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MRI-based volumetric measurement of the substantia innominata in amnesic MCI and mild AD

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Abstract

The substantia innominata (SI) contains the nucleus basalis of Meynert, which provides the major cholinergic innervation to the entire cortical mantle and the amygdala; degeneration of nucleus basalis neurons correlates with cognitive decline in Alzheimer's disease (AD). However, whether SI atrophy occurs in individuals with amnesic mild cognitive impairment (aMCI) has not been examined thoroughly *in vivo*. In the present study, we developed a new protocol to measure volumetric changes in the SI from magnetic resonance imaging (MRI) scans. Participants consisted of 27 elderly controls with no cognitive impairment (NCI); 33 individuals with aMCI; and 19 patients with mild AD. SI volumes were traced on three consecutive gapless 1 mm thick coronal slices. Results showed that SI volume was significantly reduced in the mild AD group compared to both NCI and aMCI participants; however, the NCI and aMCI groups did not differ from each other. Furthermore, a decrease in SI volume was related to impaired performance on declarative memory tasks even when attention was controlled.

Keywords

Aging; basal forebrain; cholinergic; dementia; imaging; memory

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Conflict of interest

The authors do not have any actual or potential conflicts of interest.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder manifested by cognitive decline, progressive impairment of activities of daily living, and various neuropsychiatric and behavioral dysfunctions. Although there is widespread decline in several neurotransmitter-containing perikarya in end-stage AD, the basal forebrain region of the substantia innominata (SI), containing the cholinergic neurons of the nucleus basalis (Mesulam et al., 1983), undergoes progressive degeneration during the progression of AD (Whitehouse et al., 1981; Rossor et al., 1982; Bird et al., 1983; Gilmor et al., 1999). Nucleus basalis cholinergic neurons compose the Ch4 subgroup that provides cholinergic innervation to the entire cortical mantle and the amygdala (Mesulam et al., 1983; Selden et al., 1998; Mufson et al., 2003). Although some studies using selective neurotoxic cholinergic lesion of the nucleus basalis in animals demonstrate learning and memory deficits (Berger-Sweeney et al., 1994; Fine et al., 1997), other investigations that have dissociated attention and memory suggest that this forebrain region plays a major role in regulating attentional processes (Chiba et al., 1995; Baxter et al., 1995; Shen, et al., 1996; Han et al., 1999).

Post-mortem pathological investigations report the accumulation of neurofibrillary tangles and A β depositions (Whitehouse et al., 1981; Sassin et al., 2000; Mesulam, 2004), as well as significant neuronal atrophy (Whitehouse et al., 1981; Arendt et al., 1985; Vogels et al., 1990) within the nucleus basalis of patients with AD. In addition to neuronal loss, several studies have confirmed the loss of choline acetyltransferase (ChAT) activity (Perry et al., 1978; Rossor et al., 1982; DeKosky et al., 1992; Davis et al., 1999) and a decrease in acetylcholine release (Nilsson et al., 1986) in the cortical projection sites of nucleus basalis neurons in patients with end-stage AD. Furthermore, cholinergic deficits have been shown to positively correlate with cognitive decline in AD (Wilcock et al., 1982; Collerton, 1986; DeKosky et al., 1992; Auld et al., 2002).

Recently, researchers have begun to examine individuals with a clinical diagnosis of mild cognitive impairment (MCI) who exhibit cognitive decline, but do not meet criteria for dementia (Petersen et al., 2004). The cholinergic nucleus basalis neurons undergo metabolic (Dubelaar et al., 2006) and chemical phenotypic (Counts et al., 2004) alterations in MCI, but frank cell loss has not been demonstrated in this condition (Gilmor et al., 1999). In addition, *post-mortem* tissue investigations have shown an increase in cortical ChAT activity in MCI (DeKosky et al., 2002). However, individuals with MCI comprise a heterogeneous cohort consisting of those with only memory impairments classified as amnesic MCI (aMCI), those with impairment in a non-memory domain designated as non-amnesic MCI, as well as those with impairments in multiple cognitive domains (Petersen, 2004). Since individuals with aMCI develop AD at a rate of 10% to 12% per year (Petersen et al., 1999) and represent a transitional stage between normal aging and AD (Petersen et al., 1999; Petersen, 2004), these participants are valuable in identifying *in vivo* brain changes in prodromal AD.

High-resolution magnetic resonance imaging (MRI) allows examination and quantitation of alterations in brain anatomy *in vivo*, which would be useful for determining which regions are affected during the progression of AD. Recent *in vivo* imaging studies (Sasaki et al., 1995; Hanyu et al., 2002a; Hanyu et al., 2002b; Hanyu et al., 2007) used T2-weighted MR images to calculate the thickness of the SI in patients with AD. These investigators measured the thickness on either a 2 mm or 3 mm thick coronal section at the level of the crossing of the anterior commissure and found significant atrophy of the SI in AD, compared to healthy elderly controls (Sasaki et al., 1995; Hanyu et al., 2002a; Hanyu et al., 2007; Moon et al., 2008). Apart from a recent publication (Muth et al., 2009), this region of interest has not been well studied *in vivo* during the prodromal stage of AD.

In the present cross-sectional study, we developed a new protocol using T1-weighted high resolution MR images to measure volumetric changes in the SI region of the basal forebrain that contains the nucleus basalis of Meynert. MRI scans of individuals with aMCI and mild AD were compared to healthy elderly controls with no cognitive impairment. In addition, we analyzed performance on various neuropsychological tests that assess declarative memory and frontal lobe function in order to determine their relationship with SI volume. We also determined whether the APOE $\epsilon 4$ allele status has an effect on SI volume.

2. Materials and methods

2.1. Subjects

The data reported in this study were obtained from the following three groups: 1) 27 elderly controls with no cognitive impairment (NCI) (mean age = 76 ± 6.1 years), 2) 33 participants with aMCI (mean age = 78 ± 8.1 years), and 3) 19 patients with mild AD (mean age = 77 ± 7.6 years). Participants were recruited from the Rush Alzheimer's Disease Center (RADC) clinic, Religious Order Study (ROS; Mufson et al., 1999; Kordower et al., 2001; Bennett et al., 2002) and the Rush Memory and Aging Project (MAP; Bennett et al., 2005). It should be noted that individuals who came to the RADC for a clinical work-up, but did not show cognitive impairment were not recruited as controls. All control participants were recruited from the community, the ROS or MAP. ApoE genotype was determined for all individuals using DNA extracted from cheek swab samples, as previously published (Stoub et al., 2005).

2.2. Clinical evaluation

All clinical evaluations were carried out by the RADC clinic as previously described (Bennett et al., 2002; detoledo-Morrell et al., 2004). Briefly, the standard evaluation incorporated the Consortium to Establish a Registry for Alzheimer's Disease (CERAD; Morris et al., 1989) procedures and included a medical history, neurological examination, neuropsychological testing, in addition to blood tests and informant interview as needed.

The clinical diagnosis of mild probable AD followed National Institute of Neurological and Communication Disorders and Stroke (NINCDS)/Alzheimer's Disease and Related Disorders Association (ADRDA) guidelines (McKhann et al., 1984). It required a history of cognitive decline and neuropsychological test evidence of impairment in at least two cognitive domains, one of which had to be memory. Participants who entered the study with a diagnosis of aMCI received the standard evaluation used at the RADC, but did not meet criteria for dementia; they were found to have a deficit only in the memory domain (Petersen et al., 2004).

Individuals who entered the study as elderly controls received the same clinical work-up as that used for the diagnosis of aMCI and AD. Selection as an elderly control subject required a normal neurological examination, normal cognition and a Mini Mental State Examination (MMSE) score of ≥ 27 .

Exclusion criteria for entry into the study were age < 65 , evidence of other neurological, psychiatric or systemic conditions that could cause cognitive impairment (e.g., stroke, Parkinson's disease, a history of temporal lobe epilepsy, alcoholism, and major depression). Informed consent was obtained from all participants according to the rules of the Institutional Review Board of Rush University Medical Center.

2.3. MRI acquisition parameters

All structural MR images were acquired on a 1.5 Tesla General Electric Signa scanner using a 3D Fourier-transform spoiled gradient recalled echo pulse sequence (SPGR) with the

following parameters: 124 contiguous images in the coronal plane, 1.6 mm thick sections, matrix = 256×192 , field of view = 22 cm, repetition time (TR) / echo time (TE) = 33.3/7 ms, flip angle = 35° , signals averaged = 1.

2.4. Volumetric Determination of SI

The Analyze software (Mayo Clinic Foundation, Rochester, MN) was used to determine SI volume. This region of interest was manually segmented on T1-weighted 1.0 mm thick coronal slices reformatted to be perpendicular to the anterior commissure-posterior commissure (AC-PC) line using the Analyze software package. The volume was derived from three consecutive gapless 1mm thick slices. To correct for individual differences in brain size, volumes were normalized by dividing with total intracranial volume derived from sagittally formatted 5 mm slices. Intracranial volume was calculated by tracing the inner table of the cranium in consecutive sagittal sections spanning the entire brain. At the level of foramen magnum, a straight line was drawn from the inner surface of the clivus to the occipital bone. Normalized SI volume was computed using the following formula: total SI volume (mm^3)/total intracranial volume (mm^3) $\times 1000$.

2.5. MRI tracing of the SI

SI was outlined separately for each hemisphere in a rostral-caudal direction on three consecutive gapless 1mm thick sections. A matching series of coronal sections from a celloidin embedded brain harvested from a 74 year old male who died of heart failure were used to verify the location of the SI delineated on MRI scans (see Fig. 1).

The first section traced was at the level of the crossing of the anterior commissure (Fig. 1A). The ventral aspect of the globus pallidus demarcated the dorsal border of the SI, while the ventral border was the base of the brain containing the anterior perforated space. The medial border of the SI was operationally defined by a vertical line extending from the ventrolateral border of the bed nucleus of the stria terminalis to the base of the brain. The lateral border of the region of interest extended to the lateral aspect of the putamen. In the second section analyzed, the anterior commissure might be uncrossed. The third section evaluated was at the level of the emergence of the anterior commissure from the temporal lobe (Fig. 1B). The anatomical landmarks used to define the borders of SI were applied to all three consecutive sections. The region was best visualized and defined in these three sections, with partial voluming being common more rostrally. The total SI volume calculated included both the right and left hemispheres. The most posterior subregion of the of the SI was not included in the analysis as this region is less well defined on an MRI and is wedged between the globus pallidus and the putamen (Mesulam et al., 1983; Mufson et al., 1989).

All tracings were carried out by S.G.; the intra-rater reliability was determined by making a second measurement on 10 cases, after a period of at least four weeks. The correlation coefficient of the two measurements for S.G. was 0.95. Investigators involved in the MRI analyses were blinded to clinical information until all volumetric determinations were completed.

2.6. Neuropsychological Testing

All participants were administered neuropsychological tests that comprised of declarative memory and frontal lobe dependent tasks. As previously described by Stoub et al. (2008), the declarative memory tests consisted of the East Boston Story and Story A from the Wechsler memory scale-Revised. Both immediate and delayed recall was assessed for both of these tasks. In addition, Word List Memory, Word List Recall and Word List Recognition tests from the CERAD battery were included. Summary scores were calculated for combined performance on declarative memory tasks by standardizing each of the seven

declarative memory scores. For the standardization, we used the means and standard deviations of each test from the baseline visits of the first wave of 86 participants entered into our ongoing longitudinal project and averaged the standardized values to obtain a declarative memory *z*-score. The frontal lobe dependent tasks consisted of category fluency, digit span backwards and progressive matrices. The method used to obtain a declarative memory *z*-score was applied for standardizing the scores for the frontal tasks to obtain a frontal *z*-score.

Since memory deficits on the neuropsychological tasks can be a result of impaired attention or inadequate strategies in acquiring information, we administered the verbal and spatial portions of the Buschke controlled learning task (Buschke & Grober, 1986; Grober & Buschke, 1987), which tests for declarative memory, to all participants. The Buschke task has been shown to distinguish between “apparent” and “genuine” memory deficits in elderly individuals (Buschke & Grober, 1986; Grober & Buschke, 1987), since it controls for attention. Participants were asked to learn a list of 16 items presented four at a time as previously described (deToledo-Morrell et al., 2000). Items were shown as line drawings with one picture in each quadrant of a card. When a category cue was given verbally, the subject had to search, point to and name the object from that category. After this was done for four items, immediate cued recall of the four items was tested by presenting each category cue to the subject. If the subject failed to recall an item in response to its cue, the item was shown again, and the entire process was repeated until immediate cued recall was correct for the four items. Then, the next set of four items was presented until all 16 items were correctly retrieved during immediate cued recall. The search and naming procedure ensured that all participants used the same strategy in processing information and that the items have been correctly encoded.

After the subjects learned the items, three trials of free recall were administered, with each trial being preceded by 20 seconds of interference. On each trial, subjects were allowed a maximum of 2 minutes to name as many of the learned items as possible. Next, a category cue was provided for each item missed on that trial. If the subject still failed to recall the item with the cue, the participant was reminded of the missed item which he or she then repeated. An additional trial of free recall was administered after approximately 60 minutes to test for delayed recall.

Memory for the spatial location of the learned items was also examined immediately after the third trial of free recall, as well as after delayed free recall. Each of the 16 items was presented one at a time and the subject was asked to identify which of the four quadrants it originally appeared in. The scores for both the verbal and spatial versions of the task are expressed as the percentage of correct items. The data reported for the verbal portion of the task included the scores for the third trial of free recall and for delayed free recall.

2.7. Statistical Analyses

Group differences in intracranial volume, SI volume and performance on neuropsychological tests were evaluated using either a one-way analysis of variance (ANOVA) or a two factor repeated measures ANOVA followed by Scheffé's *post hoc* comparisons. A Chi-square test was carried out to compare APOE $\epsilon 4$ allele distribution and a t-test to determine volumetric differences based on the presence or absence of the $\epsilon 4$ allele. Pearson's correlations were obtained to assess the relationship between SI volume and performance on the declarative memory and frontal lobe dependent tasks. A *p*-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Demographic data

Demographic and other data for the three groups examined are presented in Table 1. Separate one-way ANOVAs revealed that there were no differences between the three groups in age or education. However, there was a significant group effect for MMSE scores [$F(2,76) = 33.9$, $p < 0.001$]. *Post hoc* comparisons revealed that both the aMCI and mild AD groups differed significantly from the NCI group ($p < 0.001$ for both), as well as from each other ($p = 0.001$).

3.2. Comparison of SI volumes among the groups

A one-way ANOVA demonstrated no significant differences in intracranial volume among the three groups. Mean normalized SI volumes derived from the MRI scans for the three groups are plotted in Fig. 2. Group differences in the volumes were assessed with a two factor repeated measures ANOVA, with group and hemisphere as between-subject and within-subject factors, respectively. The analysis showed significant group [$F(2, 76) = 19.63$, $p < 0.001$] and hemisphere [$F(1,76) = 9.98$, $p = 0.002$] effects, but no significant interaction between these factors. *Post hoc* comparisons demonstrated that the total mean SI volume was significantly reduced in the mild AD group compared to both the NCI ($p < 0.001$) and aMCI groups ($p < 0.001$). However, the NCI and aMCI groups did not differ from each other. The hemispheric effect corresponds to a slightly higher right SI volume compared to the left in the NCI, aMCI, and AD groups (by 1.31%, 3.20%, and 3.90%, respectively).

3.3. APOE $\epsilon 4$ status

APOE $\epsilon 4$ allele status was available for 72 of 79 participants (Table 1). Of the 72 individuals, 32 (44%) had one or more $\epsilon 4$ allele; the remaining 40 did not have an $\epsilon 4$ allele. Patients with mild AD were more likely to have an $\epsilon 4$ allele, compared to individuals with aMCI and control participants ($\chi^2[2] = 7.94$, $p = 0.019$). We compared SI volume based on the presence or absence of the $\epsilon 4$ allele and found no volumetric differences between the $\epsilon 4$ carriers and the non-carriers ($t = -0.561$, $df = 70$, $p = 0.58$).

3.3. Neuropsychological Tests

Group differences in performance on neuropsychological tests were assessed with separate one-way ANOVAs. There were significant group effects for declarative memory z -scores [$F(2,75) = 47.33$, $p < 0.001$] and frontal z -scores [$F(2,75) = 7.69$, $p = 0.001$]. *Post hoc* comparisons for the declarative memory z -scores demonstrated that both the aMCI and the mild AD groups differed from the NCI group ($p < 0.001$ for both), as well as from each other ($p = 0.002$), with the mild AD group having the lowest scores. However, for the frontal z -scores, the aMCI group and the mild AD group differed significantly from the elderly controls ($p = 0.043$ and $p = 0.001$, respectively), but not from each other.

Since there were significant group differences in the neuropsychological tests, we wanted to determine whether there was a relationship between the SI volumes and performances on these tests. Analysis showed a significant positive correlation between declarative memory z -scores and the total SI volume ($r = 0.353$, $p = 0.002$, see Fig. 3). The correlation between performance on the frontal lobe tasks and volume ($r = 0.22$, $p = 0.059$) approached but did not reach significance.

Since a decreased SI volume was associated with lower declarative memory z -scores, we examined whether the observed memory deficits were “genuine” or due to impairment in attention. Therefore, we evaluated performance on the Buschke task, which tests for

declarative memory function by controlling attention. Separate ANOVAs showed significant group effects for free verbal recall [$F(2,76) = 24.45$, $p < 0.001$], as well as delayed verbal recall [$F(2,76) = 35.17$, $p < 0.001$]. *Post hoc* comparisons indicated that for free verbal recall, both aMCI and mild AD groups differed significantly from the NCI group ($p < 0.001$ for both), but not from each other. However, for delayed verbal recall, both the aMCI and mild AD groups differed from NCI participants ($p < 0.001$ for both), as well as from each other ($p = 0.004$), with the mild AD group having the lowest score. There were also significant group effects for immediate spatial recall [$F(2,76) = 12.26$, $p < 0.001$] and delayed spatial recall [$F(2,76) = 16.22$, $p < 0.001$]. *Post hoc* comparisons demonstrated that both the aMCI and mild AD groups differed from the NCI participants ($p < 0.001$), but not from each other in both immediate and delayed spatial recall.

Examination of the relation between SI volume and performance on the Buschke controlled learning task showed a significant positive correlation between volume and free verbal recall ($r = 0.35$, $p = 0.002$), as well as delayed verbal recall ($r = 0.42$, $p < 0.001$). In the spatial version of the Buschke task, immediate recall of spatial location did not correlate with volume, but delayed spatial recall showed a significant positive correlation ($r = 0.23$, $p = 0.042$). It should be noted that in both the verbal and spatial measures, the delayed scores were more strongly related to volume than the free recall scores.

4. Discussion

The present study compared *in vivo* volumetric changes in the SI, which contains the cholinergic neurons of the nucleus basalis, in individuals clinically diagnosed with aMCI and mild AD, compared to healthy elderly subjects. The basal forebrain region evaluated extended from the crossing of the anterior commissure to the level of the emergence of this fiber bundle from the temporal lobe. The outline of the region of interest on MR images shows consistent concordance with the areas visualized on Nissl-stained sections of *post-mortem* human brain tissue (Fig. 1), as well as with the probabilistic maps of the nucleus basalis cholinergic cell group (Zaborsky et al., 2008). Volumetric analysis revealed a significant decrease in SI volume among patients with mild AD compared to control participants. In addition, volumes of the aMCI cohort differed significantly from the mild AD group, but not from NCI participants.

The present volumetric results showing SI atrophy in patients with mild AD support and expand previous *in vivo* MRI studies (Sasaki et al., 1995; Hanyu et al., 2002a; Hanyu et al., 2002b; Teipel et al., 2005; Hanyu et al., 2007; Hall et al., 2008; Moon et al., 2008; Muth et al., 2009). Some of the earlier investigations found significant atrophy of the SI in patients with AD by measuring its thickness on a single coronal slice at the level of the crossing of the anterior commissure using T2-weighted MRI scans (Sasaki et al., 1995; Hanyu et al., 2002a; Hanyu et al., 2007; Moon et al., 2008). Recently, Muth et al. (2009) manually traced three T2-weighted MRI images of the SI and found significant volumetric changes in AD. Since the anterior commissure atrophies in AD (Moon et al., 2008), it is possible that changes in this as well as other extra-innominate structures could affect the present as well as other volumetric analyses of the SI showing a decrease in volume in AD. This issue remains to be investigated in future studies. Others using automated voxel-based morphometry (VBM) demonstrated similar alterations in patients with AD (Teipel et al., 2005; Hall et al., 2008). In addition, a study using a longitudinal design combined with VBM showed basal forebrain atrophy prior to the onset of cognitive symptoms in normal elderly participants who converted to AD (Hall et al., 2008). However, VBM techniques are susceptible to processing artifacts, especially due to individual differences in brain anatomy that can confound the volumetric analysis of a small region such as the SI. Direct comparisons of automated analytic techniques with anatomically accurate manual volumetry

have demonstrated manual volumetry to be more accurate in detecting atrophy (Kennedy et al., 2009; Pardoe et al., 2009).

In contrast to some previous *in vivo* MRI studies (Sasaki et al., 1995; Hanyu et al., 2002a; Hanyu et al., 2002b; Teipel et al., 2005; Hanyu et al., 2007; Hall et al., 2008; Moon et al., 2008), the present investigation also evaluated whether SI atrophy occurs in people with a clinical diagnosis of aMCI, since such individuals are at high risk for developing AD (Petersen et al., 1999). Our MRI based volumetric analysis revealed that the aMCI group differed significantly from the mild AD group, but not from healthy elderly controls. This finding is in contrast to a recent report showing basal forebrain atrophy in individuals with aMCI compared to controls (Muth et al., 2009). The differences between the two studies may be related to the area of interest analyzed or the populations examined. For example, an analysis of the supplementary data provided by Muth et al. (2009) suggests that the most rostral level of the basal forebrain measured included the olfactory tubercle. Since the olfactory system undergoes degeneration early in the disease process (Devanand et al., 2000; Attems et al., 2006), the inclusion of the olfactory tubercle may account for the discrepancy between the two sets of findings. The present study focused exclusively on the basal forebrain region containing the SI.

The lack of change in SI volume in individuals with MCI in the present study is consistent with *post-mortem* tissue investigations showing the preservation of cholinergic neuron number in this region (Gilmore et al., 1999). Although there is no frank cell loss, these neurons undergo metabolic (Dubelaar et al., 2006) and chemical phenotypic (Counts et al., 2004) alterations, as well as neurofibrillary tangle formation (Mesulam et al., 2004) even at this early stage of the disease. The lack of reduction in the SI volume in people with a clinical diagnosis of aMCI compared to healthy aged subjects suggests that the pathological burden required to disrupt the viability of these cholinergic neurons situated within the SI does not occur at this stage of the disease.

The preservation of SI volume in individuals with aMCI suggests that the SI, unlike mesial temporal lobe structures, may not provide a sensitive MRI marker of risk of AD among non-demented individuals. Previous MRI-based studies have found a significant decrease in hippocampal and entorhinal cortex volumes among aMCI cases compared to the elderly controls (Jack et al., 2000; Killiany et al., 2002; Pennanen et al., 2004; Devanand et al., 2007; Stoub et al., 2008). In addition, entorhinal cortex volume was highly accurate in predicting the likelihood of conversion from aMCI to AD (deToledo-Morrell et al., 2004; Tapiola et al., 2008). These findings corroborate *post-mortem* tissue studies that confirm the early neuropathological involvement of entorhinal and transentorhinal cortex in individuals with MCI (Mufson et al., 1999; Kordower et al., 2001). Therefore, AD related pathology might start in the mesial temporal lobe structures and then progress to other regions of the brain, including the basal forebrain. Since our results demonstrate that SI atrophy occurs later in the disease process, MRI-derived volume of this region might not be as effective in predicting the likelihood of conversion from a non-demented status to AD. Interestingly, despite the observation that the current AD group had the highest proportion of the $\epsilon 4$ allele, a risk factor for AD (Corder et al., 1993), we found no difference in SI volumes based on the presence or absence of the $\epsilon 4$ allele, a result similar to that reported by Muth et al. (2009).

A major strength of the present study is the investigation of the relationship between SI volume and performance on neuropsychological tests that assess memory. Our initial analysis showed a positive relationship between SI volume and declarative memory z -scores with a decrease in SI volume being associated with impaired performance on declarative memory tasks. Since the memory deficits observed could be attributed to impaired attention, we also tested our subjects on the Buschke controlled learning task (Buschke & Grober,

1986; Grober & Buschke, 1987), which controls for attention, to determine whether SI volumetric changes were associated with “genuine” memory deficits or were a result of changes in attention. Our results revealed that free verbal recall, delayed verbal recall and delayed spatial recall scores in the Buschke task were all significantly related to SI volume. These findings indicate that SI atrophy is associated with “genuine” memory deficits. Although there was a trend towards an association between SI volume and performance on frontal lobe dependent tasks, the results were not significant. The lack of a significant association between decreased SI volume and performance on frontal lobe tests may be due to the type of tasks that were used in the clinical evaluations.

The observation that SI volume is associated with genuine memory deficits is supported by animal studies showing that selective neurotoxic cholinergic lesion of this region induces learning and memory deficits (Berger-Sweeney et al., 1994; Fine et al., 1997). Although our data lend support to a true memory deficit associated with SI volume changes, other animal studies suggest that this region regulates attentional processes, as well (Chiba et al., 1995; Baxter et al., 1995; Shen, et al., 1996; Han et al., 1999). Further studies are needed to clarify the relationship between SI volume and memory and attentional deficits during the progression of AD.

The relation between SI volume and declarative memory deficits reported here is not surprising since there is a dense cholinergic projection from the nucleus basalis to the basolateral amygdala (Mesulam et al., 1983; Selden et al., 1998; Mufson et al., 2003). Both the hippocampus and the entorhinal cortex, brain regions important for declarative memory function that become pathologically involved very early in AD, receive direct projections from the basolateral amygdala (Pikkarainen et al., 1999). Thus, the basolateral amygdala may modulate hippocampal dependent learning and memory.

In summary, the present MRI findings demonstrate a significant reduction in SI volume in the early stages of AD. More importantly, the preservation of volume in individuals with aMCI suggests that MRI analysis of this region may not be a sensitive biomarker of incident AD. However, more advanced imaging methods in the future may allow for a more detailed analysis of the SI and its relation to cognitive decline during the progression of AD. Overall, this is the first MRI-based study to analyze the relationship between volumetric changes in the SI and performance on behavioral tests that assess memory when attention is controlled, showing that decreased SI volume is associated with true memory decline and not attention deficits.

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Fig. 1.

(A) MRI scan showing the region of interest at the level of crossing of the anterior commissure (left). Corresponding Nissl-stained coronal section of the left hemisphere of a *post-mortem* aged control brain showing the region of interest (right). (B) MRI scan showing the caudal section at the level of the emergence of the anterior commissure from the temporal lobe (left). Corresponding Nissl-stained coronal section of the left hemisphere of a *post-mortem* brain showing the region of interest (right). The outline (white, black) illustrates the segmentation of the region containing the SI. ac: anterior commissure; cd: caudate nucleus; ic: internal capsule; oc: optic chiasm; pt: putamen; vgp: ventral globus pallidus.

Fig. 2.

Mean normalized total SI volumes at baseline of elderly individuals with no cognitive impairment (NCI), participants with amnesic mild cognitive impairment (aMCI) and patients with mild AD. The total volume includes both the right and left hemispheres. Vertical bars represent the standard error of the mean.

* Significantly different from NCI ($p < 0.001$); † significantly different from aMCI ($p < 0.001$).

Fig. 3. Relationship between the mean normalized total SI volumes and declarative memory z-scores. Separate symbols are used for each diagnostic group.

Table 1

Demographic and other characteristics of participants

	NCI	aMCI	Mild AD
<i>N</i>	27	33	19
Gender			
Male	10	13	6
Female	17	20	13
Age (in years)	76 ± 6.1	78 ± 8.1	77 ± 7.6
(Mean ± S.D.)			
MMSE	29 ± 0.9	26.8 ± 2.1 *	24.9 ± 1.6 **
(Mean ± S.D.)	(27–30)	(22–30)	(23–28)
(Range)			
Education (in years)	16 ± 2.6	16 ± 3.1	16 ± 6.0
(Mean ± S.D.)			
Intracranial volume	1529329 ± 1.4 × 10 ⁵	1526123 ± 1.4 × 10 ⁵	1512172 ± 1.3 × 10 ⁵
(Mean ± S.D.)			
APOE genotype			
4/4	0	0	1
3/4	7	12	11
3/3	15	16	5
2/4	0	1	0
2/3	4	0	0
2/2	0	0	0
Not available	1	4	2

* Significantly different from NCI ($p < 0.001$) and mild AD ($p < 0.001$).

** Significantly different from NCI ($p < 0.001$) and aMCI ($p < 0.001$).