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MRI-derived entorhinal and hippocampal atrophy in incipient and very mild Alzheimer's disease☆

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Abstract

With high resolution, quantitative magnetic resonance imaging (MRI) techniques, it is now possible to examine alterations in brain anatomy *in vivo* and to identify regions affected in the earliest stages of Alzheimer's disease (AD). In this study, we compared MRI-derived entorhinal and hippocampal volume in healthy elderly controls, patients who presented at the clinic with cognitive complaints, but did not meet criteria for dementia (non-demented), and patients with very mild AD. The two patient groups differed significantly from controls in entorhinal volume, but not from each other; in contrast, they differed from each other, as well as from controls, in hippocampal volume, with the mild AD cases showing the greatest atrophy. Follow-up clinical evaluations available on 23/28 non-demented patients indicated that 12/23 had converted to AD within 12–77 months from the baseline MRI examination. Converters could be best differentiated from non-converters on the basis of entorhinal, but not hippocampal volume. These data suggest that although both the EC and hippocampal formation degenerate before the onset of overt dementia, EC volume is a better predictor of conversion.

Keywords: Mesial temporal lobe; Parahippocampal gyrus; Aging; Mild cognitive impairment; Memory

1. Introduction

Quantitative, high resolution magnetic resonance imaging (MRI) techniques provide a unique tool for examining alterations in brain anatomy *in vivo* during healthy aging and in age-related degenerative diseases. Such techniques are especially useful in identifying the anatomical origins of Alzheimer's disease (AD).

The entorhinal cortex (EC) and the hippocampal formation (HF) are part of the mesial temporal lobe memory system [42,50]; the EC connects the neocortex with the HF via the perforant path, thereby providing the latter with multimodal sensory information. These brain regions have received special attention in investigations on the pathophysiology of AD, since memory dysfunction is one of its earliest hallmarks.

Post mortem pathological studies have implicated the EC and the transentorhinal region as early sites of involvement in AD and in individuals with mild cognitive impairment [4–6,18,21,35,46]. However, the EC has received less attention in *in vivo* investigations, partly due to the fact that MRI-derived quantitative protocols for this structure were only recently developed [2,22].

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Atrophy of the HF, a region important for the acquisition of certain types of new information (i.e., declarative knowledge), has been well documented in AD using quantitative volumetric MRI analyses [7,12–14,23,24,29–32,38,40,48,49]. More recently, atrophy of the EC was demonstrated in AD with MRI-based quantitation of its volume [27,49]. Yet, while *in vivo* anatomical investigations using structural MRI are rapidly proliferating, relatively few volumetric studies have focused strictly on patients in the earliest stages of AD or on those with mild cognitive impairment (see, for example, 31,49).

Two studies that measured the entire parahippocampal gyrus failed to detect significant atrophy in this structure in patients with a clinical diagnosis of *very mild* AD [14,24]. In these mild cases, however, there was significant hippocampal atrophy. These results were initially somewhat surprising, given the early pathological involvement of the entorhinal and transentorhinal cortices in the disease process. In the two studies cited above, parahippocampal gyrus volume included both white and gray matter and, in the case of the report from our laboratory [14], measurements continued beyond the anatomical boundaries of the entorhinal and perirhinal cortices. As a result, any changes in the EC itself may have been masked. It was, therefore, important to use newly developed protocols for the quantitation of the EC in order to re-examine its involvement in patients with very mild or incipient AD.

In addition to patients with very mild AD, individuals at high risk for AD are now being studied in order to identify anatomical changes that precede a clinical diagnosis and to develop sensitive *in vivo* markers that might be predictive of conversion to AD. One such group consists of those patients who have cognitive complaints, cognitive impairment, or both, but who do not meet diagnostic criteria for dementia after a thorough evaluation. Such groups are of special interest, since they provide information on the transitional state between normal aging and AD, although definitions and designations of this subgroup have varied [8,15,25,36,37]. A number of investigations have suggested that when followed longitudinally, these individuals are at increased risk for developing AD [1,3,9,15,31,36,43]. Furthermore, there is increased risk for incident dementia among elderly people with subjective (or informant corroborated) memory complaints, even if their baseline cognitive assessment is normal [17,26,39,44,45].

Of the few MRI studies that have examined the volumes of mesial temporal lobe structures in elderly patients with mild cognitive impairment or age-associated memory impairment, some have found hippocampal atrophy [7,10,25,49], while others have not [32,41]. Two recent investigations that assessed MRI-based entorhinal volume in such patients found significant EC atrophy compared to controls [31,49].

The present research was undertaken to compare the extent of MRI-derived hippocampal and entorhinal atrophy in very mild and incipient AD in an attempt to determine the earliest sites of pathological involvement in the disease process and to develop *in vivo* anatomical markers.

2. Materials and methods

2.1. Subjects

Data reported here were obtained from the following three groups of participants: 1) 34 healthy elderly normal control subjects (NC), 2) 28 patients who were evaluated for cognitive complaints, but who did not meet clinical criteria for dementia; they are referred to here as non-demented patients or ND, and 3) 16 patients with very mild probable AD. The major difference between the ND patients and the elderly controls is that the patients were recruited from a clinic where they were being evaluated for possible dementia, whereas the elderly controls were recruited from the general population and had to meet the criteria described below.

2.2. Clinical work-up

All evaluations were carried out at the Rush Alzheimer's Disease Center (RADC, Chicago, IL) as previously described [14,48]. Briefly, the evaluation incorporated the Consortium to Establish a Registry for Alzheimer's Disease (CERAD, 34) procedures and included a medical history, neurological examination, neuropsychological testing, informant interview and blood tests.

The clinical diagnosis of probable AD followed NINCDS/ADRDA guidelines [33]; 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. In the present study, we only included patients with a diagnosis of probable AD whose Mini Mental State Examination (MMSE, 17) score was $\geq$26 (i.e., those with very mild AD).

Patients in the non-demented group presented at the clinic with cognitive complaints, received the same standard evaluation as the AD patients, but did not meet clinical criteria for dementia. Exclusion criteria for both AD and ND patients were evidence of other neurologic, psychiatric or systemic conditions that could cause cognitive impairment (e.g., stroke, alcoholism, major depression). Neuropsychological test results indicated that the ND group included 13 members with isolated memory impairment, two members with impairment of attention, one with language deficits and 12 individuals without demonstrable cognitive deficits on clinical testing. It should be noted that no individual patient in this group had a deficit in more than one domain. Because elderly individuals with complaints of memory are at high risk of developing AD even if they do not show significant deficits on formal testing, we did not exclude those ND patients without demonstrable cognitive deficits for the purpose of this study.

Control subjects were recruited from friends and family members of patients and the RADC staff, as well as from hospital volunteers. Selection as an elderly control subject required a normal neurological examination, an MMSE score ≥28 and CERAD delayed list recall ≥6. Exclusion criteria for the controls were the same as those used for the patient groups. Informed consent was obtained from all subjects according to the rules of the Human Investigation Committee of Rush Medical College.

2.3. Acquisition and quantitation of MRI data

All MR images were acquired on a 1.5 Tesla General Electric Signa scanner. Gapless 5 mm coronal slices were taken perpendicular to the long axis of the hippocampal formation with the following parameters: matrix = 256 × 256, field of view = 16 cm, eight acquisitions, TR = 400, TE = 13–16. In addition, gapless 5 mm sagittal slices were taken spanning the entire brain with the following parameters: matrix = 256 × 128, field of view = 24 cm, one acquisition, TR = 200, TE = 12.[1]

Manual segmentation with a PC-based image analysis program (Amersham Image Analysis System with software designed by Loates Associates) was used to compute volumes of regions of interest. To correct for individual differences in brain size, entorhinal and hippocampal volumes were normalized by dividing with intracranial volume derived from sagittal slices. To compute intracranial volume, the inner table of the cranium was traced in consecutive sagittal sections spanning the entire brain. At the level of the foramen magnum, a straight line was drawn from the inner surface of the clivus to the most anterior extension of the occipital bone.

EC volume was quantified with the use of a new protocol developed and validated in our laboratory, technical details of which are presented in Goncharova et al. (19, accompanying manuscript). The advantage of this protocol is that EC volume is measured from the same oblique coronal sections most commonly used for hippocampal volumetry to avoid overestimation of one of these two adjacent structures at the expense of the other.

Briefly, both entorhinal and hippocampal volumes were computed separately for the right and left hemispheres from coronal slices taken perpendicular to the long axis of the HF. For the EC, tracing began with the first section in which the gyrus ambiens, amygdala and the white matter of the parahippocampal gyrus first appeared visible. The superomedial border in rostral sections was the sulcus semiannularis and in caudal sections the subiculum. The shoulder of the collateral sulcus was used as the lateral border. The latter is somewhat of a conservative criterion that allowed consistency in tracings and avoided the use of different lateral borders depending on individual differences in the depth of the collateral sulcus (see, for example, ref. 22). In the majority of cases, tracings were carried out on 4–5 sections. Validation procedures, as well as inter- and intra-rater reliability scores for EC measurements are described in Goncharova et al. (19, accompanying paper).

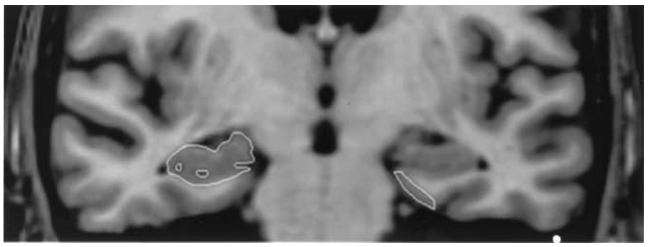

Fig. 1. A single coronal slice illustrating an example of the tracings used to measure the volumes of the entorhinal cortex (right) and hippocampal formation (left).

The protocol and validation procedures used for quantifying hippocampal volume were published previously [14, 48]. Tracings of the HF started with the first section where the dentate gyrus could be clearly identified, and included the fimbria, dentate gyrus, the hippocampus proper and the subiculum. All sections in which the hippocampus could be clearly seen without partial volume averaging were included (usually 6–7 slices).

Fig. 1 shows sample tracings of both the EC and HF from a single MR image.

2.4. Statistical analyses

Group differences were evaluated with the use of either one-way or two-way repeated measures analyses of variance (ANOVA) followed by post-hoc comparisons with the studentized range test. Logistic regression analyses were performed to determine how well hippocampal and entorhinal volume could predict group membership.

3. Results

As indicated above, all patients in the ND group sought formal evaluations due to complaints of cognitive decline. Because there were no significant differences in EC or HF volumes based on the presence or absence of cognitive deficits on formal testing (data not shown), the group of ND patients was considered as a whole in all subsequent analyses.

Demographic and MMSE data are presented in Table 1. Separate one-way ANOVAs showed that the three groups did not differ in age or level of education. There was, however, a significant group effect for MMSE scores [$F(2,75) = 18.0$, $p < 0.001$]. Pairwise comparisons indicated that the non-demented patients and those with a clinical diagnosis of mild AD differed significantly from controls ($p < 0.05$), but not from each other.

Mean normalized entorhinal and hippocampal volumes for the three groups of participants are plotted in Figs. 2 and 3 respectively. Group differences in the volumes of the two

Table 1
Demographic characteristics of participants

	Elderly controls	Patients with very mild AD	Non-demented patients
N	34	16	28
Age (in years)	70.3 ± 6.6 (61–84)	71.4 ± 9.1 (49–82)	68.6 ± 8.6 (51–82)
Education (in years)	13.6 ± 2.7	14.5 ± 2.9	15.2 ± 3.1
Female/Male	20/14	12/4	9/19
MMSE Score	29.2 ± 0.7	27.3 ± 1.1*	27.0 ± 2.2*

* Significantly different from controls ($p < 0.05$).

regions of interest were assessed with separate two-way repeated measures ANOVAs with group and hemisphere as the two factors. The analysis on entorhinal volume showed significant group [$F(2,75) = 15.73$, $p < 0.001$] and hemisphere [$F(1,75) = 13.11$, $p < 0.001$] effects, but no significant interaction between them. The hemisphere effect can be accounted for by a larger right EC in all three groups. Pairwise comparisons indicated that both the ND patients and those with mild AD differed significantly from controls in total EC volume ($p < 0.05$), but not from each other.

The ANOVA on hippocampal volume also showed significant group [$F(2,75) = 16.65$, $p < 0.001$] and hemisphere [$F(1,75) = 14.02$, $p < 0.001$] effects, without a significant interaction between them. However, in this case, pairwise comparisons demonstrated that the two patient groups differed in total hippocampal volume from controls, as well as from each other ($p < 0.05$), with the very mild AD group showing the greatest atrophy.

Logistic regression analyses were then performed to determine how well hippocampal and entorhinal volume could predict group membership. In these analyses, total EC and HF volumes were used as predictors to determine the extent to which each region of interest contributed separately to describing group differences with models used for two groups at a time. Scatter plots showing the logistic regression lines for two groups at a time are displayed in Fig. 4.

The results of the logistic regressions were consistent with the ANOVA findings. EC volume was better than hippocampal volume at predicting membership in the ND group compared to controls, even with hippocampal volume

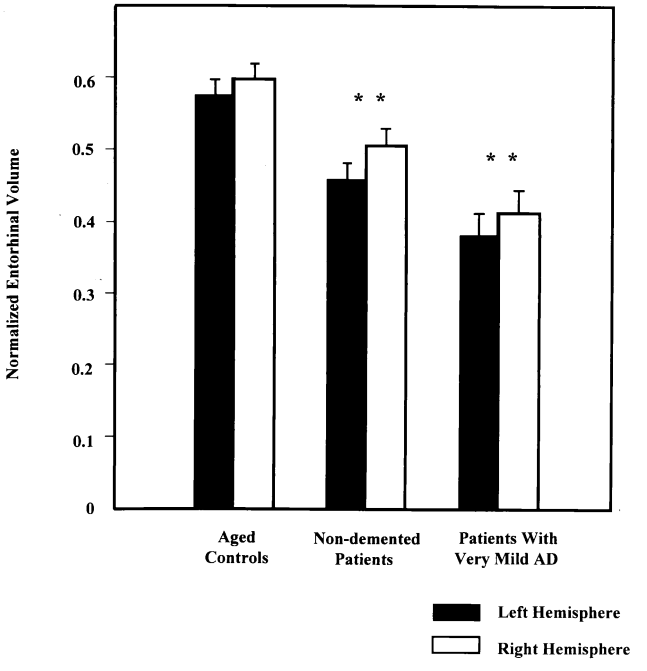

Fig. 2. Mean normalized entorhinal volume (absolute volume in mm^3/intracranial volume in $mm^3 \times 1000$) for elderly control subjects, patients with very mild AD and those who did not meet criteria for dementia. Volumes are shown for each hemisphere separately. Vertical bars represent the standard error of the mean. Note that the two patient groups were significantly different from controls (indicated by **), but not from each other.

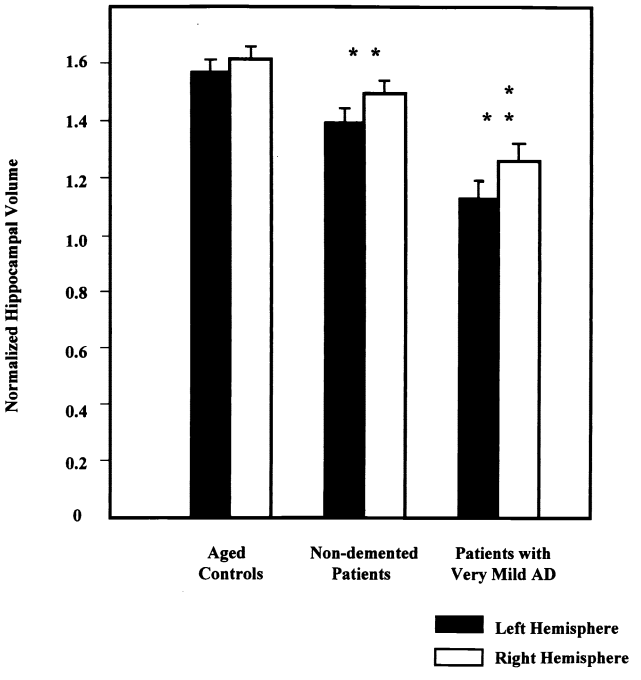

Fig. 3. Mean normalized hippocampal volume (absolute volume in mm^3/intracranial volume in $mm^3 \times 1000$) for the three groups of subjects plotted as a function of hemisphere. Vertical bars represent the standard error of the mean. Note that the two patient groups were significantly different from controls (indicated by **) and from each other (indicated by *).

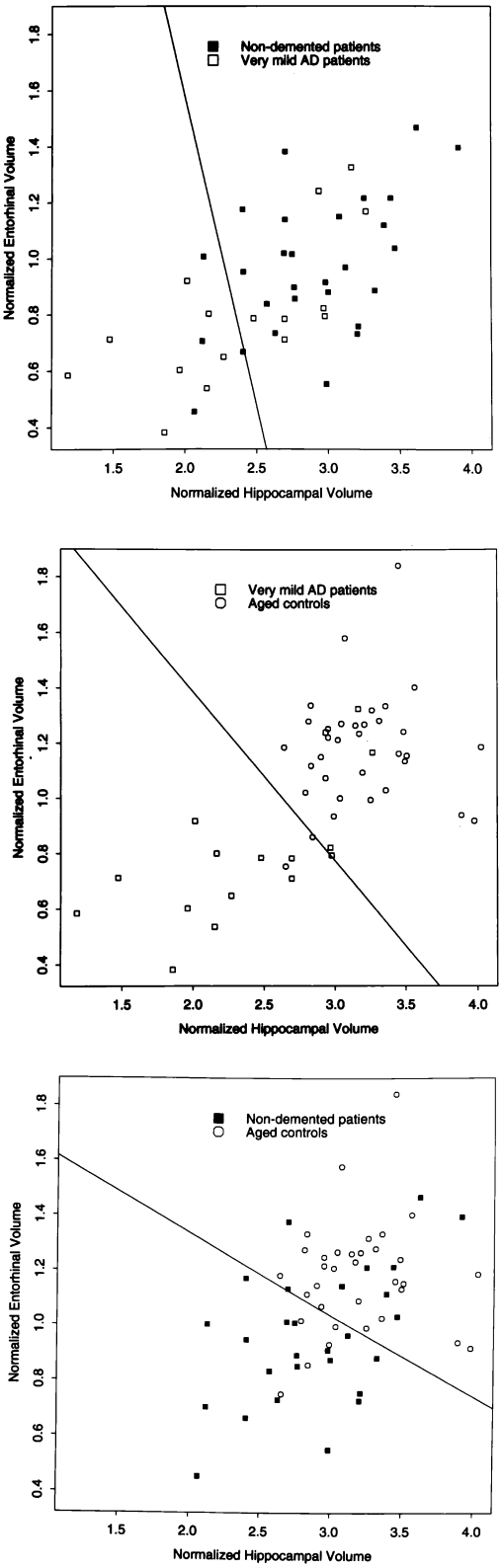

Fig. 4. Scatterplot of total entorhinal and hippocampal volume (absolute volume in mm^3/intracranial volume in $mm^3 \times 1000$) shown for two groups at a time, including regression line derived from logistic regression models using both volumes. Upper panel: elderly control subjects and non-demented patients; middle panel: non-demented patients and those with very mild AD; lower panel: control subjects and patients with very mild AD.

Table 2
Follow up period in months for each of the converters and non-converters

	Non-Converters		Converters
1	11.97	1	20.10
2	15.25	2	21.51
3	29.05	3	23.15
4	30.92	4	27.80
5	32.82	5	29.51
6	33.05	6	32.39
7	41.21	7	32.39
8	51.31	8	34.53
9	56.39	9	56.46
10	56.82	10	59.21
11	58.03	11	63.48
		12	77.21

in the model. In this case, hippocampal volume did not add significantly to predictions based on EC volume; the latter alone correctly classified 69% of all subjects in the two groups ($X^2 = 12.7$, $p < 0.001$).

In contrast, when comparing patients with very mild AD to ND cases, the prediction based on hippocampal volume alone suggested improvement over the prediction based on EC volume alone ($X^2 = 3.76$, $p = 0.052$). Entorhinal volume did not contribute any additional ability to classify patients with AD. Again, the volume of each area of interest predicted group membership significantly when examined alone, but more subjects could be classified accurately using hippocampal volume (75%; $X^2 = 8.64$, $p = 0.003$).

The comparison of patients with very mild AD and control subjects indicated that both entorhinal and hippocampal volume contributed significantly to the model. The model with both predictors classified 86% of the cases correctly ($X^2 = 29.8$, $p < 0.001$).

The results of the logistic regression analyses did not change when adjusted for age or sex. Taken together, they indicate that the primary difference between the controls and ND patients is in entorhinal volume, while hippocampal atrophy plays a more important role in differentiating AD patients from the ND cases.

Follow-up clinical evaluations available on 23 of the 28 ND participants included in this study indicated that 12 of the 23 had converted since the initial clinical and MRI evaluation and received a clinical diagnosis of probable AD. The follow-up period ranged from 12 to 77 months (mean, 39), and was equivalent for converters and non-converters [$t(22) = 0.63$, $p > 0.05$. See Table 2)]. Entorhinal and hippocampal volumes derived from the baseline MRI showed that converters differed significantly from non-converters in total (right + left) entorhinal volume [$t(22) = 2.94$, $p < 0.008$], but not hippocampal volume (see Fig. 5). Further, in logistic regressions using total hippocampal and entorhinal volume as predictors, only EC volume was a significant predictor of the likelihood of conversion (odds ratio = 0.993 per normalized unit volume, $p = 0.046$).

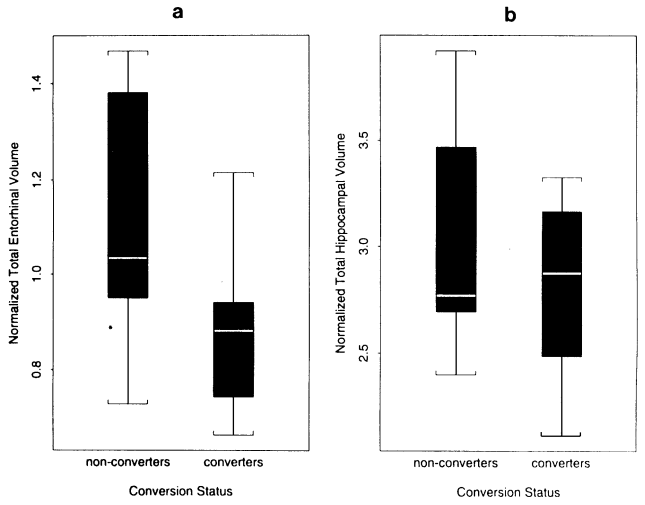

Fig. 5. Box plot comparing MRI-derived entorhinal (left hand side) and hippocampal (right hand side) volume in non-demented participants who converted to a diagnosis of Alzheimer's disease, in contrast to those who did not. The central box shows the data between the upper and lower quartiles, with the median represented by the line. The height of the line is the interquartile range (IQR); the "whiskers" extend from the upper and lower quartiles to a distance of 1.5 IQR away or to the most extreme data point within that range, whichever is closer.

Thus, for every 10 unit decrease in EC volume, the chances of converting to AD increased by 7%. Results were not changed by adjustment for age or follow-up interval. When entorhinal volume alone was used as a predictor, 83.3% of converters and 72.7% of non-converters were correctly classified.

It is interesting to note that among the 12 ND patients who converted, 9 had a memory impairment, 2 had attentional deficits and one did not show any demonstrable cognitive deficits on clinical testing. Of the 11 non-demented participants who did not convert to AD, four had a memory impairment, and seven had no demonstrable cognitive impairment on clinical testing.

4. Discussion

A major strength of our study is the inclusion of extremely mild cases of AD and a comparison of the anatomical changes detected *in vivo* in these mild cases with those observed in patients who do not meet diagnostic criteria, but are at risk for AD.

Individuals at risk for AD are of interest since they provide valuable information on the transitional state between normal aging and AD. Different investigators have used different definitions to characterize this population, although recently criteria for mild cognitive impairment have been more stringently formulated [36,37]. These criteria include objective memory impairment. Thus, the difference between the ND group in the present study and mild cognitive impairment as defined by Petersen and colleagues [36,37] is that we included some individuals who presented at the clinic with subjective cognitive complaints, but did not show objective deficits. Our reason for including them was that they have been shown to be at high risk for AD as well [17]. Although we recognize the limitations of our approach, it is of interest to note that the rate of conversion observed in the ND group in this study is very similar to the rate reported by Petersen [36] for those diagnosed with mild cognitive impairment.

The results described above showed that both patient groups (mild AD and ND) differed from controls in entorhinal volume, but not from each other. In contrast, the two patient groups differed from controls in hippocampal volume, as well as from each other, with the AD cases showing the greatest atrophy. Taken together, these findings suggest that degeneration of the EC and HF occurs before the onset of obvious dementia and that HF atrophy is associated with the progression from mild subjective or objective cognitive impairment to AD. Although the ND group showed significant atrophy in both structures, logistic regression models demonstrated that entorhinal volume was better than hippocampal volume in differentiating these patients from controls, suggesting that the EC becomes pathologically involved before the HF. On the other hand, the extent of hippocampal atrophy was better at differentiating patients with very mild AD from ND cases; atrophy of both structures played an equivalent role in differentiating patients with very mild AD from controls.

The lack of a significant difference between the two patient groups in entorhinal volume and the fact that the ND cases could best be differentiated from controls by EC volume further suggest that MRI-based entorhinal atrophy may provide an *in vivo* marker of incipient AD. This suggestion is strongly supported by the fact that converters to AD among the ND participants could be best differentiated from non-converters on the basis of entorhinal volume.

Our findings on hippocampal atrophy in patients with very mild AD are in general agreement with most of the published literature to date. In numerous studies on MR-based volumetric estimates of mesial temporal lobe structures in AD, HF atrophy has been shown to be one of the most robust and consistently documented findings [11,12, 14,23–25,28–30,32,40,48,49]. However, only a few groups have focused their investigations on patients with very mild AD.

The literature is less clear cut with respect to alterations in hippocampal volume in patients with isolated memory complaints or mild cognitive impairment compared to controls. Our finding of hippocampal atrophy in the ND group is consistent with results reported by two other laboratories [7,25,49]. However, two studies published by another group failed to find significant hippocampal atrophy in such patients [32,41].

A likely reason for this inconsistency is selection criteria. Most groups reporting on MR volumetric studies of elderly patients with mild cognitive impairment (or age-associated memory impairment) have selected their subjects by neuro-

psychological test scores; some have focused on clinic populations, while others have studied community-derived samples. The investigators reporting an absence of significant hippocampal atrophy obtained their sample from a community-based population, which was later shown to have only a 10% incidence of dementia in 3.5 years of follow-up [20]. Therefore, it is likely that their group contained a greater number of subjects who were not in the incipient phases of AD at the time MRI scans were acquired.

Our volumetric results on EC atrophy in AD are in agreement with those reported by other laboratories [27,31, 49]. They are also in agreement with two very recent papers reporting that individuals with mild memory impairment show significant atrophy of the EC compared to controls [31,49]. However, Killiany et al. [31] found that among the participants with mild memory impairment, what discriminated converters from non-converters was the volumes of the superior temporal sulcus and anterior cingulate, but not the EC. One possible factor contributing to the difference between our findings on converters and theirs may be the way in which EC volume was derived. The estimate of EC volume in the Killiany et al. [31] paper was based on tracings from three 1.5 mm coronal sections, while ours was based on a much larger portion of the structure.

In summary, the *in vivo* anatomical results presented here are in agreement with *post mortem* pathological studies and underscore the early involvement of the entorhinal cortex in AD. The results further suggest that the progression to a diagnosis of AD requires significant atrophy of the HF in addition to the EC. Taken together, our findings demonstrate the potential of sensitive neuroimaging techniques for the development of early anatomical markers of AD. Such predictive biological markers could be of great help for the development and monitoring of early interventions designed to retard disease progression.

Acknowledgment

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Notes

1. We now use a new protocol that acquires 1.6 mm coronal images of the entire head with an SPGR pulse sequence. For purposes of consistency and to have large enough group sizes, the present study was restricted to those patients and controls scanned with our old protocol.

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