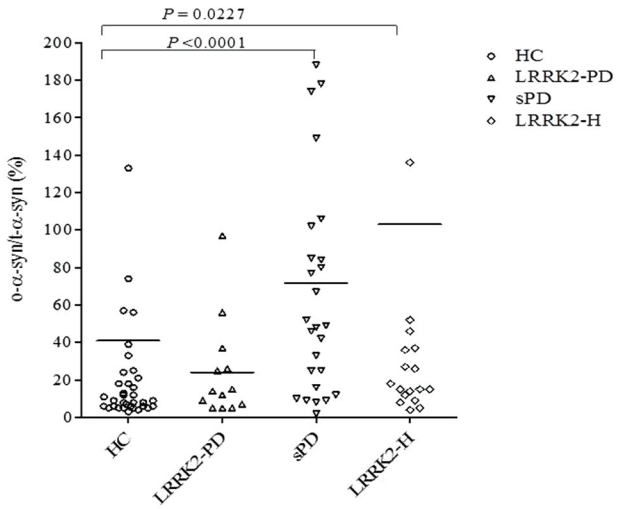

FIGURE 3 | Scatter plot presenting individual values for the ratio of o-α-syn to t-α-syn (o-α-syn/t-α-syn ratio,%) in CSF from healthy controls (HC; open circles), symptomatic PD patients with LRRK2 point mutations (LRRK2-PD; open triangles), patients with (sPD; inverted open triangles), and healthy asymptomatic LRRK2 mutation carriers (LRRK2-H; open squares). Each bar represents the mean value. The *P* value for each regression line is shown in each subfigure.

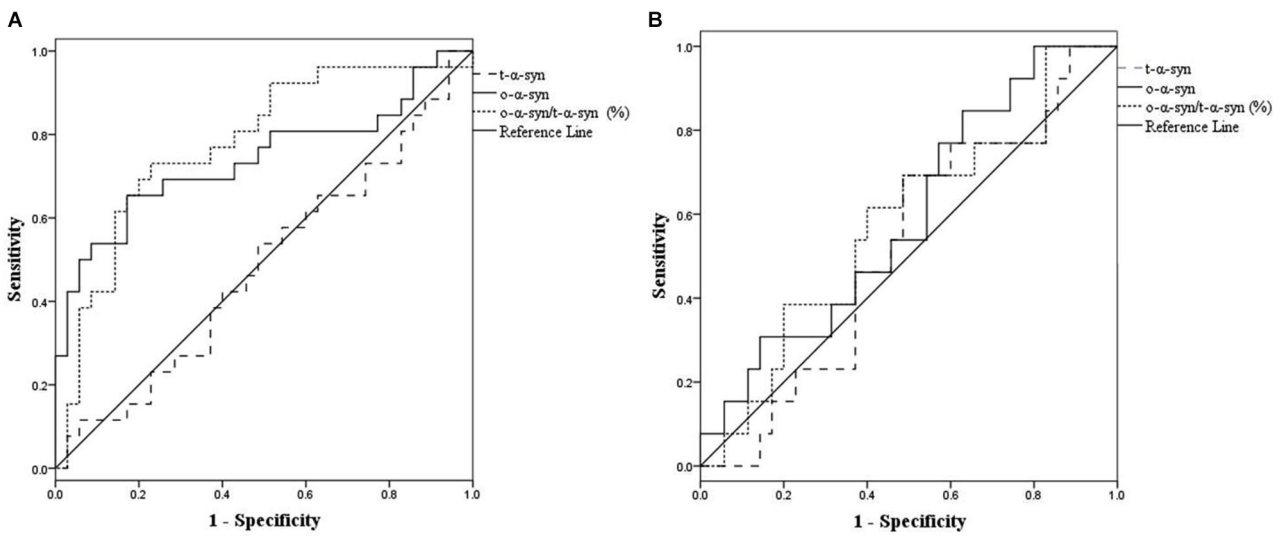

FIGURE 4 | Use of receiver operating characteristic (ROC) curves for the levels of CSF t-α-syn, o-α-syn and o-α-syn/t-α-syn ratio (%) to discriminate between (sPD) patients and controls (A), and healthy asymptomatic LRRK2 mutation carriers (LRRK2-H) patients and controls (B).

changes in the nigrostriatal dopaminergic system. In addition, compensatory responses in PD may further confound the correlation between CSF biomarkers and the severity of PD motor symptoms (Shi et al., 2011). Moreover, DA replacement therapy provided to PD patients enhances motor function while showing little or no effect on CSF protein concentrations (Hong et al., 2010; Shi et al., 2011).

It has been recently reported that the most common *LRRK2* point mutation, G2019S, initiates and enhances the formation of α-syn aggregates (Lin et al., 2009), possibly by impairing degradation pathways such as the autophagy-lysosomal pathway (Ferree et al., 2012; Tong et al., 2012). Overall, the potential interactions of LRRK2 and α-syn have not been clearly established. Although most *LRRK2*-related PD cases are pathologically and clinically undistinguishable from sPD, *LRRK2* mutation carriers exhibit considerable clinical and pathological variability (Wider et al., 2010). Our results support the hypothesis that mutations in LRRK2 may lead to the formation of the toxic oligomeric forms of α-syn critical for the pathogenesis of PD. However, our findings need

to be confirmed in prospectively planned, independent cohort, particularly in samples where PD has been longitudinally assessed such as the ongoing Parkinson's Progression Markers Initiative.

In conclusion, our current pilot study suggests for the first time that quantification of o-α-syn in CSF has strong potential value as a tool for PD diagnosis and presymptomatic screening of high-risk individuals who are good candidates for clinical trials. However, large-scale, prospective, and well-controlled studies, especially those that include subjects at genetic risk, are necessary to validate the use of CSF o-α-syn as a biomarker for PD.

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Apathy in Parkinson's disease is related to executive function, gender and age but not to depression

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Deficits in executive functions occur in up to 93% of patients with Parkinson's disease (PD). Apathy, a reduction of motivation and goal-directed behavior is an important part of the syndrome; affecting both the patients as well as their social environment. Executive functions can be subdivided into three different processes: initiation, shifting and inhibition. We examined the hypotheses, (1) that apathy in patients with Parkinson's disease is only related to initiation and not to shifting and inhibition, and (2) that depression and severity of motor signs correlate with apathy. Fifty-one non-demented patients (19 = female) with PD were evaluated for apathy, depression and executive functions. Executive function variables were summarized with an index variable according to the defined executive processes. Linear regression with stepwise elimination procedure was used to select significant predictors. The significant model ($R^2 = 0.41$; $p < 0.01$) revealed influences of initiation ($b = -0.79$; $p < 0.01$), gender ($b = -7.75$; $p < 0.01$), age ($b = -0.07$; $p < 0.05$) and an age by gender interaction ($b = 0.12$; $p < 0.01$) on apathy in Parkinson's disease. Motor signs, depression and level of education did not influence the relation. These results support an association of apathy and deficits of executive function in PD. Initiation strongly correlates with apathy, whereas depression does not. We conclude, that initiation dysfunction in a patient with Parkinson's disease heralds apathy. Apathy and depression can be dissociated. Additionally, apathy is influenced by age and gender: older age correlates with apathy in men, whereas in women it seems to protect against it.

Keywords: apathy, Parkinson's disease, executive functions, depression, gender, age

INTRODUCTION

Apathy is defined as a primary loss of motivation, a loss of interest and reduced goal-directed behavior (Levy and Dubois, 2006; Starkstein et al., 2012). Apathy is a predictive factor for cognitive deterioration in Parkinson's disease (PD) (Dujardin et al., 2009); and has significant impact on quality of life (Zesiewicz et al., 2010; Chaudhuri et al., 2011).

Executive dysfunctions are highly prevalent in PD, affecting up to 93% of the patients, depending on the disease stage (Emre, 2003) and correlate with apathy (Pluck and Brown, 2002; Zgaljardic et al., 2007; Dujardin et al., 2009).

Executive functions comprise several processes which may be independently impaired (Miyake et al., 2000; Drechsler, 2007; Stuss and Alexander, 2007). Drechsler (2007) identified three executive processes: initiation, inhibition and shifting. Initiation is defined as the ability to start intentional actions self-motivated. Impairments in this process are associated with tests, in which patients are self-generating a processing speed or starting an action. Inhibition is characterized by the ability to suppress a reaction. Patients with impaired inhibition are showing impulsive response behavior and a high level of distractibility. Shifting means the ability to relocate the focus of attention from one to another target. It is reduced if a subject shows cognitive inflexibility and tests measuring this executive process are characterized by changing criteria within the task (Drechsler, 2007).

Major depression is present in up to a third of all patients with PD (Reijnders et al., 2010) and overlaps with apathy (Pluck and Brown, 2002; Kirsch-Darrow et al., 2006; Starkstein et al., 2009). However, there is evidence, that the two syndromes can be dissociated (Levy et al., 1998; Kirsch-Darrow et al., 2006).

Apathy due to frontal lesions shows a decreased performance in tests measuring the initiation process (Drechsler, 2007). We assume that dysfunction of initiation also predicts apathy in PD. Furthermore, we expected apathy to be related to depression. Several examinations have shown a relation between apathy and motor signs (Pedersen et al., 2010; Cubo et al., 2012), and therefore, we expected motor signs to be predictive for apathy. The aim of this study is to investigate the association of neuropsychological, psychiatric and motor factors with apathy in PD.

MATERIALS AND METHODS

PATIENTS

Fifty-eight patients with PD were recruited between October 2011 and April 2013 from the movement disorders clinic of the Basel University Hospital or through advertisements. The patients were participants of a Cognitive training-study (Zimmermann

et al., 2014) and underwent neuropsychological, psychiatric and neurological assessment. Inclusion criteria for the study were idiopathic PD according to UK Parkinson's disease Brain Bank Criteria (Gibb and Lees, 1988) and written informed consent. Patients were excluded if they had moderate or severe dementia (Mini Mental State Examination (MMSE) $\leq$ 24; Folstein et al., 1975), insufficient knowledge of the German language, other severe brain disorders, alcohol or drug dependency. For this study, the data of $n = 51$ patients (19 female, 32 male) has been analyzed. Seven patients were excluded because of having deep brain stimulation ($n = 3$), an unfulfilled apathy questionnaire ($n = 3$) or because German was not their native language ($n = 1$) (see Supplementary Figure 1). All patients were on dopaminergic medication and were tested in the ON state.

PSYCHIATRIC AND PSYCHOLOGICAL ASSESSMENT

Apathy

Apathy was measured with the German version of the Apathy Evaluation Scale (AES_D; Lueken et al., 2006) filled out by a relative or a person close to the patients. This questionnaire consists of 18 items, which are evaluated on a four-point Likert scale, ranging from *not at all* (i.e., 0 points) to *a lot* (i.e., 3 points). For each patient, a total value was calculated with a maximum of 54 and minimal of zero point with higher values indicating more severe apathy.

Depression

Symptoms of depression were measured with a self-rating scale, the German version of Beck Depression Inventory II (BDI_D; Hautzinger et al., 1995). The 21 items are answered on a four-point Likert scale, ranging from zero (e.g., *I do not feel sad*) to three (e.g., *I am so sad and unhappy that I can't stand it*). Based on the evaluation of each item, a total score was calculated. Maximum score is 63 and minimal score is zero, whereby higher values are indicating more severe depression.

NEUROLOGICAL ASSESSMENT

The severity of motor signs was assessed using Unified Parkinson's disease Rating Scale (UPDRS; Fahn and Elton, 1987), subscale III, applied by trained neurologists. This scale consists of 13 items which are evaluated on a four-point Likert scale. The patients are interrogated and examined about their motor issues (e.g., speech, mimicry, rest tremor, bradykinesia etc.). A total score is calculated with zero being minimal and 54 being the maximum value.

NEUROPSYCHOLOGICAL TESTS OF EXECUTIVE FUNCTIONS AND CLASSIFICATION OF VARIABLES

The classification of the variables follows Drechsler's (2007) definition of executive functions (see **Table 1**). The executive variables were classified each to one executive process by the consensus of two raters, and were then converted into z-scores and summarized to an index variable; initiation, shifting and inhibition. Additional information about the tests and analyzed variables is annexed in the Supplementary Material (see Supplementary, description of tests and measures).

Initiation

The initiation process was measured with:

- Semantic Fluency (Morris et al., 1989)
- Phonemic Fluency (Thurstone and Thurstone, 1948)
- 5 Point Test (Regard et al., 1982)
- Trail Making Test (Reitan, 1958)
- Stroop Test (Stroop, 1935)

Shifting

The shifting process was measured with:

- Modified Wisconsin Card Sorting Test (Nelson, 1976)
- California Verbal Learning Test (Delis et al., 1987)
- Trail Making Test (Reitan, 1958)
- Flexibility [Test of Attentional Performance (TAP); Zimmermann and Fimm, 2007]

Inhibition

The inhibition process was measured with:

- Stroop Test (Stroop, 1935)
- Trail Making Test (Reitan, 1958)
- Divided Attention (TAP: Zimmermann and Fimm, 2007)
- Working Memory (TAP: Zimmermann and Fimm, 2007)

STATISTICAL ANALYSIS

Level of statistical significance was set to $p < 0.05$. R version 3.0.1 was used for the analysis (R Core Team, 2012). All variables were

Table 1 | Performance in tests measuring executive functions.

Executive process[a]	Test	Variable	*Mdn [quantile]*
Initiation	Phonemic fluency	Correct answers	12 [10, 15]
	Semantic fluency	Correct answers	21 [17.5, 23]
	5 point test	Correct figures	23 [18.5, 28.5]
	TMT	TMT A	45 [37, 58.5][b]
	Stroop	Naming colors	14 [13.7, 17][b]
Shifting	mWCST	Perseverative errors	1 [0, 2.5]
	CVLT	Perseverative errors	2 [0.5, 3]
	TMT	TMT B/A (ratio)	2.9 [1.9, 2.7]
	Flexibility	Reaction time	4 [1, 9][c]
Inhibition	Stroop	Interference	1.8 [1.6, 2.1]
	TMT	TMT B, errors	0 [0, 1]
	Divided attention	Errors	3 [1, 6]
	Working memory	Errors	5 [2, 7.5]

[a] *Classification according to Drechsler (2007). All medians (quantiles) represent raw values. Quantile refer to 25th and to the 75th percentile. TMT, Trail Making Test; mWCST, modified Wisconsin Card Sorting Test; CVLT, California Verbal Learning Test.*

[b] *Seconds.*

[c] *Milliseconds.*

averaged over the patients and z-scored; variables indicating good performance in smaller values were inversed.

The apathy total score was not normally distributed (Kolmogorov Smirnow Test (KST): $p < 0.01$) therefore, this variable was square root transformed, leading to a non-significant p-value ($p = 0.34$) of the KST. After z-score transformation, the executive function variables were averaged per each executive process: initiation, shifting and inhibition. To check the validity of the sub processes of executive functions, the intercorrelations between all the collected z-transformed cognitive variables and internal consistency (Cronbach's alpha) were calculated. As defined by George and Mallery (2003), acceptable internal consistency was set at Cronbach's α of greater or equal to 0.70. A linear regression model with Akaike Information Criterion (AIC) based stepwise backwards elimination procedure (Venables and Ripley, 2002) was applied to select the relevant predictors of apathy. The analyses were performed using initiation, inhibition, switching, age, gender, education and subscale III of the UPDRS as potential predictors for AES_D total score. Multiple R^2 (coefficient of determination) and F-statistics were used for characterization of the overall model. The R package relaimpo (Grömping, 2006) was used to evaluate the relative importance of the regressors. Relaimpo splits the explained variance (R^2) according to the importance of the predictors (Grömping, 2006).

RESULTS

PATIENTS

Low average total scores of apathy and depression were found in our sample of 51 PD patients. According to the proposed cut-off score of 19 points in the AES_D (Leentjens et al., 2008), 8% of the patients met the criteria for clinical relevant apathy. In 8% of the included patients, a total score in the BDI_D of ≥14 was observed. Therefore, most of the patients had sub-syndromal depression as defined by Schrag et al. (2007). **Table 2** shows the properties of the sample.

INTERNAL CONSISTENCY OF INITIATION, INHIBITION, AND SHIFTING

The internal consistency for initiation was Cronbach's α = 0.78. This finding indicates acceptable internal consistency for this executive process. In contrast, it was inadequate for inhibition (Cronbach's α = 0.48) and shifting (Cronbach's α = 0.41).

The significant model ($R^2 = 0.41$; $p < 0.01$) revealed influences of initiation ($b = -0.79$; $p < 0.01$), gender ($b = -7.75$; $p < 0.01$), age ($b = -0.07$; $p < 0.05$) and an age by gender interaction ($b = 0.12$; $p < 0.01$) on apathy in PD. Motor signs, depression and level of education did not influence the relation.

PREDICTORS FOR APATHY

By stepwise elimination the following variables were excluded from the model: depression, inhibition, shifting and education. The parameters initiation ($b = -0.79$; $p < 0.01$), age ($b = -0.07$; $p > 0.05$), gender ($b = -7.75$; $p < 0.01$) and the interaction of age by gender ($b = 0.12$; $p < 0.01$) remained in the model explaining apathy. Furthermore, the UPDRS subscale III was not significant in the prediction of the AES_D total score ($b = -0.01$; $p = 0.18$), but remained in the model according to AIC. The overall model was significant ($p < 0.01$; $R^2 = 0.41$); adjusted $R^2 = 0.35$; $F_{(5,\ 44=6.24)}$. According to Cohen (1992) an adjusted R^2 of 0.35 can be interpreted as a strong effect. The calculation of the relative importance of the regressors in the model showed, that initiation and the interaction age by gender were the most important variables predicting apathy in PD. Initiation explained 17%, the age by gender interaction 15%, gender 6% and age 2% of the variance of apathy. The age by gender interaction showed that, in men, the AES_Dtotal score was positively correlated with age ($b = 0.06$; $p < 0.05$) while it was negatively correlated in women ($b = -0.06$; $p < 0.05$) (see **Figure 1**).

INTERCORRELATIONS

Table 3 shows the intercorrelations of apathy and the executive functions variables. With exception of phonemic fluency, all variables categorized as initiation (Semantic Fluency, Five Point Test, Trail Making Test, Stroop Test) correlated significantly and negatively with apathy (see **Figure 2**).

DISCUSSION

Apathy is related to executive dysfunction in patients with PD but not to depression or motor signs. As hypothesized, the executive process of initiation is the most influential factor in this association. Incongruent with our initial hypothesis, neither depression nor

Table 2 | Sample description.

	Mdn [quantile][a]
Patients ($n = 51$, 19 Female)	
Age (years)	67 [32, 73]
Education (years)	15 [13, 16]
AES_D	6 [2, 13]
BDI_D	8 [4, 11.5]
MMSE	29 [28, 30]
UPDRS, subscale III	14.5 [7.6, 21]
Hoehn and Yahr stage	2 [0, 2]
LED (mg/day)	600 [300, 947]
Disease duration (years)	4 [2, 6.5]

[a] *All values represent median [quantiles]. Quantiles refer to 25th and to the 75th percentile. AES_D, Apathy Evaluation Scale; BDI_D, Beck Depression Inventory; UPDRS, Unified Parkinson's Disease Rating Scale; LED, L-Dopa- Equivalent Dose.*

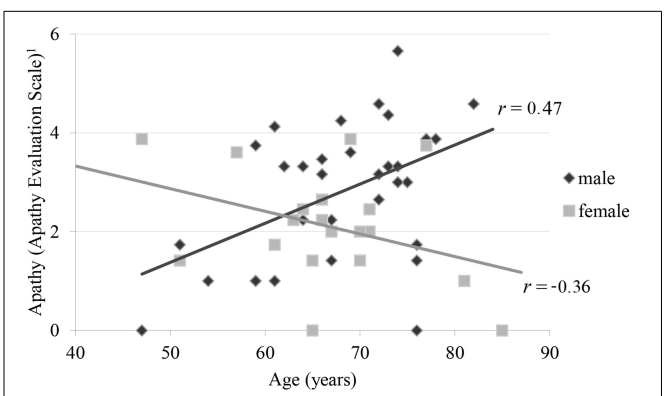

FIGURE 1 | Correlation of apathy and age by gender [1]Apathy Evaluation Scale represents root transformed values.

Table 3 | AES_D, Apathy Evaluation Scale; 5PT, Five Point Test; TMT, Trail Making Test; mWCST, modified Wisconsin Card Sorting Test; CVLT, California Verbal Learning Test; RT, reaction time.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. AES_D, total score	1													
Initiation	**−0.44****													
2. S-words, correct words	−0.13	1												
3. Animals, correct words	−0.35**	−0.45**	1											
4. 5PT, correct figures	−0.36**	0.32*	0.56**	1										
TMT, A	−0.41**	0.18	0.43**	0.40**	1									
Stroopt test, naming colors	−0.35**	0.29*	0.42**	0.54**	0.58**	1								
Shifting	**−0.26**													
7. mWCST, perseverative errors	−0.25	−0.11	0.25	0.29*	0.26	0.09	1							
8. CVLT, perseverative errors	0.03	0.20	0.00	−0.02	−0.21	0.13	−0.22	1						
9. TMT, B/A (ratio)	−0.08	0.35*	0.37**	0.42**	0.04	0.24	0.25	0.00	1					
10. Flexibility RT	−0.24	−0.30*	0.37**	0.35*	0.31*	0.38**	0.12	−0.14	0.26	1				
Inhibition	**−0.23**													
11. Stroop test, interference	0.01	0.34*	0.23	0.24	−0.04	−0.16	−0.08	0.07	0.22	0.18	1			
12. TMT, part B errors	−0.25	0.35*	0.35*	0.22	0.36*	0.43**	0.22	−0.14	0.57**	0.35*	−0.03	1		
13. Divided attention, errors	−0.22	0.17	0.38*	0.27	0.26	0.33*	0.27	−0.02	0.44**	0.32*	0.14	0.38**	1	
14. Working memory, errors	−0.09	0.02	0.24	0.22	0.12	−0.07	0.31*	−0.08	0.38**	0.15	0.06	0.34*	0.23	1

$^{**}p < 0.01$, $^{*}p < 0.050$.

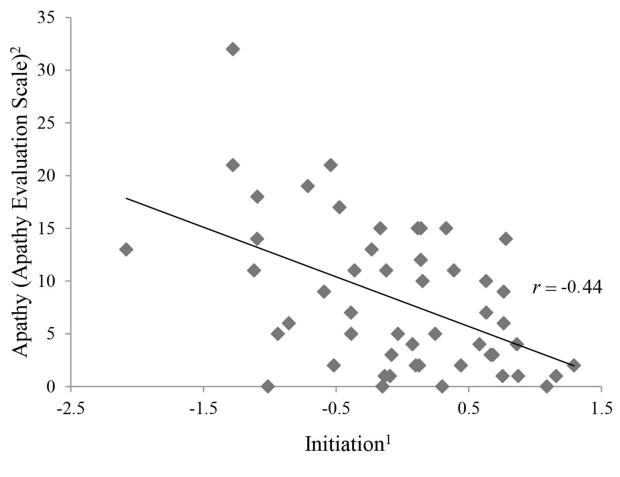

FIGURE 2 | Correlation between apathy and initiation.[1]The ability to start an intentional actions self-motivated. [2]Apathy Evaluation Score represents raw values.

motor symptoms significantly predict apathy. In our study, not all executive processes were predictive of apathy. Neither the single executive variables categorized as shifting, nor inhibition process variables nor the z-scored variables correlate with apathy (see **Table 3**).

In contrast to previous investigations (Aarsland et al., 2009; Pedersen et al., 2010; Varanese et al., 2011), the patients in this study were only slightly affected by apathy and depression. While this fact may be a limitation for the exclusion of weak but still existing relations, it helps to differentiate between distinct psychopathological syndromes; less severely affected patients are more likely to suffer from only one, instead of several disorders simultaneously.

The result regarding the relation between apathy and executive functions are in accordance with the existing literature (Pluck and Brown, 2002; Zgaljardic et al., 2007; Dujardin et al., 2009). Levy and Dubois (2006) propose dysfunctions in the prefrontal cortico-cortex-basal ganglia circuits to be involved in apathy. Dysfunctions in these circuits are also responsible for executive dysfunctions (Alvarez and Emory, 2006). Thus, it is possible that both initiation and apathy are caused by dysfunction in the prefrontal cortico-basal-ganglionic loop. However, some severely affected patients with PD do not suffer from apathy. From this, we can infer that dysfunction of the different cortico-basal loops (like the motor loop as opposed to the cingular-basal and other fronto-basal loop) is disparate.

An alternative explanation for the association between apathy and initiation might be that behavioral symptoms (i.e., lack of motivation, a loss of interest and reduced goal-directed behavior as defined by Levy and Dubois, 2006) are leading to a worse performance in initiation tasks.

Although "executive functions" are a widely accepted neuropsychological construct, the categorization of tests and variables measuring this dysfunction occur mostly in an arbitrary manner. In this study, we used the approach by Drechsler (2007) (i.e., initiation, inhibition, shifting) whereas Stuss and Alexander (2007) for example propose three different processes, i.e., *energization* (initiating and maintaining a response), *task setting* (ability of if-then setting and adjustment of contention scheduling) and *monitoring* (prolonged fore period effect and an increase of all subtypes of errors, including false negatives). This concept of executive processes is based on the analyses of the effects of lesions of the frontal lobes (Stuss and Alexander, 2007). Interestingly, the Drechsler's concept of initiation and Stuss' concept of energization seem to be congruent.

Contrary to our hypothesis we did not find an association between apathy and depression. This is consistent with the assumption that apathy and depression are distinct disorders (Kirsch-Darrow et al., 2006). It is also interesting to note that severity of motor signs is not related to apathy.

In this study, we find an unexpected interaction of age and gender on apathy in PD. The literature regarding the relation of gender and apathy in PD is incongruent. Some studies reported male patients to be more severely affected by apathy (Ready et al., 2004; Pedersen et al., 2010). In contrast the female patients in a multicenter review by Martinez-Martin et al. (2012) were predominantly affected by apathy compared to male patients. Kirsch-Darrow et al. (2006) have detected a significant association between age and apathy in PD, but they did not determine an effect of gender. None of the cited studies reported an interaction of age and gender on apathy in PD. It could possibly be related to the fact that apathy was rated by a relative or a person close to the patients. Thus, this age by gender interaction has not been reported so far and needs validation.

A limitation of this study is the insufficient internal consistency for shifting and inhibition. It might be criticized, that only initiation is predictive for apathy because it has highest internal consistency. However, this possibility is unlikely as the intercorrelations of the single executive variables composing shifting and inhibition were not significantly correlated with apathy, while the single variables used for initiation were. A further limitation of this study is the relatively small sample size. In addition, only a low number of patients are strongly pronouncing apathy respectively depression. Therefore, these results have to be replicated in a larger sample of patients with PD.

In conclusion, our data support an influence of initiation, gender and age on apathy in PD.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fnagi.2014.00350/abstract

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Emotion recognition in early Parkinson's disease patients undergoing deep brain stimulation or dopaminergic therapy: a comparison to healthy participants

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Parkinson's disease (PD) is traditionally regarded as a neurodegenerative movement disorder, however, nigrostriatal dopaminergic degeneration is also thought to disrupt non-motor loops connecting basal ganglia to areas in frontal cortex involved in cognition and emotion processing. PD patients are impaired on tests of emotion recognition, but it is difficult to disentangle this deficit from the more general cognitive dysfunction that frequently accompanies disease progression. Testing for emotion recognition deficits early in the disease course, prior to cognitive decline, better assesses the sensitivity of these non-motor corticobasal ganglia-thalamocortical loops involved in emotion processing to early degenerative change in basal ganglia circuits. In addition, contrasting this with a group of healthy aging individuals demonstrates changes in emotion processing specific to the degeneration of basal ganglia circuitry in PD. Early PD patients (EPD) were recruited from a randomized clinical trial testing the safety and tolerability of deep brain stimulation (DBS) of the subthalamic nucleus (STN-DBS) in early-staged PD. EPD patients were previously randomized to receive optimal drug therapy only (ODT), or drug therapy plus STN-DBS (ODT + DBS). Matched healthy elderly controls (HEC) and young controls (HYC) also participated in this study. Participants completed two control tasks and three emotion recognition tests that varied in stimulus domain. EPD patients were impaired on all emotion recognition tasks compared to HEC. Neither therapy type (ODT or ODT + DBS) nor therapy state (ON/OFF) altered emotion recognition performance in this study. Finally, HEC were impaired on vocal emotion recognition relative to HYC, suggesting a decline related to healthy aging. This study supports the existence of impaired emotion recognition early in the PD course, implicating an early disruption of fronto-striatal loops mediating emotional function.

Keywords: emotion recognition, early-stage Parkinson's disease, healthy aging, DBS, levodopa, dopamine

INTRODUCTION

While Parkinson's disease (PD) is traditionally regarded as a neurodegenerative motor disorder, there is growing evidence for significant cognitive and emotional impairment, particularly as the disease progresses (for review see Owen, 2004). Degeneration of the substantia nigra pars compacta and the subsequent loss of dopaminergic input to the rest of the basal ganglia affects cortex through several corticobasal ganglia-thalamocortical circuits (Alexander et al., 1986). These structurally and functionally segregated loops connecting the basal ganglia to cortex are described as having motor, association (cognitive), or limbic functions (Alexander and Crutcher, 1990; Middleton and Strick, 2002; Le Jeune et al., 2008). Disruption of associative and limbic loops is thought to be one factor contributing to the accompanying cognitive decline, including poor executive functioning and memory, as well as impaired emotion recognition ability in PD (Owen, 2004).

The ability to recognize and identify emotional cues in others is a crucial component of human social interaction. Deficits in emotion recognition have been tied to poor social competence, interpersonal functioning, and communication, as well as a reduced quality of life (Ruffman et al., 2008). In regard to PD, as disease advancement leads to increased dependance on caregivers, intact emotion recognition is imperative for maintaining these intimate and essential relationships.

There is a growing literature evidencing impaired facial and vocal emotion recognition in PD and other diseases of the basal ganglia, including progressive supranuclear palsy (Gray and Tickle-Degnen, 2010; Péron et al., 2012; Pontieri et al., 2012). With respect to PD, neuroimaging work has found this

impairment to be associated with bilateral orbitofrontal cortex (OFC) gray matter loss in PD (Ibarretxe-Bilbao et al., 2009). Importantly, this emotion recognition deficit is separable from an underlying visuoperceptual and cognitive dysfunction (Gray and Tickle-Degnen, 2010; Herrera et al., 2011). Deficits in vocal emotion recognition (i.e., prosody), in contrast, may not be completely separable from underlying deficits in working memory and executive function (Gray and Tickle-Degnen, 2010). While there is support for this remaining deficit for emotion recognition after factoring out dementia or other gross cognitive impairments in PD, few studies have investigated emotion recognition ability earlier in the disease progression.

Patients in the early stage of disease progression (Early PD, or EPD) are less likely to have developed cognitive impairment or dementia than their later-staged PD counterparts, as cognitive impairment is positively correlated with neuropathological staging in PD (Braak et al., 2005). A recent study documented that 70% of EPD patients showed normal cognitive performance (Elgh et al., 2009) and what deficits they do have tend to be mild (Lees and Smith, 1983; Owen, 2004). In contrast, prevalence of dementia in PD patients surviving past 90 years of age is over 80% (Buter et al., 2008). It is therefore more likely that performance of EPD patients on tasks of emotion recognition reflects the specific integrity of these emotion-related processes. The literature on emotion recognition in EPD is mixed, with several studies finding impairment similar to that of later disease stages (Sprengelmeyer et al., 2003; Dujardin et al., 2004a; Ibarretxe-Bilbao et al., 2009; Poletti et al., 2013) and others finding it generally intact (Pell and Leonard, 2005; Péron et al., 2009; Roca et al., 2010). Likely contributing to these differential findings is variation in treatment type and medication status across PD samples studied.

Two effective and common therapies for the motor symptoms of PD are dopaminergic medication and deep brain stimulation (DBS) of the subthalamic nucleus (STN). STN-DBS surgery was first reserved for advanced PD, but now is increasingly considered for those earlier in the disease progression (deSouza et al., 2013; Schuepbach et al., 2013; Charles et al., 2014) as it allows for the reduction of dopaminergic medication (Vingerhoets et al., 2002; Charles et al., 2004). Although both DBS and dopaminergic medication therapies are effective in controling motor symptoms of PD, their effects on emotion are not well understood.

A recent meta-analysis reports that the effect size for PD emotion recognition impairment to be large regardless of medication state (Gray and Tickle-Degnen, 2010). Two studies testing affect recognition in early PD samples also found no difference in task performance between medicated and unmedicated patients (Péron et al., 2009; Roca et al., 2010). However, many studies have observed either evidence for beneficial and deleterious effects of levodopa on emotion recognition in PD. Divergent findings may be explained by understanding the specific brain regions supporting the particular emotion task at hand. For instance, Cools et al. (2001, 2002, 2003) hypothesize that medication is likely to lead to enhanced performance (relative to "off" medication) for tasks involving DLPFC, where DA loss is significant. However, for tasks engaging OFC or other more ventral areas of PFC that are relatively unaffected by DA loss, medication may impair performance through an overdose action.

The literature is similarly inconclusive on the effects of STN-DBS on emotion recognition. Several studies have found no effect of STN stimulation on emotion processing (Schneider et al., 2003; Péron et al., 2010b; Brück et al., 2011; Albuquerque et al., 2014). However, others have found specific deficits in the recognition of fear (Biseul et al., 2005; Drapier et al., 2008; Péron et al., 2010a), sadness (Dujardin et al., 2004b; Drapier et al., 2008; Péron et al., 2010a), as well as anger and disgust (Dujardin et al., 2004b) post-STN-DBS surgery compared to pre-surgery. Mondillon et al. (2012) discusses possible reasons for such divergent findings on the effects of medication and stimulation on emotion recognition, and hypothesizes that STN-amygdala modulation may correct the levodopa overdose on OFC. Therefore the best emotion recognition performance may result from the combination of both levodopa and STN-DBS therapies. Additionally, methodological differences likely account for some of the variability in these findings. Interpreting results from studies comparing pre and post DBS surgery is difficult because there is often change in medication dose as well as the effect of invasive nature of the surgical procedure. Therefore, to understand the effects of stimulation itself, direct comparison of post-surgery performance with STN stimulation turned "on" and "off" is most appropriate.

The aim of this study is to investigate the ability of PD patients early in the disease course to recognize and identify emotion. By capitalizing on an ongoing prospective study examining the safety and tolerability of STN-DBS surgery in early-stage PD patients, we sought to expand our understanding of the development of neuropsychological sequelae of PD and possible effects of therapy. First, we tested emotion recognition performance across three stimulus domains (face, voice, face + voice), thereby increasing the generalizability of our findings to real life emotion perception. Second, we contrasted the effects of two kinds of therapy on emotion processing. Specifically, we capitalized on the randomized nature of the larger study to compare EPD patients who were randomly assigned to receive optimal drug therapy (ODT) to those randomly assigned to receive drug therapy plus STN-DBS (ODT + DBS). The washout nature of the larger study additionally allowed a within-subject approach of on vs. off either therapy. Third, we utilized two healthy control groups in order to separate emotion recognition impairments due to PD pathology from impairments arising from typical aging.

METHODS

PARTICIPANTS

Demographic information is summarized in **Table 1**. Sixteen EPD patients were recruited from the Vanderbilt Clinic of Neurological Surgery from an ongoing pilot clinical trial testing the safety and efficacy of DBS therapy in early-stage PD at Vanderbilt University Medical Center (NCT00282152, IRB 040797, FDA investigational device exemption G050016) (Charles et al., 2014). Seven of these patients were recruited from a group of patients randomized to receive optimal drug therapy only (ODT group), while nine

Table 1 | Participant clinical and demographic information and performance on control tasks.

	Healthy adults		Early Parkinson's disease patients	
	HYC	**HEC**	**ODT**	**ODT + DBS**
N	21	23	7	9
Sex	12 F, 9 M	10 F, 13 M	2 F, 5 M	1 F, 8 M
Age (years)	20.00 ± 1.87	61.96 ± 7.76	62.29 ± 9.09	62.22 ± 7.97
Education (years)	13.24 ± 1.30	15.13 ± 2.80	15.86 ± 2.73	14.22 ± 2.49
WTAR IQ	110.25 ± 5.89	112.26 ± 11.94	108.29 ± 7.18	108.44 ± 9.81
WASI IQ	–	119.30 ± 11.04	111.86 ± 12.58	108.44 ± 16.76
LEDD (mg)	–	–	551.1 ± 197.2	348.7 ± 240.3
UPDRS ON[a]	–	–	21.43 ± 9.88	26.13 ± 14.43
UPDRS OFF[b]	–	–	37.57 ± 8.24	36.75 ± 16.99
BFRT[c]	48.62 ± 2.85	48.18 ± 3.88	42.57 ± 4.16	46.71 ± 3.73
DTT[d]	94.80 ± 6.22	91.54 ± 9.79	82.42 ± 16.89	89.74 ± 12.16

Mean ± standard deviation (SD) reported where appropriate. [a]UPDRS motor scores in the ON state (ODT: on medication; ODT+ DBS: on medication and on stimulation) and [b]OFF state (ODT: off medication; ODT+ DBS: off medication and off stimulation). [c]BFRT has a maximum score of 54. [d]DTT performance presented as percent accuracy ± SD. EPD patients, DTT scores from ON therapy state are reported.

patients were recruited from a group randomized to receive STN-DBS surgery in addition to drug therapy (ODT + DBS group). Prior to completing our tasks, ODT + DBS patients were previously implanted with bilateral electrodes in the dorsolateral part of the STN using methods identical to those used in later-stage patients (Kahn et al., 2012; Camalier et al., 2014). At the time of testing, DBS stimulation amplitude averaged 1.70 V (±0.31) in left electrode, and 1.64 V (±0.25) in right electrode. All ODT + DBS patients had settings of 130 Hz and 60 μs bilaterally. Consistent with study criteria (Charles et al., 2014), all EPD patients were between the ages of 50 and 75, had a clinical diagnosis of PD, had not passed Hoehn and Yahr stage II of the disease (Hoehn and Yahr, 1967), had been treated with levodopa for more than 6 months but less than 4 years, and had not developed on/off motor fluctuations (for full inclusion criteria see Charles et al., 2014). Twenty-three healthy elderly control participants (HEC) who were matched to the EPD patients on age ($t_{(37)} = -0.11$, $p = 0.91$), sex ($\chi^2 = 2.60$, $p = 0.11$), estimated premorbid IQ ($t_{(37)} = 1.1$, $p = 0.27$), and years of education ($t_{(37)} = 0.22$, $p = 0.83$), were recruited from Nashville area. HEC had higher current IQ than EPD patients ($t_{(37)} = 2.27$, $p = 0.03$), but mean current IQ for EPD patients was still above average. In addition, 21 healthy young control participants (HYC) were recruited from the Vanderbilt University student subject pool. HYC and HEC were matched on estimated premorbid IQ ($t_{(34.00)} = 0.70$, $p = 0.49$) and sex ($\chi^2 = 0.82$, $p = 0.37$). HEC had more years of education than HYC ($t_{(31.67)} = -2.91$, $p = 0.006$), who were still in school.

All procedures were in accordance with the ethical standards of Vanderbilt University Institutional Review Board and with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants after they were given a complete description of the study. HEC were paid and HYC received course credit for their participation.

NEUROPSYCHOLOGICAL TESTING

Pre-morbid IQ (e.g., estimated IQ before onset of disease) was estimated for all participants using the Wechsler Test of Adult Reading (WTAR; Wechsler, 2001). EPD patients and HEC also completed the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) in order to obtain an estimated current IQ. The difference between estimated premorbid and current IQ was used to screen for evidence of early dementia (if WTAR > WASI by more than 1 standard deviation (15 index points) patient would be excluded). Consistent with the early-stage nature of the disease in this group, no EPD or HEC participants were excluded from the present study using these criteria. All testing took place at the Vanderbilt Neurosurgery Clinic or Vanderbilt Clinical Research Center.

PROCEDURE

EPD patients underwent 8-day inpatient evaluations at baseline optimal therapy with a 24-h period of washout from drug therapy and stimulation (see Charles et al., 2014 for full methods description). EPD patients were tested first in the ON therapy condition on day 1. In the ON condition, both groups of EPD patients were receiving optimized doses of medication, ODT + DBS patients were also receiving optimum STN stimulation as set by the clinical trial. After day 1, EPD patients began a medication washout period. In this study, OFF therapy testing occurred at least 24-h into the washout period (mean = 2.5 days, sd = 0.73). In the OFF condition, all therapy was discontinued (i.e., all patients had discontinued medications and ODT + DBS patients had also discontinued stimulation). Due to fixed study design of the larger clinical trial, testing order of therapeutic state was not counterbalanced and all patients were tested first in the ON therapy condition, and second in the OFF therapy condition. Potential effects due to practice are addressed in Section Discussion. In order to avoid interference with larger clinical study, we attempted to minimize testing time for the current study. As such, the entire testing battery was not completed for some patients. To aid in interpretation of results, the number of participants for which data was available is reported by task. Levodopa equivalent daily dose (LEDD) at study enrollment was calculated using the formula from Tomlinson et al. (2010). Average LEDD for ODT and ODT + DBS patients is reported in **Table 1**. Four patients (2 ODT, 2 ODT + DBS)

were taking levodopa only, five patients were taking dopamine agonists only (1 ODT, 4 ODT + DBS) and seven patients were taking a combination of levodopa and dopamine agonists (4 ODT, 3 ODT + DBS). HEC and HYC participants completed all testing in one session, approximately 90 min. Unlike the EPD patients, healthy control participants completed all tasks only once. HYC completed the WTAR, but not WASI, in order to reduce testing time, as dementia was not a concern for this population.

TESTING BATTERY

The battery included three tasks assessing emotion recognition ability across three stimulus domains: Montreal Affective Voices Task (MAV, voice; Belin et al., 2008), the Baron-Cohen Reading the Mind in the Eyes Task (RMET, face; Baron-Cohen et al., 2001), and the Awareness of Social Inference Test (TASIT, face + voice; McDonald et al., 2003, 2006). The testing battery also included two control tasks, the Benton Facial Recognition Test (BFRT; Benton, 1983) and the Distorted Tunes Test (DTT; Drayna et al., 2001).

The MAV is a validated set of 90 nonverbal affect bursts corresponding to the emotions of anger, disgust, fear, pain, sadness, surprise, happiness, and pleasure, and neutral. The voices belong to five male and five female actors, each actor vocalizing the nine emotions (Belin et al., 2008). After hearing each stimulus, participants chose between the nine options on the screen. MAV has been previously used to assess prosodic emotion recognition in PD (Eitan et al., 2013), progressive supranuclear palsy (Ghosh et al., 2012) as well as schizophrenia (Alba-Ferrara et al., 2013) and major depressive disorder (Naranjo et al., 2011). Neuroimaging results show poor MAV performance associated with gray matter loss in right inferior frontal and left middle frontal gyri (Ghosh et al., 2012). Electrophysiological evidence also points to involvement of ventral area of the right STN during this task (Eitan et al., 2013).

The RMET is a set of 36 black and white images of the eye region of faces, designed to assess the affective component of Theory of Mind (Tom; Baron-Cohen et al., 2001). Each stimulus is presented with four adjective answer choices. Participants are instructed to choose the word that best describes what the person in the image is thinking or feeling. Answer choices extend beyond the Ekman (1972) six emotions generally considered to be universal, with complex options such as *fantasizing, contemplative, playful*, and *amused*. While not a classic emotion recognition task, RMET has been previously used to assess affective processes in PD (Péron et al., 2009, 2010c; Bodden et al., 2010; Roca et al., 2010; Poletti et al., 2011; Tsuruya et al., 2011), autism (Baron-Cohen et al., 2001), and schizophrenia (meta-analysis: Bora et al., 2009). Posterior superior temporal sulcus and inferior frontal gyrus are among the cortical areas thought to support RMET performance (Adams et al., 2010; Moor et al., 2012).

The TASIT consists of videotaped vignettes of everyday social interactions designed to assess various components of social perception. TASIT has been used in clinical populations including traumatic brain injury (TBI; McDonald and Flanagan, 2004; McDonald and Saunders, 2005; Williams and Wood, 2010), frontotemporal dementia (Kipps et al., 2009), chronic depression (Ridout et al., 2007), and schizophrenia (Kern et al., 2009; Roberts and Penn, 2009). In the current study, we used a TASIT subtest, the Emotion Evaluation Test (EET), to assess the recognition of spontaneous emotional expression (happy, surprised, sad, anxious, angry, disgusted, and neutral). Participants were shown 28 audiovisual vignettes, with a seven forced-choice response following the end of each trial. TASIT consists of forms A and B, which were counterbalanced by testing session in the EPD groups, and by participant in the HEC and HYC groups. Impaired emotion recognition performance on TASIT has been found in patients with nonspecific TBI (McDonald et al., 2003; McDonald and Flanagan, 2004) as well as those who have undergone anterior cingulotomies for depression (Ridout et al., 2007).

The BFRT is a standardized measure with 27 items used to assess the ability to discriminate and match different faces. It is often used to screen for lower-level visuoperceptual impairments, particularly in studies assessing facial affect recognition (Hayes et al., 2009; Yamada et al., 2009; Hot et al., 2013). Poor performance on BFRT has been linked to lesions to right posterior-inferior parietal and right ventral occipitotemporal cortex (Tranel et al., 2009). Due to the small sample size we did not use BFRT as a tool to screen and exclude subjects, but we included the measure in the testing battery in order to quantify facial processing ability across the groups. For EPD groups, BFRT was administered once, during the ON therapy state for each patient.

The DTT is an auditory processing task with 26 items that requires participants to judge whether simple popular melodies contain notes with an incorrect pitch (Drayna et al., 2001; Braun et al., 2008). Used in other clinical populations as a tool to assess sensory deficits (Yang et al., 2012), poor performance on DTT is associated with deficits in processing speech sounds (Jones et al., 2009). Paired with MAV, DTT allows us to determine whether impairment in vocal emotion recognition is attributable to lower-level auditory processing impairment.

All tasks, with the exception of BFRT, were presented on a computer screen. In order to avoid confounds due to alterations in motor state, participants provided verbal responses, which were recorded by experimenter, for all tasks apart from RMET. Due to verbal response format and instructed emphasis on accuracy, reaction time was not collected or measured for any task.

DATA ANALYSIS

Mean percent accuracy was calculated for DTT, TASIT, RMET, and MAV. BFRT was scored out of a possible total long form score of 54. Statistical tests were performed using SPSS. Due to the small number of participants in ODT and ODT + DBS groups, for a subset of the analyses these groups were combined to form one EPD group for comparison with HEC. HEC performance and that of EPD patients in the ON condition (first testing condition) were compared to investigate the effect of PD pathology on emotion recognition. To elucidate effects of PD therapy type (ODT/ODT + DBS) as well as state (ON/OFF), ODT and ODT + DBS emotion recognition performances were compared using repeated-measures ANOVA design. HEC and HYC performances

Table 2 | Task performance in the ON and OFF therapy state for ODT and ODT + DBS patients.

Task	Therapy state	ODT		ODT + DBS	
		N	Mean ± SD	*N*	Mean ± SD
DTT[a]	*ON*	7	82.42 ± 16.89	9	89.74 ± 12.16
	OFF	7	77.47 ± 13.22	9	89.32 ± 13.43
TASIT	*ON*	6	72.02 ± 7.30[b]	9	71.43 ± 13.60
	OFF	4	74.11 ± 10.26	9	71.03 ± 15.09
RMET	*ON*	6	67.13 ± 6.90	8	63.22 ± 12.93[c]
	OFF	6	70.37 ± 5.46	7	65.62 ± 21.18
MAV	*ON*	7	62.22 ± 8.19	9	59.38 ± 9.38
	OFF	7	63.49 ± 6.92	9	59.63 ± 10.57

Means shown are % accuracy ± SD. N = Number of subjects per condition and task. [a]$p < 0.05$ for effect of therapy state. For within-subjects analyses comparing ON/OFF performance, patients with data available from only one testing session were not included. For [b]TASIT, two ODT patients were excluded from within-subject analysis for this reason and the adjusted mean ± SD for the remaining four subjects in the ON therapy state is 69.64 ± 6.84, and for [c]RMET, one ODT + DBS patient was excluded and the adjusted mean ± SD for the remaining seven subjects in the ON therapy state is 66.25 ± 10.46.

were compared to isolate effects of typical aging on these tasks. For comparisons between EPD and HEC, results are reported with and without controling for group differences in current IQ (WASI) and BFRT scores.

RESULTS

Clinical and demographic information for all participants is provided in **Table 1**.

PERFORMANCE ON CONTROL TASKS: BENTON AND DTT

HEC had higher BFRT scores than ODT ($t_{(27)} = 3.28$, $p = 0.003$) but not DBS ($t_{(27)} = 0.88$, $p = 0.17$) patients. Although they made more errors than HEC, on average ODT patients performed in the "normal" range on BFRT (score of ≥41; Benton, 1983). HEC also had higher DTT scores than ODT, though this did not reach statistical significance ($t_{(25)} = 1.75$, $p = 0.09$). HEC and ODT + DBS patients had very similar DTT scores ($t_{(27)} = 0.42$, $p = 0.68$). Means ± standard deviation (SD) for BFRT and DTT performance are reported in **Table 1**.

EFFECT OF PD THERAPY TYPE AND STATE

For each emotion recognition task, a 2 (treatment type: ODT/ODT + DBS) × 2 (treatment state: ON/OFF) repeated-measures ANOVA was conducted on the mean percent accuracy. There was no significant effect of treatment type ($p > 0.4$ for all tasks) or treatment state ($p > 0.5$ for all tasks) on emotion recognition tasks. There was, however, a significant effect of therapy state on DTT performance ($F_{(1,14)} = 4.82$, $p = 0.045$). This was the result of ODT performing worse on DTT during OFF state, as ODT + DBS patients showed no ON/OFF differences in performance. For the DTT interaction between therapy type and state, there was a moderate trend toward significance ($F_{(1,14)} = 3.41$, $p = 0.09$). Means and SD for these analyses are reported in **Table 2**.

EFFECT OF HEALTHY AGING ON EMOTION RECOGNITION

Means ± SD and *t*-values for emotion recognition tests are reported in **Table 3**. HEC and HYC performed similarly overall on TASIT ($t_{(37)} = 0.65$, $p = 0.52$). However, with respect to specific emotion recognition performance, HEC performed significantly better than HYC at identifying disgusting videos ($t_{(23.18)} = -2.13$, $p = 0.04$). The opposite was true for neutral videos ($t_{(37)} = 2.63$, $p = 0.01$). There was no effect of age on RMET performance ($t_{(36)} = 0.35$, $p = 0.73$). There is a significant effect of age on overall MAV performance ($t_{(29.65)} = 4.57$, $p < 0.001$), due to HEC performing worse than HYC. This age-related impairment was evident in specific emotion conditions including anger ($t_{(34)} = 2.98$, $p = 0.005$), fear ($t_{(34)} = 4.79$, $p < 0.001$), happy ($t_{(20.00)} = 4.18$, $p < 0.001$), neutral ($t_{(22.79)} = 4.26$, $p < 0.001$), and pain ($t_{(34)} = 2.42$, $p = 0.02$).

EFFECT OF EPD PATHOLOGY ON EMOTION RECOGNITION

Because we found no effect of PD therapy type on emotion recognition performance between the ODT and ODT + DBS groups, we combined these two groups into one (EPD) so that we would be better powered to detect group differences on tasks with HEC. Means ± SD and *t*-values for HEC and EPD performance on emotion recognition tests are reported in **Table 3**.

TASIT (emotion recognition from faces + voices): *T*-tests found that EPD patients performed worse than HEC on TASIT overall ($t_{(33)} = 3.70$, $p = 0.001$). Specific affect impairment was found for disgusted, anxious, and neutral. Statistical trends for specific affect impairments were found for sad ($p = 0.07$), happy ($p = 0.06$), and surprised ($p = 0.05$). Group effect remained significant after controling for current IQ ($F_{(1,32)} = 7.76$, $p = 0.009$). BFRT performance was found to be unrelated to TASIT accuracy ($F_{(1,30)} = 1.02$, $p = 0.32$).

RMET (emotion recognition from faces): EPD patients also performed worse than HEC on RMET ($t_{(31)} = 2.98$, $p = 0.006$). This effect remained significant at trend level ($F_{(1,32)} = 3.73$, $p = 0.06$) after controling for current IQ. BFRT performance was unrelated to RMET accuracy ($F_{(1,27)} = 0.04$, $p = 0.84$).

MAV (emotion recognition from voices): *T*-tests showed EPD patients performed worse than HEC on MAV ($t_{(35)} = 2.02$, $p = 0.05$). The groups also diverged in performance for angry ($t_{(35)} = 2.57$, $p = 0.02$), and surprised ($t_{(35)} = 3.24$, $p = 0.003$) conditions. After controling for current IQ, EPD and HEC performed similarly overall on MAV ($F_{(1,34)} = 0.679$, $p = 0.42$).

DISCUSSION

We found that EPD patients are impaired on emotion recognition across three types of stimuli relative to matched healthy controls. Impairment on facial emotion recognition tasks (RMET, TASIT) was not accounted for by underlying differences in ability to process basic facial features. EPD patients' vocal emotion recognition impairment (MAV) was less pronounced, though specific deficits in processing of angry and surprised voice stimuli were found. Similar performance on DTT between EPD patients and healthy aged matched controls indicate this impairment is not accounted for by differences in low-level pitch perception. These group effects were differentially affected by controling for current IQ (WASI), though this potential confound is tempered by the

Table 3 | Performance for HYC, HEC, and EPD patients on emotion recognition tests TASIT, MAV, and RMET.

		HYC vs. HEC *t*	HYC	HEC	EPD	HEC vs. EPD *t*
TASIT	*N*		19	20	15	
	Overall	−0.65	86.47 ± 7.95	84.64 ± 9.56	71.67 ± 11.17	3.70**
	Neutral	−2.63*	92.11 ± 11.94	81.25 ± 3.75	56.67 ± 19.97	4.32**
	Disgusted	2.13*	81.58 ± 26.14	95.00 ± 10.26	81.67 ± 17.59	2.62*
	Anxious	−1.05	89.47 ± 15.17	83.75 ± 18.63	66.67 ± 29.48	2.10*
	Angry	−1.66	88.16 ± 15.29	80.00 ± 15.39	83.33 ± 24.40	−0.50
	Sad	0.77	80.26 ± 17.83	85.00 ± 17.01	73.33 ± 20.00	1.86
	Happy	−0.37	80.26 ± 17.83	77.50 ± 27.98	60.00 ± 24.64	1.93
	Surprised	−0.89	93.42 ± 11.31	90.00 ± 12.57	80.00 ± 16.90	2.01
MAV	*N*		15	21	16	
	Overall	−4.57**	78.37 ± 4.56	66.98 ± 10.06	60.62 ± 8.71	2.02
	Neutral	−4.26**	98.67 ± 5.16	76.67 ± 22.88	68.75 ± 25.53	0.99
	Disgusted	−0.76	82.00 ± 11.46	77.62 ± 19.98	74.38 ± 11.53	0.58
	Afraid	−4.79**	66.00 ± 11.83	41.43 ± 17.11	37.50 ± 18.80	0.66
	Angry	−2.98**	54.67 ± 19.95	36.67 ± 16.23	23.13 ± 15.37	2.57*
	Sad	−1.72	96.00 ± 6.32	90.00 ± 14.14	88.75 ± 10.25	0.30
	Pain	−2.43*	62.67 ± 16.24	47.62 ± 19.72	43.75 ± 18.93	0.60
	Happy	−3.65**	100.00 ± 0.00	92.86 ± 7.84	89.38 ± 10.63	1.15
	Pleasure	−0.39	76.00 ± 23.84	72.86 ± 23.48	73.75 ± 13.60	−0.14
	Surprised	−0.36	69.33 ± 17.51	67.14 ± 18.75	46.25 ± 20.29	3.24**
RMET	*N*		19	19	14	
	Overall	0.12	77.98 ± 11.05	76.68 ± 11.69	64.90 ± 10.60	2.98**

*Means shown are % accuracy ± SD. *p < 0.05, **p < 0.01.*

fact that the EPD patients in this study had an average IQ of 108 and thus had typical cognitive functioning.

Our finding of facial emotion recognition impairment in EPD replicates that of a meta-analysis showing this impairment in later stages of PD (Gray and Tickle-Degnen, 2010). Evidence of brain regions supporting the affect recognition tasks in the present study partially overlap with known projections from basal ganglia to frontal cortex via the associative and limbic loops. Thus, our findings suggest these loops may be dysfunctional early in the disease process. Our finding of impaired emotion recognition from eyes (RMET) does conflict with two studies (Péron et al., 2009; Roca et al., 2010), which have found intact RMET performance in early PD. One important methodological difference between these former studies and the current study, which may account for divergent results, is the other studies' use of abbreviated version of the RMET, with half the number of stimuli used in the current study. Most recently, Poletti et al. (2013), using the same full 36-item RMET used in the current study, also found impaired performance in EPD patients relative to matched healthy controls. While the EPD patients included in this study were also impaired on emotion recognition from voice stimuli (MAV), the effect was weaker than those for face stimuli (RMET, TASIT). This is in contrast to a meta-analysis that found larger effect size for PD emotion recognition impairment for vocal stimuli compared to that of faces (Gray and Tickle-Degnen, 2010). It is possible that prosody recognition deteriorates over time as PD progresses, but that remains to be tested longitudinally.

Our results indicate that dopaminergic medication and STN-DBS do not differentially affect emotion recognition performance in EPD. Likely playing a part in many studies that contrast these two therapies is underlying patient clinical and neuropsychological differences between the treatment groups. For example, some PD patients are not willing to undergo surgery for treatment, or surgery is contraindicated due to other medical concerns. Furthermore, existing neurocognitive deficits such as impairment in semantic fluency, or problems with impulse control, affect the decision to provide surgical intervention and certainly the surgical target for DBS (e.g., STN v. globus pallidus interna (GPi)). An important feature of the current study is that EPD patients were assigned to their treatment groups through a completely randomized process. Perhaps, then, these patient characteristics that often clinically influence a therapy choice are not a factor for this study and our results reflect this.

We do not see an effect of STN stimulation on emotion recognition in this sample. This finding is in contrast to previous findings that STN-DBS surgery leads to deficits in emotion recognition, particularly for negative emotions (Dujardin et al., 2004b; Drapier et al., 2008; Péron et al., 2010a). However, methodological differences between previous studies and ours are of note. In this study, patients receiving STN stimulation (ODT + DBS) were of early PD progression, not past Hoehn and Yahr stage II. Additionally, this study isolates the effect of STN-DBS stimulation from that of DBS surgery, which may confer its own effects on emotion recognition processes (Brück et al., 2011) as well as executive function (Okun et al., 2009). There is extremely limited evidence for the effect of STN-DBS stimulation in early PD, and for this reason it is very difficult to compare our finding to the published literature on STN stimulation effects. In comparison to published studies examining STN stimulation

in advanced PD patients, stimulation levels in this study (ODT + DBS group) were relatively low; under 2 V. It is possible that this low setting is efficacious for controling motor symptoms but does not exert measurable extra-motor side effects. Another possibility is potential disruptive effects of stimulation, exhibited in the other studies, is only evidenced as disease progression itself produces greater deficits in emotional processing. In view of the concerns for cognitive change associated with STN-DBS in advanced PD, this study seems to indicate that STN-DBS, when applied early in the therapy, does not worsen the pre-existing decline in emotional processing already present early in the disease.

A similar lack of ON/OFF effect for emotion recognition was found on dopaminergic medication therapy in the ODT group. The literature on the effect of dopaminergic medication on emotion processing in PD (most of which has been conducted in moderate to severe PD populations) is mixed. It is possible that early in the disease progression, levodopa or dopamine agonists do little to affect emotion recognition processes. Within the EPD literature, our finding that emotion recognition is unaffected by medication status is in accordance with other studies (Péron et al., 2009; Roca et al., 2010). Separate from the emotion recognition battery used in this study, the DTT did reveal an effect of therapy state, potentially an effect of medication. DTT was employed in this study as a perceptual control task, not expected to be affected by treatment state. This finding certainly requires further investigation.

Our finding of EPD impairment on tests of emotion recognition is consistent with previous work documenting altered structure and function of OFC and amygdala in early disease stages. Ouchi et al. (1999) observed reduced density of dopamine transporter binding sites in OFC and amygdala in levodopa naïve, early PD patients. Consistent with progression of Lewy-body pathology to neocortex (Braak et al., 2003), OFC (Ibarretxe-Bilbao et al., 2009; Tinaz et al., 2011) and amygdala (Ibarretxe-Bilbao et al., 2009) volumes are decreased by Hoehn and Yahr stage II. OFC volume is strongly correlated with emotion recognition performance in EPD patients (Ibarretxe-Bilbao et al., 2009). However, functional MRI study has suggested that abnormal amygdala activation during perception of emotional faces is present in EPD prior to manifestation of behavioral impairment in emotion recognition (Tessitore et al., 2002).

It is important to consider how neuropsychological assessments for emotion recognition or discrimination abilities may be included in neuropsychological assessments for PD. Early detection of an emotion recognition deficit could direct the patient to interventions, for instance, social cognition training, to remediate the impairment before it worsens. While not yet studied in PD, such interventions are found to be effective in improving social cognition in disorders such as schizophrenia (Horan et al., 2008; Roberts and Penn, 2009) and autism (Turner-Brown et al., 2008). Emotion recognition tests employed in research could be easily adapted for neuropsychological assessment, assuming sufficient normative data exists. For example, Ekman and Friesen's (1975) classical facial affect recognition test is now a subtest in the Social Cognition and Emotional Battery (SEA; Bertoux et al., 2012; Funkiewiez et al., 2012), used for early diagnosis of the behavioral variant of frontotemporal dementia (bvFTD), where aberrant social behavior is often the presenting symptom. The RMET (Baron-Cohen et al., 2001) is already widely used in research for assessing ToM in different clinical populations. While there is some normative data available (Baron-Cohen et al., 2001), we know little about how healthy older adults perform on this task, preventing present adoption of the task in neuropsychological assessment of the elderly. Although not greatly impaired in this EPD sample, deficits in vocal emotion recognition are more striking than for facial emotion recognition in later stages of PD (Gray and Tickle-Degnen, 2010). To our knowledge, no neuropsychological testing includes vocal emotion recognition tests. However, the Montreal Affective Voices Battery (Belin et al., 2008) could be standardized and eventually included alongside tests of facial affect recognition in neuropsychological assessments.

Finally, it is still relatively unknown how deficits on tests of emotion recognition translate into patients' every day social behaviors, so future research should endeavor to link test performance with a real-life measure of social functioning. Additionally, the development of more ecologically valid measurements of social cognition, such as gaze behavior, is warranted (Sturm et al., 2011; see also Kumfor and Piguet, 2013).

EFFECT OF AGING ON EMOTION RECOGNITION

By including two healthy control groups in this study, we were able to capture effects of typical aging on emotion recognition. Our finding of a robust HEC impairment on MAV, relative to HYC, indicates that decoding of emotional prosody deteriorates with age. This is in accordance with previous studies investigating the affect of aging (Ruffman et al., 2008; Mill et al., 2009), reporting deficits in vocal emotion processing in older adults. Importantly, there was no difference between HYC and HEC in performance on DTT, our auditory control task. This suggests that the finding cannot be attributed to age-related hearing loss, which is also in line with previous research (Orbelo et al., 2005; Mitchell, 2007). However, other studies (Mill et al., 2009; Ruffman et al., 2008, 2009) have also reported that facial affect recognition also deteriorates with age, particularly that of negative emotions. This HEC sample performed similarly to HYC on our facial emotion recognition tasks, TASIT and RMET, with the exception of neutral and revolted videos in the TASIT. However, most previous studies investigating facial affect recognition use static face stimuli, different from that used in the current study. TASIT requires the integration of contextual verbal and non-verbal information from social interaction. RMET provides only the eye region of faces, so perhaps it is easier focusing on only this region, though studies have observed aging related impairment on this task as well (see Kemp et al., 2012 for review). Additionally, the answer choices for RMET are dissimilar to those used in typical affect recognition tasks, as it was originally designed to assess affective ToM. It is possible that these methodological differences could account for the differences between our study and previous studies investigating the effects of healthy aging.

LIMITATIONS

It is important to note certain limitations to our study. Due to study constraints, we were unable to counterbalance the therapeutic state of the testing sessions in the EPD patients; ON testing was always first and OFF testing always second. This limitation is shared by many studies investigating the effect of STN-DBS on emotion recognition, as a pre vs. post surgery approach is frequently employed to investigate effects of stimulation. Future studies investigating the effect of PD therapy on emotion recognition using a similar within-subject design would ideally counterbalance testing sessions. We were unable to compare all possible therapy states for EPD-DBS group (e.g., DBS-ON/ MED-ON, DBS-ON/MED-OFF, DBS-OFF/MED-ON, DBS-OFF/MED-OFF). Our ability to compare all possible testing states was limited by concerns for EPD patients' comfort and desire to minimize potential practice effects. Future studies should compare all possible testing states in order to completely separate stimulation from medication effects in PD-DBS patients. Additionally, as this study was initiated after the larger clinical trial was initiated, pre-surgical testing of emotion recognition was not possible. Furthermore, EPD patients were not blinded to therapy state, making placebo effects a possible confound in our results. Finally, our EPD sample is small, which may have hindered our ability to detect group differences in such a heterogeneous disease. As such, the study may have been underpowered to detect effects of therapy type and state on emotion recognition. Unfortunately, given the constraints of the larger clinical trial, a larger group of EPD patents could not be sampled. Interpretation of these results would benefit from replication in a larger sample.

GENERAL CONCLUSIONS

This study finds emotion recognition impairment in early PD. This impairment was strongest for tasks of facial affect recognition. These deficits are not accounted for by lower-level impairment in visual or auditory processing. It is interesting to find such deficits in patients who otherwise exhibited a normal cognitive profile. It suggests that alterations of the basal ganglia circuitry effects both motor and limbic domains before broader cognitive effects are observed in later PD (Braak et al., 2005). We found no difference treatment type (ODT/ODT + DBS) on emotion recognition performance in this early PD sample. The patients who participated this study were randomized to treatment groups as part of a larger study, which allowed us to test early PD patients receiving DBS stimulation; a rare population. This randomized design also avoids many confounds in patient characteristics that may otherwise have affected task performance, such as neurocognitive deficits or problems in impulse control. Nevertheless, this finding would benefit from replication in a larger sample. By utilizing two healthy control groups (young and elderly), we were able to replicate the finding that emotional prosody identification decays with age, and provide evidence that facial affect recognition remains intact in individuals in their 60 s. Our results support the existence of altered functioning in non-motor corticobasal loops early in the PD progression, which is likely associated with documented disturbances in OFC and amygdala in EPD patients. Early deficits in the ability to interpret social cues seen in this study are especially concerning as progressive motoric impairment in PD often leads to increased reliance on caregivers. Researchers and clinicians in this field should work toward the inclusion of emotion perception tests into neuropsychological assessments to aid in identifying patients who are significantly impaired in this domain. Provision of education and support surrounding these interpersonal deficits as a symptom of the disease may be warranted in an effort to preserve important relationships with loved ones. Whether these deficits could be improved through intervention is a critical area that is yet to be explored in PD.

AUTHOR CONTRIBUTION

Lindsey G. McIntosh collected and analyzed data, wrote, and revised the final manuscript. Sishir Mannava collected and analyzed data, and wrote a first draft of the manuscript. Corrie R. Camalier contributed to study conception and design, analyzed data, revised and critiqued the manuscript and provided supervision. Bradley S. Folley obtained funding, contributed to study conception and design, and reviewed and critiqued the manuscript. Aaron Albritton collected data and reviewed and critiqued the manuscript. Sohee Park contributed to study conception and design and reviewed and critiqued the manuscript. Peter E. Konrad obtained funding, reviewed and critiqued manuscript, and provided supervision. David Charles obtained funding, reviewed and critiqued manuscript, and provided supervision. Joseph S. Neimat obtained funding, contributed to study conception and design, reviewed and critiqued manuscript, and provided supervision.

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Effects of combined MAO-B inhibitors and levodopa vs. monotherapy in Parkinson's disease

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Background: Prior studies report that monoamine oxidases inhibitors (MAO-I) when used as an adjunct to levodopa ameliorate motor symptoms in Parkinson's disease (PD), but this was not tested in relation to cognitive or psychiatric measures.

Objective: Here, we tested the effects of MAO-I as an adjunct to levodopa, in comparison to levodopa or dopamine (DA) agonists alone, on various cognitive, affective and quality of life measures.

Methods: We studied three groups of subjects: healthy controls, PD patients on combined levodopa and MAO-I, and PD patients on levodopa or DA agonists only.

Results: We found that compared to monotherapy, combined MAO-I and levodopa seemed to improve cognition, including probabilistic learning, working memory and executive functions. There were no differences between the different medication regimes on deterministic learning, attention or memory recall. It was also found that MAO-I as an adjunct to levodopa improves affective measures such as depression, apathy, anxiety and quality of life. Interestingly, this enhancing effect of combined levodopa and MAO-I was more pronounced in PD patients with severe akinesia, compared to patients with severe tremor.

Conclusion: Our data are in agreement with (a) the Continuous Dopaminergic Stimulation (CDS) theory which states that continuous stimulation of the basal ganglia enhances motor, psychiatric and cognitive functions in PD patients; and/or (b) findings that MAO-I increase the bioavailability of monoamines that have beneficial effects on motor and behavioral dysfunction in PD.

Keywords: Parkinson's disease, MAO inhibitors, cognition, working memory, depression, anxiety, quality of life, learning

INTRODUCTION

PHARMACOLOGICAL THERAPY FOR PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive and neurodegenerative movement disorder associated with a substantial loss of dopamine (DA) neurons in the substantia nigra (Kish et al., 1988), which are essential for regulating the function of the striatum (a major input structure of the basal ganglia), and the control of voluntary movement.

Standard pharmacological medications for PD include levodopa, various kinds of DA agonists, and Monoamine Oxidases Inhibitors (MAO-I), among others. These medications are prescribed as either monotherapy (taken alone) or polytherapy (using more than one medication in combination) (Rinne, 1987). Levodopa is a DA precursor, taken up by DA cells and converted into DA (Trugman et al., 1991; Muriel et al., 2002; Grace, 2008). MAO- I are a class of chemicals that inhibit the activity of monoamine oxidase enzymes, thus preventing the breakdown of monoamine neurotransmitters, including DA (O'Carroll et al., 1983; Dewey, 2004; Robottom, 2011). MAO-B inhibitors are often administered in the earlier stages of PD, including selegiline and rasagiline. Both these drugs are used either as monotherapy or in combination with levodopa (Caslake et al., 2009; Riederer and Laux, 2011; Fabbrini et al., 2012). While the beneficial effects of these drugs on the motor symptoms of PD are well established, their effects on cognitive and affective symptoms are not as thoroughly investigated.

EFFECTS OF MAO-B INHIBITORS ON MOTOR, COGNITIVE, AND AFFECTIVE PROCESSES IN PARKINSON'S DISEASE

As monotherapy, MAO-B inhibitors may be more effective at the early stages of PD and can delay the need for levodopa. When taken in combination with levodopa (especially as it is usually done in advanced stages of PD), they are known to prolong the effectiveness of levodopa, reduce the amount of levodopa

required to control the symptoms and reduce motor fluctuations (Henchcliffe et al., 2005; Riederer and Laux, 2011). While the effectiveness of selegiline in controlling the motor symptoms of PD has been reported as early as 1975 with the first clinical trial by Birkmayer et al. (1975), the effect of rasagiline on motor processes was recently confirmed by several large multicenters as well as smaller clinical trials (Parkinson Study Group, 2002; Olanow et al., 2009). Notable among these studies which used rasagiline as a monotherapy are the ADAGIO and TEMPO studies. The TEMPO study showed the effectiveness of rasagiline on motor processes (as measured by the UPDRS scores), over a 26-week double blind placebo controlled clinical trial on 404 patients with early PD (Parkinson Study Group, 2002). Further, the ADAGIO study noted that 1 mg of MAO-B-I might have a disease modifying effect as observed in activities of daily living scale and the rate of change in the UPDRS scale (Olanow et al., 2009). Importantly, a recent meta-analysis study found that MAO-B inhibitors as an adjunct to levodopa is superior to levodopa alone at reducing PD symptoms in PD patients (Talati et al., 2009).

Research on the effect of MAO Inhibitors on cognitive, behavioral as well as emotional functions in PD has been limited. Most of these studies focus on the effectiveness of the drugs to reduce neuropsychiatric symptoms (Hindmarch et al., 1992; Parkinson Study Group, 1993; Elmer et al., 2006). MAO-B inhibitors such as moclobemide, tranylcypromine, seligiline, and rasagiline (taken alone or in combination with other antidepressants) have been found to be useful in treating depression and anxiety in PD (Steur and Ballering, 1997; Fahn and Chouinard, 1998; Nayak and Henchcliffe, 2008; Korchounov et al., 2012).

Due to its amphetamine-like derivatives, MAO-B Inhibitors have been suggested to improve cognitive performance in cognitively-impaired rats and other subjects (Yasar et al., 1996). Studies conducted on mouse models with derivatives of MAO Inhibitors have shown neuroprotective effects that additionally lend support to the hypothesis of the potential of MAO inhibitors in affecting cognitive, behavioral and emotional measures in patients with PD (Youdim, 2006, 2013; Kupershmidt et al., 2012). Further, Nickel et al. (1990) have shown that in rats, l-deprenyl and l-amphetamine (metabolites of MAOC-I, such as selegiline) increase EEG theta rhythms, indicating that the drug has facilitory effects on learning and memory.

The purpose of this present study is to examine the combined effects of MAO-B inhibitors and levodopa vs. monotherapy involving either DA agonists or levodopa on the motor, psychiatric, and cognitive processes in patients with PD. For this we designed an exploratory study where we recruited three groups of subjects: healthy controls, PD patients on MAO-I and levodopa, and PD patients on levodopa or DA agonists only. All groups were tested on various cognitive, neuropsychological, and affective processes, as described below.

METHODS

SUBJECTS

The study was approved by the local medical ethics committee, and informed consent was obtained from all subjects, in compliance with research standards for human research at Ain Shams and Cairo Universities. All subjects were recruited from the clinics associated with the Institute of Psychiatry, Ain Shams University as well as Cairo University. The patients diagnosed by a neurologist as having idiopathic PD according to UK Brain Bank diagnostic criteria for PD and they were in three different treatment regimes such as: (1) a monotherapy of levodopa; (2) monotherapy of DA agonists; and (3) combined therapy of levodopa and MAO- I (selegiline or rasagiline). PD patients were assigned different medications to manage their symptoms (tremor, akinesia, gait disturbance, and postural instability). Most patients were initially prescribed levodopa. If levodopa did not manage the symptoms (e.g., based on patients' distress or caregiver's observations), they were given MAO-I in addition to it. Some patients were taken off levodopa and assigned DA agonists to manage their symptoms.

We tested healthy controls, PD patients on levodopa and MAO-I (selegiline or rasagiline), and PD patients on monotherapy (levodopa or DA agonists only), using a between-subject design (see **Table 1**). Among PD patients on monotherapy, 35 subjects were on levodopa while 4 subjects were on DA agonists only (Pramipexole and requip). Among 37 PD patients on levodopa and MAO-I, 19 patients were on selegiline and levodopa and were 18 patients on rasagiline and levodopa. In neurological and clinical practice, the dosage of each selegiline and rasagiline is different. Most of our patients were on daily dose of selegiline of 10 mg (except one patient was on 20 mg daily dose); other patients were on daily dose of either 0.5 or 1 mg of rasagiline. As in our prior studies (Moustafa et al., 2008a,b; Piray et al., 2014), most of our healthy control subjects were spouses of patients, who tended to be fairly well matched demographically. Other healthy control subjects were recruited from the community. The total testing time took approximately 105–120 min for healthy controls, and 115–135 min for PD patients (over two sessions of testing).

Each patient's disease severity was measured using the Hoehn and Yahr stages (Hoehn and Yahr, 1967) and the Unified Parkinson's Disease Rating Scale (UPDRS).The severity of freezing of gait episodes was measured using the Freezing of Gait Questionnaire (FOGQ; Giladi et al., 2000) and The National Adult Reading Test was used to measure the premorbid intellectual functioning (predicted intelligence quotient (IQ); Bright et al., 2002).

All subjects were screened for intact general cognitive function and absence of dementia with the Mini-Mental Status Exam (MMSE; Folstein et al., 1975) and were required to obtain a score of at least 26 to be considered for the study. Patients who were on combined levodopa and DA agonist therapy, cholinergic, or serotonergic medications were excluded. Altogether, nine subjects were excluded based on these criteria. In addition, two subjects did not learn one of the learning tasks, so their data was not included in the statistical analysis. Subjects who were on multiple levodopa and DA agonists were also excluded from further testing. The final study sample consisted of 43 healthy controls, 37 PD patients on MAO-I and levodopa, and 39 PD patients on monotherapy, which is either levodopa or DA agonists only (see **Table 1**). Levodopa equivalent daily dose (LEDD) was calculated as in prior studies (Hobson et al., 2005; Ecker et al., 2009; Weintraub et al., 2010; Moustafa et al., 2013).

The motor symptoms of the selected patients varied. Therefore to avoid this factor confounding the results, we categorized the

Table 1 | Subject demographic, clinical and data for healthy controls, PD patients on MAO-I and levodopa, and PD patients on monotherapy (levodopa or dopamine agonists only).

	PD Patients on MAO-I and Ldopa	PD Patients on Ldopa or DA agonists only	Healthy Controls	*P*-value
N	37	39	43	
Age	65.2 (4.6)	67.3 (4.3)	66.9 (5.2)	0.304
Sex (M/F)	26/11	27/12	29/14	0.48
PD (akinetic-rigid/Tremor)	21/16	22/17	–	0.734
Education (years)	13.1 (2.3)	12.9 (2.2)	13.2 (3.1)	0.21
NART-predicted IQ	109.5 (12.7)	114.5 (13.1)	116.5 (9.7)	0.53
MMSE	27.4 (1.1)	27.7 (1.4)	28.2 (1.9)	0.420
NAART	34.2 (11.8)	34.8 (11.4)	36.9 (7.3)	0.312
H and Y	2.61 (0.4)	2.53 (0.5)	–	0.601
UPDRS	18.9 (5.9)	23.8 (5.2)	–	0.094
FOGQ	2.6 (0.6)	3.1 (0.7)	–	0.09
Disease duration	8.61 (4.1)	8.46 (3.8)	–	0.732

Values here represent Mean (S.D). Abbreviations: H and Y, Hoehn and Yahr staging of PD; NAART, North American Adult Reading Test; MMSE, Mini-Mental State Examination; FOGQ, Freezing of Gait Questionnaire; UPDRS, Unified Parkinson's Disease Rating Scale.

subjects into tremor-predominant and akinetic-rigid subtypes, using their scores on the UPDRS and included the motor symptoms as an independent variable in the analysis. Specifically, a ratio was computed based on the UPDRS part III tremor score (average for items 20 and 21) and the mean UPDRS akinetic/rigid score (average for items 22–27 and 31). A ratio of >1.0 was considered tremor-dominant, <0.80 akinetic-rigid and 0.80–1.0 mixed. Subjects with mixed rigidity-akinesia and tremor (for similar methods used to subtype patients, see Jankovic et al., 1990; Vakil and Herishanu-Naaman, 1998; Poletti et al., 2012) were excluded from the study.

One-way ANOVA was used to compare demographic and neuropsychological measures between the PD patients on MAO-I and levodopa, PD patients on monotherapy, and healthy control subjects. Chi square statistic was used to compare the gender ratio and the PD type (akinesia vs. tremor-dominant), between the medicated PD patients, unmedicated PD patients, and healthy controls groups (**Table 1**). There was no significant difference between the groups on any of the demographic or motor measures. Although statistically nonsignificant, patients on MAO-I and levodopa had lesser motor dysfunction (as measured by UPDRS) and gait abnormalities (as measured by FOGQ) than the patients who was on a monotherapy of levodopa (**Table 1**).

BEHAVIORAL TASKS AND SCALES

All subjects performed two learning tasks in a counterbalanced order. All tasks were administered on a PC laptop computer.

Probabilistic learning task

Subjects were administered a computer-based probabilistic learning task. On each trial, subjects viewed one of six stimuli, and were instructed to make a right or left button-press (e.g., response X or Y). The feedback was probabilistic. So, a correct response results in either gaining points or no feedback with 20 and 80% probability, respectively. An incorrect response results in gaining points or no feedback with 80 and 20% probability, respectively. The correct response (X or Y) varies across stimuli, and the task had 120 trials.

Deterministic learning task

The deterministic learning task was similar to the probabilistic learning task, except that the stimuli were different and the stimulus-feedback relationship was deterministic (i.e., stimuli were 100% predictive of a feedback).

Neuropsychological assessment

We used the following tests to measure neuropsychological functions such as verbal and visual attention, learning and memory, working memory as well as executive functions.

Trail making test- A and B (Reitan and Wolfson, 1985). This test which consists of a set of numbered and lettered dots which a subject must connect as fast as possible in a serial order. The test consists of two parts, with Part B requiring more cognitive flexibility than Part A. The test provides information about visual attention, scanning, speed of processing and mental flexibility, a component of executive functioning.

Digit span test (D. Wechsler, 1958). This test taken from the Wechsler Adult Intelligence Scale (Digit Span forward and backward) assesses verbal attention and working memory. The subject has to recall immediately a set of numbers presented in either the order in which it was presented or in a backward order. In this study, both forward and backward tests were administered.

Controlled oral word association test (Troyer et al., 1997). This test which assesses verbal fluency requires a subject to generate words starting with the letters F, A and S. In this study the patients were asked to generate the names of animals starting with the above letters.

Logical memory test (Wechsler, 1987). This subtest from the Wechsler Memory Scale–III, assesses verbal memory through immediate recall, delayed recall and recognition tasks following the examiner reading aloud a passage containing a short story to the subject.

California verbal learning test (CVLT) (Delis et al., 1987). This test of verbal learning and memory involves the oral presentation

of a 16 word list and immediate recall of the same over five trials. Free recall of the words was assessed again after a delay of approximately 20 min. Afterwards, cued recall is tested by offering the names of the four semantic categories to guide memory retrieval, and yes–no recognition is assessed by embedding the 16 targets among 28 distractors.

n-back. The *n*-back task tests the effects of working memory load on performance (Cohen et al., 1997; Perlstein et al., 2003; Owen et al., 2005). In this task, a sequence of letters is presented to the subjects, one at a time. Here, working memory load was either two or three items, that is, subjects had to evaluate the similarity of each item to the one presented *n*-items previously ($n = 2$ or 3). In the two- and three-back conditions, a target was any letter that was identical to the one presented two or three trials preceding it, respectively. Stimulus encoding and response demands were constant across conditions; only requirements to maintain and update increasingly greater amounts of information at higher loads differed. Pseudorandom sequences of single consonants were presented, and subjects responded to each stimulus, pressing one button to targets and another to no targets. Most subjects did not learn the three-back condition, so we do not analyze it any further in the study here, and focus on group differences and medication effects on the two-back task.

Stroop color word test (Stroop, 1935). This test consists of a white sheet of paper with blocks of colors printed on it in a matrix of rows and columns. The subject in the first trial has to read out the names of the colors in an order across rows or columns. In the second trial, the subject is shown a paper with the names of colors written in incongruous colors, again arranged in a matrix. The subject has to suppress reading the print, but name the color in which it is printed, again in the same serial order as before. Each trials are timed separately and scores computed to assess the color-word interference to study the executive functions such as inhibition and cognitive flexibility.

Wisconsin card sorting test (WCST) (Heaton et al., 1993). This test is used to assess the executive functions- set shifting and category formation. The test consists of cards with geometric patterns which the subject has to match based on a principal he/she thinks is right. On receiving a feedback of right or wrong across several trials, a subject learns to match the cards. The category for matching is then shifted to another category at a certain stage in the test without informing the subject. Number and pattern of errors and correct responses reveals learning about category formation and set shifting abilities.

Frontal assessment battery (FAB) (Dubois et al., 2000). The FAB consists of six subtests assessing different functions related to the frontal lobes such as: (1) conceptualization and abstract reasoning (similarities test); (2) mental flexibility (verbal fluency test); (3) motor programming and executive control of action (Luria motor sequences); (4) resistance to interference (conflicting instructions); (5) inhibitory control (go–no go test); and (6) environmental autonomy (prehension behavior). The FAB has shown a good validity (correlation of $\rho = 0.82$ with the Mattis Dementia Rating Scale) and interrater reliability ($\kappa = 0.87$).

Affective and quality of life measures

We should not say additionally here as in the study design we have already said we are looking into the effects/association of different treatment on/with affective measures Beck Depression Inventory was used to assess depressive symptoms (Beck et al., 1987), Beck Anxiety Inventory (Beck et al., 1988) to assess anxiety, Apathy evaluation scale (Marin et al., 1991) to assess apathy, and the PDQ-39 (Jenkinson et al., 1995) questionnaire to assess quality of life.

STATISTICAL ANALYSIS

For all analyses, we used SPSS as well as SAS v8.0 PROC MIXED. One-way ANOVA was used to compare demographic and neuropsychological measures between the PD patients on MAO-I and levodopa, PD patients on monotherapy, and healthy control subjects. Chi square statistic was used to compare the gender ratio and the PD motor subttype (akinesia vs. tremor-dominant), between the medicated PD patients, unmedicated PD patients, and healthy controls groups.

Between-subject differences were examined using unstructured covariance matrices which do not make any strong assumptions about the variance and correlation of the data unlike structured covariances. Where indicated, we tested for specific planned contrasts. In these contrasts, the number of degrees of freedom reflects the entire sample, and not just the subjects involved in the particular contrast, because the mixed procedure analyses between-subject effects, and controls for other variables of interest that apply across all subjects. This procedure uses all of the data to provide a more stable estimate of the error term. Finally, we conducted interaction analysis of medication regime (MAO-I and levodopa vs. monotherapy) and subtype of PD patients (akinesia- vs. tremor-dominant) with all neuropsychological and psychiatric measures.

RESULTS

Here, we first present results on the association of medication types (multiple vs. monotherapy) on cognition. We then discuss their differential effects on affective and quality of life measures. Finally, we discuss the association of tremor vs. akinesia severity on the same measures.

EFFECTS OF MAO-I AS AN ADJUNCT TO LEVODOPA ON COGNITION

Results show that PD patients on monotherapy were more impaired than healthy subjects and PD patients on combined MAO-I and levodopa in the probabilistic learning task (all p's $<$ 0.01, **Figure 1A**). There was no significant difference among the healthy controls and PD groups in the deterministic learning task ($p > 0.1$, **Figure 1B**).

In the case of patients who had MAO-I as an adjunct to levodopa, their neuropsychological measures were better than patients on monotherapy (**Table 2**). A significant difference was noted on *n*-back task ($p < 0.01$), Backward digit span ($p = 0.04$), Trail Making B ($p = 0.031$), FAB ($p = 0.031$), and WCST-64 ($p = 0.04$). There was no statistically significant difference among the healthy controls and PD groups on other neuropsychological measures (see **Table 2**).

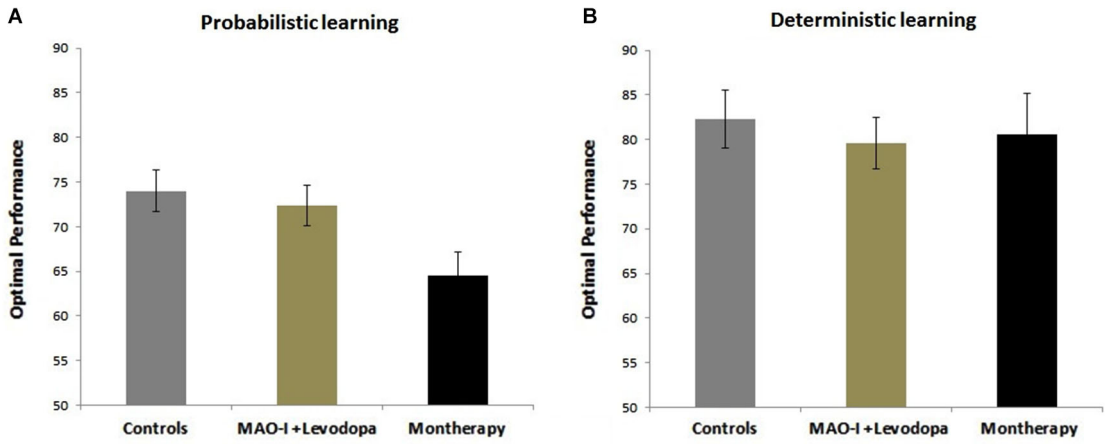

FIGURE 1 | Effects of combined MAO-B Inhibitors vs. monotherapy on probabilistic and deterministic learning tasks. (A) Probabilistic learning task. Data show that healthy subjects and PD patients on combined MAO-I and levodopa show better performance than PD patients on monotherapy. **(B)** Deterministic learning task. There was no significant difference among PD and the healthy control groups in the deterministic learning task.

ASSOCIATION OF MAO-I AS AN ADJUNCT TO LEVODOPA ON AFFECTIVE AND QUALITY OF LIFE MEASURES

As in the case of neuropsychological measures, patients who had MAO-I as an adjunct to levodopa had better scores on motivational processes than patients on monotherapy (**Table 3**). Specifically, we found that compared to PD patients on monotherapy, MAO-I as adjunct to levodopa show more beneficial effects on mental health on the following measures: apathy ($p = 0.032$), BDI ($p = 0.031$) and quality of life, as measured using the PD-39 scale ($p = 0.041$). Although statistically nonsignificant, unlike monotherapy, MAO-I as adjunct to levodopa seem to better ameliorate anxiety ($p = 0.09$).

We further separated the levodopa and MAO-I group into two groups (patients on rasagiline vs. others on selegiline), and tested their performance on all questionnaires and cognitive tests. There were no significant differences among the two groups in any of the measures, except that in the rasagiline and levodopa group, UPDRS scores were slightly but not significantly lower than in patients in the selegiline and levodopa group ($p = 0.083$).

INTERACTION OF MEDICATION REGIME WITH MOTOR SUBTYPE

On conducting an interaction analysis of medication regime (MAO-I and levodopa vs. monotherapy) and subtype of PD patients (akinesia- vs. tremor-dominant), the ameliorating effects of MAO-I were more pronounced in PD patients with severe akinesia than PD patients with predominant tremor. A significant interaction was found between medication regime and subtype

Table 2 | Effects of combined MAO-B Inhibitors vs. monotherapy on cognition.

	PD Patients on MAO Inhibitors and Ldopa	PD Patients on Ldopa or DA agonists only	Healthy Controls	*P*-value
Forward digit span	7.1 (1.7)	6.9 (1.4)	6.8 (1.9)	0.37
Backward digit span	7.9 (1.6)	6.12 (1.7)	8.2 (1.8)	0.04
Trail Making A	57.9 (6.3)	75.4 (7.0)	55.8 (6.9)	0.21
Trail Making B	104.1 (18.1)	142.3 (18.1)	94.4 (14.3)	0.031
Logical Memory, Delayed Recall	19.7 (7.2)	19.2 (8.1)	18.9 (6.8)	0.54
Logical Memory, Encoding	34.7 (10.9)	35.2 (11.2)	34.1 (12.1)	0.623
Verbal Fluency	18.7 (4.7)	19.6 (4.1)	20.3 (4.2)	0.58
Stroop Errors	5.31 (3.3)	5.1 (2.9)	4.9 (3.1)	0.41
FAB	13.69 (1.4)	10.6 (1.4)	14.1 (1.1)	0.031
CVLT-short delay free	7.0 (1.3)	6.9 (1.7)	7.2 (1.3)	0.21
CVLT-long delay free	6.9 (1.2)	6.81 (1.3)	6.9 (1.5)	0.29
CVLT-long delay cued	7.1 (1.0)	7.01 (1.4)	7.1 (1.3)	0.28
CVLT-delayed recognition	8.2 (0.8)	7.9 (0.9)	7.89 (1.3)	0.48
WCST-64	42.8 (10.1)	35.1 (9.3)	44.8 (12.2)	0.04
n-back	80.3 (3.35)	74.2 (3.54)	86.2 (3.71)	0.007

Abbreviations: FAB = Frontal Assessment Battery; NART Predicted IQ = National Adult Reading Test predicted intelligence; CVLT = California Verbal Learning Test; WCST-64 = Wisconsin Card Sorting Test-64 cards version.

Table 3 | Effects of combined MAO-B Inhibitors and levodopa vs. monotherapy on affective and quality of life measures.

	PD Patients on MAO-B Inhibitors and Ldopa	PD Patients on Ldopa or DA agonists only	Healthy Controls	*P*-value
Apathy (Apathy evaluation scale)	32.6 (6.2)	39.8 (5.1)	33.4 (5.7)	0.032
Depression (BDI)	6.2 (1.2)	8.9 (1.1)	6.5 (1.9)	0.031
Anxiety (BAI)	14.3 (3.0)	17.2 (4.3)	13.3 (3.1)	0.09
Quality of life (PDQ-39)	29.8 (2.7)	25.3 (2.4)	–	0.041

Abbreviations: BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory.

of PD patients on measures of Trail-Making B ($p < 0.02$), FAB ($p < 0.01$), WCST ($p < 0.04$), BDI ($p < 0.02$), and apathy ($p < 0.009$). All other interaction effects (with neuropsychological and psychiatric measures) were not significant (all p's > 0.15).

DISCUSSION

Our results show MAO-B inhibitors as an adjunct to levodopa provide a more enhancing effect than the use of monotherapy (levodopa or DA agonists alone) on most cognitive, affective and quality of life measures used in our study. Below, we discuss the effects of MAO-B inhibitors as an adjunct to levodopa on neuropsychiatric and cognitive measures.

EFFECTS OF MAO-B INHIBITORS ON COGNITIVE MEASURES

As mentioned above, there are very few studies that have investigated the effects of MAO-B inhibitors (taken alone or as an adjunct to levodopa) on neuropsychological and cognitive measures. For example, rasagiline has been used to treat mild cognitive impairment in PD (Goldman and Holden, 2014). A small study involving seven patients on selegiline showed some improvement in memory and motor speed without progressive memory loss compared to those with dementia (Portin and Rinne, 1983). Subsequently, Hietanen (1991) tested 18 patients with idiopathic PD on selegiline as monotherapy for 8 weeks in a double blind randomized placebo controlled trial, using a battery of neuropsychological tests. The study found some improvement in learning easy word associations, but did not find any significant specific cognitive effect. As part of a multicenter trial of Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP), 800 patients with early untreated PD were administered tests that measured memory, visuospatial, and frontal lobe functions (Kieburtz et al., 1994). The study did not find any significant effect of either deprenyl (selegiline) or tocopherol on cognitive test performance (Kieburtz et al., 1994). Further, in an 8-week study, it was found that selegiline did not have an effect on executive function using the Wisconsin Card Sorting Test (Dalrymple-Alford et al., 1995). Recently, Hanagasi et al. (2011) found that compared to placebo, the MAO-B inhibitor rasagiline has a better effect on attention and executive function in nondemented PD patients. In this double blind placebo controlled multicenter trial of 48 non-demented patients with PD and cognitive impairment, significant improvement was noticed in the rasagiline group on scores of digit span backward, verbal fluency, semantic fluency, Stroop, and attentional measures (Hanagasi et al., 2011).

Our results extend these findings by showing that MAO-B inhibitors as an adjunct to levodopa have a better effect on cognitive function (including probabilistic learning, forward and backward digit span, *n*-back, WCST-64, FAB, Trail Making B) in PD patients than levodopa or DA agonists monotherapy. Unlike the Hanagasi et al. study, the present study employed computerized cognitive tasks to assess learning in PD patients. Our data show that patients who had MAO-B inhibitors as adjunct to levodopa had better probabilistic but not deterministic learning. Our results suggest that learning impairment is exacerbated when the learning task involves uncertain feedback and response conflict, as in the probabilistic, but not deterministic, learning task. This interpretation is also in agreement with prior research showing that PD patients show impairments in conflict-based decision making tasks (Farooqui et al., 2011; Vandenbossche et al., 2012). This also points to the finding that probabilistic learning has a better potential to reveal differences in drug effects than deterministic learning tasks.

EFFECTS OF MAO-B INHIBITORS ON AFFECTIVE AND QUALITY OF LIFE MEASURES

MAO inhibitors have been generally used as traditional antidepressant drugs (Johnson et al., 2010). For example, many studies show that selegiline transdermal system help reduce depressive symptoms in various patient populations (Bodkin and Amsterdam, 2002; Amsterdam and Bodkin, 2006; Feiger et al., 2006). In our study, we found that PD patients who were on MAO-I and levodopa have lower BDI scores than patients on levodopa or DA agonists alone, suggesting a beneficial effect of MAO inhibitors on depression. Although not statistically significant, we found that PD patients on MAO-I and levodopa have lower scores on anxiety measure (as used by the Beck anxiety Inventory questionnaire), when compared to patients on monotherapy.

Interestingly, we also found that PD patients who were on MAO-I and levodopa have lower apathy and higher quality of life scores than patients on levodopa or DA agonists alone, suggesting a beneficial effect of MAO inhibitors on these measures.

INTERACTION BETWEEN MOTOR SUBTYPE IN PD AND MEDICATION REGIME

We also found an interaction effect in the motor subtype, medication and cognitive functions. Better cognitive functions were seen more in patients with severe akinesia, in comparison to patients with severe tremor. Our results extend prior studies showing relationships between motor and cognitive measures in PD (Riggeal et al., 2007; Colman et al., 2009; Wylie et al., 2012; Smulders et al., 2013), and further show that the effects of MAO-I

on neuropsychological, affective, and quality of life measures in PD depends on the subtypes of PD.

Our results are also in line with prior studies showing that PD patients with tremor are usually less cognitively impaired than PD patients with akinesia or gait dysfunction (Vakil and Herishanu-Naaman, 1998; Burn et al., 2006; Lyros et al., 2008; Oh et al., 2009; Domellof et al., 2011). In addition, prior studies have suggested that akinesia in PD patients is related to basal ganglia dysfunction (Kassubek et al., 2002; Probst-Cousin et al., 2003; Weinberger et al., 2009; Zaidel et al., 2009; Mure et al., 2011). It is possible that in PD patients with severe akinesia, the combined therapy of MAO inhibitors and levodopa has a more ameliorative effect on basal ganglia dysfunction than levodopa alone. We hypothesize that this effect can perhaps be due to findings that tremor in PD is related to damage to brain areas such as the cerebellum, while akinesia has been consistently seen to be related to basal ganglia dysfunction (Mure et al., 2011). Further, it is possible that MAO-B inhibitors ameliorate the function of the basal ganglia and DA and thus ameliorate impairment in PD patients with akinesia that is patients with more cognitive damage get better effects from treatment.

CONTINUOUS DOPAMINERGIC STIMULATION THEORY AND BIOAVAILABILITY OF MONOAMINES

The beneficial effects of MAO-I can be due to the Continuous Dopaminergic Stimulation (CDS) of the basal ganglia and/or an increase in the bioavailability of monoamines. The CDS theory posits that sufficient dopaminergic stimulation of the basal ganglia (and particularly the striatum) reduces the occurrence of motor complications (such as wearing-off phenomenon) and dyskinesia (Nyholm, 2007; Silverdale, 2007). A multitude of studies have shown that these motor complications are associated with the administration of levodopa medications, and that combined therapies (levodopa with DA agonists, COMT inhibitors, or MAO inhibitors) can reduce these motor fluctuations (Jankovic and Stacy, 2007; Stocchi et al., 2008).

Alternatively, many studies have shown that MAO-I can increase the levels of many monoamines including DA, serotonin, and norepinephrine (Riederer and Laux, 2011). Most of these monoamines are known to impact cognitive and psychiatric measures in various patient populations, and thus the beneficial effects of MAO-I on our patients could be due to an increase of monoamine levels in the brain (Hamon and Blier, 2013).

Our results extend these findings, and show that the beneficiary effects of levodopa and MAO-I can also be observed in cognitive, affective and quality of life measures. It is possible that MAO-I and levodopa enhance DA and other monoamine neurotransmission and thus provide a continuous stimulation of the basal ganglia, which in turn, ameliorate neuropsychological, affective and quality of life dysfunction in PD patients. None of our patients were on COMT inhibitors, but future studies should evaluate whether COMT inhibitors (taken alone or in combination with levodopa) also ameliorate neuropsychiatric and neuropsychological function in PD patients, in comparison to monotherapy.

LIMITATIONS

Our study is not without limitations. Our samples included a small number of subjects to compare PD patients on levodopa to patients on DA agonists alone. There are very few number of PD patients on DA agonists (either alone or in combination with other medications). A future study recruiting patients on DA agonists and/or MAO-B inhibitors can help understand its effects on motor, psychiatric, and cognitive processes in comparison to levodopa and/or MAO-B inhibitors. Similarly, we did not have a large sample of PD patients to compare differential effects of selegiline vs. rasagiline on neurocognitive and neuropsychiatric measures. Further, a longitudinal study is needed to confirm findings that the administration of MAO-I and levodopa better ameliorate and cognitive abnormalities than the administration of levodopa alone.

Overall, our results show that combination therapy of MAO inhibitors and levodopa are associated with better neuropsychological, cognitive, and affective function in PD patients than levodopa or DA agonists alone. To our knowledge, this is the first study to investigate the effects of MAO inhibitors on cognitive as well as classical neuropsychological tests in PD patients using computerized learning tests.

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Dopaminergic modulation of emotional conflict in Parkinson's disease

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Neuropsychiatric fluctuations in Parkinson's disease (PD) are frequent and disabling. One way to investigate them is to assess the ability to inhibit distractive emotional information by a modified emotional Stroop (ES) task. We compared non-depressed, non-demented PD patients with healthy controls. During an acute levodopa challenge, patients performed a modified ES task during functional MRI and a neuropsychological assessment including Visual Analog Mood (VAMS) and Apathy scales. Ten patients and 12 controls completed the study. The VAMS scores were significantly improved by the acute intake of levodopa ($p = 0.02$), as was the apathy score ($p = 0.03$). Negative ES task (i.e. fearful facial expressions with the words "happy" or "fear" written across them), induced a lengthening of the mean reaction time during the incongruent trials compared with the congruent trials in controls (relative difference = 2.7%, $p < 0.001$) and in ON patients (relative difference = 5.9%, $p < 0.001$), but not in OFF patients (relative difference = 1.7%, $p = 0.28$). Controls and ON patients displayed greater activation than OFF patients within the right pregenual anterior cingulate cortex (pACC), an area specifically involved in emotional conflict resolution ($p < 0.001$ and $p < 0.008$ respectively, $k > 5$ uncorrected). No difference in the activation of the pACC was found between controls and ON patients, suggesting a normalization of the activation following levodopa administration. These results suggest that emotional conflict processes could be dopamine-dependent. Pregenual ACC hypoactivation could be directly due to the degeneration of dopaminergic mesocorticolimbic pathway. Our results propose that neuropsychiatric fluctuations in PD patients could be partially explained by pACC hypoactivation and that adjustments of dopaminergic medication might be helpful for their treatment.

Keywords: emotional stroop, dopamine, Parkinson's disease, cingulate cortex, non-motor fluctuations

INTRODUCTION

Although non-motor fluctuations (NMF) occur frequently throughout the course of Parkinson's disease, their prevalence is often underestimated. They can be more disabling than motor symptoms. NMF frequently manifest in relationship to motor fluctuations (Witjas et al., 2002) and fluctuate in parallel with them (Storch et al., 2013). Forms of NMF include neuropsychiatric, dysautonomic, and sensory symptoms (Riley and Lang, 1993). Neuropsychiatric fluctuations encompass apathy, anxiety, sadness, slowness of thinking, fatigue during drug-off condition, and mood elation with euphoria and increased alertness in

Abbreviations: ACC, anterior cingulate cortex; BA, Brodmann area; BDI, Beck Depression Inventory; BOLD, Blood-Oxygen-Level Dependent; C, congruent trial; CI, confidence interval; DRS, Dementia Rating Scale; ES, emotional Stroop; FAB, Frontal Assessment Battery; FOV, field of view; fMRI, functional MRI; HC, healthy controls; I, incongruent trial; k, cluster size; LEDD, levodopa-equivalent daily dosage; MDD, major depressive disorder; MNI, Montreal Neurological Institute; N, negative facial expression; NMF, non-motor fluctuations; OFC, orbitofrontal cortex; OCD, obsessive-compulsive disorder; P, positive facial expression; PTSD, posttraumatic stress disorder; R1, region 1; R2, region 2; rACC, rostral anterior cingulate cortex; RT, reaction time; SAS, Starkstein Apathy Scale; SD, standard deviation; t, *t*-value (against chance level); TE, time echo; TR, time repetition; UPDRS, Unified Parkinson's Disease Rating Scale; VAMS, Visual Analog Mood Scale; VTA, Ventral Tegmental Area.

drug-on state (Witjas et al., 2002; Fox and Lang, 2008; Lhommée et al., 2012). Previous publications have demonstrated that mood and anxiety fluctuations are related to levo-dopa dosing (Friedenberg and Cummings, 1989; Maricle et al., 1995b; Richard et al., 2005). Different mechanisms have been proposed to explain neuropsychiatric fluctuations such as mesocorticolimbic dopaminergic denervation underlying off-period related neuropsychiatric symptoms (Thobois et al., 2010), sensitization related to severity of the mesocorticolimbic lesion in combination with pulsatile dopaminergic treatment with levodopa (Voon et al., 2011; Castrioto et al., 2013) and/or relatively selected stimulation of mesocorticolimbic dopamine D3 receptors with dopamine agonists (Thobois et al., 2013). Both mood and anxiety disorders have a high prevalence among parkinsonian patients (Reijnders et al., 2008; Dissanayaka et al., 2010). Certain authors suggest that their impact on quality of life is more important than the impact of motor signs (Schrag, 2006; Chaudhuri and Schapira, 2009). In the "NMF in Parkinson's disease study," anxious and depressive symptoms were highly associated with drug-off state. Beck Depression Inventory (BDI) scores, obtained while parkinsonian patients were on chronic dopaminergic medication, only correlated with depression severity in drug-off but not in drug-on state, suggesting that depression scoring in fluctuating patients mainly reflects the mood in drug-off state during NMF (Storch et al., 2013). Consequently, a better understanding of neuropsychiatric fluctuations physiopathology seems necessary in order to improve parkinsonian patient's mood symptoms management and quality of life.

Patients with depression or anxiety disorders show a higher sensitivity toward negatively valenced stimuli, which results in slower processing of negative emotional information (Williams and Nulty, 1986; Becker et al., 2001). A method for investigating inhibition of distractive emotional information (i.e. emotional interference) is the emotional Stroop (ES) task (Williams et al., 1996). Variants of the ES task have been developed such as the word-face Stroop task (Stenberg et al., 1998) or the modified ES task (Etkin et al., 2006). In this latter task, subjects are asked to identify the affect of faces with fearful or happy expressions while ignoring the words "happy" or "fear" written across them. Words are either congruent or incongruent with faces. Emotional conflict arises when the word is not in agreement with the facial expression. It induces an emotional interference resulting in a decision-making slowdown with slower reaction time to name the affect of faces if the emotion of the word written across the face is incongruent with the facial expression. Serra-Mestres and Ring (2002) demonstrated that non-depressed parkinsonian patients presented with significantly greater emotional interference than did controls, despite matching for depression score, supporting the hypothesis that non-depressed parkinsonian patients were more vulnerable to the interfering effects of negative emotional stimuli. This vulnerability could be a factor contributing to the increased risk of developing depression in parkinsonian patients, because in the presence of sadness, patients will remain in that state for longer periods.

Neuroimaging studies suggested that the pregenual part of the anterior cingulate cortex (ACC), also known as the rostral anterior cingulate cortex (rACC), plays a major role in ES task performance (Whalen et al., 1998; Etkin et al., 2006; Egner et al., 2008). The rACC is located in the medial surface of the brain and is anterior to the genu of the corpus callosum. It is a key structure for the integration of emotion and cognition (Pessoa, 2008). It is considered to be a part of the affective subdivision of the ACC (together with the subgenual ACC) because it has connections with the limbic and paralimbic regions, such as the amygdala, the orbitofrontal cortex (Devinsky et al., 1995) and the nucleus accumbens (Johansen-Berg et al., 2008). The rostral ACC is specifically associated with the resolution of emotional conflict (i.e., overcoming of conflict), whereas the lateral prefrontal cortex resolves exclusively non-emotional conflict (Egner et al., 2008). Conversely, a common area of the dorsal ACC, which is located in the posterior region of this structure, detects and signals the occurrence of conflict in information processing (Botvinick et al., 1999; Kerns et al., 2004). This region is activated by both emotional and non-emotional conflict monitoring (Egner et al., 2008).

The neurotransmission involved in ES processes is largely unknown. The ACC is involved in the regulation of attention (Posner and DiGirolamo, 1998). As it has been suggested that dopaminergic signals serve to draw attention to salient events of all sorts (Gray et al., 1997) and that dopamine is a critical component involved in the attribution of salience of attractive or aversive valence to stimuli (Bressan and Crippa, 2005), it is possible that dopamine plays a role in ES processes. Parkinson's disease is primarily due to degeneration of dopaminergic neurons and offers the opportunity to investigate the pharmacological manipulation of the dopaminergic system in humans. Abnormal behavioral performances to the ES task have been found in Parkinson's disease (Serra-Mestres and Ring, 1999, 2002) and in psychiatric conditions such as cocaine or heroin addiction in which dopaminergic dysfunction is well demonstrated (Marissen et al., 2006).

We hypothesized that dopamine would play a role in emotional interference processes and that mood disturbances during neuropsychiatric fluctuations in parkinsonian patients could be explained by an abnormal rACC functioning. We assessed fluctuating non-depressed parkinsonian patients during their drug-on and drug-off states while performing a modified ES task during functional MRI (fMRI), in order to better understand the pathophysiology of neuropsychiatric fluctuations.

MATERIALS AND METHODS

PARTICIPANTS

Patients were recruited from the Grenoble University Hospital Movement Disorders Clinic. The selection criteria were as follows: presence of clinically diagnosed Parkinson's disease according to the United Kingdom Parkinson's Disease Society Brain Bank clinical criteria for Parkinson's disease (Hughes et al., 1992), presence of motor and non-motor fluctuations, age < 70 years, the ability to tolerate the experimental period in the MRI machine during a drug-off state (i.e., $\geq$11 h after the patient's last dopaminergic drug dose the night before) and during a drug-on state (i.e., 1 h after a dose of levodopa which efficiently controls parkinsonian symptoms without disabling dyskinesia) and the absence of a contraindication to an MRI. The patients were compared to healthy

volunteers who were recruited from the community. Healthy controls (HC) had no history of neurological or psychiatric disorders. They were chosen to match the patient group as closely as possible for age, sex, and education level.

Exclusion criteria for both groups were: the presence of dementia as indicated by a score of ≤130 on the Mattis Dementia Rating Scale (DRS) (Mattis, 1976; Schmidt et al., 1994) or a score ≤12 on the Frontal Assessment Battery (FAB) (Dubois et al., 2000; Kaszás et al., 2012), the presence of moderate to severe depression as indicated by a score ≥20 on the BDI-II (Beck et al., 1996), the presence of psychosis, and/or the presence of impaired face recognition as indicated by a score < 41 on the long-form of the Benton Recognition Test (Levin et al., 1975; Benton, 1994). Subjects with fMRI-related issues (i.e. unsatisfactory picture quality caused by participants moving within the scanner during acquisition or other scanner-specific technical complications) were excluded. The Ethics Committee of the Grenoble University Hospital approved the study. After a complete description of the study, the participants gave written informed consent in accordance with the Grenoble University Hospital Review Board guidelines and the Declaration of Helsinki.

PROCEDURES

Each participant completed a modified ES task during fMRI followed by a neuropsychological assessment. Each parkinsonian patient was studied twice in a counterbalanced manner during a levodopa challenge where the drug-off and the drug-on states were performed on two consecutive mornings. The drug-off state followed an overnight withdrawal of all antiparkinsonian drugs. The drug-on state was performed in a fasting state in order to improve levodopa absorption and to enable more precise timing of the maximum therapeutic effect. Levodopa was administrated orally using a levodopa-benserazide dispersible formulation. For each patient, the dose of levodopa used for the challenge was calculated as 100% of his levodopa equivalent first morning dose. The equivalent dose was calculated according to Tomlinson et al. (2010). We decided against the use of suprathreshold doses of levodopa (i.e., 130–150% of the levodopa equivalent first morning dose) in order to not prime dyskinesias, which could have induced fMRI movement artifacts, and to avoid modifying cognition. Before each MRI scan, all patients underwent a neurological examination to rate the severity of their motor function using the Unified Parkinson's Disease Rating Scale (UPDRS) motor rating items 18–31 in drug-on and drug-off conditions (Fahn and Elton, 1987). Levodopa and dopamine agonist doses were expressed as total levodopa-equivalent daily dosage (LEDD) (Tomlinson et al., 2010). All participants were asked to refrain from nicotine and caffeine for ≥4 h prior to the fMRI studies.

fMRI stimuli

The modified ES task consisted of 136 presentations of black and white happy and fearful facial expression photographs drawn from the Montreal Set of Facial Displays of Emotion (Beaupré and Hess, 2005). This battery consists of emotional facial expressions by men and women of European, Asian, Hispanic and African decent. Each expression had been coded according to the Facial Action Coding System (Ekman and Friesen, 1978) to assure identical expressions across actors. The words "FEAR" or "HAPPY" were prominently written in red bold "Arial" size 45, centered on the middle of faces (i.e., on the "nose" position). The face–word association created either a congruent condition (32 happy faces with the word "HAPPY," 32 fearful faces with the word "FEAR") or an incongruent condition (32 happy faces with the word "FEAR," 32 fearful faces with the word "HAPPY") (**Figure 1**). Eight additional faces in the incongruent condition (happy faces with the word "FEAR") were added, in order to obtain 136 presentations to allow the construction of a pseudoaleatory design with blocks of 17 pictures presented 8 times. All faces were resized to 1024 × 717 pixels and size was controlled by e-prime2 software (100% of the initial size). Gender, ethnic group (Caucasian, Asian, African and Hispanic), and facial expressions (fear and happiness) were counterbalanced across conditions (congruent or incongruent) and across trial sequences. There were neither direct repetitions of the same face with varying word distracters, in order to avoid negative priming effects, nor direct repetitions of exact face-word-distracter combination, in order to avoid repetition priming effects (Etkin et al., 2006). All conditions (i.e., congruent and incongruent) were displayed in pseudorandom order.

Emotional stroop task

Participants were instructed to judge as fast and as accurately as possible whether facial expressions represented fear or happiness while ignoring the word. After receiving instructions, participants underwent a short training session immediately prior to the experiment, with items that were not shown during the real experiment.

The ES task was presented with the E-Prime 2 software (E-Prime Psychology Software Tools Inc., Pittsburgh, USA) and was displayed on a back-projection screen that was viewed by the subjects via a mirror attached to the head-coil. Each trial began with a stimulus (face-word association) displayed for 1000 ms, followed by a fixation cross for 2000 ms (interstimulus interval of 3000 ms) (**Figure 2**). The responses (*fear* and *happy*) were recorded by means of two response keys pressed by the index finger of the subject's dominant hand. The mean response time and percentage of correct responses were recorded for each participant. The duration of the ES task was 7 min.

FIGURE 1 | Examples of stimuli used in the modified emotional Stroop task.

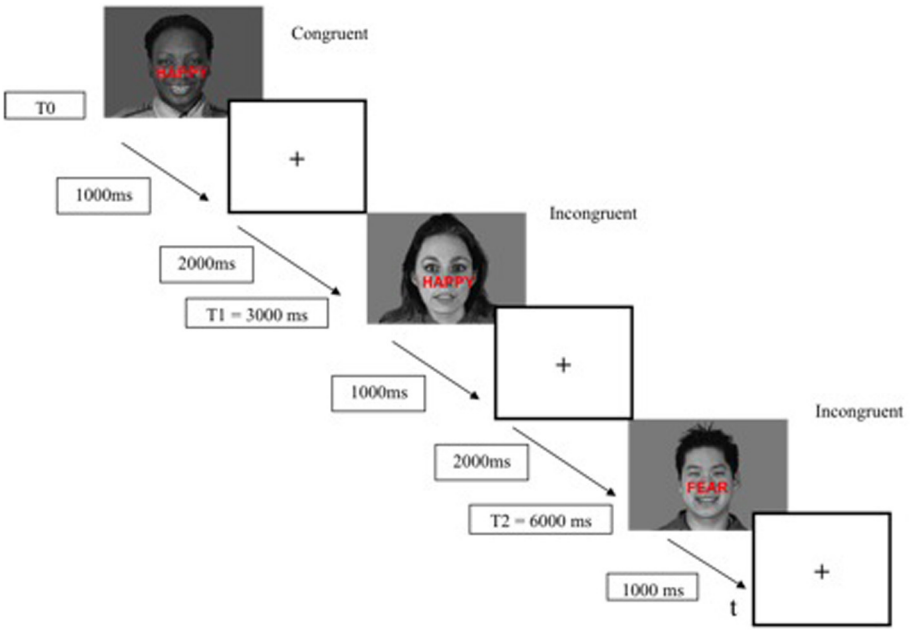

FIGURE 2 | Example timeline used in the modified emotional Stroop task fMRI paradigm.

fMRI data acquisition

BOLD fMRI data were collected while subjects performed the ES task in a 3Tesla Bruker Medspec S300 MRI scanner using a gradient–echo T2*–weighted Echo Planar Imaging (EPI) sequence covering 39 axial, interleaved slices (3.2 mm thick, 0 mm gap), beginning at the cerebral vertex and encompassing the entire cerebrum and the cerebellum (time repetition (TR) = 2500 ms; time echo (TE) = 30 ms; flip angle = 77°; field of view (FOV) = 216 × 216 mm^2; matrix = 72 × 72; voxel size = 3 × 3 × 3.2 mm). Six dummy scans were done before image acquisition to avoid the effects of signal saturation. All scanning parameters were selected to optimize the quality of the BOLD signal while maintaining a sufficient number of slices to acquire whole-brain data. For structural whole-brain images, three-dimensional T1–weighted sagittal images of the whole-brain were also acquired (TR = 2500 ms; TE = 4.3 ms; TI = 908.1 ms; flip angle = 8°; FOV = 256 × 224 × 176 mm^3; voxel size = 1.33 ×1.75 × 1.37 mm^3).

Neuropsychological assessment

All participants performed a neuropsychological evaluation including the Mattis DRS for global cognitive assessment, the FAB for dysexecutive dysfunction assessment, the BDI for depression assessment, the State-Trait Anxiety Inventory for Adults (Spielberger, 1983) to assess their anxiety level and the Benton Recognition Test for facial perception assessment. Patients continued their standard dopaminergic medication during this evaluation. The presence and the intensity of NMF were evaluated with the Ardouin Scale (Ardouin et al., 2009). The NMF, which patients experienced in daily life on their usual treatment, were assessed by adding the two items "drug-on NMF" and "drug-off NMF." A score ≥ 1 was taken as indicative of the presence of NMF.

During their drug-off and drug-on states, participants performed a second neuropsychological assessment in a counterbalanced manner, comprising a Visual Analog Mood Scale (VAMS) (Norris, 1971) to measure the acute levodopa effects on patient's subjective states, an Starkstein Apathy Scale (SAS) where a score ≥ 14/42 corresponded to the diagnosis of apathy (Starkstein et al., 1992), and an Ekman Facial Affect Test (Ekman and Friesen, 1976) to assess the ability to identify facial expressions of emotions (happiness, sadness, fear, disgust, surprise, anger, neutral).

STATISTICAL ANALYSIS

Statistical analysis of behavioral data

Accuracy (% of correct responses) and reaction time (RT) latencies were recorded during the fMRI experiment. Mean task accuracy was calculated as the average percentage of trials correctly identified relative to the sum of trials. Error trials (wrong answers and omissions) were excluded from the RT analysis. Different conditions were distinguished: incongruent trials with negative or positive faces and congruent trials with negative or positive faces. RT was log transformed to normalize the data. Differences in performances between groups (HC, drug-on and drug-off patients), congruency (congruent or incongruent) and emotion (negative or positive faces) were assessed using linear mixed effects models with a random effect on subject. Interaction terms between congruency and emotion, and between congruency and group were introduced in the model. ES effect was defined as a lengthening of the mean RT during the incongruent trials compared with the congruent trials. Statistical significance was assessed at the 0.05 level for all analyses.

fMRI statistical analysis

Data analysis was performed by using the general linear model as implemented in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) where each event is modeled using a hemodynamic function model. Data analysis started with several spatial pre-processing steps. First, the functional volumes were time-corrected with the second slice as reference. Subsequently, all volumes were realigned to correct head motion using rigid body transformations. After discarding the four first slices, the first volume of the fMRI session was taken as the reference volume. To correct for interaction between head movements and EPI distortions, unwarping was performed by using the individually acquired fieldmaps (Andersson et al., 2001). Weighted anatomical volume was co-registered to mean images created by the realignment procedure and was normalized to the Montreal Neurological Institute (MNI) space. The anatomical segmentation parameters were subsequently used for the normalization of anatomical and functional volumes. Finally, each functional volume was smoothed by an 8-mm Full Width at Half Maximum Gaussian kernel to ameliorate differences in intersubject localization. Time series for each voxel were high-pass filtered (1/128 Hz cutoff) to remove low-frequency noise and signal drift.

With regards to congruence [Congruence (C) vs. Incongruence (I)] and emotion [Positive for facial expression of happiness (P), Negative for facial expression of fear (N)] for each participant, we defined four experimental conditions (CP, CN, IP, IN). These conditions were modeled as four regressors and convolved with the canonical form of the hemodynamic response function. Moreover, movement parameters derived from realignment corrections (3 translations and 3 rotations) were also taken into account in the design matrix to remove the movement-related variance.

Next, several statistical analyses were performed. At the first level analysis (within-group comparisons), the following effects were evaluated: the ES effect with all faces (I vs. C) [I (P+N) vs. C (P+N)] as well as the ES effect with only the positive faces analyzed [I (P) vs. C (P)] and the ES effect with only the negative faces analyzed [I (N) vs. C (N)] (i.e., Negative ES contrast), using a one-sample *t*-test, in order to identify cerebral regions involved in emotional conflict. At the second level analysis (between-group level), we performed a random-effect between-group analysis by using paired *t*-tests (Friston et al., 1998) according to the Negative ES contrast defined at the individual level analysis in order to characterize cerebral differences between parkinsonian patients in their drug-off and drug-on states, and HC.

RESULTS

Twelve parkinsonian patients and twelve HC were included in the study. One patient was not able to complete the SE task in the MRI machine during his drug-off state because of a painful dystonia and was excluded from all analyses. Another patient was excluded because of unsatisfactory picture quality caused by participant movements during image acquisition in the MRI. Consequently, data from ten patients (seven males, age range: 51–66 years, mean age: 60, *SD*: 4.2) and twelve HC (8 males, age range: 46–69 years, mean age: 60.1, *SD*: 6.4) were included in the analyses. Clinical characteristics of each group are reported in **Table 1**.

Table 1 | Demographic and clinical characteristics of participants.

	Patients ($n = 10$)	Controls ($n = 12$)	*p*-value
Age (years)	60 ± 4.2	60.1 ± 6.4	ns
Sex ratio (male:female)	7:3	8:4	
Education duration (years)	10.7 ± 5	12.4 ± 5.2	ns
Disease duration (years)	9 ± 3.1		
Levodopa-equivalent daily dose (mg/day)	917.7 ± 367.6		
Drug-off UPDRS motor score (/108)	33.8 ± 10.8		
Drug-on UPDRS motor score(/108)	11.4 ± 7.4*		
Non-motor fluctuation Ardouin score (/8)	2.1 ± 0.9		
Mattis DRS score** (/144)	138.2 ± 4.5	141.2 ± 2.0	ns
FAB** (/18)	15.8 ± 1.8	17.3 ± 0.9	0.03
Benton** (/54)	47.3 ± 2.3	47.6 ± 2.5	ns
BDI** (/63)	11.5 ± 7.9	5.4 ± 5.5	0.04
STAI state** (/80)	34 ± 17.1	24.5 ± 4.4	ns
STAI trait** (/80)	37.6 ± 8.4	32.75 ± 6.8	ns

Values are expressed as mean (±SD) scores. BDI, beck depression inventory; FAB, frontal assessment battery; Mattis DRS, dementia rating scale; n = number of participants; ns = non-significant; STAI, State-Trait Anxiety Inventory for Adults; UPDRS motor score, motor scale of Unified Parkinson's Disease Rating Scale.

*p significant if <0.05 when compared with controls (Student test for age and education level; Mann-Whitney test for score on the Mattis, FAB, Benton, BDI, STAI tests). *p = 0.005 for difference between the drug-off and drug-on UPDRS motor scores (Wilcoxon's test for paired data). **Scores obtained from patients on chronic dopaminergic medication.*

All patients were on dopamine replacement medications. Patients and HC did not statistically differ in terms of age, education, gender distribution, global cognitive performance, facial perception or anxiety level. The parkinsonian group scored significantly lower on the FAB score than the controls ($p = 0.03$), although the averaged score fell within the non-pathological range (>14/18). Patients scored significantly higher on the BDI score than the controls ($p = 0.04$), although the averaged score fell within the non-depressed range (<12/63). In terms of psychotropic medications, two patients were taking antidepressant drugs (sertraline or mirtazapine) and four were taking benzodiazepines (clonazepam or prazepam). The psychotropic drug dosage was low and had been stable for at least 6 months in all patients. None of the control subjects was on psychotropic medication.

NEUROPSYCHOLOGICAL RESULTS DURING THE LEVODOPA CHALLENGE

The results are presented in **Table 2**.

In patients, apathy, as measured by the SAS, significantly improved with L-dopa intake between drug-off and drug-on conditions ($p = 0.03$). The affective and asthenia subscores of the

Table 2 | Neuropsychological characteristics of patients during the levodopa-challenge.

	Group 1	Group 2	*p*-Value
	Drug-off patients Mean ± *SD*	**Drug-on patients Mean ± *SD***	**Group 1 vs. Group 2**
SAS (/42)	13 ± 2.6	9.5 ± 3.6	0.03
Norris VAS			
Asthenia subscore (/80)	42.3 ± 14.2	12.2 ± 11.2	0.02
Affective subscore (/80)	25.7 ± 16.3	11.8 ± 8.6	0.02
EKMAN FAT (% CR)			
Happiness	100 ± 0	97.1 ± 9.0	ns
Fear	52.8 ± 17.9	64.3 ± 28.7	0.08
Surprise	90 ± 13.6	91.4 ± 10	ns
Sadness	72.84 ± 23.8	62.8 ± 21.5	ns
Disgust	80 ± 23.5	77.1 ± 20.5	ns
Anger	61.4 ± 27	78.6 ± 22.6	ns
Neutral	81.4 ± 16.6	78.6 ± 31	ns

Values are expressed as mean (±SD) scores. Ekman FAT, Ekman Facial Affect test; ns, non-significant; SAS, Starkstein Apathy Scale; VAS, Visual Analog Mood Scale; %CR, percentage of correct response. p significant if <0.05 (Wilcoxon test for paired data for the intra-group comparisons).

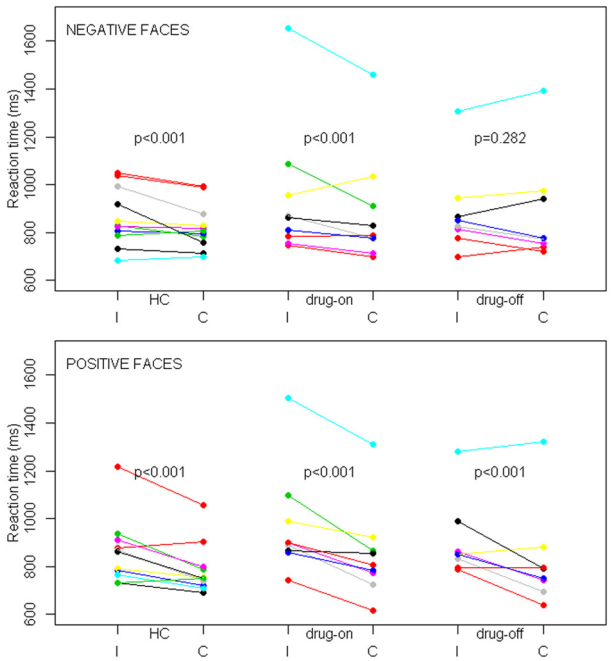

FIGURE 3 | SE task behavioral results in controls (HC), drug-off and drug-on parkinsonian patients. Data are patients' mean reaction times expressed in milliseconds (ms) depending on the congruence (I, incongruent; C, congruent) and facial emotion conditions among the different groups. The top panel represents trials with negative faces and the bottom panel represents trials with positive faces. *p* represents the statistical significance of the reaction time difference between incongruent and congruent conditions. Differences were assessed with a linear mixed effects model.

VAMS were significantly improved by the acute intake of levodopa ($p = 0.02$). In the drug-off condition, patients had a tendency to suffer from a selective impairment in facial emotion recognition of fear compared with drug-on patients ($p = 0.08$) while there was no difference in correct responses for other emotional expressions.

BEHAVIORAL DATA

The percentage of correct responses and RT were unavailable for two drug-off patients, one drug-on patient and one healthy control, due to technical difficulties during MRI. The mean task accuracy was not statistically different between the three groups with an overall accuracy of 80%. The percentage of correct responses was higher during congruent trials than during incongruent trials, regardless of the group ($p = 0.002$). Facial emotions did not influence the percentage of correct responses. The reaction time results are presented in **Figure 3**. In trials with positive faces, our multivariate mixed effect model indicated a significant effect of congruency on performances in each group ($p < 0.001$ for all three groups), with a shorter RT in the congruent condition compared with the incongruent condition (in HC, drug-on and drug-off patients, the mean relative decrease in RT was 8.6% [95%CI: 6.2–11.0], 11.7% [95%CI: 9.1–14.2] and 7.7% [95%CI: 4.9–11.4], respectively). In trials with negative faces, there was a significant effect of congruency on performances in HC (relative difference = 2.7% [95%CI: 0–5.3], $p < 0.001$) and drug-on patients (relative difference = 5.9% [95%CI: 3.1–8.7], $p < 0.001$). No significant effect of congruency was found in the drug-off patients in trials with negative faces (relative difference = 1.7% [95%CI: -1.4–4.6], $p = 0.282$). The congruence effect was not statistically different between HC and the drug-on or drug-off patients, whereas it was significantly larger for the drug-on patients compared to the drug-off patients ($p = 0.02$), regardless of the facial emotion.

fMRI WITHIN-GROUP COMPARISONS

ES task validation in healthy participants

In HC, the network of cerebral activation elicited by the Incongruent vs. Congruent [I-C] contrast is shown in **Figure 4** and **Table 3**. A significant activation within the postgenual ACC (x, y, z MNI coordinates: −9, 16, 20; $p < 0.001$; $k > 5$ uncorrected) was identified. No significant activation was found in the rACC or in the amygdala, areas specifically activated in emotional conflict.

Given that no significant activation was found in the rACC, and because numerous studies have reported rACC activation during "sad" ES tasks (i.e., classic ES task using negatively valenced words written in different ink colors) (Whalen et al., 1998, for review), we decided to decorrelate the analysis depending on the emotion of the face. For the contrast [I-C] for negative faces, the rACC was consistently activated. Two regions, R1 and R2 were activated within this area (R1: x, y, z MNI coordinates: 6, 50, 4; Brodmann area (BA) = 32; $p < 0.001$; $k > 5$ uncorrected, and R2: x, y, z: 6, 41, 7; $BA = 24$; $p < 0.001$; $k > 5$ uncorrected)

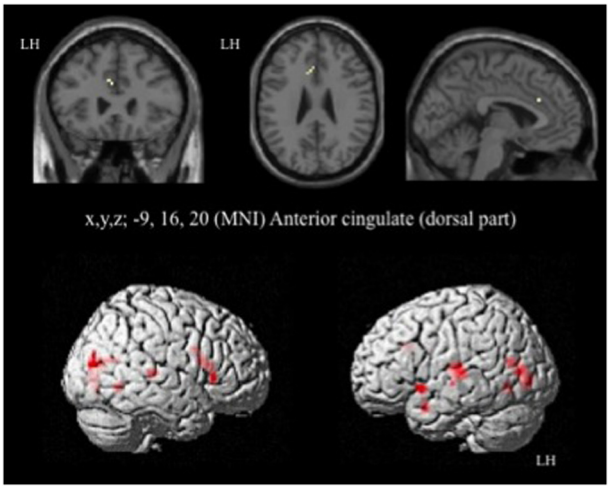

FIGURE 4 | Brain activation during the emotional Stroop task (contrast: Incongruent vs. Congruent with all faces) in healthy subjects. $p < 0.001$, $k > 5$, $n = 12$, uncorrected. LH, left hemisphere; MNI, Montreal Neurological Institute.

(**Figure 5A** and **Table 4**). Other areas less specifically involved in emotional conflict were also activated, such as the superior parietal gyrus and the superior temporal gyrus, which has been involved in the perception of emotion in facial stimuli. The right inferior frontal gyrus, which has been typically implicated in go/no go tasks (Aron et al., 2004), was engaged. The amygdala was not activated.

For the contrast [I-C] for positive faces, a significant activation within the caudal ACC was found (x, y, z: 0, 14, 30; $BA = 24$; $p < 0.001$; $k > 5$ uncorrected) but not in the rACC (**Figure 5B** and **Table 5**). The contrast [I-C] for positive faces also activated other areas commonly activated in non-emotional conflict (Egner et al., 2008), such as the anterior prefrontal cortex, the precuneus, the lingual gyrus and the inferior parietal gyrus. No suprathreshold voxels were activated for [C-I] contrast for positive, negative or all faces.

ES task in parkinsonian patients

Considering the results obtained in HC, we decided to study the ES effect with the contrast [I-C] for negative faces, as this was only contrast that activated the rACC. The cerebral sites of hemodynamic response during the ES task are shown in **Table 6** for the drug-off patients and in **Table 7** for the drug-on patients. Both patient groups activated less cerebral areas and recruited a decreased number of voxels for all activated brain areas compared to controls. Drug-off patients exhibited additional areas of activation not seen in HC and drug-on patients, such as the left superior medial frontal gyrus (MNI coordinates $x = -3$, $y = 32$, $z = 46$; $BA = 8$; $p < 0.001$, $k > 5$), the left hippocampus ($x = -24$, $y = -37$, $z = -8$; $p < 0.001$, $k > 5$), and the right cuneus ($x = 12$, $y = -94$, $z = 14$; $p < 0.001$, $k > 5$). No suprathreshold voxels were activated for [C-I] contrast for negative faces in any patients groups.

Table 3 | Brain regions activated during the emotional Stroop task using all faces in healthy subjects.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (all faces)	**Cingulate cortex**					
	Anterior cingulate	L	24	−9, 16, 20	9	4.95
	Frontal cortex					
	Inferior frontal triangularis	R	45	54, 23, 4	8	4.74
	Temporal cortex					
	Fusiform	L	19	−24, −64, −2	9	5
	Superior temporal	L	41	−51, −22, 8	49	7.82
		R	41	51, −28, 8	4.75	6
	Superior temporal pole	L	13	−27, 8, 21		
		L	38	−54, 8, −8	10	4.69
	Middle temporal	L	19	−45, −64, 14	10	4.69
	Inferior temporal	R	37	39, −58, −5	6	4.71
	Insula	R	47	33, 26, 1	32	6.1
	Occipital cortex					
	Middle occipital	L	19	−45, −76, 8	5	6.31
	Lingual	L	17	−24, −76, 11	49	8.27
	Calcarine	R	19	36, −82, 14	41	6.88
		R	18	15, −79, 1	11	4.60
		L	18	−6, −70, 14	22	6.83
[I-C] (all faces)	*No suprathreshold voxels*					

Cerebral activation locations refer to maximal hemodynamic response sites. $p < 0.001$, $k > 5$, $n = 12$, uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.

fMRI BETWEEN-GROUP COMPARISONS

For the contrast [I-C] with negative faces, HC displayed significantly greater activation than off-drug patients within the right rACC (x, y, z: 6, 47, 4.4; $BA = 32$; $p < 0.001$; $k > 5$ uncorrected) (**Figure 6**), the right pre- and post-central gyri and the right thalamus (**Table 8A**). Drug-off patients vs. HC did not yield any suprathreshold clusters (**Table 8B**).

Comparisons between HC and drug-on patients (HC vs. drug-on patients and drug-on patients vs. HC) did not yield any suprathreshold clusters. Drug-on patients had significantly greater activation than drug-off patients in the right inferior temporal gyrus (x, y, z: 42, −61, −8; $BA = 37$; $p < 0.001$; $k > 5$ uncorrected), an area involved in visual recognition. No significant activation was found in the ACC for a $p < 0.001$. However, by reducing the threshold to $p < 0.005$, drug-on patients displayed greater activation than drug-off patients in the left postgenual ACC (x, y, z: −3, 23, 19; $BA = 24$; $T = 4.28$; $p < 0.005$; $k > 5$ uncorrected). For a $p < 0.008$, drug-on patients displayed greater activation than drug-off patients in the right rACC (x, y,

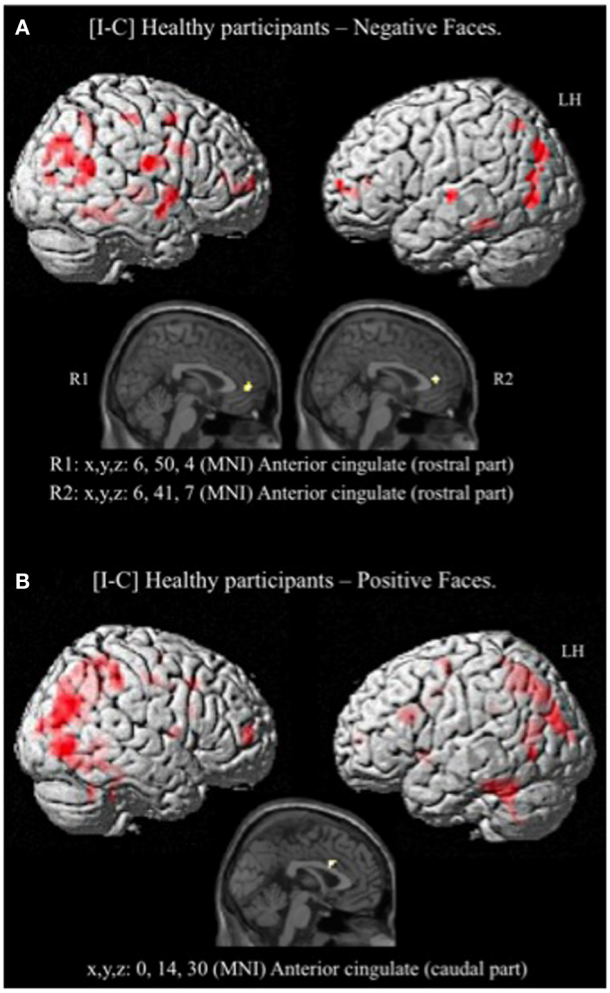

FIGURE 5 | Brain activation during the emotional Stroop task (contrast: Incongruent vs. Congruent) with separate analysis of negative (A) and positive (B) faces, in healthy subjects. $p < 0.001$, $k > 5$, $n = 12$, uncorrected. C, congruent; I, incongruent; LH, left hemisphere; MNI, Montreal Neurological Institute; R1, region 1; R2, region 2; x, y, z: mediolateral, rostrocaudal, and dorsoventral.

z: 9, 38, 1; $BA = 32$; $p < 0.008$; $k > 5$ uncorrected) (**Table 9**). Drug-off patients did not exhibit any suprathreshold clusters when compared with drug-on patients at a $p < 0.001$ threshold. No activation was seen in the ACC when the threshold was increased to $p < 0.008$ (**Table 9**). No suprathreshold voxels were activated for [C-I] contrast for negative faces in any between-group comparisons.

DISCUSSION

The main finding of this study is the demonstration of a dopaminergic modulation in the rostral anterior cingulate cortex, an area specifically implicated in emotional conflict resolution. During our negative emotional Stroop task, drug-off parkinsonian patients displayed a relative hypoactivation in their rACC compared with healthy controls. No significant difference within the rACC activation was found between the HC and the drug-on patients, suggesting a normalization of the rACC activation deficit after levodopa administration.

Table 4 | Brain regions activated during the emotional Stroop task when only negative faces were analyzed in healthy subjects.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (negative faces)	**Cingulate cortex**					
	Anterior cingulate	L	32	6, 50, 4	55	6.73
		R	24	6, 41, 7	11	5.46
	Frontal cortex					
	Inferior frontal opercularis	R	9	36, 11, 30	17	4.56
	Middle frontal	R	6	33, 2, 49	9	4.70
	Parietal cortex					
	Superior parietal	L	7	−18, −64, 52	13	4.80
	Postcentral	R	3	45, −22, 52	8	4.90
	Precuneus	R	30	15, −52, 11	18	5.09
	Temporal cortex					
	Fusiform	L	20	−36, −37, −18	17	5.34
		R	20	27, −37, −18	50	6.70
	Superior temporal	L	22	−63, −16, 1	7	8.48
	Insula	R	13	45, 5, −2	37	6.49
	Thalamus	R		15, −16, 1	51	8.64
	Occipital cortex					
	Middle occipital	L	19	−27, −76, 33	42	9.01
	Middle occipital	L	18	−42, −73, 1	30	8.86
	Middle occipital	R	39	51, −52, 14	146	7.76
[C-I] (negative faces)	*No suprathreshold voxels*					

$p < 0.001$, $k > 5$, $n = 12$, uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.

Our behavioral results indicated an abnormal negative ES effect in drug-off patients. Controls and drug-on patients presented a negative ES effect, whereas drug-off patients did not, suggesting that drug-off patients were less sensitive to negative emotional interference. Based on the anxiety and depressive literature (Williams and Nulty, 1986; Becker et al., 2001), we would have expected that the drug-off state, which increases anxiety and depressive mood, would be linked to an increased ES effect. We would also have expected that drug-off patients took longer to solve the ES conflict because of the abnormal activation of the rACC and the recruitment of additional brain circuits. Serra-Mestres and Ring (2002) showed that non-depressed parkinsonian patients presented with greater interference to sad words than did HC. However, their patients were tested while on dopaminergic medication and it is difficult to compare their study to ours, due to methodological differences such as the use of another type of ES task and an alternative method for calculating the ES effect. An explanation to understand the diminished emotional interference in our drug-off patients would be that, although most dopamine neurons provide a reward signal, a few

Table 5 | Brain regions activated during the emotional Stroop task when only positive faces are analyzed in healthy subjects.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (positive faces)	**Cingulate cortex**					
	Anterior cingulate	L	24	0, 14, 30	31	5.49
	Middle cingulate	L	24	0, −1, 43	13	4.60
	Frontal cortex					
	Superior frontal	R	10	21, 56, 8	34	6.63
	Parietal cortex					
	Inferior parietal	R	7	27, −49, 56	61	5.34
	Precuneus	L	19	33, −70, 27	862	8.85
	Temporal cortex					
	Fusiform	L	37	−36, −46, −28	80	7.29
	Middle temporal	L	37	−48, −64, −2	7	5.19
	Inferior temporal	R	37	51, −43, −15	9	4.98
	Basal ganglia					
	Caudate nucleus	L		−12, 5, −5	13	5.70
	Pallidum	L		−12, 5, −5	13	5.70
	Putamen	L		−12, 5, −5	13	5.70
		R		36, 5, 11	8	4.96
	Occipital cortex					
	Superior occipital	L	18	−12, −91, 11	9	5.97
	Middle occipital	L	19	−36, −82, 24	36	6.58
	Lingual	R	19	15, −49, −2	84	7.51
	Calcarine	L	31	−12, −64, 17	60	8.40
	Cerebellum	L		−18, −52, −21	18	5.14
		R		27, −31, −21	15	4.70
[C-I] (positive faces)	*No suprathreshold voxels*					

p < 0.001, k > 5, n = 12, uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.

Table 6 | Brain regions activated during the emotional Stroop task with negative faces in drug-off patients.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (negative faces)	**Frontal cortex**					
	Superior medial frontal	L	8	−3, 32, 46	10	5.82
	Temporal cortex					
	Inferior temporal	L	37	−51, −55, −8	7	6.02
	Hippocampus	L	28	−24, −37, −8	15	6.28
	Occipital lobe					
	Middle occipital	R	19	42, −79, 14	11	8.32
		R	19	30, −85, 20	7	5.09
	Cuneus	R	18	12, −94, 14	8	4.98
[C-I] (negative faces)	*No suprathreshold voxels*					

p < 0.001, k > 5, n = 10, uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.

Table 7 | Brain regions activated during the emotional Stroop task with negative faces in drug-on parkinsonian patients.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (negative faces)	**Frontal cortex**					
	Middle frontal	R	46	45, 38, 17	5	5.05
	Temporal cortex					
	Middle temporal	L	22	−63, −31, 4	5	4.98
	Inferior temporal	R	19	39, −64, −8	13	7.71
[C-I] (negative faces)	*No suprathreshold voxels*					

p < 0.001, k > 5, n = 10, uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.

dopamine neurons (5–15%) are activated by primary aversive stimuli (Schultz, 2013) This may partially explain why drug-on patients are more sensitive to negative stimuli/distractors than drug-off patients. Another hypothesis could be that Parkinson's disease enhances distractor resistance when patients are in their drug-off state and that dopaminergic medication reinstates susceptibility to distraction (Cools et al., 2010). This could explain the absence of negative distractor-related slowing in drug-off patients, leading to relatively faster responding after distraction than in controls and drug-on patients. Slowing was reinstated by dopaminergic medication, as evidenced by the finding that the patient responses in their drug-on state did not differ from that of controls.

Lastly, another hypothesis to explain the decreased vulnerability to emotional interference could be that the presence of apathy in our drug-off patients enhances their negative emotional blunting, making them less sensitive to negative distractors. Our drug-off patients displayed a tendency to have impaired fearful expression recognition and presented higher scores on the Apathy scale. Numerous studies have demonstrated an impaired recognition of facial expression in Parkinson' disease, especially for negative emotions such as fear, disgust, anger, and sadness (Peron et al., 2012). Clinically, apathy is identified as a reduction of goal-directed behavior because of a lack of feeling, interest, emotional reactivity and motivation (Marin, 1991). Apathetic patients have been shown to have a diminished capacity to process, identify and differentiate between favorable (positive stimuli) and unfavorable (negative stimuli) outcomes and to adjust subsequent behaviors accordingly (Holroyd et al., 2002; Cohen and Ranganath, 2007; Martínez-Horta et al., 2014). Research in the field of apathy has shown impaired emotion recognition and reward processing in the absence of deficits of higher cognition

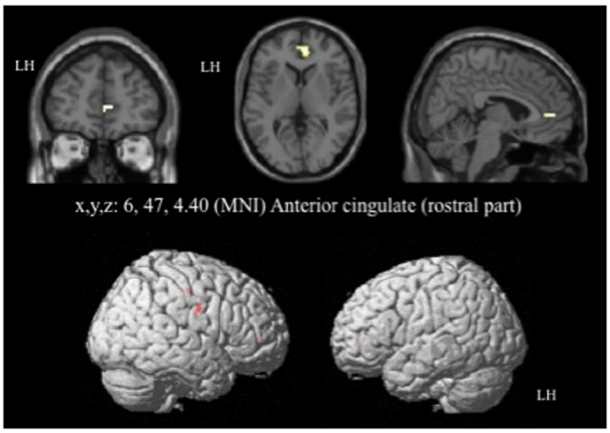

FIGURE 6 | Brain activation during the emotional Stroop task with negative faces (contrast [I-C]) in the between group comparison (Control vs. drug-off patients). $p < 0.001$, $k > 5$, $n_{healthy} = 12$, $n_{patients} = 10$, uncorrected. I; incongruent; C; congruent; LH, left hemisphere; x, y, z: mediolateral, rostrocaudal, and dorsoventral; MNI, Montreal Neurological Institute.

Table 8 | Comparisons between controls and drug-off patients: brain regions activated during the emotional Stroop task with negative faces.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (negative faces)						
A. Controls vs. Drug-off patients						
	Cingulate cortex					
	Anterior cingulate	R	32	6, 47, 4.4	16	4.75
	Frontal cortex					
	Precentral	R	4	39, −16, 49	6	3.67
	Parietal cortex					
	Postcentral	R	6	54, −7, 30	5	3.78
	Thalamus	R		12, −10, 1	11	4.76
B. Drug-off patients vs. Controls						
	No suprathreshold voxels					
[C-I] (negative faces)						
A. Controls vs. Drug-off patients						
	No suprathreshold voxels					
B. Drug-off patients vs. Controls						
	No suprathreshold voxels					

$p < 0.001$, $k > 5$, $n_{healthy} = 12$, $n_{patients} = 10$; uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.

(Martínez-Corral et al., 2010; Lawrence et al., 2011; Robert et al., 2014). In conclusion, apathetic drug-off patients are more impaired than drug-on patients in distinguishing the valence of emotional stimuli and in negative emotion recognition, which could make them less sensitive to negative distractors. It is noteworthy that the ES effect was absent during negative trials in hypodopaminergic patients but present during positive trials, probably because the emotional blunting in PD predominantly affects negative emotions.

The abnormal negative emotional interference in our drug-off patients could be linked with their relative rACC hypoactivation, because this area is specific to emotional conflict and was hypoactive in drug-off patients compared with drug-on patients and HC. Whalen et al. (1998) proposed that rACC activation reflects the successful processing of emotional stimuli in the service of facilitating task performance. The rACC appears to decrease the weighting of irrelevant affective information in the service of optimizing cognitive performance. In healthy volunteers, Bishop et al. (2004) found that the rACC was strongly activated by infrequent threat-related distractors, consistent with a role of this area in responding to unexpected processing conflict caused by salient emotional stimuli. Egner et al. (2008) demonstrated that the rACC was specifically implicated in the resolution of emotional conflict. In our patients, failure to activate the rACC might then reduce the rACC control over negatively salient task-irrelevant emotional stimuli, leading to a reduced recruitment of the normal brain circuitry to overcome emotional conflict and increase the cognitive processing load. In our study, the dorsal ACC, a region implicated in both emotional and non-emotional monitoring, was activated in HC in the Incongruent vs. Congruent trials using all faces, negative faces and positives faces. It was also more activated in drug-on than in drug-off patients, suggesting an abnormal conflict monitoring in hypodopaminergic parkinsonian patients. This relative dorsal ACC hypoactivation might explain why drug-off patients presented with rCCA hypoactivation via probable top-down mechanisms.

The abnormal emotional interference effect and the rACC hypoactivation seen in our drug-off patients were reversed after dopamine administration, suggesting a dopaminergic involvement in emotional conflict. To our knowledge, our study is the first to evoke a specific dopaminergic modulation in the rACC in parkinsonian patients. The ACC hypoactivation in drug-off patients could be directly induced by the degeneration of mesocortical dopaminergic fibers. PD alters the mesocorticolimbic dopaminergic system which consequently impairs the function of the cingulate striatofrontal loop (Czernecki et al., 2002). Drug-on patients did not differ from HC in terms of MRI activation probably because dopatherapy doses were sufficient to restore dopamine function in the mesocortocolimbic pathways in our patients. The ACC receives one of the richest dopaminergic innervations of any cortical area (Gaspar et al., 1989). The source of ACC dopaminergic input comes from cell bodies located in the ventral tegmental area (VTA). Using immunohistochemicals methods, Raghanti et al. (2008) demonstrated a dense dopaminergic innervation in the dorsal ACC (BA 32) in humans. The mesocortical dopaminergic degeneration could then induce a lack of rACC dopaminergic activation. The rACC hypoactivation in drug-off patients could also be explained more indirectly by the degeneration of dopaminergic cells originating from the VTA and projecting to the orbitofrontal cortex (OFC). This denervation could induce a dysfunction in the limbic loop of basal ganglia. This circuit originates in the OFC and the ACC, and projects

Table 9 | Comparisons between drug-on and drug-off patients: cerebral sites of hemodynamic response during the emotional Stroop task with negative faces.

Contrast	Cerebral areas	H	BA	MNI coordinates (x, y, z)	k	*T*
[I-C] (negative faces)						
A. Drug-on vs. Drug-off patients						
	Temporal cortex					
	Inferior temporal*	R	37	42, −61, −8	5	6.92
	Cingulate cortex					
	Dorsal anterior cingulate**	L	24	−3, 23, 19	21	4.28
	Rostral anterior cingulate***	R	32	9, 38, 1	5	3.59
B. Drug-off vs. Drug-on patients						
	Parietal cortex					
	Inferior parietal**	L	40	−42, −52, 52	14	4.25
	Temporal cortex					
	Inferior temporal***	L	37	−45, −52, −8	5	5.18
[C-I] (negative faces)						
A. Drug-on vs. Drug-off patients						
	No suprathreshold voxels					
B. Drug-off vs. Drug-on patients						
	No suprathreshold voxels					

*$p < 0.001$, **$p < 0.005$, ***$p < 0.008$, $k > 5$, $n = 10$, uncorrected. Cerebral areas are defined by the Automatic Anatomical Labeling. BA, Brodmann areas; C, congruent; H, hemisphere; I, incongruent; k, cluster size (number of voxels); L/R, left/right; MNI, Montreal Neurological Institute; x, y, z, mediolateral, rostrocaudal, and dorsoventral.*

to the nucleus accumbens and the most rostral portions of the ventral striatum (Oades and Halliday, 1987). The ventral striatum projects in turn to the ventral pallidum, which then projects to the substantia nigra *pars reticulata*, which finally projects to the mediodorsal thalamic nucleus. This circuit is then closed by thalamic projections to the ACC and the medial OFC (Alexander et al., 1986; Hamani et al., 2004). The rACC being a structure involved in the limbic loop of the basal ganglia, the relative rACC hypoactivation demonstrated in our drug-off patients could induce a dysfunction of this circuit.

During the incongruent trials of our ES task, there was conflict between the automatic reading of emotional words (distractors) and the recognition of facial emotion. This situation requires an implicit analysis, which is automatic, subconscious, and rapid. This analysis probably involves the basal ganglia, which are responsible for the automatic execution of learned motor, cognitive, and emotional plans (Marsden, 1982; Alexander et al., 1986). A basal ganglia limbic loop dysfunction would induce an abnormal emotion regulation by cingulate failure to implement control processes over affective distractive stimuli.

Neuropsychiatric fluctuations have been shown to be an important component of levodopa-induced fluctuations (Maricle et al., 1995a). They are related to levodopa dosing (Maricle et al., 1995b). Parkinson's disease behavioral disturbances can be described on a continuous spectrum ranging from hypodopaminergic behaviors (apathy, anxiety, depression and fatigue) to hyperdopaminergic behaviors (mania, impulse control disorder, dopamine dysregulation syndrome, punding and appetitive behaviors) (Ardouin et al., 2009; Lhommée et al., 2012; Rieu et al., 2012; Castrioto et al., 2014). In our patients, acute intake of dopamine replacement therapy improved the Apathy and VAMS scores and was concomitant with normalized ES task behavioral results and rACC activation. This finding could provide a neurobiological basis for the physiopathology of neuropsychiatric fluctuations. During their drug-off state, our patients displayed higher apathy scores and VAMS affective and asthenia subscores, than drug-on patients. We have discussed above how apathy could lead to decreased negative interference in drug-off patients. The VAMS is designed to measure anxiety, mood changes and physical and mental sedation in a non-specific way after the administration of drugs. It is possible than the higher level of anxiety and sedation in our drug-off patients was associated with the rACC hypoactivation through disrupted attentional control over negatively salient task-irrelevant emotional stimuli. This would be consistent with studies addressing anxiety disorders such as Shin et al. (2001) and Kim et al. (2008) who reported that posttraumatic stress disorder patients presented decreased rACC functioning when exposed to situations eliciting negative emotional conflict. In terms of neuroanatomical network, our hypothesis that rACC hypoactivation could explain at least in part the presence of neuropsychiatric fluctuations, is consistent with Thobois et al.'s study (2010) that demonstrated that patients with neuropsychiatric fluctuations had greater mesocorticolimbic dopaminergic denervation than patients without neuropsychiatric fluctuations.

Our drug-off patients exhibited additional areas of cerebral activation not seen in HC and drug-on patients, such as the left superior medial frontal gyrus (BA 8) and the left hippocampus. BA 8 is involved in the management of uncertainty. Increased activation has been shown when subjects experience increasing uncertainty (Volz et al., 2005), which appears to be the case in our drug-off patients. The hippocampus is crucial for long-term episodic memory (Bird and Burgess, 2008) and it has been suggested that it facilitates predictions about upcoming events (Buckner, 2010). It is possible that the limbic basal ganglia loop dysfunction in drug-off patients is compensated by hyperactivation of these two structures. When basal ganglia are inefficient, they cannot execute learned plans in an automatic manner (Marsden, 1982) and drug-off patients rely on compensatory cortical areas based on slow intracortical connections. Compensatory activations have been frequently observed in drug-off patients and impaired patterns of cortical activation tend to normalize with both dopaminergic treatment and subthalamic deep brain stimulation (Jenkins et al., 1992; Limousin et al., 1997).

A number of limitations of our study should be acknowledged. Firstly, we report uncorrected statistics for our fMRI

results. The small sample size did not allow for significant brain activation when we applied correction of multiple comparisons. Consequently, our results should be considered preliminary and need to be repeated in a larger population. However, we adopted a more stringent statistical threshold ($p < 0.001$) than would normally be applied for corrected analyses. Each patient served as his own control, meaning that differences between drug-on and drug-off state may be highly significant in relatively small samples. Secondly, we did not include a non-emotional Stroop task for comparison with our ES task. It would have been interesting to use the non-emotional Stroop task described by Egner et al. (2008). Without this task, it is difficult to dissociate the proportion of behavioral slowdown during incongruent trials, due to non-emotional and to emotional interferences. It is also difficult to dissociate the neuroanatomical networks recruited in conflict depending on the nature (emotional or non-emotional) of the conflict. Nevertheless, numerous studies, such as the paper by Egner et al. (2008) have precisely defined which regions are specifically activated in non-emotional and emotional conflict tasks, making the rACC hypoactivation in our drug-off patients specific for emotional conflict resolution.

In conclusion, our study suggests that emotional conflict processes could be dopamine-dependent. Drug-off parkinsonian patients demonstrated a relative underactivity in the affective subdivision of the ACC (rACC) during a negative emotional interference task, which was not seen in the same patients in the drug-on condition, nor in healthy controls. However, our results should be considered preliminary, as further trials with larger sample sizes are required. Rostral ACC hypoactivation could be due to the degeneration of dopaminergic mesocorticolimbic pathways.

In light of the role of the rACC in mediating affective and motivational behaviors (in particular in emotional conflict resolution), we suggest that deficient rACC activation in hypodopaminergic parkinsonian patients might explain their adaptational difficulties in response to negative emotional distractors and promote neuropsychiatric fluctuations such as apathy, anxiety or depression. Our data support the hypothesis stipulating that neuropsychiatric fluctuations have a dopaminergic substrate and reinforces the knowledge that adjustments of dopaminergic medication might be helpful for the treatment of neuropsychiatric fluctuations in Parkinson's disease.

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Graph analysis of verbal fluency test discriminate between patients with Alzheimer's disease, mild cognitive impairment and normal elderly controls

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Verbal fluency is the ability to produce a satisfying sequence of spoken words during a given time interval. The core of verbal fluency lies in the capacity to manage the executive aspects of language. The standard scores of the semantic verbal fluency test are broadly used in the neuropsychological assessment of the elderly, and different analytical methods are likely to extract even more information from the data generated in this test. Graph theory, a mathematical approach to analyze relations between items, represents a promising tool to understand a variety of neuropsychological states. This study reports a graph analysis of data generated by the semantic verbal fluency test by cognitively healthy elderly (NC), patients with Mild Cognitive Impairment—subtypes amnestic (aMCI) and amnestic multiple domain (a+mdMCI)—and patients with Alzheimer's disease (AD). Sequences of words were represented as a speech graph in which every word corresponded to a node and temporal links between words were represented by directed edges. To characterize the structure of the data we calculated 13 speech graph attributes (SGA). The individuals were compared when divided in three (NC—MCI—AD) and four (NC—aMCI—a+mdMCI—AD) groups. When the three groups were compared, significant differences were found in the standard measure of correct words produced, and three SGA: diameter, average shortest path, and network density. SGA sorted the elderly groups with good specificity and sensitivity. When the four groups were compared, the groups differed significantly in network density, except between the two MCI subtypes and NC and aMCI. The diameter of the network and the average shortest path were significantly different between the NC and AD, and between aMCI and AD. SGA sorted the elderly in their groups with good specificity and sensitivity, performing better than the standard score of the task. These findings provide support for a new methodological frame to assess the strength of semantic memory through the verbal fluency task, with potential to amplify the predictive power of this test. Graph analysis is likely to become clinically relevant in neurology and psychiatry, and may be particularly useful for the differential diagnosis of the elderly.

Keywords: semantic verbal fluency, graph analysis, elderly, Alzheimer's disease, mild cognitive impairment

INTRODUCTION

Language and semantic memory tend to remain stable across the human lifespan in contrast to other cognitive domains, like episodic memory and attention, which usually decline after the 5th decade (Craik and Bialystok, 2006). They are also usually spared in the initial stages of neurodegenerative disorders, such as Alzheimer's disease (AD), though we can still observe milder deficits, e.g., anomia or reduced semantic verbal fluency, which can be identified in a comprehensive neuropsychological evaluation (Henry et al., 2004; Garrard et al., 2005; Nutter-Upham et al., 2008; Taler and Phillips, 2008).

Verbal fluency is the ability to produce a satisfying sequence of spoken words during a given time interval (Nickles, 2001). Verbal fluency tests are experimentally designed to assess this ability through the production of words starting with a specific letter (Phonemic Verbal Fluency) or belonging to a category of knowledge (Semantic Verbal Fluency). Semantic verbal fluency is one of the most commonly used tasks to evaluate language and semantic memory skills in older adults. This task depends on

the preservation of language (e.g., words can be spoken correctly during the task), though it is significantly influenced by semantic memory (e.g., the knowledge of the category asked must be intact) and executive function (e.g., the ability to search the asked knowledge) domains (Adlam et al., 2006; Unsworth et al., 2011). This task often activates the temporal lobe, a region broadly related to conceptualization, general information and knowledge about names (Patterson et al., 2007). Semantic verbal fluency contributes to predict future cognitive and functional impairments in the elderly (Salmon et al., 2002; Amieva et al., 2005; Hodges et al., 2006; Aretouli et al., 2011), and predict the progression from MCI to AD (Saxton et al., 2004).

Despite being widely used for neuropsychological assessment in the elderly, the standard measure of the verbal fluency test is restricted to the total of correct words produced in the task (Lezak et al., 2004; Strauss et al., 2006), and does not take into account other clinically-relevant information that may be contained in the patient's specific performance. This task requires the production of words belonging to a specific category, and each subject produced the words following an order of exemplars during the 1-min task. This order of words produced allows the construction of a network based on the temporal link between the words. These temporal links may inform that words produced in a specific temporal sequence are probably conceptually related, as suggested by the semantic association models (McClelland and Rogers, 2003; Griffiths et al., 2007).

Goni et al. (2010) constructed a semantic network using the verbal fluency task applied to an adult sample, and represented the semantic memory as a graph ruled by conceptual constraints. A normal semantic verbal fluency network is represented by a directed graph with only one occurrence for each word. Lerner et al. (2009) investigated the network properties of subjects with MCI and AD, and found that the path lengths of the network decline while the clustering coefficient increases in the MCI and AD subjects compared to healthy elderly controls. These results showed that the normal characteristics of the semantic verbal network are significantly changed in the continuum from normal aging to AD.

The analysis of network properties helps understanding the dynamics and organization of the cognitive and behavioral processes. A graph represents a network with nodes linked by edges (Mota et al., 2012). Formally, a graph is a mathematical representation of a network G = (N, E), with N = $\{w_1, w_2, \ldots w_n\}$ a set of nodes and E = $\{(w_i, w_j)\}$ a set of edges or links between words w_i in N and w_j in N. The interpretation of the meaning of a graph depends on what is being represented (Butts, 2009; Mota et al., 2012). We carried out an analysis of the network properties of the semantic verbal fluency of subjects with MCI or AD. We hypothesize that the analysis of the semantic verbal fluency network properties can help to better discriminate between older adults with normal cognitive performance, mild cognitive impairment or Alzheimer's disease. This approach had been used

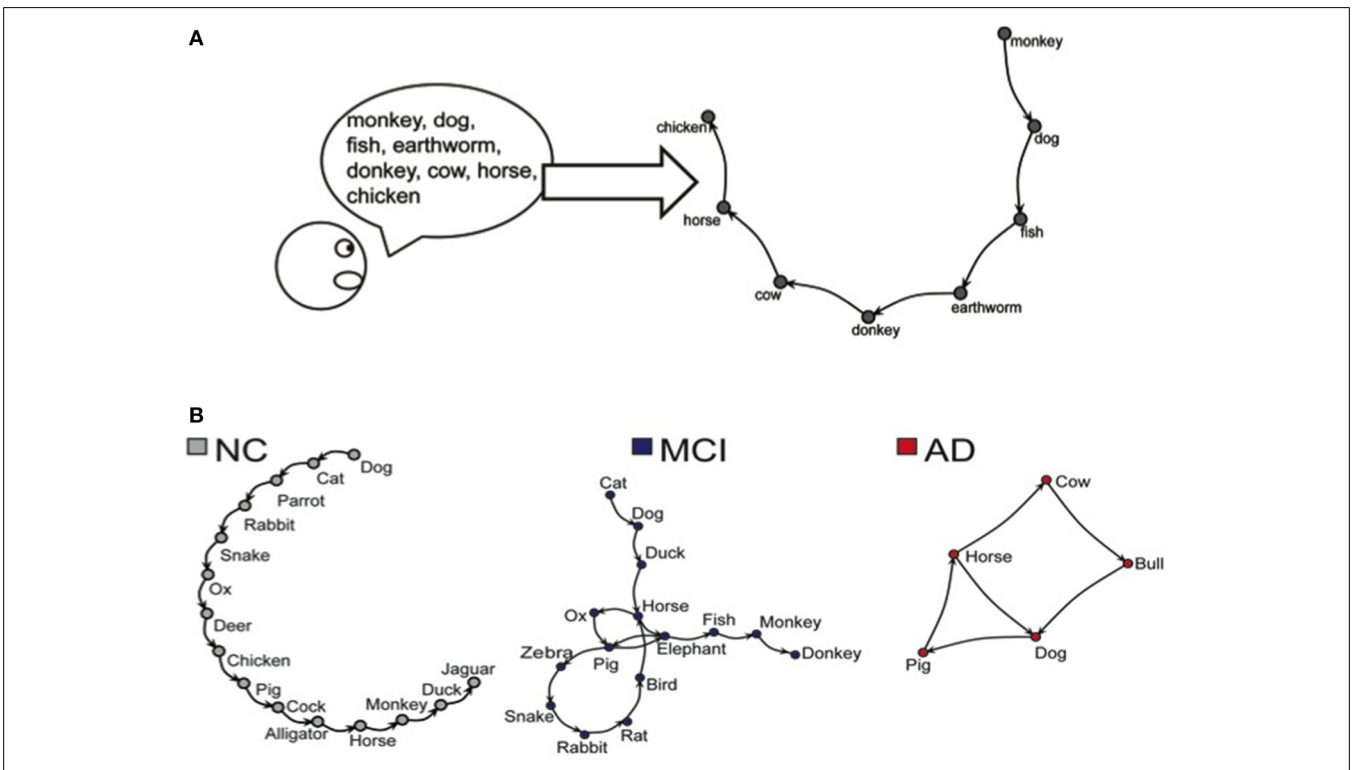

FIGURE 1 | (A) Representation of the word sequence produced on the Semantic Verbal Fluency task. **(B)** Representations of networks generated by NC, MCI, and AD subjects during the Semantic Verbal Fluency task.

with success to identify patients with schizophrenia and bipolar disorder (Mota et al., 2012, 2014).

MATERIALS AND METHODS

SUBJECTS

One hundred older adults were included in this study. All subjects were assessed in the Centro de Referência à Saúde do Idoso Jenny de Andrade Faria, Clinical Hospital, Federal University of Minas Gerais. All the participants underwent a comprehensive clinical and neuropsychological assessment. The neuropsychological protocol included the following tests: Mini Mental State Exam, Frontal Assessment Battery, Category Verbal Fluency of Animals and Fruits, Letter Fluency of S, Digit Span, Stick Design Test, Clock Drawing Test, Rey Auditory Verbal Learning Test, Naming Test (TN-LIN), and Token Test. This protocol has been validated for the neuropsychological assessment of older adults with low educational status (de Paula et al., 2013). After the clinical and neuropsychological assessment, and adjudication meeting was held and the final diagnosis was reached by consensus. The AD diagnosis was based on the proposed criteria of McKhann et al. (1984) and the patient should present general and worsening cognitive impairment, in two or more cognitive domains, and functional impairment in the daily living activities. The MCI diagnosis followed the criteria proposed by Winblad et al. (2004), were the older adult presents cognitive decline in one or more cognitive domains but is preserved in basic and instrumental daily living activities or presents a minimal impairment. The MCI subgroup division considered the amnestic MCI (aMCI) classification for participants that only present memory impairment, and amnestic multiple-domain MCI (a+mdMCI) for participants that present impairment in memory and other cognitive domain, though fulfilling all the MCI criteria established by Winblad et al. (2004).

The project was approved by the Research Ethics Committee of the Federal University of Minas Gerais (COEP-334/06). The subjects were divided into four groups: (1) normal cognitive performance (NC), $n = 25$; (2) amnestic single-domain MCI (aMCI), $n = 25$; (3) amnestic multiple-domain MCI (a+mdMCI), $n = 25$; (4) AD, $n = 25$.

VERBAL FLUENCY TEST

The participants performed the Semantic Verbal Fluency test, category of animals, for which they were asked to produce the maximum names of animals within 60 s; explicit/implicit instructions were given to avoid repetitions. All the words were recorded, including repetitions and errors. The scoring procedure included: total of words produced, total of correct words, total of errors, total of repetitions, and the fraction of repetitions according to the total of words produced by each participant. The scores in this task were not taken into account in the diagnosis adjudication of each participant.

STATISTICAL ANALYSIS

The study design involved two stages of analysis, considering three (NC, MCI, AD) or four groups (NC, aMCI, a+mdMCI, AD), and the same statistical analysis and graph measures were performed for comparing the three or four groups. The MCI group comprised both the aMCI and the a+mdMCI groups.

We performed the Shapiro-Wilk test of normality of the sample, and since the majority of the variables did not fit the assumption of normality, we used the Kruskal-Wallis test of differences between several independent groups and the Wilcoxon Rank sum test for two independent samples. Bonferroni correction was applied to all analyses.

Group sorting was implemented with a Naïve Bayes classifier, which shows superior performance with small samples (Singh and Provan, 1995; Kotsiantis, 2007). The choice of attributes for the classifier was based on significant correlations of the attributes with established clinical measures of differential diagnosis (global cognitive status and daily living functionality). Sensitivity, specificity and the area under the receiver operating characteristic curve (AUC) were used to estimate classification quality, which was considered excellent when AUC was higher than 0.8, good when AUC ranged from 0.6 to 0.8, and poor (not above the chance), when AUC was smaller than 0.6.

GRAPH MEASURES

The word sequence produced on the Semantic Verbal Fluency test was represented as a speech graph, using the software *SpeechGraphs* (Mota et al., 2014). The program represents a text (in this case, the sequence of words produced by the verbal fluency test) as a graph, representing every word as a node, and the temporal link between words as an edge (**Figure 1**).

We then calculated word count (WC) and 13 additional Speech Graph Attributes (SGA) comprising general attributes: total of nodes (N) and edges (E); connected components: the largest strongly connected component (LSC); recurrence attributes: repeated (RE) and parallel edges (PE), cycles of one (L1), two (L2), or 3 nodes (L3); global attributes: average total degree (ATD), density, diameter, average shortest path (ASP) and clustering coefficient (CC) (for more detailed information see Supplementary Table and Figure on Supplementary Material).

Given the task instructions, we expected the subjects to produce a linear network, i.e., a sequence in which each correct word

Table 1 | Socio-demographic data, verbal fluency and Speech Graph Attributes of NC, MCI, and AD groups, with Bonferroni-corrected significant differences across groups established by the Kruskal-Wallis comparison.

	NC			MCI			AD			*p*
	Median	IQR		Median	IQR		Median	IQR		
		Q1	Q3		Q1	Q3		Q1	Q3	
Age	76	72	80	76	71	81	78	67	81	0.9785
Education	4	3	4	4	2	4	4	3	4	0.8400
Katz	0	0	0	–	–	–	0	0	0	0.0105
Lawton	0	0	0	0	0	1	6	4	8	0.0000
MMSE	27	24	29	25	23	27	20	17	23	0.0000

Katz, Katz Index; Lawton, Lawton Index; MMSE, Mini Mental State Exam; IQR, Interquartile Range; Q1, 1th Quartile; Q3, 3trd Quartile. Red values have significance p = 0.0167.

was followed by a different correct word, without repetitions. A correct performance in this test should yield graphs with identical number of nodes (N) and words (WC), N-1 edges, no recurrence (i.e., without parallel edges, repeated edges or loops), and zero strongly connected components (LSC). In addition, the average total degree (ATD) should be close to 2, with a very small density, very low clustering coefficient (CC), and large distances (diameter should be equal to E).

RESULTS

Table 1 shows data for socio-demographic data, Mini Mental State Exam (MMSE), total number of produced words in the

Table 2 | Verbal fluency and Speech Graph Attributes of NC, MCI, and AD groups, with Bonferroni-corrected significant differences across groups established by the Kruskal-Wallis comparison.

	NC			MCI			AD			*p*
	Median	IQR		Median	IQR		Median	IQR		
		Q1	Q3		Q1	Q3		Q1	Q3	
VF.E	0	0	0	0	0	0	0	0	0	1.0000
VF.PR	0	0	0.07	0	0	0.13	0	0	0.1	0.2330
VF.R	0	0	1	0	0	1	0	0	1	0.4462
VF.C	14	12	15	11	10	14	9	7	10	0.0000
VF.TT	15	13	15	12	10	15	9	8	10	0.0000
WC	15	13	15	12	10	15	9	8	10	0.0000
N	14	13	15	11	10	14	9	7	10	0.0000
E	14	12	14	11	9	14	8	7	9	0.0000
RE	–	–	–	0	0	0	0	0	0	0.6034
PE	0	0	0	0	0	0	0	0	0	0.6591
L1	–	–	–	0	0	0	–	–	–	0.6065
L2	0	0	0	0	0	0	0	0	0	0.6942
L3	0	0	0	0	0	0	0	0	0	0.0265
LSC	1	1	7	1	1	6	1	1	4	0.7568
ATD	1.86	1.85	2.00	1.87	1.82	2.00	1.80	1.75	2.00	0.2584
Diameter	12	9.00	13.00	9	6.00	12.00	7	5.00	8.00	0.0001
ASP	4.66	3.67	5.20	3.66	2.91	4.67	3	2.29	3.33	0.0001
CC	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0.2479
Density	0.07	0.06	0.08	0.08	0.07	0.10	0.10	0.10	0.14	0.0000

VF.E, errors; VF.PR, percentage of repetitions; VF.R, repetitions; VF.C, corrects words; VF.TT, total of words; WC, word count; N, nodes; E, edges; RE, repeated; PE, parallel edges; L1, L2, L3, cycles of one, two or 3 nodes; LSC, largest strongly connected component; ATD, average total degree; ASP, average shortest path; CC, clustering coefficient. Red values have significance p = 0.0167.

Table 3 | Pairwise group comparison with Bonferroni-corrected significant differences between groups established by Wilcoxon Ranksum test.

	NC × MCI			NC × AD			MCI × AD		
	W	*Z*	*p*	W	*z*	*p*	*W*	*Z*	*p*
Katz	1875.0	1.385	0.1658	598.5	−1.388	0.1651	1800.0	2.815	0.0049
Lawton	707.0	−3.396	0.0007	325.0	−6.254	0.0000	1327.5	6.358	0.0000
MMSE	1712.5	2.114	0.0345	394.0	4.732	0.0000	558.5	−4.412	0.0000
VF.C	1626.0	3.088	0.0020	396.5	4.776	0.0000	658.5	−3.427	0.0006
VF.TT	1689.5	2.375	0.0175	410.5	4.513	0.0000	656.5	−3.432	0.0006
WC	1689.5	2.375	0.0175	410.5	4.513	0.0000	656.5	−3.432	0.0006
N	1626.0	3.091	0.0020	394.0	4.824	0.0000	640.5	−3.629	0.0003
E	1689.5	2.375	0.0175	410.5	4.513	0.0000	656.5	−3.432	0.0006
L3	1862.5	1.225	0.2205	600.0	−1.137	0.2552	1787.5	2.613	0.0090
Diameter	1686.5	2.405	0.0161	425.5	4.238	0.0000	720.0	−2.783	0.0054
ASP	1667.0	2.618	0.0088	414.5	4.433	0.0000	720.0	−2.773	0.0055
Density	653.0	−3.338	0.0008	388.5	−4.924	0.0000	1596.5	3.558	0.0004

W, Wilcoxon Ranksum; z, = z score. Red values have significance p = 0.0167.

verbal fluency test, total number of correct words produced, total number of repetitions performed during the task, the percentage of repetitions performed according to the total of produced words, and the errors produced.

The groups did not differ in age and education, and only the control group had a significant difference in gender distributions ($X^2 = 6.76$, $df = 2$, $p = 0.009$) (**Table 1**). The results of the groups' comparison on the daily living activities, the global cognitive status are also reported on **Table 1**. Verbal fluency measures and the Speech Graph Attributes are reported on **Table 2**.

Despite the lower number of correct words produced by the NC group, it is similar to those observed to Brazilian normative data (Brucki et al., 1997). Moreover, the scores on the verbal fluency test were not taken into account for participant classification into the diagnostic groups.

The groups significantly differed in the performance on ADLs, in general cognitive status, number of correct words and total words produced, and in the Speech Graph measures of word count, nodes, edges, loops of 3 nodes, diameter, average short path and density. As expected, the NC group performed better at ADLs, had higher scores on the MMSE, produced more nodes, a network with larger diameter and less dense, when compared with the MCI and AD groups. The MCI group showed an intermediate performance between NC and AD groups in all measures.

Table 3 and **Figure 2A** show pairwise comparisons of the 3 diagnosis groups. Statistical significance was set at $p < 0.0167$, after Bonferroni correction for multiple comparisons.

The comparison of the variables between NC and MCI groups demonstrate that the groups differ in the index of instrumental daily living activities, in the number of correct words produced,

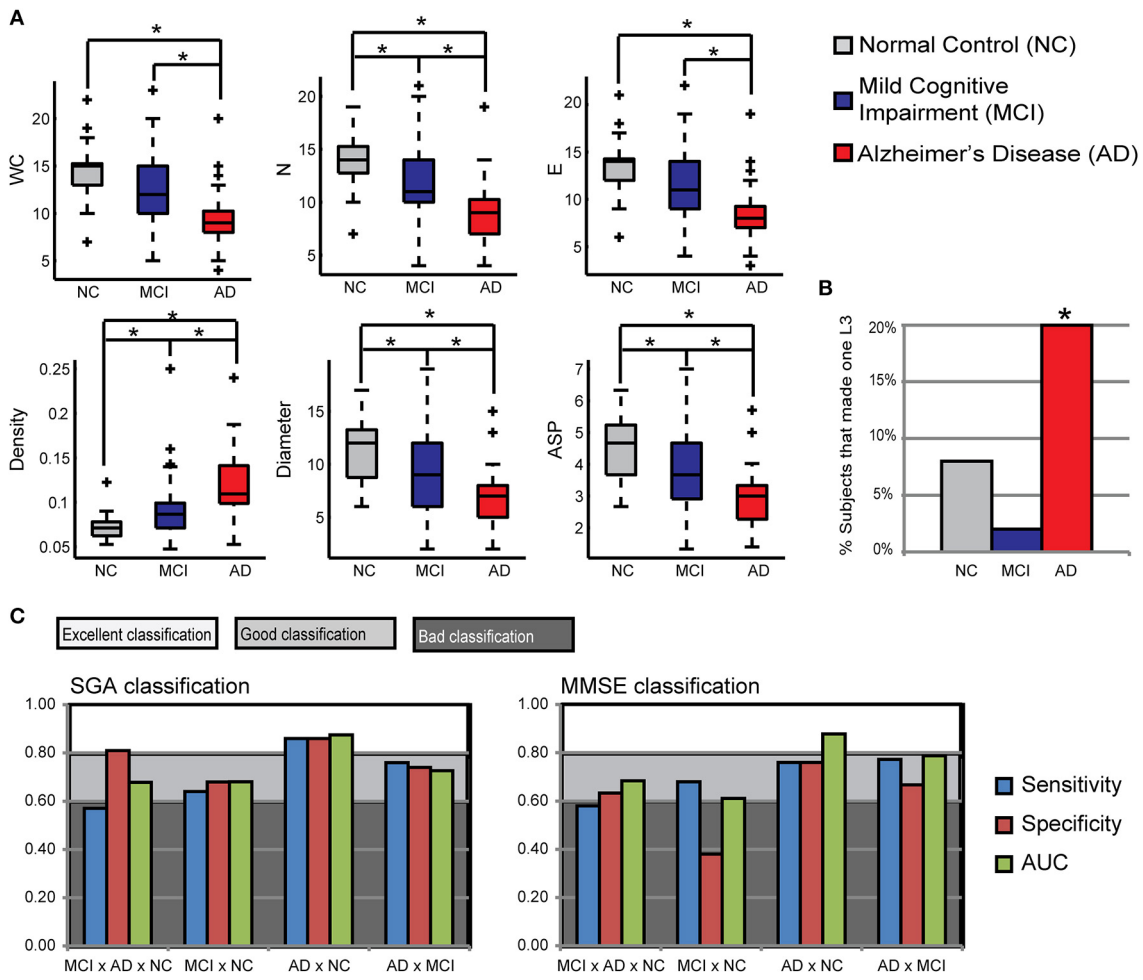

FIGURE 2 | Speech Graph Attributes (SGA) differentiates psychopathological groups. (A) SGA boxplots with significant differences among Alzheimer Disorder (AD), Moderate Cognitive Impairment (MCI) and control groups ($N = 25$ on AD and C group, $N = 50$ on MCI group; Kruskal-Wallis test followed by two-sided Wilcoxon Rank-sum test with Bonferroni correction with alpha = 0.0167). **(B)** Percentage of subjects in each group that made one L3 on the verbal fluency test. AD subjects showed more L3 than MCI subjects (Wilcoxon Rank-sum test with Bonferroni correction with alpha = 0.0167, $p = 0.0090$). **(C)** Rating quality measured by AUC, sensitivity and specificity, using MMSE or SGA correlated with clinical symptoms measured with MMSE and Lawton scales (**Table 3**) (attributes: WC, N, E, Density, Diameter, and ASP). Notice that SGA was more specific than MMSE on triple group sorting, and on MCI diagnosis against the control group. $^*p = 0.0167$.

number of nodes, diameter, average short path and density of the network. The NC produced less dense graphs with more nodes, and larger Diameter and ASP than the MCI and AD. Furthermore, NC made more edges, total words produced, and had a better general cognitive status than the AD group. The MCI and AD groups differ in all measures, demonstrating that a change in the general cognitive status, functionality, verbal fluency measures and the speech graph attributes (WC, N, E, L3, Diameter, ASP, and Density) (**Figure 2B**) almost follow a continuous modification as the diagnosis impairs.

Table 4 shows the Spearman correlations between the SGA and the clinical measures of differential diagnosis (global cognitive status—MMSE—and daily living functionality—Katz and Lawton Index). The significance level was established in $p = 0.0012$ after a Bonferroni correction for 42 comparisons.

We found significant correlations between the MMSE and the SGA of Nodes and Density, indicating that the more cognitively preserved elderly produced a larger number of unique nodes, and networks with a smaller density than cognitively impaired subjects. The correlation between the attributes and the Lawton Index of instrumental daily living activities revealed that the more functionally dependent were the elderly, the less words, nodes and edges they produced, showing networks with a smaller diameter and average short path, but a higher density. These results indicate that functional autonomy correlate more with SGA than with the general cognitive status.

The Naïve Bayes classifier results (**Figure 2C**) show that a selection of SGA correlated with functional and cognitive impairment measured by other instruments, provided good to excellent classification power, being similar to the MMSE classification power, or even better for the distinction between the NC and MCI groups. When the SGA were associated to the Lawton Index or the MMSE, the power of classification increased; a combination of the 3 measurements provided maximal classification quality (**Table 5**). Overall, the combination of graph measures and functional dependence yielded very accurate differential classification of the AD (1.00) and MCI (0.78) against the NC group, and between the MCI and AD (0.84).

Table 4 | Spearman correlation (RHO and *p*-values) between SGA scores and the Katz, Lawton or MMSE scores.

	Katz		Lawton		MMSE	
	RHO	***P***	**RHO**	***P***	**RHO**	***p***
WC	−0.1762	0.0796	−0.4519	0.0000	0.3161	0.0014
N	−0.1811	0.0714	−0.4963	0.0000	0.3335	0.0007
E	−0.1762	0.0796	−0.4519	0.0000	0.3161	0.0014
RE	0.3014	0.0023	0.0698	0.4900	−0.0050	0.9609
PE	0.1031	0.3075	−0.0239	0.8137	0.0958	0.3432
L1	−0.0230	0.8199	−0.0888	0.3797	−0.0943	0.3505
L2	−0.0579	0.5670	−0.0673	0.5062	0.1116	0.2690
L3	0.1048	0.2993	0.2737	0.0059	−0.1557	0.1219
LSC	−0.0349	0.7305	−0.0545	0.5905	0.1171	0.2458
ATD	−0.0352	0.7279	−0.1220	0.2267	0.1611	0.1094
Diameter	−0.1339	0.1842	−0.3897	0.0001	0.2433	0.0147
ASP	−0.1480	0.1418	−0.4017	0.0000	0.2549	0.0105
CC	0.0692	0.4941	0.1786	0.0755	−0.1379	0.1713
Density	0.1766	0.0788	0.4933	0.0000	−0.3239	0.0010

Red values have significance p = 0.0012.

The additional description of the socio-demographic data, Mini Mental State Exam (MMSE), verbal fluency measures of the two subgroups of MCI are reported on **Table 6**, and also the results of the four groups' comparison on the sociodemographic variables. **Table 7** shows the four group comparison on the verbal fluency and Speech Graph Attributes.

A comparison of the four groups showed significant differences in daily functionality, general cognitive status, total and correct words produced, and in the SGA word count, nodes, edges, diameter, ASP and density (same attributes found in the three-group comparison).

Table 8 and **Figure 3A** compare the four groups of elderly, with Bonferroni correction for multiple comparisons (alpha = 0.0083).

The pairwise comparison detected no significant differences between MCI subtypes in the measures selected in this study. The difference between the NC and aMCI groups occurred only in instrumental daily living functionality, i.e., NC are more independent than aMCI. The significant differences between the NC and AD and between aMCI and AD are similar; the NC and aMCI groups are less functionally dependent, have better cognitive status, produce more total and correct words, a higher word count, more nodes and edges, higher Diameter and ASP, and less dense networks when compared to the AD group. The NC are more functionally independent, produce more total and correct words, a higher word count, more nodes and edges, and a network less dense than the a+mdMCI group. AD subjects, comparable to the a+mdMCI group, were more functionally dependent, showed general cognitive impairment, and produced fewer nodes and a denser network.

The Naïve Bayes classifier results (**Figure 3B**) indicate that the selected SGA has a good classification power to the diagnosis of MCI subtypes against cognitive healthy aging, and also a good classification against the dementia group. On the other hand, SGA yielded a poor classification when used to distinguish between the two subtypes of MCI. When SGA were combined with the Lawton Index, we observed an increase in the power of classification across the four groups, except between the two MCI subtypes.

The combination of the SGA with the MMSE, showed less power when compared to the combination with the Lawton index; the combination of these three variables barely improved the classification beyond the SGA and Lawton combination. These results indicate that the combination of graph measures and functional dependence again provides for good classification across the three groups (AUC = 0.71–0.85), except between the MCI subtypes (AUC = 0.47).

DISCUSSION

The aim of the present study was to assess graph-theoretical differences in the execution of a verbal fluency task among elderly with normal and pathological aging. Our results demonstrate that SGA differed significantly among the AD, MCI, and NC groups and it could be used to classify the groups. The present results

Table 5 | Rating quality measured by AUC, using SGA (attributes: WC, N, E, Density, Diameter, and ASP) correlated with clinical symptoms measured with MMSE and Lawton scales, in addition with Lawton, MMSE or both, classifying AD and MCI from NC, AD from MCI, and also classifying subtypes of MCI (aMCI or a+mdMCI) from NC or AD, or from each other.

		SGA	SGA+MMSE	SGA+ Lawton	SGA+MMSE+Lawton	MMSE	Lawton
NC x	MCI	0.681	0.716	0.780	0.793	0.638	0.649
	aMCI	0.619	0.618	0.710	0.714	0.586	0.612
	a+mdMCI	0.710	0.738	0.803	0.822	0.694	0.746
	AD	0.875	0.886	1.000	1.000	0.888	1.000
aMCI x	a+mdMCI	0.486	0.470	0.472	0.483	0.631	0.494
	AD	0.767	0.824	0.856	0.856	0.854	0.957
a+mdMCI x	AD	0.652	0.717	0.814	0.811	0.772	0.959
MCI x	AD	0.727	0.793	0.849	0.858	0.813	0.958

Table 6 | Additional description of socio-demographic data for the MCI subtypes, and the four groups comparison.

	aMCI			a+mdMCI			*p*
	Median	IQR		Median	IQR		
		Q1	Q3		Q1	Q3	
Age	75	71	79	79	73	81	0.7561
Education	4	2	5	3	2	4	0.4662
Katz	0	–	–	0	–	–	0.0279
Lawton	0	0	1	1	0	2	0.0000
MMSE	26	23	28	24	23	26	0.0000

p group comparison (NC; aMCI; a+mdMCI; AD). Red values have significance p = 0.0083.*

Table 7 | Additional description of verbal fluency and Speech Graph Attributes for the MCI subtypes, and the four groups comparison.

	aMCI			a+mdMCI			*p*
	Median	IQR		Median	IQR		
		Q1	Q3		Q1	Q3	
VF.E	0	0	0	0	0	0	1.0000
VF.PR	0.083	0	0.125	0	0	0.1	0.1300
VF.R	1	0	2	0	0	1	0.2084
VF.C	11	10	14	11	9	13	0.0000
VF.TT	13	11	16	11	10	14	0.0000
WC	13	11	16	11	10	14	0.0000
N	11	11	14	11	9	13	0.0000
E	12	10	15	10	9	13	0.0000
RE	0	0	0	0	–	–	0.5682
PE	0	0	0	0	0	0	0.7670
L1	0	–	–	0	0	0	0.3916
L2	0	0	0	0	0	0	0.8658
L3	0	0	0	0	–	–	0.0567
LSC	4	1	7	1	1	5	0.5115
ATD	2	1.810	2.095	1.857	1.8	2	0.1998
Diameter	9	8	12	9	6	11	0.0003
ASP	3.666	3.309	4.666	3.666	2.666	4.333	0.0002
CC	0	0	0	0	0	0	0.3936
Density	0.082	0.071	0.090	0.9	0.075	0.109	0.0000

Red values have significance p = 0.0083.

show the potential of graph analysis of verbal fluency task to discriminate between these groups in clinical practice.

The correlation between the SGA and the MMSE or the Lawton Index indicate that the SGA are associated with the general cognitive status and functional performance, two important clinical measures used in geriatric assessment. Patients with worse scores in the MMSE produced fewer numbers of nodes and a less dense network. As the functional performance decreases, indicating more severe cognitive impairment stages, the networks became denser, with a smaller diameter and average short path and with fewer numbers of nodes and edges. Their networks became smaller in the number of words, with a small path through the first word to the last one, and their animals have more connection with different neighbors than would be necessary. Subjects more cognitively impaired tended to perform more dependently on their daily activities. Importantly, some attributes of SGA could indicate the progression of cognitive impairment and functional decline, as shown by denser and smaller networks, with a fewer number of nodes, in subjects with more severe cognitive impairment.

Application of speech graph analysis for sorting the groups showed moderate to good classification quality. When selected SGA were combined to the Lawton Index, better classification were obtained, suggesting that the combination of these two simple tools of network measure and functionality can provide to the clinician a good indication of differential diagnosis, except for the contrast between the two MCI subtypes, which spanned a continuum and did not allow the differentiation and classification of the two groups.

The differences prevalent across all groups were in the global attributes of diameter, density and average shortest path (ASP). The results indicate that the networks built by the normal control elderly were more direct, without reoccurrence of words, resulting in a less dense network. Conversely, cognitive impairment corresponded to denser and less direct networks. The density

Table 8 | Pairwise group comparison in the variables with significant difference across the four groups.

	NC × aMCI			NC × a+mdMCI			NC × AD		
	W	***z***	***p***	***W***	***z***	***p***	***W***	***z***	***p***
Katz	625	−0.960	0.3371	625	−0.960	0.3371	598.5	−1.388	0.1651
Lawton	548.0	2.581	0.0099	484	3.758	0.0002	325	−6.254	0.0000
MMSE	583.5	−1.046	0.2956	504	−2.597	0.0094	394	4.733	0.0000
VF.C	525	−2.186	0.0288	476	−3.140	0.0017	396.5	4.777	0.0000
VF.TT	563.5	−1.440	0.1498	501	−2.661	0.0078	410.5	4.514	0.0000
WC	563.5	−1.440	0.1498	501	−2.661	0.0078	410.5	4.514	0.0000
N	526	−2.170	0.0300	475	−3.159	0.0016	394	4.824	0.0000
E	563.5	−1.440	0.1498	501	−2.661	0.0078	410.5	4.514	0.0000
L3	625.0	−0.566	0.5714	612.5	−1.400	0.1614	600	−1.138	0.2552
Diameter	546	−1.778	0.0753	515.5	−2.367	0.0179	425.5	4.239	0.0000
ASP	534.5	−1.994	0.0462	507.5	−2.518	0.0118	414.5	4.433	0.0000
Density	510.5	2.460	0.0139	467.5	3.296	0.0010	388.5	−4.924	0.0000
	aMCI × a+mdMCI			**aMCI × AD**			**a+mdMCI × AD**		
Katz	637.5	NaN	NaN	587.5	−2.001	0.0454	587.5	−2.001	0.0454
Lawton	577.5	−1.294	0.1958	352	−5.423	0.0000	350.5	−5.335	0.0000
MMSE	545	1.797	0.0723	416	4.301	0.0000	467.5	3.284	0.0010
VF.C	582	1.074	0.2828	471.5	3.334	0.0009	512	2.561	0.0105
VF.TT	573.5	1.238	0.2157	466.5	3.418	0.0006	515	2.486	0.0129
WC	573.5	1.238	0.2157	466.5	3.418	0.0006	515	2.486	0.0129
N	574.5	1.223	0.2213	460.5	3.551	0.0004	505	2.692	0.0071
E	573.5	1.238	0.2157	466.5	3.418	0.0006	515	2.486	0.0129
L3	625	0.960	0.3371	587.5	−1.654	0.0981	575	−2.268	0.0233
Diameter	600.5	0.712	0.4764	495.5	2.901	0.0037	549.5	1.884	0.0595
ASP	602.5	0.671	0.5023	502.5	2.755	0.0059	542.5	2.010	0.0444
Density	597	−0.778	0.4367	467	−3.415	0.0006	504.5	−2.700	0.0069

Red values have significance p = 0.0083.

differences across the groups were, among all comparisons, the most uniform result, except for the comparison between the two MCI subgroups, which yielded a pattern of continuous performance. The progressive worsening of cognitive performance within the MCI subtypes is consistent in the literature, indicating that a group of subtle deficits underlie the differential diagnosis (Diniz et al., 2007; Radanovic et al., 2009).

Even the groups that did not differ in total number of word repetitions differ in the occurrence of loops of 3 nodes (L3). Nearly all subjects, as expected, managed to avoid recurrences, but 20% of the AD subjects repeated the same word with only two words of interval (e.g., dog-cat-horse-dog). According to Huntley and Howard (2010), subjects with AD already have working memory deficits at the earliest stages of the disease. The impairment in central executive and episodic buffer functions of working memory probably stems from the difficulty of keeping information in mind while keeping the search for new information. These deficits probably explain the repetition of words in verbal fluency tasks with a very small interval.

The results outline a field that needs to be further explored in future studies, involving the density of the networks and the strength between the words in the semantic memory of elderly with pathological aging. The Parallel Distributed Processing Approach of Semantic Cognition predicts that the decrease in strength of the links between words in a semantic network may allow connections between pairs of words that would not be preferential under normal circumstances (McClelland and Rogers, 2003). Another aspect that deserves further investigation is the absence of difference across the groups in the connectivity attributes (LSC, ATD, and CC). This raises the hypothesis that even very different networks can share a similar structure of local connections, in which a small portion of the words are highly connected with other less connected words, maintaining the integrity of the network's general connection (Bales and Johnson, 2006; De Deyne and Storms, 2008).

Considering the graph analysis performed in this study, build-up in a co-occurrence of the words and based on the temporal link between them, future studies should consider multidimensional scaling and hierarchical clustering analysis. These types of analyses will represent the relation between the variables and combine it into groups, enhancing the results. Future studies should also address the differences between MCI patients and other neurological conditions in which cognitive impairments are quite similar, for example, Temporal Lobe Epilepsy (Holler and Trinka, 2014), as well as the potential association between graph analysis, neuroimaging and other diagnosis instruments. Furthermore, longitudinal studies are also necessary to evaluate whether SGA can help to identify MCI subjects with higher risk of progressing

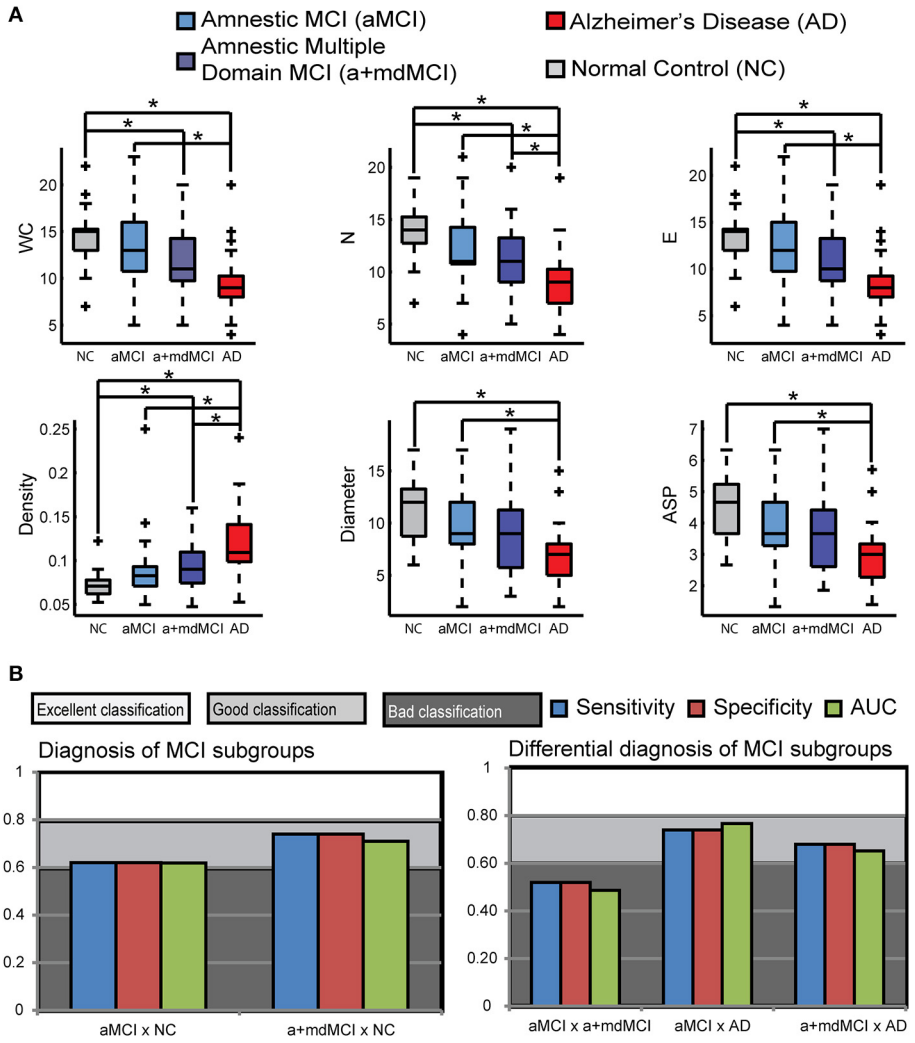

FIGURE 3 | Speech Graph Attributes (SGA) differentiates psychopathological MCI subgroups. (A) SGA boxplots with significant differences among Alzheimer Disorder (AD), Amnesic Moderate Cognitive Impairment (aMCI), Multiple Domain Moderate Cognitive Impairment (a+mdMCI), and control groups indicated ($N = 25$ per group; Kruskal-Wallis test followed by two-sided Wilcoxon Rank-sum test with Bonferroni correction with alpha = 0.0083). **(B)** Rating quality measured by AUC, sensitivity and specificity, using SGA correlated with clinical symptoms measured with MMSE and Lawton scales (**Table 4**) (attributes: WC, N, E, Density, Diameter, and ASP). Notice that it is possible to sort the MCI subgroups from the NC or AD groups, but not one from another. Classification quality was considered excellent when AUC was higher than 0.8, good when AUC ranged from 0.6 to 0.8, and poor (not above the chance), when AUC was smaller than 0.6. $^*p = 0.0083$.

to Alzheimer's disease. In conclusion, the results suggest that SGA may be a useful tool to help in the differential diagnosis between MCI and AD.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Journal/10.3389/fnagi.2014.00185/abstract

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Impaired generation of new subcategories and switching in a semantic verbal fluency test in older adults with mild cognitive impairment

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The semantic verbal fluency task is broadly used in the neuropsychological assessment of elderly subjects. Even some studies have identified differences in verbal fluency clustering and switching measures between subjects with normal aging and a clinical condition such as mild cognitive impairment (MCI) and Alzheimer's disease, the results are not always consistent. This study aimed to compare clustering and switching measures of an animal's semantic verbal fluency task among normal controls (NC, $n = 25$), amnestic mild cognitive impairment (aMCI; $n = 25$), amnestic multiple domain Mild Cognitive Impairment (a+mdMCI; $n = 25$) and Alzheimer's disease (AD; $n = 25$) Brazilian subjects. The analyses were executed considering three (unifying the MCI subtypes) and four groups. As the data were not normally distributed, we carried out non-parametric tests (Kruskal-Wallis and Mann-Whitney tests) to evaluate the differences in performance in the measures of the verbal fluency test among the groups. The comparison demonstrated that the groups differed in the total of correct words produced, number of clusters and switching but the measure of new subcategories was the only with significant difference among the NC and all the clinical groups. The measure of new subcategories is the number of original subcategories inside the higher category of animals that the subject produced, such as farm, domestic, African animals. Our results indicate that semantic memory impairment is a visible and recent deficit that occurs even in non-demented subjects with very MCI and the implications of these findings are discussed.

Keywords: semantic verbal fluency, clustering, switching, subcategories, mild cognitive impairment, Alzheimer's disease

INTRODUCTION

Mild cognitive impairment (MCI) is common among older adults with prevalence estimates ranging from 3 to 42% (Yesavage et al., 2002; Ward et al., 2012), and subjects with MCI have increased risk of developing dementia (Han et al., 2012). The gold standard for the identification of MCI usually relies on comprehensive neuropsychological assessment. Nonetheless, neuropsychological assessments are expensive, time-consuming, need highly trained professionals to do and, thus, may not be readily available in clinical practice. Cognitive screening tests are widely available, have low costs and need no specialized training for its correct administration and interpretation of results. Though being routinely used in clinical practice to evaluate subjects with cognitive complaints, they are not sensitive to identify mild cognitive deficits (Diniz et al., 2007). As a consequence many older adults may not be correctly identified as MCI delaying the diagnosis until they reach the threshold for dementia.

Semantic verbal fluency (SVF) tests are one of the most common cognitive screening used in clinical and research settings. This task requires the initialization of a specific verbal behavior, the search for specific verbal information, and self-monitoring to avoid mistakes and repetitions. Additionally, it requires the availability of a specific semantic knowledge (Unsworth et al., 2011). The traditional scoring system is the sum of correct words produced within one minute (Strauss et al., 2006). The scores on this task has a good sensitivity and specificity to discriminate older adults with no cognitive impairment from those with dementia (Radanovic et al., 2009). Despite not useful to differentiate MCI from AD patients (Radanovic et al., 2009), lower scores on SVF is a predictor of progression from MCI to AD (O'Dowd et al., 2004; Saxton et al., 2004; Amieva et al., 2005; Cottingham and Hawkins, 2009).

In addition to the traditional score, other information provided by the SVF can add valuable information about cognitive

status of an individual. Clustering is the grouping of words belonging to the same subcategory and is a measure of semantic memory knowledge. Switching is the change of subcategory across all possibilities and measure the efficiency to retrieve the stored information (i.e., measure of executive functioning). Previous studies found that AD patients produced smaller clusters and switched less frequently from subcategories than normal controls (Troyer et al., 1998; Murphy et al., 2006; Fagundo et al., 2008). Recently, Price et al. (2012) showed that subjects with that amnestic MCI produced, smaller cluster sizes and fewer new subcategories when compared to controls, but the frequency of switches was not significantly different. Murphy et al. (2006) reported similar results, except that amnestic MCI subjects did not differ in the mean cluster size from the healthy subjects.

As SVF is commonly used in clinical practice and the analysis of other aspects of this task can add important information on the cognitive status of a patient, we aim to evaluate whether measures of clustering, switching, and number of subcategories can help to distinguish MCI and AD patients from healthy older adults with no cognitive decline. In addition, we aim to compare these measures between subgroups of MCI subjects. We hypothesize that patients with MCI and AD will show significant differences in these measures in comparison to healthy controls; and that there is gradual decline in these measures as the subjects progress from amnestic single domain MCI to amnestic multiple domain MCI, and finally to AD.

MATERIALS AND METHODS

PARTICIPANTS AND ASSESSMENT

One hundred older adults were included in this study. These participants were referred to a secondary outpatient geriatric unit for a comprehensive geriatric clinical assessment. All participants underwent a comprehensive clinical interview done by a geriatrician, and evaluated with a standardized neuropsychological protocol (de Paula et al., 2013). In brief, this protocol included the tests for the principal cognitive domains. General cognitive status (Mini Mental State Exam), executive functions (Frontal Assessment Battery, Letter Fluency of S and Digit Span), visioconstructional ability (Stick Design Test and Clock Drawing Test), episodic memory (Rey Auditory Verbal Learning Test), semantic memory [Naming Test (TN-LINC) and Category Verbal Fluency (Fruits)], and language (Token Test). The caregivers answered the General Activities of Daily Living Scale (GADL) that evaluates performance on activities of daily living (de Paula et al., 2014).

The neurocognitive status of each participant was adjudicated taking into account all information from the clinical and neuropsychological assessments. The participants were classified into three groups: normal controls ($n = 25$), MCI ($n = 50$), and mild AD ($n = 25$). MCI was diagnosed according to the Mayo Clinic Criteria (Petersen et al., 2001); AD was diagnosed according to the NINCDS-ADRDA criteria (McKhann et al., 1984). The normal control (NC) group included older adults without cognitive complains and scores above −1.5 SD of the mean according to local norms, adjusted for age and educational level.

The MCI participants was further subdivided into two groups: amnestic single domain—aMCI ($n = 25$) and amnestic multiple domain—a+mdMCI ($n = 25$) (Petersen, 2004). Amnestic MCI is defined by significant memory impairment and normal performance on other cognitive domains, no evidence of impairment in activities of daily living and preserved global cognition (Petersen et al., 2001). Amnestic multiple domain MCI is defined by significant memory impairment and of one or more additional cognitive domains (e.g., executive function, language), no evidence of impairment in activities of daily living and preserved global cognition. The local Ethics Committee approved this study and all participants and their families gave written consent.

VERBAL FLUENCY TEST

All participants did the category verbal fluency test (animals). They were asked to say names of animals within 1 minute, and were advised to not repeat already spoken animals. All the words were recorded, including repetitions and errors (when other words that not animals were spoken).

Standard scores

The scoring procedure included the number of correct words, excluding number of errors (occurrence of words that do not refer to any animal) and number of repetitions (animals that were spoken more than once).

Clustering and switching scores

The scores for clustering and switching were obtained according to Troyer et al. (1997). Clusters were formulated according to the shared attributes between animals (e.g., farm animals, pet animals, zoo), or when an animal appeared alone. For example, the following animals compose one cluster: cow, pig, horse (farm animals). The cluster sizes were computed after a second word of the same subcategory if generated in sequence (cluster size = total of animals in a given cluster −1). For example, cluster with two words receive a size score of 1, with 3 words a size score of 2. If the word appeared alone, this cluster receives a size score of 0. The mean cluster size is the sum of all clusters sizes generated (including single words, repetitions, and errors) divided by the number of clusters. Switching is the number of changes in cluster generation during the task (Troyer et al., 1997). For example, a subject can say: cow, pig, horse (farm animals), whale, and fish (see animals). This subject produced one switching since he changed between the clusters of farm animals to a cluster of sea animals. We also included the measures of new subcategories (number of original subcategories produced, excluding reoccurrence of animals belonging to the same subcategory, but produced in a non-sequence way), as suggested by March and Pattison (2006). In the given example the subject produced two original subcategories. Other subject could saw: horse, cow, dog, cat, and pig. These second example shows that the subject first produced a cluster of farm animals (horse and cow), second a cluster of domestic animals (dog, cat), and third another cluster of farm animals (pig). In total these subject produces only two new subcategories of animals once he produced twice clusters of farm animals.

Additionally we proposed the scoring of the number of effective clusters that were developed, in which the subject produced more than one exemplar for that subcategory (horse and cow, instead of only saying horse), and the mean size of these developed clusters.

STATISTICAL ANALYSIS

We did Kolmogorov-Smirnov tests to evaluate the pattern distribution of the data. As the data were not normally distributed (data not shown), we carried out non-parametric tests (Kruskal-Wallis and Mann-Whitney tests) to compare for differences in the performance in the neuropsychological tests and measures of the verbal fluency test between groups. We also calculated the corresponding effect size (r) for each comparison. Chi-square tests were performed to analyze differences in the distribution of dichotomous variables between the groups. Statistical significance was set according to the Holm-Bonferroni correction for multiple comparisons. All analyses were performed with the Statistical Package for Social Science (SPSS), v.21 for Windows (IBM Corp. Released, 2012).

We carried out the same set of analysis dividing the MCI group according MCI subtype (groups: normal control, aMCI, a+mdMCI, AD).

RESULTS

NC, MCI, AD

The AD, MCI, and control participants did not differ in most demographic data, except that the normal control group showed a significant higher frequency of women according to the Chi-square test ($X^2 = 6.76$, $df = 2$, $p = 0.009$). Participants with AD had worse performance in all neuropsychological measures and activities of daily living (**Table 1**). Participants with MCI had intermediate performance between AD and controls (**Table 1**).

Table 2 shows the data from SVF variables. The groups significantly differed in total of words, number of clusters, new subcategories, and switching (**Table 2**).

Pairwise comparison using the Mann-Whitney non-parametric test showed that all the three groups differed from each other on the number of correct words produced (**Table 2**). The NC group produced more new subcategories than MCI and AD, but there were no difference in these measure between the clinical groups ($p = 0.072$). The NC group performed significantly better than AD also for number of clusters and switching (**Table 2**).

NC, aMCI, a+mdMCI, AD

After we divided the MCI group in aMCI and a+MCI we found a significant difference between the groups in in daily living activities and general cognitive status (**Table 3**), and in the SVF measures of correct words, number of clusters, new subcategories, and switching (**Table 4**).

Pairwise comparison showed that the NC group significantly differed from all clinical groups in the number of new subcategories (**Table 4**). NC group performed significantly better than a+mdMCI and AD subjects also in the SVF variables of correct words, number of clusters and switching. There were no significant differences between aMCI, a+mdMCI and AD for all SVF variables, except a difference between aMCI and AD in the number of correct words produced (**Table 4**).

The number of new subcategories is the only significant difference found between the NC and all the clinical groups, including the aMCI subtype (**Figure 1**). There were no differences between the four groups in the mean cluster size.

We found a marginal significant difference between the NC and AD groups in the number of developed clusters ($p = 0.05$) and the mean size of the developed clusters ($p = 0.05$).

Table 1 | Demographic description of NC, MCI, and AD groups.

	NC		MCI		AD		K–W	
	Median	**Q1–Q3**	**Median**	**Q1–Q3**	**Median**	**Q1–Q3**	**H(2)**	***p***
Age	76	(70–81.5)	76	(70.5–81)	78	(67–81.5)	0.04	0.979
Education	4	(3–4)	4	(2–4)	4	(2.5–4)	0.34	0.840
GADL	26	(26–26)	26	(25–26)	20	(17.5–22)	62.74	**0.000**†
MMSE	27	(23.5–29)	25	(23–27)	20	(17–23.5)	29.18	**0.000**††

GADL, General Activities of Daily Living Scale; MMSE, Mini Mental State Exam. †*NC < MCI < AD;* ††*NC > MCI > AD.*

Table 2 | Verbal fluency measures description of NC, MCI, and AD groups.

	NC		MCI		AD		K–W		Pairwise comparison†
	Median	**Q1–Q3**	**Median**	**Q1–Q3**	**Median**	**Q1–Q3**	**H(2)**	***P***	
Corrects	14	(12–15.5)	11	(9.75–14)	9	(7–10.5)	26.28	**0.000**	NC > MCI > AD
Number of clusters	8	(6.5–9)	6	(4–8.25)	5	(4–6.5)	11.70	**0.003**	NC > AD
Mean clusters size	0.78	(0.50–1.14)	0.82	(0.52–1.47)	0.75	(0.5–1)	0.64	0.724	
Developed clusters	3	(2–4.5)	3	(2–3)	3	(2–3)	4.45	0.108	
Mean developed clusters size	1.80	(1.55–2.41)	1.71	(1.31–2.81)	1.33	(1–2.16)	3.81	0.148	
New subcategories	6	(5–7)	4	(3–6)	3	(2–4.5)	20.01	**0.000**	NC > MCI = AD
Switching	7	(5.5–8)	5	(3–8)	4	(3–5.5)	11.18	**0.004**	NC > AD

†*Mann-Whitney of verbal fluency measures between NC, MCI, and AD groups (significant p-value < 0.016 after Holm-Bonferroni correction for multiple analysis). Bold values of p means that the p-value was statistically significant.*

Table 3 | Demographic description of NC, aMCI, a-mdMCI, and AD groups.

	NC		aMCI		a+mdMCI		AD		K–W	
	Median	Q1–Q3	Median	Q1–Q3	Median	Q1–Q3	Median	Q1–Q3	H(3)	*p*
Age	76	(70–81.5)	75	(70–79.5)	79	(71–81)	78	(67–81.5)	1.18	0.756
Education	4	(3–4)	4	(1.5–5)	3	(2–4)	4	(2.5–4)	2.55	0.466
GADL	26	(26–26)	26	(25–26)	25	(24–26)	20	(17.5–22)	63.67	**0.000**†
MMSE	27	(23.5–29)	26	(23–28)	24	(22.5–26)	20	(17–23.5)	31.57	**0.000**††

GADL, General Activities of Daily Living Scale; MMSE, Mini Mental State Examination. †*NC < aMCI; a+mdMCI < AD;* ††*NC > a+mdMCI > AD; aMCI > AD.*

Table 4 | Verbal fluency measures description of NC, aMCI, a-mdMCI, and AD groups.

	NC		aMCI		a+mdMCI		AD		K-W		Pairwise comparison†
	Median	Q1–Q3	Median	Q1–Q3	Median	Q1–Q3	Median	Q1–Q3	H(3)	*P*	
Corrects	14	(12–15.5)	11	(10–14.5)	11	(9–13.5)	9	(7–10.5)	27.24	**0.000**	NC > a+mdMCI, AD; aMCI > AD
Number of clusters	8	(6.5–9)	8	(4.5–9)	6	(4–7)	5	(4–6.5)	14.30	**0.003**	NC > a+mdMCI, AD
Mean clusters size	0.78	(0.50–1.14)	0.67	(0.41–1.32)	1	(0.65–1.63)	0.75	(0.5–1)	2.36	0.501	
Developed clusters	3	(2–4.5)	3	(2–3.5)	3	(2–3)	3	(2–3)	4.62	0.202	
Mean developed clusters size	1.80	(1.55–2.41)	2	(1–3.41)	1.67	(1.36–2.41)	1.33	(1–2.16)	3.81	0.282	
New subcategories	6	(5–7)	4	(3–6)	4	(3–5)	3	(2–4.5)	20.82	**0.000**	NC > aMCI; a+mdMCI; AD
Switching	7	(5.5–8)	7	(3.5–8)	5	(3–6)	4	(3–5.5)	13.07	**0.004**	NC > a+mdMCI, AD

†*Mann-Whitney of verbal fluency measures between NC, MCI, and AD groups (Holm-Bonferroni corrected significant differences across groups was established in p = 0.008). Bold values of p means that the p-value was statistically significant.*

DISCUSSION

The present study found that the number of animal subcategories produced in the SVF showed a significant decline in the amnestic MCI group compared to healthy controls. However, we did not observe significant differences in these measures between amnestic MCI, amnestic multiple-domain MCI and AD subjects. This may suggest that impairments in semantic memory are present in the early stages of the continuum of healthy cognitive aging, MCI, and dementia. In addition, we found that the number of clusters and switching were significantly reduced only in the amnestic multiple-domain and AD groups. These findings suggest that the latter subjects present with progressive difficulty to access the bulk of knowledge stored in semantic memory, possibly reflecting the presence of executive dysfunction in these subjects. Overall, these findings highlight the relevance of the assessment of other variables that can be extracted from an SVF task to understand the pattern of cognitive changes in the health cognitive aging, MCI and AD continuum.

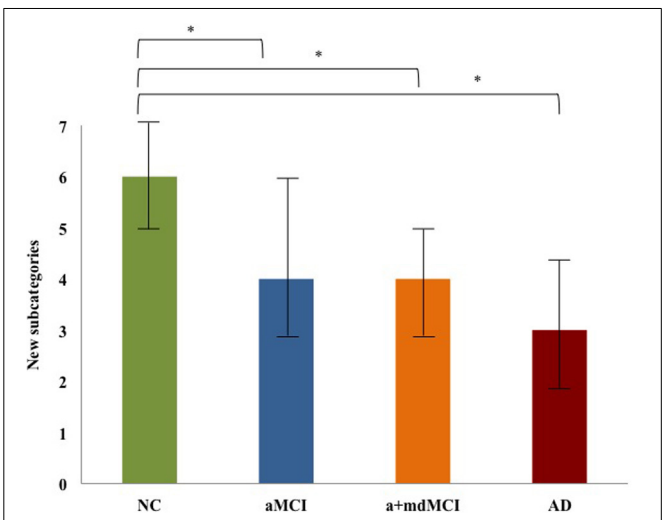

FIGURE 1 | New subcategories comparison among NC, aMCI, a-mdMCI, and AD groups. *Indicates the significant ($p < 0.008$) difference between the groups.

The production of new subcategories was described as an alternative measure of semantic memory (March and Pattison, 2006). The generation of subcategories of animals depends on the knowledge of how a given animal relates to others or the shared attributes between them (e.g., pet animals or animals that are grown in farms) (Hoffman and Lambon Ralph, 2013). As animals in the same subcategory are closely related in the semantic memory system, once a person retrieves one animal it is easier to retrieve animals from that same subcategory following a structured semantic network (McClelland and Rogers, 2003). In our study we found that despite there were no significant differences in total of words produced in the SVF between controls and amnestic MCI participants, the latter showed a significant lower

generation of animal subcategories. This finding suggests that the semantic memory system may be already disrupted in the earliest stages of the transition between normal cognitive aging and dementing disorders. Our findings are in line with the literature and highlight the importance of specifically evaluating semantic memory changes in addition to episodic memory in these subjects (Chan et al., 2001; Adlam et al., 2006; Joubert et al., 2008; Cuetos et al., 2009; Price et al., 2012).

It is worth noting that the switching measure were not significantly different between healthy controls and amnestic MCI participants, though were significantly lower in the amnestic multiple-domain MCI and AD. Switching is a measure of mental flexibility and indicates the ability to search and access novel information in the semantic memory system (Troyer et al., 1998; Nutter-Upham et al., 2008). These findings are in line with the theoretical decline in cognitive performance observed in the transition between amnestic MCI, multiple-domain MCI, and AD (Forlenza et al., 2009). Furthermore, our findings may suggest that the progressive decline in executive functions in subjects with MCI may indicate a higher risk of progression to AD (Rozzini et al., 2007).

The present results should be viewed in light of some limitations. We included a relatively small sample size what may have influenced the current analysis. Our sample has a low educational status. It is widely accepted that education is one of the main factors that influence the performance on a broad range of cognitive tests, including SVF (Radanovic et al., 2009). Also, education influences on how we store and retrieve information in the semantic memory (Reis and Castro-Caldas, 1997; Mathuranath et al., 2003). Therefore, we cannot exclude the possibility that some of the results are biased due to the educational status of our sample. Furthermore, our control group has a significant difference of gender distribution, which may be a limitation for the study. Nonetheless, previous studies did not find a significant effect of gender in clustering and switching (Troyer and Moscovitch, 2006; Weiss et al., 2006). Therefore, additional studies, including greater sample sizes, including subjects with more years of education and with a prospective design, are necessary to evaluate whether SVF variables (i.e., generation of new subcategories, clustering, and switching) can help to differentiate between healthy controls, MCI and AD subjects, as well as to identify those subjects of progressing to dementia upon follow-up. Our results also present overlapping values of SVF measures across the groups. Future research should address whether these SVF attributes can complement the neuropsychological assessment to differentiate older adults with distinct levels of cognitive impairment.

Our study highlights the importance of evaluating other variables from the SVF tests, like the generation of new subcategories, cluster and switching, in subjects with MCI and AD. The impairment of production of new subcategories indicates the presence of semantic memory impairment in amnestic MCI subjects. Difficulties in SVF switching measures harbinger the presence of executive dysfunction and the diagnosis of multiple-domain MCI and AD. Finally, these measures may help to identify those subjects at a higher for dementia.

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