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ORIGINAL ARTICLE

Volume Loss of the Nucleus Basalis of Meynert is Associated with Atrophy of Innervated Regions in Mild Cognitive Impairment

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Abstract

Extensive research suggests that basal forebrain (BF) cholinergic neurons are selectively vulnerable to Alzheimer's disease (AD). However, it remains unknown whether volume loss of BF cholinergic compartments parallels structural changes of their innervated regions in prodromal AD. To this aim, we have correlated volume of each BF compartment with cortical thickness and hippocampus/amygdala volume in 106 healthy older (HO) adults and 106 amnestic mild cognitive impairment (aMCI) patients. Correlations were limited to regions affected by atrophy in aMCI. The volume of the nucleus basalis of Meynert (NBM/Ch4) was positively correlated with thickness of the temporal cortex in aMCI, and with volume of amygdala in HO and aMCI, separately. Volume of the medial septum/diagonal band of Broca (Ch1–Ch3) was also positively correlated with volume of the hippocampus within the 2 groups. Only correlations between the NBM and their innervated regions showed diagnostic value. Unlike men, aMCI women showed a stronger association between volume of the NBM and thickness of the temporal lobe when compared with HO women. Altogether, these results reveal, for the first time in humans, that atrophy of NBM is associated with structural changes of their innervated regions in prodromal AD, being this relationship more evident in women.

Key words: amygdala, basal forebrain, cholinergic system, gender, hippocampus, mild cognitive impairment, neocortex, nucleus basalis of Meynert

Introduction

The basal forebrain (BF) complex plays a pivotal role in the production of acetylcholine (ACh), a neurotransmitter involved in corticogenesis (Hohmann et al. 1988), regulation of neuronal excitability (Yang et al. 2010), sleep–wake cycle (Jones 1993), and distinct cognitive functions (Hasselmo and Bower 1993; Sarter et al. 2005; Gritton et al. 2016). The BF cholinergic system contains 4 overlapping cell groups (Ch1–Ch4) that provide ACh to the neocortex, hippocampus, and amygdala (Mesulam et al. 1983; Mesulam and Geula 1988). Specifically, the nucleus basalis of Meynert (NBM/Ch4) sends cholinergic projections to the entire cerebral cortex and the amygdala, whereas the hippocampus predominantly receives cholinergic innervation from the medial septum (Ch1) and the vertical limb of the diagonal band of Broca (Ch2).

Evidence suggests that BF cholinergic neurons are selectively vulnerable to degeneration in Alzheimer's disease (AD). Accordingly, *postmortem* AD studies have shown neuronal loss in the NBM (Whitehouse et al. 1981; McGeer et al. 1984; Arendt et al. 1985; Vogels et al. 1990) and reduced cortical choline

acetyltransferase activity associated with the degree of cognitive decline (Nagai et al. 1983). Downregulation of cholinergic markers has been further reported in asymptomatic elderly subjects, suggesting that cholinergic denervation occurs relatively early in the course of the disease (Palmer and Gershon 1990). *Postmortem* evidence has been confirmed by in vivo magnetic resonance imaging (MRI) findings, showing reduced volume of BF cholinergic nuclei in AD (Teipel et al. 2005; Kilimann et al. 2014) and mild cognitive impairment (MCI) (Grothe et al. 2010; Teipel et al. 2011; Kilimann et al. 2014). Interestingly, recent studies have further found that BF atrophy is correlated with high neocortical amyloid-beta burden in both AD-MCI (Kerbler et al. 2014) and cognitively intact elderly subjects (Grothe et al. 2014).

Postmortem evidence suggests that cerebral regions receiving cholinergic projections from BF nuclei (i.e., the neocortex, hippocampus, and amygdala) are severely affected in AD (Braak and Braak 1991; Haroutunian et al. 1998; Scheff et al. 2006). However, the association between structural changes of specific BF nuclei and their cholinergically innervated regions has been unexplored with in vivo MRI techniques in prodromal AD. Because the NBM/Ch4 is the main source of cholinergic input to neocortex and amygdala in humans (Mesulam and Geula 1988), we hypothesize that the relationship between volume loss of the NBM/Ch4 and atrophy of their innervated regions will allow us distinguishing aMCI from cognitively normal elderly subjects. Given that the cholinergic depletion is more accentuated within the temporal lobe (i.e., inferotemporal, midtemporal, and entorhinal cortex) and amygdala (Emre et al. 1993; Geula and Mesulam 1994), we would expect that correlations between volume loss of the NBM/Ch4 and cerebral atrophy in MCI patients were restricted to these prominently affected AD brain regions.

Materials and Methods

Patients

Two hundred twelve participants, 106 healthy older (HO) (43 females: 66.9 ± 7.3 years; 63 males: 68.1 ± 5.5 years) and 106 aMCI (51 females: 69.9 ± 7.1 years; 55 males: 70.4 ± 6.9 years) were included in the study. Participants were primarily recruited from senior citizen's associations, memory screening programs, and hospital dementia services. Patients gave informed consent before their participation in the study, which was approved by the Ethical Committee for Human Research at the Pablo de Olavide University.

All participants received neurological and MRI examinations. Only those who met established criteria (see below) were included in the study. Cerebral MRI was previously examined to rule out lesions such as territorial cerebral infarction, brain tumor, hippocampal sclerosis, and/or vascular malformations. Those patients with large periventricular and/or deep white matter (WM) lesions, confirmed by scores ≥2 on the Fazekas ischemic scale (Fazekas et al. 1987), were excluded from the study. Cardiovascular risk factors (i.e., hypertension, diabetes, overweight, family history of coronary heart disease, or stroke) were also exclusion criteria.

Patients were diagnosed with aMCI if they met Petersen's criteria (Petersen et al. 1999) and showed an idiopathic amnestic disorder with the absence of impairment in cognitive areas other than memory. The absence of secondary causes of cognitive deficits was confirmed by laboratory tests, including complete blood count, vitamin B12/folate, and thyroid function. Elderly depression was excluded (scores ≤5) with the shorter version of the Geriatric Depression Scale (Yesavage et al. 1983). None of the participants were taking cholinesterase inhibitors and/or psychiatric medication at the time of recruiting or during the study. Inclusion criteria for HO subjects were normal cognitive performance relative to appropriate reference values for age and education, CDR global score of 0 (no dementia), and normal independent function.

MRI Acquisition and Cortical Thickness Estimation

Structural cerebral images were acquired on a Philips Achieva 3T MRI scanner equipped with an 8-channel head coil. High-resolution magnetization-prepared rapid gradient echo T1-weighted anatomical brain images were obtained for each participant. Acquisition parameters were empirically optimized for grey/white contrast (0.8 mm^3 isotropic voxel resolution, no gap between slices, TR = 11 ms, TE = 4.5 ms, flip angle = 8°, acquisition time = 9.1 min).

MRI data were preprocessed using the analysis pipeline of Freesurfer v5.3 (http://surfer.nmr.mgh.harvard.edu/) involving intensity normalization, registration to Talairach, skull stripping, segmentation of WM, tesselation of the WM boundary, and automatic correction of topological defects (Fischl and Dale 2000). Pial/WM boundaries were manually corrected on a slice-by-slice basis in each participant to enhance the reliability of cortical thickness measures. Special attention was paid to cortical regions at the border with cerebrospinal fluid (CSF) to avoid partial volume effects. Cortical thickness maps were finally smoothed using nonlinear spherical wavelet-based denoising schemes, which have demonstrated enhanced specificity and sensitivity at detecting local and global changes in cortical thickness (Bernal-Rusiel et al. 2008).

Volume Estimation of BF, Hippocampus, and Amygdala

MRI data were preprocessed using the voxel-based morphometry (VBM) approach integrated in SPM12 (Wellcome Trust Center for Neuroimaging; www.fil.ion.ucl.ac.uk/spm) and implemented in Matlab R2013a (MathWorks, Natick, MA, USA). Briefly, T1-weighted images were manually reoriented to the anterior commissure and further segmented into grey matter (GM), WM, CSF, and skull/scalp compartments following the unified segmentation of SPM12. Next, the diffeomorphic anatomical registration through an exponentiated lie algebra (DARTEL) algorithm was applied to segmented brain images to obtain an enhanced inter-subject registration with improved realignment of smaller inner structures (Ashburner 2007). GM images were spatially normalized into the Montreal Neurological Institute space with an isotropic voxel resolution of 1.5 mm^3, and normalized modulated GM images were further smoothed with a Gaussian kernel of 4 mm.

Volume of BF cholinergic nuclei (i.e., Ch1–Ch3 and Ch4) was obtained with cytoarchitectonic probabilistic maps of BF magnocellular compartments (Zaborszky et al. 2008). For the hippocampus and amygdala, we also used cytoarchitectonic probabilistic maps of each structure (Amunts et al. 2005). Maximum probabilistic maps of each brain region were used for statistical purposes (Eickhoff et al. 2005).

Statistical Analysis

Group differences in demographic data were assessed with unpaired *t*-tests (continuous variables) and the chi-square test (categorical variables) using SPSS v21 (SPSS Inc., Chicago, IL, USA).

By using a previously validated hierarchical statistical model (Bernal-Rusiel et al. 2010), we assessed vertex-wise differences in cortical thickness between HO and aMCI patients. An analysis of covariance (ANCOVA) was performed for each hemisphere, including group as the main factor, and age and gender as covariates. The significance threshold was set at $P < 0.05$ after correcting for multiple comparisons, with a cluster extent threshold of 90 vertices. We further assessed group differences (HO vs. aMCI) in the volume of the BF complex, hippocampus, and amygdala, separately, using the modulated VBM approach implemented in SPM12 (ANCOVA with age and gender as covariates, corrected for family-wise error (FWE), $P < 0.05$; cluster extend threshold of 15 mm^3).

Vertex-wise linear regression analyses were performed to investigate if volume changes in each BF cholinergic compartment (i.e., Ch1–Ch3 and Ch4) were correlated with variations in cortical thickness. These analyses were adjusted for age and gender, and corrected for multiple comparisons ($P < 0.05$; cluster extend threshold of 90 vertices). We further performed voxel-wise linear regression analyses to assess correlations between volume changes in each BF cholinergic compartment (i.e., Ch1–Ch3 and Ch4) and volume changes in hippocampus and amygdala. These analyses were also adjusted for age and gender, and corrected for FWE ($P < 0.05$; cluster extend threshold = 15 mm^3). It is important to note that regression analyses were restricted to those regions showing significant volume/thickness loss in aMCI patients when compared with HO subjects, and that correlations were further performed in each group separately. If at least 1 of the 2 groups showed significant correlations, we then assessed group differences between correlation coefficients applying the same adjustments and correction procedure mentioned above.

Although AD pathology is more likely to be clinically expressed as dementia in women than in men (Barnes et al. 2005), other studies have found the opposite (O'Dwyer et al. 2012). To assess gender differences in structural changes of BF cholinergic compartments and innervated regions in HO and aMCI, we applied a full factorial design with 2 independent factors (diagnostic and gender) and 1 covariate (age). Next, we evaluated whether the relationship between volume loss of BF cholinergic compartments and innervated regions was affected by gender after controlling for age.

Results

Demographic Characteristics

Patients with aMCI were significantly older than HO ($P < 0.005$), although both groups were statistically homogeneous in gender and ApoE4 genotype.

Group Differences in Volume of BF Cholinergic Compartments, Hippocampus, Amygdala, and Cortical Thickness

The aMCI group showed bilateral reductions of BF cholinergic compartments when compared with HO. Significant group differences in the volume of each BF cholinergic compartment are shown in Table 1 and illustrated in Figure 1 (upper left panel). By using probabilistic maps of BF magnocellular compartments (Zaborszky et al. 2008), we found the most pronounced atrophies in the NBM (i.e., Ch4) bilaterally (left: $P < 10^{-15}$; right: $P < 10^{-12}$), although significant reductions were also evident in the right Ch1–Ch3 ($P < 10^{-5}$) corresponding to the medial septum and the diagonal band of Broca.

Table 1 Extent of atrophy of BF magnocellular compartments, amygdala, and hippocampus in aMCI patients compared with HO patients

Brain region	CS (mm^3)	x	y	z	T	P
HO > aMCI						
BF						
Left Ch4	156	−20	−5	−14	9.4	10^{-15}
Right Ch4	99	−18	−5	−14	8.2	10^{-12}
Right Ch1–Ch3	58	2	6	−9	5.2	10^{-5}
Amygdala						
Left amygdala	1209	−23	−3	−17	10.3	10^{-17}
Right amygdala	1053	24	−3	−17	9.0	10^{-14}
Hippocampus						
Left hippocampus	1261	−24	−11	−20	8.8	10^{-13}
Right hippocampus	1384	29	−11	−18	8.3	10^{-11}

CS = cluster size; coordinates x, y, and z are in the MNI space and correspond to the peak-effect within the cluster; P = exact P value (FWE corrected, age and gender as covariates).

Table 1 and Figure 1 (upper middle-right panel) show significant volume reductions of the amygdala and hippocampus in aMCI patients when compared with HO subjects. Atrophies were bilateral in both the amygdala (left: $P < 10^{-17}$; right: $P < 10^{-14}$) and hippocampus (left: $P < 10^{-13}$; right: $P < 10^{-11}$).

Table 2 and Figure 1 (bottom panel) show significant differences in cortical thickness between HO and aMCI. As expected, aMCI exhibited the most significant patterns of cortical thinning over the temporal lobe bilaterally (left: $P < 10^{-6}$; right: $P < 10^{-5}$) affecting the entorhinal cortex (BA28), portions of the temporal pole, inferior and superior temporal cortex (BA38), parahippocampal gyrus (BA36), fusiform gyrus (BA20), and middle aspects of the temporal cortex (BA21). Significant patterns of cortical thinning were also observed in the orbitofrontal cortex (BA10) ($P < 10^{-4}$) and insula ($P < 0.001$) of the right hemisphere in aMCI patients.

Gender showed a significant effect on BF cholinergic compartments and volume/thickness of target structures ($P < 0.05$, FWE corrected; women showing lower volume and thinner cortex than men). However, the lack of gender × diagnosis interaction suggested that the effect of diagnosis was independent of gender.

Structural Relationships Between BF Magnocellular Compartments and their Innervated Regions

Table 3 shows significant group differences in the strength of correlations between volume loss of each BF magnocellular compartment and structural changes of regions receiving their cholinergic projections. The aMCI group showed a significant positive correlation between volume reduction of Ch4 and regional thinning of the temporal lobe (left: $P < 10^{-10}$; right: $P < 10^{-8}$). Further analyses demonstrated that this pattern of correlation was significantly stronger in aMCI than in HO (left: $P < 10^{-6}$; right: $P < 10^{-3}$) (Fig. 2). We also found positive correlations between volume changes in Ch4 and amygdala in HO (left: $P < 10^{-18}$; right: $P < 10^{-15}$) and aMCI (left: $P < 10^{-16}$; right: $P < 10^{-16}$). This relationship was also stronger in the aMCI than in the HO group (right: $P < 10^{-5}$) (Fig. 3). Variations of volume in Ch4 and hippocampus were correlated neither in HO nor in aMCI patients.

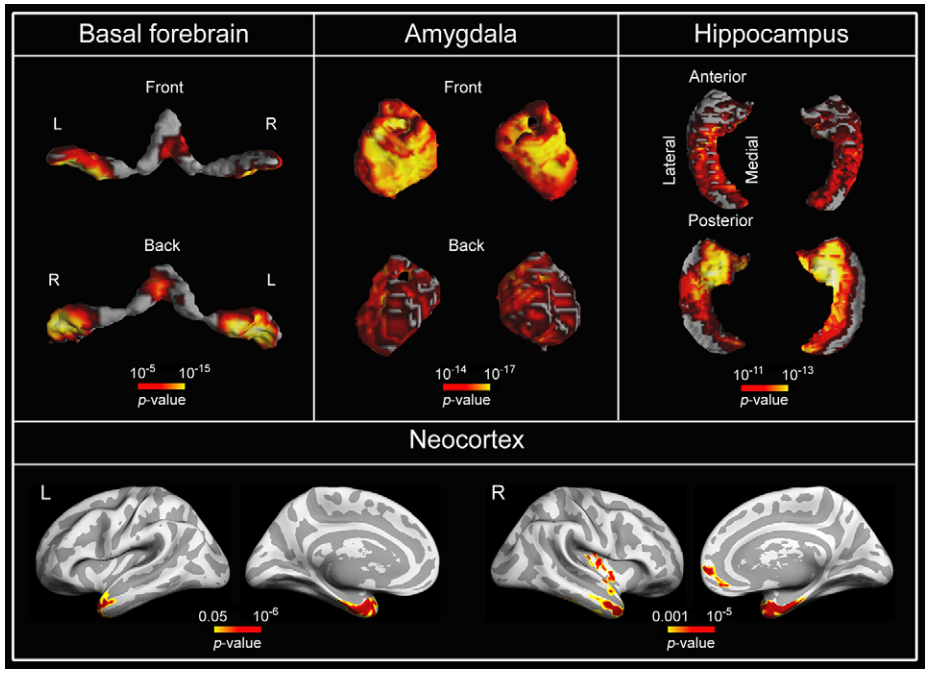

Figure 1. Group differences in volume of BF magnocellular compartments, amygdala, and hippocampus (upper panel), and patterns of cortical thickness (bottom panel). Volume results were previously corrected for FWE ($P < 0.05$), whereas thickness results were derived from applying a hierarchical statistical model that corrects for multiple comparisons (Bernal-Rusiel et al. 2010). L = left; R = right. Note that aMCI patients showed significant atrophies in all cerebral structures when compared with HO subjects.

Table 2 Extent of cortical thinning in aMCI patients compared with HO

Cortical region	CS (mm^2)	Mean ± SD thickness		% Atrophy	*P*
HO > aMCI					
Left temporal	2408	3.18 ± 0.26	2.75 ± 0.43	14	10^{-6}
Right temporal	2665	3.23 ± 0.25	2.82 ± 0.41	13	10^{-5}
Right orbitofrontal	314	2.30 ± 0.27	2.00 ± 0.26	14	10^{-4}
Right insula	273	3.06 ± 0.27	2.75 ± 0.32	11	0.001

SD = standard deviation; CS = cluster size; *P* = exact *P* value (corrected, age and gender as covariates).

Table 3 Group differences in the strength of correlation between volume loss of the NBM and cortical thinning/atrophy of amygdala

Brain region	CS	*T*	*P*
aMCI > HO			
Neocortex			
Left temporal	307	4.2	10^{-6}
Right temporal	971	3.3	0.001
Amygdala			
Right amygdala	301	5.4	10^{-5}

CS = cluster size, expressed in mm^2 for the neocortex and in mm^3 for the amygdala; *P* = exact *P*- value (corrected, age and gender as covariates).

Volume changes in the BF (Ch1–Ch3) cholinergic compartment were associated with hippocampal volume variations in both groups (HO: $P < 10^{-6}$; aMCI: $P < 10^{-5}$), although the strength of this relationship did not differ between groups. No significant correlations were found between the volume of Ch1–Ch3 and their remaining innervated regions in any group.

Gender only affected associations between volume loss of Ch4 and thinning of the right temporal lobe; women showing stronger positive correlations in aMCI than in HO ($P < 0.001$) (see Supplementary Fig. 1).

Discussion

The present study has shown a significant relationship between the pattern of atrophy of particular BF cholinergic compartments and their innervated regions in aMCI patients. As expected from the cholinergic projections of the different BF nuclei, volume loss of Ch1–Ch3 was associated with lower hippocampal volume, whereas NBM/Ch4 volume loss was correlated with atrophy of amygdala and temporal regions. However, only correlations between the NBM and their innervated regions allowed us distinguishing aMCI from normal aging. Collectively, these results suggest that cholinergic depletion of NBM/Ch4 may contribute to target AD pathology in prodromal stages of the disease.

BF Magnocellular Compartments and their Target Regions Are Affected in aMCI

The aMCI group showed significant volume reductions of BF cholinergic compartments, as previously confirmed in different cohorts (Grothe et al. 2010; Zhang et al. 2011; Kilimann et al. 2014). These changes were remarkable in the NBM bilaterally and modest in the most rostral BF nuclei. Although our findings are only indirect markers of cholinergic degeneration, they fit well with the loss of BF cholinergic neurons reported in *post-mortem* AD brains (Whitehouse et al. 1981; McGeer et al. 1984; Arendt et al. 1985; Vogels et al. 1990) and represent a robust approach to unveil incipient degeneration in BF cholinergic compartments regardless of the sample size (e.g., Grothe et al.

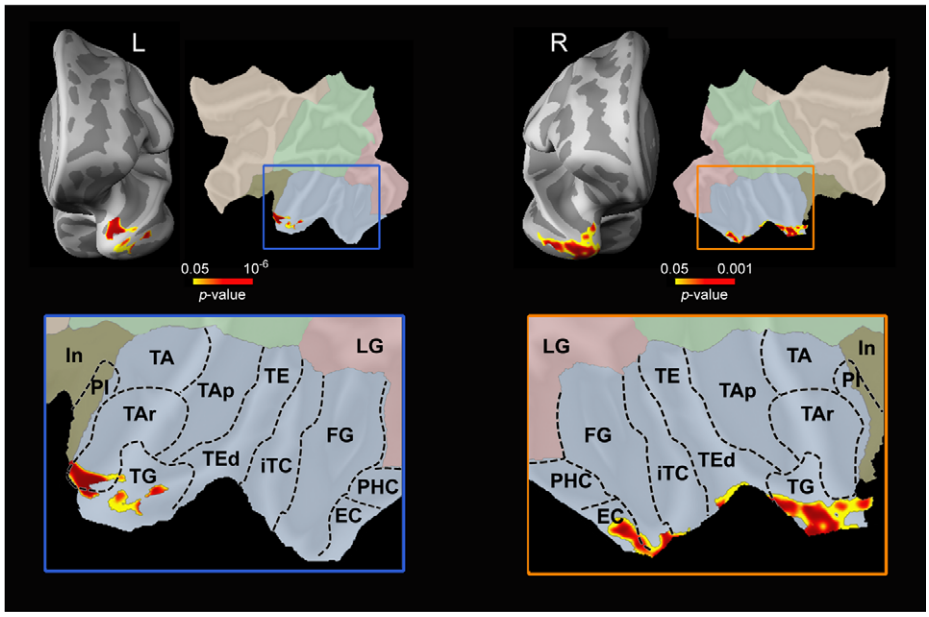

Figure 2. aMCI patients showed stronger correlations between volume reduction of Ch4/NBM and regional thinning of the temporal lobe than HO subjects. *Upper panel*: Results were displayed on inflated and flattened cortical surfaces of the same hemisphere. *Bottom panel*: Flattened cortical maps were zoomed and major cytoarchitectonic subdivisions of affected regions were delimited. Abbreviations for the temporal lobe (McDonald et al. 2000; Ding et al. 2009): FG = fusiform gyrus; EC = entorhinal cortex; PHC = parahippocampal cortex; LG = lingual gyrus; iTC = inferior temporal cortex; TE = temporal area; TAp = polysensory cortex; TEd = temporal dorsal area; TG = temporopolar area; TA = primary auditory cortex; TAr = rostral auditory cortex; PI = parainsular cortex; In = insula. L = left; R = right.

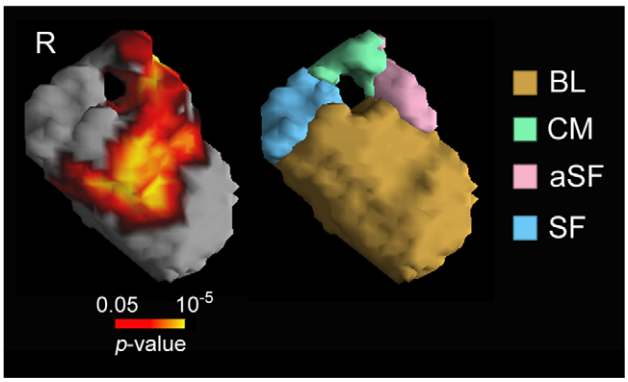

Figure 3. aMCI patients showed stronger correlations between volume reduction of Ch4/NBM and amygdala atrophy than HO subjects. *Left panel*: Significant correlations corrected for FWE ($P < 0.05$). *Right panel*: Anatomical delimitation of amygdala nuclei based on stereotaxic, probabilistic maps obtained from microscopic architectonic parcellations in 10 human *postmortem* brains. BL = basolateral; CM = centromedial; aSF = anterior superficial nuclei; SF = superficial nuclei. Superficial nuclei include the anterior amygdaloid area, the amygdalopyriform transition area, the amygdaloid-hippocampal area, and the ventral and posterior cortical nuclei (Amunts et al. 2005). R = right.

2010; Kilimann et al. 2014). Because aMCI patients were significantly older than HO, age effects on structural brain changes cannot be fully ruled out in this study. However, this possibility seems unlikely because age was included as a covariate in all statistical analyses performed. Furthermore, previous evidence suggests that age-related changes in the NBM are particularly modest and commonly occur in advanced senescence (Geula and Mesulam 1994). In either case, age is the most important risk factor for AD, and thus it is an important potential confounding variable that should be adequately controlled in future experiments.

In vivo MRI patterns of cortical thinning are reliable signatures of prodromal AD (Singh et al. 2006; Seo et al. 2007; Julkunen et al. 2010), which is mainly supported by the significant loss of large pyramidal neurons in AD cortical regions (Terry et al. 1981; Hof et al. 1990) and the low cytoarchitectonic variability of the cortical ribbon (Pakkenberg and Gundersen 1997; Regeur 2000). By using an enhanced approach to detect changes in cortical thickness (Bernal-Rusiel et al. 2008, 2010), we found bilateral thinning of canonical AD cortical regions (Price et al. 1991; Gomez-Isla et al. 1996) as well as atrophy of the right orbitofrontal and insular cortex (Van Hoesen et al. 2000; Ye et al. 2014). *Postmortem* evidence has revealed widespread neurofibrillary tangle (NFT) pathology in the orbitofrontal cortex of AD patients paralleling widening of sulci and atrophy of gyri (Van Hoesen et al. 2000). Moreover, greater NFT pathology in the orbitofrontal cortex has been correlated with aggravation of agitation in AD patients (Tekin et al. 2001), suggesting that thinning of this region may precede behavioral symptoms in AD. It has recently been shown that patterns of cortical thinning progress from early to late aMCI; evolving from medial temporal and insular regions to dorsolateral prefrontal cortex, temporal lobe, temporo-parietal association cortices, and the precuneus (Ye et al. 2014). Our results fitted well with the early phase of this continuum, and revealed that atrophy of BF cholinergic compartments is a noticeable even at this stage.

We further found bilateral reductions of the volume of the hippocampus and amygdala in the aMCI group. Evidence from different sources has extensively reported a selective vulnerability of the hippocampal formation to early AD lesions (Gomez-Isla et al. 1996; Scheff et al. 2006) that correlates with hippocampal atrophy (Chetelat and Baron 2003; Tapiola et al. 2008) and with associative memory deficits in aMCI (Atienza et al. 2011). However, few studies have confirmed the involvement of

amygdala in prodromal AD. Autopsy studies have revealed a gradual accumulation of NFT pathology in the amygdala of early AD (Braak and Braak 1991; Scott et al. 1992; Haroutunian et al. 1999; Markesbery et al. 2006) together with an association between the density of neuritic plaques in the amygdala and the severity of AD pathology in the neocortex (Yilmazer-Hanke 1998). In line with these results, volume reductions of the amygdala have been found to predict the risk of AD progression with a sensitivity of 76% and specificity of 68% (Liu et al. 2010). In fact, both hippocampus and amygdala are critically involved in memory processes and present a similar degree of volume loss in AD (Horínek et al. 2007; Klein-Koerkamp et al. 2014). Although in vivo MRI studies reporting atrophy of amygdala in MCI patients are infrequent, changes were noticeable when analysis were specifically focused on this structure (Miller et al. 2015). Our study confirms this aspect and extends this evidence to aMCI.

Structural Relationship Between BF Magnocellular Compartments and their Innervated Regions Determines Different Trajectories of Aging

Previous AD research has extensively documented a severe loss of cholinergic neurons within the NBM (Whitehouse et al. 1981; McGeer et al. 1984; Arendt et al. 1985; Vogels et al. 1990), the main source of cholinergic afferents to neocortex and amygdala (Mesulam et al. 1983; Mesulam and Geula 1988). These findings, mostly derived from *postmortem* AD studies, have been indirectly confirmed by in vivo MRI techniques in AD (Teipel et al. 2005; Kilimann et al. 2014) and MCI (Grothe et al. 2010; Teipel et al. 2011; Kilimann et al. 2014). Here, we have demonstrated that volume reductions of specific BF cholinergic compartments are selectively related to atrophy of their innervated regions in aMCI patients. In the case of the NBM/Ch4, this relationship allowed us to discriminate between HO and aMCI patients, providing in vivo MRI evidence that different pattern of correlations in the cerebral cholinergic circuitry determines different trajectories of aging. Previous attempts to link the NBM pathology to cortical changes in AD patients found a positive relationship between the number of Ch4 neurons and cortical volume (Cullen et al. 1997), and between neuronal loss in Ch4 and the number of neuritic plaques in neocortical regions (Arendt et al. 1985). Although these studies are purely correlational, they add support to our findings in the sense that regional thinning of temporal lobe parallels cortical denervation in aMCI. Nevertheless, it is worth noting that our results are derived from a cross-sectional study, and thus it prevents us to make conclusions on the time course of these changes. Inferences about disease trajectory and evolution of markers are only possible in the framework of longitudinal studies able of tracking the time course of the structural brain changes observed in the present study.

Evidence suggests that approximately 60% of the AD patients are women (Prince et al. 2015), and that relative to men they present accelerated rates of pathology (Barnes et al. 2005) and faster cognitive decline (Lin et al. 2015). Previous studies have further shown that female AD patients have more severe cytoskeletal alterations in the NBM than men (Salehi et al. 1998) as well as a higher reduction of mRNA levels of trkA and p75(NTR), 2 neurotrophin receptors of the nerve growth factor (Counts et al. 2011). Similar results have been obtained in animal models of AD, with women showing less BF cholinergic neurons and smaller NBM than men (Kelley et al. 2014). The present study goes a step further by extending the influence of gender to regions innervated by the NBM, particularly to the temporal lobe, where the cholinergic depletion seems to be more accentuated in AD (Geula and Mesulam 1994).

Previous studies have shown that the NBM is functionally connected, among other regions, to the orbitofrontal cortex, inferior temporal pole, insula, and amygdala (Li et al. 2014), mirroring correlations between volume loss of NBM and innervated regions reported in the present study (e.g., temporal pole and amygdala). Interestingly, the NBM and amygdala together with the orbitofrontal cortex are functionally involved in the response to salient stimuli (Morris et al. 1997; Rothkirch et al. 2012), which is affected in aMCI (Döhnel et al. 2008). Volume loss of the NBM was not significantly correlated with structural changes of the insula and/or orbitofrontal cortex, although both cortical regions have exhibited significant thinning in aMCI patients in the present study, and showed structural and functional vulnerability with aging in a previous work (Hu et al. 2014). Future experiments are needed to determine to what extent functional connectivity patterns of the NBM are affected in prodromal AD, and whether functional connectivity deficits are restricted to NBM-innervated regions or spread to other brain regions.

Our analyses further showed a significant morphometric relationship between Ch1–Ch3 and hippocampus, regardless of the aging trajectory. These findings suggest that volume loss of medial septum may have a nondegenerative origin, supporting previous evidence that the density of AD pathology in the hippocampus is not related to the loss of cholinergic terminals originated in the medial septum (Ch1) and the vertical limb of the diagonal band of Broca (Ch2) (Ransmayr et al. 1989, 1992). This hypothesis has been further supported in rats by the partial removal of medial septum cholinergic neurons before hippocampal ministrokes. In combination, but not alone, both factors caused cognitive deficits in the absence of neurodegeneration (Craig et al. 2009). Altogether, these results suggest that ACh dysfunctions do not directly damage hippocampal neurons, but instead increase hippocampal vulnerability to future insults by reducing the brain's ability to compensate for damage (Craig et al. 2011). It has recently been found that volume of human septal forebrain (Ch1–Ch2) is positively correlated with recognition memory accuracy (Butler et al. 2012), supporting a specific role of septal cholinergic nuclei in human episodic memory (Butler et al. 2012), which is significantly affected in aMCI (e.g., Atienza et al. 2011).

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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