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Longitudinal measures of cholinergic forebrain atrophy in the transition from healthy aging to Alzheimer's disease

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Abstract

Recent evidence from cross-sectional in-vivo imaging studies suggests that atrophy of the cholinergic basal forebrain (BF) in Alzheimer's disease (AD) can be distinguished from normal age-related degeneration even at pre-dementia stages of the disease. Longitudinal study designs are needed to specify the dynamics of BF degeneration in the transition from normal aging to AD. We applied recently developed techniques for in-vivo volumetry of the BF to serial MRI scans of 82 initially healthy elderly individuals (HE, 60–93 years) and 50 patients with very mild AD (vmAD, CDR=0.5) that were clinically followed over an average of 3 ± 1.5 years. BF atrophy rates were found to be significantly higher than rates of global brain shrinkage even in cognitively stable HE. Compared to healthy controls, vmAD showed reduced BF volumes at baseline and increased volume loss over time. Atrophy of the BF was more pronounced in progressive patients compared to those that remained stable. The cholinergic BF undergoes disproportionate degeneration in the aging process, which is further increased by the presence of AD.

Keywords

nucleus basalis Meynert; substantia innominata; cholinergic basal forebrain; MRI; voxel-based morphometry; VBM; MCI; OASIS; longitudinal

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1. Introduction

Degeneration of basal forebrain (BF) cholinergic cells and loss of cortical cholinergic innervation is a well established characteristic of Alzheimer's disease (AD). A range of post-mortem studies on AD found severe neurofibrillary degeneration and cell loss in the cholinergic BF, most pronounced in the nucleus basalis of Meynert (NBM), as well as a depletion of cortical choline-acetyl transferase activity (Lehericy et al., 1993; McGeer et al., 1984; Perry, 1980; Whitehouse et al., 1981). The extent of cholinergic loss was also found to correlate with dementia severity and several lines of evidence suggest that the cholinergic lesion in AD is, at least partly, responsible for specific cognitive impairments in the domains of memory and higher attentional functions (Bartus, 2000; Muir, 1997). Number and size of BF cholinergic neurons as well as activity of cortical cholinergic markers were also found to decrease along the human lifespan, suggesting that the cholinergic degeneration in AD occurs against a background of considerable age-related atrophy (Lowes-Hummel et al., 1989; Mann et al., 1984; McGeer et al., 1984; Perry, 1980).

The onset and temporal dynamics of augmented cholinergic degeneration in AD compared to normal age-related degeneration are not well understood (Mesulam, 2004). Mild cognitive impairment (MCI) is regarded a transitional state between normal aging and AD, and research has focused on this patient group to study early pathologic alterations in the course of AD progression (Gauthier et al., 2006). Post-mortem studies on MCI found no cholinergic cell loss or reduced cortical cholinergic markers when compared to cognitively healthy controls of the same age (Gilmor et al., 1999; DeKosky et al., 2002). However, cholinergic cells of the BF showed significantly increased neurofibrillary burden (Mesulam et al., 2004; Saskin et al., 2000), axonal abnormalities (Geula et al., 2008) and reduced trophic support (Mufson et al., 2007) in MCI and early stages of AD, indicating an accelerated and qualitatively different neurodegenerative process compared to normal aging.

Complementary to post-mortem studies that are usually limited to small sample sizes and do not allow for longitudinal observations, in-vivo imaging approaches help to study the course of BF atrophy in normal and pathologic aging. Previous studies have developed a structural MRI marker of BF integrity, based on cytoarchitectonic maps of BF cholinergic nuclei and deformation-based morphometry, enabling the in-vivo study of volumetric changes of the cholinergic BF in aging and AD (Grothe et al., 2010, 2012; Teipel et al., 2005, 2011; Zaborszky et al., 2008). In a recent study (Grothe et al., 2012) we applied this technique to a relatively large, publicly available dataset of cross-sectional MRI scans covering the whole adult age-range as well as early stages of AD (Open Access Series of Imaging Studies (OASIS), www.oasis-brains.org; Marcus et al., 2007). We found a decline of BF volume with advanced age that was further pronounced in early stages of AD, allowing for the use of BF atrophy as a diagnostic marker of AD.

In addition to cross-sectional analysis, in-vivo imaging allows for longitudinal observations of the same subjects, to assess subject-specific atrophy rates as well as the association between imaging measures at baseline and the clinical outcome at follow-up. Here we applied our previously established method for in-vivo assessment of BF volume to the recently released longitudinal dataset of the OASIS database (Marcus et al., 2010), including

serial MRI scans of healthy elderly subjects as well as clinically stable and clinically declining patients with very mild AD (vmAD, CDR = 0.5). The dataset also includes subjects that were cognitively normal at baseline and declined to a diagnosis of vmAD over follow-up, thus providing the possibility to study atrophic brain changes in subjects with presumptive AD pathology before the onset of cognitive symptoms. In-vivo measures of BF volume at baseline as well as rates of BF volume loss over time were compared between diagnostic groups. To the best of our knowledge, this is the first study examining longitudinal changes in BF volume in healthy elderly subjects and early stages of AD. For comparison, we also investigated cross-sectional and longitudinal measures of hippocampus atrophy as well as whole-brain patterns of atrophic changes within and across diagnostic groups, using automated hippocampus segmentation and voxel-based analysis, respectively.

2. Methods

2.1. Subjects

MRI scans were retrieved from the longitudinal collection of the OASIS database (Marcus et al., 2010; <http://www.oasis-brains.org>). Detailed inclusion- and diagnostic criteria of the OASIS database are described in the OASIS documentation (Marcus et al., 2007, 2010). Briefly, all subjects of the OASIS cohort participated in accordance with guidelines of the Washington University Human Studies Committee. Approval for public sharing of the data was also specifically obtained. Participants were clinically screened and exclusion criteria included use of psychoactive drugs, serious head injury, history of clinically meaningful stroke, active neurological or psychiatric illness as well as primary causes of dementia other than AD. Subjects underwent full clinical assessment at each visit and MRI acquisitions were typically obtained within one year before or after a subject's clinical assessment (mean=111 days, range=0 to 352 days). Twelve subjects with AD were scanned after a somewhat longer duration (mean=653 days, range=374 to 924 days) but were included because each subject had several previous clinical assessments with CDR scores greater than 0. Two subjects without dementia were scanned somewhat longer than one year prior to a clinical assessment (392 and 431 days) but were included because their subsequent clinical assessments yielded a CDR score of 0. Each subject was scanned on two or more separate occasions, with an average delay of 719 days (range = 183–1707 days) between visits.

Dementia status was established and staged using clinical interviews and the Clinical Dementia Rating (CDR) scale (McKhann et al., 1984; Morris et al., 1993). Following the staging of AD based on CDR scores, a global CDR of 0 indicates no dementia, and CDRs of 0.5, 1, 2, and 3 represent very mild, mild, moderate, and severe dementia, respectively (Berg et al., 1982, 1998). Based on a CDR score of 0.5 (questionable dementia), subjects with very mild AD present with mild consistent memory problems but show preserved function or only doubtful impairment in the other domains of the CDR interview, including orientation, judgment and problem solving, function in community affairs, home and hobbies, and personal care (Berg et al., 1982; Morris et al., 1991). Although this classification differs slightly from the Petersen-criteria for the diagnosis of MCI (Gauthier et al., 2006), autopsy studies suggest that the diagnoses of very mild AD and MCI represent similar levels of early AD pathology (Morris et al., 2001; Morris and Price, 2001; Storandt et al., 2006).

The present study included the baseline scan and the last follow-up scan of 82 healthy elderly subjects (HE; age at baseline: 75.7 ± 8.2 years) as well as 50 subjects with very mild AD (vmAD; CDR = 0.5, MMSE = 26.1 ± 2.5 , age at baseline: 75.2 ± 6.0). A total of 79.5% of the subjects, equally distributed across the diagnostic groups, overlapped with the cross-sectional OASIS sample we used in our previous study on BF degeneration in aging and AD (Grothe et al., 2012). Of the 82 healthy elderly subjects, 69 remained cognitively normal (s-HE) and 13 declined cognitively to a diagnosis of vmAD over follow-up (p-HE). In the vmAD group, 37 patients remained cognitively stable (s-vmAD) and 13 progressed to a diagnosis of clinically manifest AD (p-vmAD) over follow-up. Demographics and global neuropsychological profiles of the studied samples are summarized in Table 1. MMSE scores at baseline differed significantly between the vmAD groups and the s-HE sample (both $p < 0.001$) and between s-vmAD and p-vmAD ($p = 0.05$) but not between s-HE and p-HE ($p = 0.38$). Diagnostic groups did not differ significantly in age, with the exception of the p-vmAD group, being significantly younger than the s-vmAD group ($p < 0.02$) and showing a trend for younger age compared to the s-HE group ($p = 0.06$). Mean follow-up time in years differed significantly between s-HE (3.3 ± 1.4) and both vmAD groups (s-vmAD: 2.5 ± 1.4 ; $p = 0.004$; p-vmAD: 2.0 ± 0.9 , $p < 0.001$) and there were trends for longer follow-up times in p-HE (4.0 ± 1.5) compared to s-HE ($p = 0.06$) as well as in s-vmAD (2.5 ± 1.4) compared to p-vmAD (2.0 ± 0.9 ; $p = 0.06$). There were significantly more women than men in the s-HE group (21% male; $\chi^2 = 12.2$, $p < 0.001$). The gender distribution of the stable control group differed significantly from the s-vmAD group (Fisher's exact test, $p = 0.003$) but not from the p-HE ($p = 1$) or the p-vmAD group ($p = 0.1$). Also, gender distribution did not differ significantly between the s-vmAD and p-vmAD groups ($p = 0.7$).

2.2. MRI acquisition

All images of the OASIS-database were acquired on a 1.5-T Vision scanner (Siemens, Erlangen, Germany) using a sagittally oriented T1-weighted magnetization prepared rapid gradient-echo (MP-RAGE) sequence with a resolution of $1.25\text{mm} \times 1\text{mm} \times 1\text{mm}$ and empirically optimized parameters for gray-white contrast. For each subject, 3–4 individual T1-weighted MP-RAGE images were acquired in a single imaging session and averaged subsequently, resulting in high signal to noise ratios for the individual scans. More detailed information on the acquisition procedure and scanning parameters can be found in (Marcus et al., 2007, 2010).

2.3. MRI processing

Main processing steps and computational analyses employed in the present study followed a previously described protocol (Grothe et al., 2012), modified for the analysis of serial MRI scans (Teipel et al., 2010). MRI data were processed by using statistical parametric mapping (SPM8, Wellcome Trust Center for Neuroimaging) and the VBM8-toolbox (<http://dbm.neuro.uni-jena.de/vbm/>) implemented in MatLab R2007a (MathWorks, Natick, MA).

First, baseline and follow-up images of each subject were bias-corrected to account for magnetic field inhomogeneities within subject. Next, images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) partitions using the tissue prior free segmentation routine of the VBM8-toolbox. The GM and WM partitions of each

subject's baseline and follow-up scans were high-dimensionally registered to their anatomic mean (Intra-subject template) using DARTEL (Ashburner, 2007). The resulting flow-fields were used to intra-individually warp baseline and follow-up GM segments and voxel-values were modulated for volumetric changes, such that the total amount of GM volume present before warping was preserved. Inter-subject normalization was then performed by DARTEL-aligning the GM and WM portions of the intra-subject templates to a study population-specific template composed of 50 healthy elderly subjects and 50 subjects with very mild, mild and moderate AD from the OASIS database. For this template, representing an aging-AD specific average anatomy in native space, an affine transform to MNI standard space was estimated. This affine transform was combined with the individual flow-fields resulting from the inter-subject DARTEL registration and the combined transforms were used to warp the (intra-individually registered) baseline and follow-up GM maps to the aging-AD specific mean anatomy in MNI space at an isotropic resolution of 1 mm³. Again, voxel-values were modulated to preserve the original amount of GM volume. The creation of an intra-subject template and estimation of the spatial normalization parameters based on this template ensures both unbiased registration of the individual's baseline and follow-up scans and identical spatial normalization of both time-points (Reuter et al., 2012). All GM maps were visually inspected for segmentation and registration accuracy and only one subject (OAS2_0154) had to be excluded from further analysis due to inaccurate tissue segmentation of the follow-up scan. Example images of registration accuracy can be found in the supplemental Figures. For voxel-based analysis of volume loss over time, the intra- and inter-individually warped baseline and follow-up GM segments were subtracted to generate individual maps of GM change over time. Finally, modulated warped GM maps were smoothed with two different Gaussian smoothing kernels. Smoothing aims to account for imperfect image alignment and also renders the data more normally distributed. Following the matched-filter theorem, the optimal smoothing kernel in voxel-based analyses should be matched in size to the expected effects. Thus, for voxel-wise analysis of regional GM changes throughout the brain, GM maps were smoothed with a smoothing kernel of 8mm full-width at half maximum (FWHM). For voxel-wise analysis restricted to the BF region-of-interest (ROI) as well as for automated extraction of individual BF and hippocampus GM volumes, a smaller smoothing kernel of 4mm FWHM was applied to the GM maps. Individual GM volumes of the BF and the hippocampus at baseline and follow-up were extracted automatically by summing up the modulated GM voxel values within the respective ROI masks (see below).

2.4. Definition of the basal forebrain and hippocampus regions-of-interest

The ROI mask for the hippocampus was obtained by manual delineation of the hippocampus in the reference template of aging-AD specific anatomy in MNI space using the interactive software package Display (McConnell Brain Imaging Centre at the Montreal Neurological Institute) and a previously described protocol for segmentation of the medial temporal lobe (Pruessner et al., 2000). The cholinergic BF is composed of four groups of cholinergic cells. According to Mesulam's nomenclature, Ch1 refers to the cholinergic cells associated with the medial septal nucleus, Ch2 and Ch3 to those belonging to the vertical and horizontal limb of the diagonal band of Broca (DB), respectively, and Ch4 designates the cholinergic cells within the NBM (Mesulam et al., 1983). The NBM is the largest cholinergic nucleus of

the BF and can be further subdivided into anterior lateral (Ch4al) and medial (Ch4am), intermediate (Ch4i) and posterior regions (Ch4p). The cholinergic nuclei lack clear anatomical borders that could be easily identified on MRI scans, rendering manual delineation impractical. The BF mask used in this study was therefore based on a cytoarchitectonic map of BF cholinergic nuclei in MNI space, derived from combined histology and MRI of a postmortem brain (Teipel et al., 2005). Given that this cytoarchitectonic map was designed to match standard MNI space, we non-linearly registered the MNI152-template to the aging-AD specific template used in this study and used the resulting DARTEL parameters to warp the cytoarchitectonic map into the population specific template space.

2.5. Statistical analysis

Statistical analysis of extracted regional volumes was performed within the Statistics Software Package for the Social Sciences (SPSS v15.0). For complementary voxel-wise statistical analyses we employed the general linear model on a voxel-basis within the SPM framework.

2.5.1. Analysis of volumetric differences at baseline—The effect of diagnosis on extracted volumes of the BF, hippocampus and global GM at baseline was assessed using analysis of covariance (ANCOVA), controlling for total intracranial volume (TIV), age and gender. TIV was calculated as the sum of total volumes of the GM, WM and CSF partitions. Statistical threshold was set at $p < 0.05$.

In analogy to the analysis of extracted volumes, voxel-wise differences were assessed by subjecting the individual GM maps at baseline to a voxel-wise ANCOVA model with diagnosis as variable of interest and TIV, age and gender as confounding variables. Group-wise differences were assessed for the following contrasts of interest: p-HE < s-HE, s-vmAD < s-HE, p-vmAD < s-HE, p-vmAD < s-vmAD. The voxel-based analysis of GM differences across diagnostic groups was performed twice. First, a whole-brain analysis on smooth GM maps (8mm) was only restricted to an optimally thresholded GM mask of the group mean (Ridgway et al., 2009). A second analysis was restricted to the apriori ROI of the BF and used a smaller smoothing kernel (4mm). Minimum cluster extension thresholds of 50 and 5 contiguous voxels were applied for the analyses restricted to the GM mask and the BF ROI, respectively. These cluster extension thresholds were chosen in accordance to previous voxel-based studies by our group (Grothe et al., 2010, 2012; Teipel et al., 2005, 2011) and aim to limit the results to clusters above a minimum size of interest for the voxel-based patterns within the BF ROI and across the whole brain, respectively. Voxel-wise differences were assessed at a statistical threshold of $p < 0.05$, corrected for multiple comparisons using the false discovery rate (FDR). Given the apriori hypothesis for effects in the BF ROI and the very small search region of probably highly interdependent voxel-values, results of the analysis restricted to the BF ROI were additionally assessed at an uncorrected threshold of $p < 0.01$ (Grothe et al. 2010; Teipel et al. 2011).

2.5.2. Analysis of volume loss over time—Extracted GM volumes at follow-up were subtracted from the extracted volumes at baseline and divided by baseline volume and

follow-up time (in years) to calculate annual atrophy rates (in positive units of baseline volume) for the bilateral BF and hippocampus as well as for global GM. In the s-HE group one sample t-tests were used to assess the statistical significance of the annual atrophy rates being significantly greater than zero. Paired t-tests were used to determine differences between regional (BF and hippocampus, respectively) and global atrophy rates within groups. The effect of diagnosis on annual atrophy rates was determined using an ANCOVA model, controlling for age, gender and follow-up time. Statistical threshold was set at $p < 0.05$.

For the cognitively stable healthy individuals a complimentary voxel-wise one sample t-test on the preprocessed maps of GM change was used to examine regional pattern of GM change over time that exceeded global GM change. For this analysis, for each subject the average GM change across all voxels was calculated within the global GM mask and subtracted from the individual maps of GM change prior to statistical analysis. Given the highly significant effects of global GM loss over follow-up in the s-HE group, this analysis was chosen to detect regions of disproportionately high volume loss in the normal aging process. Group-wise differences in GM loss over time were assessed by subjecting the individual maps of GM change to a voxel-wise ANCOVA model with diagnosis as variable of interest and age at baseline, gender, TIV and follow-up time as confounding variables. Similar to the analysis of volumetric differences at baseline, all voxel-based analyses of GM change over time were performed twice, assessing voxel-wise effects throughout the entire cerebral GM as well as restricted to the BF ROI. For analysis of effects throughout the whole brain a statistical threshold of p (FDR) < 0.05 was applied. Effects within the BF ROI were evaluated at p (FDR) < 0.05 as well as $p < 0.01$, uncorrected. Minimum cluster extension thresholds of 50 and 5 contiguous voxels were applied for the analyses restricted to the GM mask and the BF ROI, respectively.

2.5.3. Atrophy and cognitive decline—Complementary to the categorical analysis of volumetric differences between diagnostic groups, associations between regional brain volumes and global cognitive function were assessed in subjects with vmAD by calculating partial correlation coefficients between BF and hippocampus volumes and MMSE scores at baseline, controlling for TIV, age and gender. In addition, partial correlations between rates of regional atrophy and rates of decline in MMSE score, controlling for age, gender and follow-up time, were calculated separately for the progressive normal elderly subjects and vmAD patients.

3. Results

3.1. Baseline volumes

Differences in baseline BF, hippocampus and global GM volumes between the diagnostic groups are summarized in Table 2. Figure 1 illustrates the reduced baseline volumes in the p-HE, s-vmAD, and p-vmAD groups, respectively, as percent volume reduction compared to the baseline volumes of the cognitively stable healthy control group (s-HE). All volumetric measures declined in the predicted direction, being highest in s-HE, intermediate in p-HE and s-vmAD and smallest in p-vmAD (BF, $F=8.41$; hippocampus, $F=15.56$; global GM, $F=6.13$; all $p < 0.001$). However, post-hoc comparisons of the estimated marginal means

controlled for age, gender and TIV revealed that none of the volumetric differences between p-HE and s-HE were statistically significant. On the other hand, both BF and hippocampus volumes were significantly reduced in s-vmAD ($p = 0.009$ and $p < 0.001$, for BF and hippocampus, respectively) and p-vmAD ($p < 0.001$, for both BF and hippocampus) when compared to the stable healthy control group. Global GM volume was significantly reduced in the progressive vmAD patients, but not in the s-vmAD patients. When compared to the p-HE group, hippocampus volume was reduced in both the s-vmAD and the p-vmAD groups, whereas BF volume as well as global GM volume was only significantly reduced in the p-vmAD group. All of the volumetric measures were significantly more reduced in p-vmAD as compared to s-vmAD ($p = 0.004$, $p = 0.02$ and $p = 0.003$ for BF, hippocampus and global GM, respectively).

Results of the complimentary voxel-based analysis are illustrated in Figure 2. Results from the analysis restricted to the BF ROI are illustrated on the left side and regional patterns of GM reductions throughout the brain on the right side. The analysis confirmed the absence of statistically robust volumetric differences between s-HE and p-HE at baseline. However, when lowering the statistical threshold for effects in the BF ROI to $p < 0.01$, uncorrected, a small cluster of reduced volume could be detected, corresponding to left anterior to intermediate NBM. On the other hand, significant effects of reduced volume in both the stable and progressive vmAD groups were found in widely distributed cortical and subcortical sites. While clusters of reduced volume in s-vmAD were most pronounced in the medial temporal lobes, reaching into the posterior BF, volume reductions in p-vmAD were much more widespread and included temporoparietal areas, perigenual and posterior cingulate cortices, precuneus and, to a lesser extent, medial and lateral frontal lobes. However, when directly comparing p-vmAD and s-vmAD, reduced volume in p-vmAD was mainly restricted to the temporal lobes and effects showed a marked right hemispheric dominance. Volume reductions in the BF ROI were restricted to posterior parts of the NBM in s-vmAD but included the whole cholinergic BF in p-vmAD. Accordingly, differences between cognitively declining and stable patients with vmAD mapped to the rostral BF in the right hemisphere, corresponding to the vertical and horizontal limb of the DB and the anterior NBM, as well as to the bilateral posterior NBM. All of the voxel-wise effects of reduced BF volume in the vmAD groups compared to the healthy control group as well as those effects between progressive and stable vmAD groups met the more stringent statistical threshold of $p(\text{FDR}) < 0.05$.

3.2. Volume loss over time

Annual atrophy rates of the BF, hippocampus and global GM are summarized for the different diagnostic groups in Table 2 and Figure 3. Cognitively stable healthy controls showed significant volume loss of all examined regions over follow-up (BF and hippocampus, $p < 0.001$; global GM, $p = 0.004$). However, atrophy rates of both BF and hippocampus were significantly higher than rates of global GM loss (BF vs global GM, $p = 0.003$; HC vs global GM, $p = 0.009$). Slightly higher atrophy rates of the BF compared to hippocampus were not statistically significant ($p = 0.3$). The complimentary voxel-based analysis of regional GM loss significantly exceeding global GM loss confirmed the disproportionate atrophy of the BF and the hippocampus and further revealed

disproportionately high gray matter loss over time in temporoparietal areas, ventromedial and lateral frontal lobes, insula, posterior cingulate and cuneus (Figure 4). All effects within the BF ROI survived the FDR-controlled statistical threshold and included the whole cholinergic forebrain. Statistically most pronounced effects mapped to anterior lateral and posterior regions of the NBM.

Patient groups showed a similar pattern of increased BF and hippocampus atrophy rates compared to global GM atrophy (BF vs global GM, $p < 0.05$ within each group; HC vs global GM, $p < 0.01$ within each group), while rates of BF and hippocampus atrophy were statistically similar within groups ($p > 0.3$ within each group). Across groups, atrophy rates of all examined regions increased in the predicted direction, being lowest in s-HE, intermediate in p-HE and s-vmAD and highest in p-vmAD. However, for atrophy rates of the BF and global GM the overall effect of diagnosis only reached trend level significance (BF, $F = 2.46$, $p = 0.07$; hippocampus, $F = 7.14$, $p < 0.001$; global GM, $F = 2.37$, $p = 0.07$). Group-wise comparisons of the estimated marginal means revealed that BF atrophy rates were only significantly increased in the p-vmAD group compared to the s-HE group ($p = 0.01$). However, the differences in BF atrophy rates between the p-HE, s-vmAD and the p-vmAD groups were not statistically significant. On the other hand, atrophy rates of the hippocampus were significantly increased in the p-HE ($p = 0.03$), s-vmAD ($p = 0.001$) and p-vmAD ($p < 0.001$) groups compared to the s-HE group. However, atrophy rates of the hippocampus, the BF or the global GM did not differ significantly between the p-HE, the s-vmAD and the p-vmAD groups.

Results of the voxel-based analysis of differences in longitudinal GM loss between diagnostic groups are illustrated in Figure 5. None of the voxel-wise group differences in BF volume loss over time survived correction for multiple comparisons. However, at a more lenient threshold of $p < 0.01$, uncorrected, p-HE showed increased atrophy rates of the bilateral posterior NBM compared to the cognitively stable controls. In the s-vmAD group only a very small cluster in the left rostral BF could be detected, whereas the p-vmAD group showed bilateral effects of increased atrophy rates in anterior lateral, intermediate and posterior parts of the NBM. A direct comparison between the two vmAD groups revealed a small cluster of increased atrophy in progressive patients, corresponding to right intermediate to posterior parts of the NBM. In the additional voxel-wise analysis of GM changes throughout the whole-brain, no effects of increased GM loss over time in the p-HE group compared to the stable controls survived the FDR-controlled threshold. The stable vmAD patients showed two small clusters of increased GM loss corresponding to right lateral temporal cortex and left posterior lateral orbital cortex/anterior ventral insula, respectively. The progressive vmAD group, on the other hand, demonstrated widespread effects of increased longitudinal GM loss throughout the temporal, parietal and frontal lobes. A direct comparison between stable and progressive vmAD groups only yielded a small cluster of increased GM loss over time in the progressive patients, mapping to the right posterior lateral temporal cortex.

3.3. Association of atrophy with cognitive decline

Reduced BF volumes at baseline, but not hippocampus volumes, correlated significantly with reduced MMSE scores in the vmAD group (BF: $r_{\text{part}} = 0.37$, $p = 0.01$; hippocampus: $r_{\text{part}} = 0.01$, $p > 0.1$). Rates of atrophy of the BF correlated strongly with rates of MMSE score decline in the group of healthy controls that progressed to vmAD over follow-up ($r_{\text{part}} = 0.70$, $p = 0.02$), but not in the pooled vmAD group ($r_{\text{part}} = 0.05$, $p > 0.1$) nor when examining s-vmAD and p-vmAD separately (s-vmAD: $r_{\text{part}} = 0.07$, $p > 0.1$; p-vmAD: $r_{\text{part}} = -0.01$, $p > 0.1$). Atrophy rates of the hippocampus did not correlate significantly with longitudinal global cognitive decline within any group (p-HE: $r_{\text{part}} = -0.14$, $p > 0.1$; pooled vmAD: $r_{\text{part}} = 0.24$, $p > 0.1$; s-vmAD: $r_{\text{part}} = 0.27$, $p > 0.1$; p-vmAD: $r_{\text{part}} = 0.12$, $p > 0.1$).

4. Discussion

In the present study we used ROI-based and voxel-based analyses of longitudinal datasets to replicate and extend previous cross-sectional findings of BF atrophy in healthy aging and early stages of AD. Specifically, BF volume was found to be highly vulnerable to normal age-related atrophy, exceeding global GM atrophy more than 2.5-fold in cognitively stable healthy elderly. When compared to the stable healthy control group, both cognitively declining patients with vmAD and those that remained relatively stable had lower BF volumes at baseline. However, BF volume at baseline was already significantly more reduced in patients with vmAD that later converted to a diagnosis of clinically probable AD (CDR = 1) than in patients that remained stable over follow-up. Clinically declining patients with vmAD, but not those that remained stable, also showed increased BF volume loss over time compared to the healthy elderly control group.

Disproportionately high longitudinal atrophy rates of the BF in healthy elderly individuals match well with previous findings from cross-sectional in-vivo imaging studies showing a marked negative effect of age on BF volume (Grothe et al., 2012; Hanyu et al., 2002). Several studies on animal models as well as human post-mortem autopsy studies demonstrated an age-related decline in cholinergic function, which is mainly mediated by cell shrinkage, axonal degeneration and synaptic loss, rather than gross neuronal loss, and is believed to contribute to cognitive impairments in memory and higher attentional functions associated with advanced age (Bartus, 2000; Lowes-Hummel et al., 1989; Mann et al., 1984; McGeer et al., 1984; Muir, 1997; Schliebs and Arendt, 2011). We suggest that the gray matter volume reductions within the cytoarchitectonic map of BF cholinergic nuclei, as measured here with in-vivo MRI, reflect these neurodegenerative processes of cholinergic cells. Although the increased BF atrophy rates in cognitively declining subjects that were normal at baseline did not reach statistical significance when compared to stable healthy controls, the decline in BF structural integrity correlated strongly with the cognitive decline in this group.

BF volume at baseline was reduced in subjects at a pre-dementia stage of AD compared to stable healthy controls and was correlated with global cognitive status. These findings are in line with previous cross-sectional studies on independent data by our group, showing that BF volume is reduced, and parallels cognitive impairment, in subjects with MCI (Grothe et al., 2010; Teipel et al., 2011). However, two recent cross-sectional volumetry studies

manually measured anterior parts of the cholinergic BF centered on the anterior commissure and reported discordant findings with respect to reduced volumes in MCI (George et al., 2011; Muth et al., 2010). This may be due to the intrinsic heterogeneity in MCI and slightly differing manual volumetry protocols. On the other hand, our voxel-based analyses in this and previous studies have consistently shown that volumetric reductions in pre-dementia stages of AD are most pronounced over posterior parts of the cholinergic BF (Grothe et al., 2010, 2012; Teipel et al., 2011). This is also in line with an early histopathologic study on the regional heterogeneity of cholinergic forebrain atrophy in AD, which found cholinergic degeneration to be most pronounced over posterior parts of the NBM (Wilcock et al., 1988). These posterior regions, however, were not covered in the manual volumetry protocols employed in the studies described above. The differences in anterior BF volume between MCI and healthy controls observed by Muth et al. (2010) may also be explained by the relatively young average age of the control group (63 ± 6 years), which also differed significantly from the average age of the MCI group (68 ± 7 years). Given the marked BF atrophy rates in healthy elderly observed in the present study, age-related atrophy should be less developed in younger control groups and thus, structural differences attributable to early AD pathology should be more pronounced and easier to detect. Accordingly, post-mortem studies found that cholinergic loss in initial stages of the disease is easier to detect in presenile than in senile AD (Bowen et al., 1982; Gilmore et al., 1999; DeKosky et al., 2002) and, likewise, it has been hypothesized that this observation may be explained by age-related cholinergic decline (Mesulam, 2004). Baseline differences in BF and hippocampus volume between healthy controls and vmAD resemble the findings of our previous cross-sectional in-vivo study on BF degeneration in aging and early AD (Grothe et al., 2012). This is expected, given the substantial overlap of the cross-sectional and longitudinal datasets of the OASIS database (Marcus et al., 2010) and the very similar methodical approach. However, the clinical follow-up data of the present dataset allows for a better characterization of the healthy control group, being cognitively stable for an average follow-up time of 3.3 years, and further allows distinguishing between vmAD subjects that imminently progress to clinically probable AD and those that remain cognitively stable. Consequently, we could show here that progressive vmAD patients had already significantly lower BF volumes at baseline compared to stable vmAD patients. Complementary voxel-based analyses revealed that this difference mainly mapped to the bilateral posterior NBM as well as to rostral parts of the BF in the right hemisphere. This agrees with the hypothesis of spatially restricted atrophy in posterior parts of the BF at pre-dementia stages and increasing involvement of the rostral BF as the disease progresses.

Here we also report the first data on longitudinal measures of BF atrophy in the transition from normal aging to early stages of AD. Both analysis of extracted BF volumes and complimentary voxel-based analysis demonstrated increased GM loss over time in the clinically declining patients with vmAD compared to healthy elderly controls, whereas stable vmAD patients showed only negligible effects in the voxel-based analysis. Although the increased BF GM loss in initially healthy elderly that declined to a diagnosis of vmAD over follow-up compared to those that remained stable was not statistically significant when considering the BF ROI as a whole, complimentary voxel-based analysis revealed bilateral clusters of increased longitudinal GM loss in posterior parts of the NBM. This finding is

interesting as it fits nicely with the hypothesis based on cross-sectional data of this and earlier studies that AD-related neurodegeneration of the BF, as opposed to normal age-related dystrophic processes, starts in posterior parts of the NBM and progresses to include rostral nuclei at a time the disease becomes clinically manifest (Grothe et al., 2010, 2012; Teipel et al., 2011). Such a sequential involvement of cholinergic nuclei in AD progression may be of functional importance, given that the different sections of the cholinergic BF innervate distinct allo- and neocortical areas, which likely underlies their differential association to cognitive functions (Grothe et al., 2010; Mesulam and Geula, 1988; Pappas et al., 2000).

In order to compare findings of BF atrophy to overall brain atrophy in aging and AD, we also assessed global GM and hippocampus atrophy as well as voxel-based patterns of regional atrophy throughout the entire brain. Within each diagnostic group longitudinal loss of BF volume was significantly higher than global GM loss but statistically similar to hippocampal volume loss, highlighting the vulnerability of BF and medial temporal lobe regions in age- and AD-related neurodegeneration. However, volume reductions of the BF in stable and progressive vmAD patients at baseline appeared to be less pronounced than volume reductions of the hippocampus when compared to the reference values of the healthy controls. Moreover, hippocampal atrophy rates were significantly increased in cognitively declining normal elderly subjects compared to those that remained stable, whereas effects of increased longitudinal BF atrophy was only significant for the posterior NBM in the voxel-based analysis. Together these data add up to previous cross-sectional evidence of a sequential pattern of spreading atrophy from the medial temporal lobes, as the sites where AD-related atrophy first manifests, to the cholinergic nuclei of the BF at pre-clinical stages of the disease (Grothe et al., 2012). In line with these findings it has been suggested that the vulnerability of BF cholinergic nuclei to AD-related neurodegeneration may be related to their anatomic location among a “basolateral cortical strip”, further including the pyriform cortex, amygdala, hippocampus and entorhinal cortex, that attracts the greatest neurofibrillary pathology in the course of aging and AD (Mesulam, 2004; Mesulam, 2012). There are no reference data for in-vivo measures of longitudinal BF atrophy, but atrophy rates as well as cross-sectional volume reductions for global GM and the hippocampus found in the present study are in the range of previously reported values from automated as well as manual volumetry studies in preclinical AD and MCI (Leung et al., 2010; Risacher et al., 2010; Stoub et al., 2010; Van de Pol et al., 2007; Shi et al., 2009; Chupin et al., 2009). For example, group-wise annual hippocampal atrophy rates in the cohort of the Alzheimer’s Disease Neuroimaging Initiative (ADNI) range from 0.9 to 2.2% in cognitively normal, 2.1 to 2.7% in stable MCI subjects and 3.6 to 4.6% in declining MCI subjects, depending on processing method and hemisphere (Risacher et al., 2010; Leung et al., 2010). Slightly smaller atrophy rates for the p-vmAD group in this study compared to declining MCI subjects in the ADNI cohort may be related to the small sample size of the p-vmAD group, but also to slightly differing diagnostic criteria and differences in the longitudinal processing stream. Likewise, the whole-brain patterns of regional atrophy in aging and AD, respectively, as revealed by voxel-based analyses are in good accordance with previous cross-sectional and longitudinal voxel-based imaging studies (Davatzikos et al., 2009; Driscoll et al., 2009; Fjell et al., 2009; Risacher et al., 2009, 2010). Of note, the whole-brain

patterns of regional differences in baseline volume and longitudinal atrophy rates, respectively, between the different diagnostic groups in the present study closely resemble the voxel-based patterns found in two recent studies on stable and declining subjects with MCI of the ADNI database (Risacher et al., 2009, 2010).

There is an ongoing discussion on how structural disturbances in the cholinergic BF relate to dysfunctional cortical cholinergic transmission, which contributes to cognitive impairments (DeKosky et al., 2002; Sarter and Bruno, 2002). It has been hypothesized that the BF cholinergic system may functionally compensate early neurodegenerative processes and that there may be a critical number of cholinergic cells below which normal cortical cholinergic transmission cannot be maintained and cognitive disturbances emerge (Lowes-Hummel et al., 1989; Schliebs and Arendt, 2006). A plausible scenario is that an increasingly burdened cholinergic system at advanced age is especially vulnerable to AD pathology-associated stressors. Thus, early AD pathology, e.g. in the form of amyloid- β aggregates, may accelerate the age-related decline in cholinergic function and structural integrity, likely contributing to the worsening of cognitive functions relative to healthy elderly of the same age (Auld et al., 2002; Potter et al., 2011). Our findings here support such a scenario and further suggest that the process of age- and AD-related dystrophic shrinkage can be monitored in-vivo using modern automated morphometry techniques.

Limitations

Firstly, it has to be emphasized that the structural in-vivo marker of BF degeneration employed in this study is necessarily an indirect marker of cholinergic degeneration. It measures volumetric gray matter changes in a brain region that is known to house the major cholinergic nuclei of the brain. The likelihood of addressing the cholinergic space is further increased by deriving the BF ROI from a direct mapping of histologically stained cholinergic cells into standard MRI space (Teipel et al., 2005). Although the cytoarchitectonic map used in the present study was derived from a single subject only, a direct comparison between this map and a recently developed probabilistic map based on ten subjects (Grothe et al., 2010; Zaborszky et al., 2008) showed high general agreement between the maps, with the only major difference being that Ch4p was located more dorsally in the probabilistic map (thresholded at 50%) than in the single-subject map. Of note, all center-of-gravity coordinates for the different cholinergic nuclei, including Ch1–2, Ch3, Ch4 and Ch4p, reported by Zaborszky et al. (2008) fall within the space of the single-subject map used in the present study, further supporting the maps correspondence with the cholinergic space of the BF. However, given the indirect character of this method, the volumetric differences may also reflect changes in other neuronal (e.g. GABAergic) or glial components of the BF. Furthermore, the in-vivo marker does not provide information whether the volumetric changes reflect tissue shrinkage or definite cell loss. Future studies will have to evaluate if in-vivo volumetric reductions of the BF are associated with a decline in cortical cholinergic markers, as assessed by molecular imaging studies (Herholz et al., 2008; Kendziorra et al., 2011), or correlate with cholinergic degeneration assessed by post-mortem histology.

Secondly, the sample size of cognitively declining normal subjects and progressive vmAD patients was relatively small, therefore limiting statistical power and inference for these diagnostic groups. Although a great advantage of the OASIS database is its monocentric character, providing highly consistent image quality, large multi-center databases like the ADNI may be more suitable for the study of rare patient populations (Risacher et al., 2009, 2010).

Furthermore, the available psychometric data in the database is limited to MMSE scores. While the mean temporal delay between the clinical and psychometric evaluations and MRI scanning is acceptable, missing information about individual delays in the OASIS data limits the reliability of the correlational analysis between regional brain atrophy and cognitive decline in the present study.

Finally, the diagnostic group membership in this study was solely based on clinical information and not further verified by biomarker evidence of AD pathology. Although the healthy control group in this study remained cognitively stable over an average follow-up of 3.3 years, previous post-mortem as well as more recent molecular imaging studies have consistently shown that a considerable proportion of cognitively normal elderly individuals harbor pre-symptomatic AD pathology in the form of amyloid plaques and neurofibrillary tangles (Knopman et al., 2003; Rabinovici and Jagust, 2009). On the other side, subjects with clinically diagnosed MCI or vmAD also show variability with regards to AD-specific pathology, and thus some subjects develop other forms of dementia or reverse to normal (Gauthier et al., 2006). Therefore, combined amyloid- and structural imaging studies may be more accurate in discerning normal age-related processes from early AD-related atrophy (Chetelat et al., 2010; Ewers et al., 2011).

In summary, using an in-vivo structural marker of cholinergic forebrain degeneration this study describes for the first time longitudinal atrophy pattern of the cholinergic BF in the normal aging process as well as in transitional stages between normal aging and clinically manifest AD. The BF undergoes disproportionately high degeneration in the normal aging process which further aggravates in pre-dementia stages of AD. AD-related atrophy of the BF appears to begin in posterior parts of the NBM at a time first cognitive impairments emerge and spreads anteriorly to affect the whole NBM and the rostral cholinergic nuclei as subjects progress to clinically manifest AD. Given the therapeutic implications of cholinergic degeneration for age- and AD-related cognitive deficits, longitudinal assessment of cholinergic forebrain atrophy may become a useful tool for improvement of diagnosis, prognosis and treatment of the elderly with first symptoms of AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Reduced volumes at baseline: ROI analysis. Group means of basal forebrain (BF), hippocampus and global gray matter (GM) volumes at baseline are plotted as percent volume reductions relative to the reference values of the healthy control group.

Figure 2.

Reduced volumes at baseline: voxel-based analysis. Results from the voxel-based analysis restricted to the BF ROI (left side of figure) are projected onto representative coronal sections of the population-specific template in MNI space, magnified to better represent the basal forebrain. The respective y-coordinates in MNI space are indicated in the upper left corner of each section. Regional patterns of GM reductions throughout the brain are illustrated as renderings on the left, right and lower cortical surfaces as well as on a sagittal section of the study-specific template (right side of figure). All results are corrected for total intracranial volume, age and gender. Voxel-wise effects of the analysis restricted to the BF ROI are thresholded at $p < .01$, uncorrected, with a minimum cluster extension threshold of 5 contiguous voxels. Voxel-wise effects of group differences in GM volume throughout the whole brain are thresholded at $p < .05$, FDR-corrected, with a minimum cluster extension threshold of 50 voxels. Statistical significance (in terms of T values, 125 degrees of freedom) is coded by a color scale from black-blue to red.

Figure 3.

Gray matter loss over time: annual atrophy rates of ROIs. Group means of annual atrophy rates (as percentage of baseline volume) for basal forebrain (BF), hippocampus and global gray matter (GM).

Figure 4.

Voxel-based analysis of disproportionately high gray matter loss over time in healthy elderly individuals. A voxel-wise one sample t-test was used to detect regions where longitudinal gray matter (GM) loss exceeded global GM loss. Results from the voxel-based analysis restricted to the BF ROI (left side of figure) are projected onto representative coronal sections of the population-specific template in MNI space, magnified to better represent the basal forebrain. The respective y-coordinates in MNI space are indicated in the upper left corner of each section. Regional patterns of GM reductions throughout the brain are illustrated as renderings on the left, right and lower cortical surfaces as well as on a sagittal section of the study-specific template (right side of figure). Voxel-wise effects of the analysis restricted to the BF ROI are thresholded at $p < .01$, uncorrected, with a minimum cluster extension threshold of 5 contiguous voxels. Voxel-wise effects throughout the whole brain GM are thresholded at $p < .05$, FDR-corrected, with a minimum cluster extension threshold of 50 voxels. Statistical significance (in terms of T values, 68 degrees of freedom) is coded by a color scale from black-blue to red.

Figure 5.

Voxel-based analysis of group-wise differences in gray matter loss over time. Results from the voxel-based analysis restricted to the BF ROI (left side of figure) are projected onto representative coronal sections of the population-specific template in MNI space, magnified to better represent the basal forebrain. The respective y-coordinates in MNI space are indicated in the upper left corner of each section. Regional patterns of GM reductions throughout the brain are illustrated on representative cortical renderings and a sagittal section of the study-specific template (right side of figure). All results are corrected for total intracranial volume, age, gender and follow-up time. Voxel-wise effects of the analysis restricted to the BF ROI are thresholded at $p < .01$, uncorrected, with a minimum cluster extension threshold of 5 contiguous voxels. Voxel-wise effects of group differences in longitudinal GM loss throughout the whole brain are thresholded at $p < .05$, FDR-corrected, with a minimum cluster extension threshold of 50 voxels. Statistical significance (in terms of T values, 124 degrees of freedom) is coded by a color scale from black-blue to red.

Table 1

Subject demographics

	s-HE	p-HE	s-vmAD	p-vmAD
Number of Subjects	69	13	37	13
Age-BL [years]	75.3 (SD 8.4)	78.0 (SD 7.1)	76.1 (SD 4.6)	72.7 (SD 6.8)
Gender (F / M)	49/20	9/4	15/22	6/7
Follow-up [years]	3.3 (SD 1.4)	4.0 (SD 1.5)	2.5 (SD 1.4)	2.0 (SD 0.9)
MMSE-BL	29.2 (SD 0.9)	29.3 (SD 0.9)	26.6 (SD 3.4)	24.8 (SD 2.8)
MMSE-FU	29.2 (SD 0.9)	27.9 (SD 1.9)	25.9 (SD 5.0)	22.3 (SD 3.5)

Age-BL = age at baseline; F = female; M = male; MMSE = Mini-Mental State Examination; SD = standard deviation.

Table 2

ROI volumes at baseline and annual atrophy rates

	s-HE	p-HE	s-vmAD	p-vmAD
<u>Volume at baseline</u>				
BF (mm ³)	1020 (SD 109)	977 (SD 120)	957 (SD 138) *	873 (SD 107) **, ##, ++
Hippocampus (mm ³)	4386 (SD 482)	4341 (SD 478)	3934 (SD 577) **, ++	3579 (SD 722) **, #, ++
Global GM (cm ³)	591 (SD 55)	577 (SD 37)	579 (SD 62)	547 (SD 41) **, ##, +
<u>Annual atrophy rates</u>				
BF	1.05 (SD 2.06)	1.61 (SD 1.55)	1.94 (SD 2.77)	2.90 (SD 4.08) *
Hippocampus	0.82 (SD 1.13)	1.97 (SD 1.11) *	2.15 (SD 2.68) **	2.90 (SD 1.37) **
Global GM	0.36 (SD 0.99)	0.72 (SD 0.85)	0.91 (SD 1.53) *	1.20 (SD 1.92) *

Baseline volumes reflect group means (and standard deviations) of the raw ROI volumes. Annual atrophy rates reflect group means (and standard deviations) of the annual decline in raw ROI volumes as percentage of baseline volume. All statistical analyses are controlled for age and gender. Analyses of baseline volumes are additionally controlled for total intracranial volume. Analyses of annual atrophy rates are additionally controlled for follow-up time. ROI = region of interest; GM = gray matter; SD = standard deviation.

- * significantly different from s-HE at $p < 0.05$
- ** significantly different from s-HE at $p < 0.005$
- # significantly different from s-vmAD at $p < 0.05$
- ## significantly different from s-vmAD at $p < 0.005$
- + significantly different from p-HE at $p < 0.05$
- ++ significantly different from s-HE at $p < 0.005$