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Atrophy of presubiculum and subiculum is the earliest hippocampal anatomical marker of Alzheimer's disease

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Abstract

Background: There is no consensus about which hippocampal subfields become atrophic earliest in the course of Alzheimer's disease (AD).
Methods: Thirty AD patients, 41 mild cognitive impairment (MCI) patients, and 38 healthy controls (HCs) underwent cerebral magnetic resonance imaging (with an automated segmentation protocol for the volumetric analysis of hippocampal subfields) and a test of immediate and delayed recall of a 15-word list.
Results: The volumes of the presubiculum and subiculum presented the most remarkable reduction in the patient's groups. In the MCI group, only the volumes of presubiculum and subiculum predicted performance on the memory tests. In AD patients, the volumes of all hippocampal subfields (with the notable exception of the CA1) predicted memory scores.
Conclusions: Our data point to a prevalent atrophy of the presubicular-subicular complex from the early phases of AD. This finding is consistent with neuropathological observations in AD patients and probably reflects the severe degeneration of the perforant pathway while penetrating the hippocampus through the subicular field in its course from the entorhinal cortex to the dentate gyrus.

Keywords: Alzheimer's disease; Mild cognitive impairment; Memory; Structural MRI; Brain atrophy

1. Introduction

Neuropathological changes in patients with Alzheimer's disease (AD) typically affect the hippocampal formation very early [1,2]. Therefore, neuroradiological indexes of hippocampal atrophy [3,4] and, consistent with the well-known role of the hippocampus in human declarative memory [5], episodic memory deficits [6,7] are among the most powerful diagnostic indexes of AD from the very early phases, corresponding to the clinical condition of amnestic mild cognitive impairment (MCI).

The hippocampus is not, however, a unitary anatomical formation. Several subfields have been identified, which can be differentiated on both histological grounds [2,8] and according to their functional role in memory encoding and retrieval [9,10]. There is no consensus about which subfields become atrophic earliest in the disease course or which neuropathological change is primarily implicated in volume reduction. According to some authors, neuronal loss is the main cause of atrophy. However, the three studies that provided neuronal counts in the hippocampal subfields of patients with AD report discrepant data. West et al. [11,12] found the most striking AD-related neuronal loss at the level of the CA1 subfield, whereas Simic et al. [13] reported a reduced number of neurons in the subiculum and dentate gyrus of AD patients but not in other subfields. On the other side,

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neuronal loss might not be the only or even the main reason for regional volume reduction of the hippocampus in AD. Indeed, Rössler et al. [14] found a very weak correlation between the observed volume reduction and neuronal loss in the overall hippocampus of AD patients, and Mizutani and Kasahara [15,16] found that hippocampal atrophy in AD was mainly caused by degeneration of the stratum lacunosum-radiatum, which contains the perforant pathway that originates in the entorhinal cortex, rather than neuronal loss in the pyramidal layer of the hippocampus.

The aims of this cross-sectional study were to delineate the in vivo progression of atrophy in the main subfields of the hippocampus in patients diagnosed with either MCI or AD, and to determine whether atrophy in one or more specific hippocampal subfields best predict severity of episodic memory impairment.

2. Materials and methods

2.1. Subjects

A cohort of 109 individuals, 30 with a diagnosis of probable AD, 41 with diagnostic characteristics of single-domain amnestic MCI, and 38 healthy matched controls (HC), were enrolled in this study. AD and MCI patients were consecutively recruited from the specialist dementia clinics of Santa Lucia Foundation (Rome, Italy).

HCs were recruited from patients' relatives or through local advertisements. None of them showed cognitive problems or evidence of cognitive deficits on neuropsychological testing.

Patients with AD met the clinical criteria for Alzheimer's dementia established by the National Institute on Aging and the Alzheimer's Association [17].

Diagnosis of single-domain amnestic MCI was made according to established criteria [18] by trained neurologists. Inclusion criteria were as follows: (1) subjective memory impairment confirmed by a pathological score on at least one memory test of the neuropsychological battery; (2) nonfulfillment of the criteria for dementia according to the recommendations of the National Institute on Aging-Alzheimer's Association work groups [17]; (3) preserved general cognitive functions as confirmed by normal scores on the Mini-Mental State Examination (MMSE) (normality cutoff score, 24 [19]) and on tests of the neuropsychological battery; (4) no or very mild impact of the memory deficit on the subject's activities, as confirmed by a normal score on the instrumental activities of daily living and by a total Clinical Dementia Rating (CDR) score = 0.5; (5) lack of any evidence of neurological or systemic pathology able to induce memory disorders; (6) brain magnetic resonance imaging (MRI) negative for focal lesions (minimal diffuse changes or minimal lacunar lesions of white matter were admitted) [20]; and (6) absence of moderate to severe depression and/or anxiety, as confirmed by scores on Beck's Depression Inventory and the Hamilton Anxiety Rating scale (14 was the highest acceptable score for both scales). Finally, for a subsample of 46 patients (12 AD and 34 MCI patients), the investigation of *APOE* ε4 allele frequency was carried out, whereas for the HCs, no genetic data were available.

The principal demographic and clinical characteristics of the studied subjects are summarized in Table 1.

After the procedures were explained to them, the subjects gave their written informed consent in a protocol approved by the Joint Ethics Committee of the Fondazione Santa Lucia.

2.2. Neuropsychological examination

Consistent with the hypothesis of the study, two declarative memory tests were selected from the comprehensive neuropsychological battery administered to all participants: the immediate and the 15-minute delayed recall of a 15-word list.

In the 15-word list learning test [21], the examiner reads 15 words aloud (at a rate of 1 word/s) five times; immediately

Table 1
Number of females and males (F/M) and mean (and standard deviation) of other demographic, clinical, and genetic characteristics of the three groups of participants

Groups	AD (n = 30)	MCI (n = 41)	HC (n = 38)	Chi-square	*P*
F/M	16/14	16/25	22/19	1.7	.44
				$F_{2,106}$ (df)	
Age	71.2 (7.3)	70.6 (6.8)	69.7 (4.4)	0.5	.61
Years of formal education	8.4 (4.1)*,†	11.7 (4.1)	11.4 (4.3)	6.3	.003
MMSE (adjusted score)	19.7 (3.8)*,†	26.3 (2.3)*	27.2 (1.7)	102.0	.0001
15-Word list test					
Immediate recall	18.3 (9.4)*,†	26.6 (6.2)*	42.7 (8.3)	78.5	.0001
Delayed recall	1.5 (2.7)*,†	3.3 (2.7)*	9.0 (2.8)	72.8	.0001
APOE, n (%)	AD (n = 12)	MCI (n = 34)			
APOE ε2/ε3	0 (0)	1 (3)	—		
APOE ε3/ε3	10 (83)	20 (59)	—		
APOE ε3/ε4	2 (17)	13 (38)	—		

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; HC, healthy control; MMSE, Mini-Mental State Examination.
*Significantly less than in the HC group.
†Significantly less than in the MCI group.

after each presentation, the patient is asked to recall as many words as possible in any order (immediate recall, score range: 0–75). After a 15-minute interval, the patient is asked to recall as many words as possible (delayed recall, score range: 0–15).

The neuropsychological screening battery included tests of logical reasoning (Raven's Coloured Progressive Matrices [21]), language (Sentence Construction [21]), selective attention and resistance to interference (Stroop test [22]), concept formation and shifting (Modified Card Sorting Test [23]), and constructional praxis and visuospatial abilities (copy of the Rey-Osterrieth Complex Figure Test [24]).

2.3. MRI protocol

Participants underwent an imaging protocol which included three-dimensional (3D) T1-weighted, T2-weighted, and FLAIR sequences, using a 3-T Allegra MR imager (Siemens, Erlangen, Germany) with a standard quadrature head coil. Whole-brain T1-weighted images were obtained in the sagittal plane using a modified driven equilibrium Fourier transform sequence (echo time/repetition time = 2.4/7.92 ms, flip angle 15°, voxel size $1 \times 1 \times 1$ mm^3).

The hippocampal segmentation was carried out by using two successive methods. The whole hippocampus was initially segmented by completing the FreeSurfer image analysis pipeline (Martinos Center for Biomedical Imaging, Boston, MA, USA) [25]. In brief, the processing relevant to this work includes removal of nonbrain tissue by using a hybrid watershed/surface deformation procedure [26], automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures. In subjects with substantial anatomical differences with respect to the template, for example, enlarged ventricles, the resulting segmentation of the subcortical structures was improved by a pairwise registration of their images to training images [27]. Next, segmentation of the hippocampus into its respective subfields was performed by using Bayesian inference and a previously described statistical model of the medial temporal lobe [28]. The Dice overlap measures between manual and automated segmentation methods were approximately 0.7 for all the substructures [28]. We focused on the volume of the CA1, CA2/3, CA4/dentate gyrus, presubiculum, and subiculum and excluded from the analyses the fimbria (which is a white matter region), hippocampal fissure, and the final portion of the hippocampal tail, which is not subdividable in any of the subfields. Total hippocampal volume was computed by summing relative volumes of the CA1, CA2/3, CA4/dentate gyrus, presubiculum, subiculum, and hippocampal tail.

2.4. Statistical methods

For statistical analysis, volumes of the left and right hippocampus and volumes of the left and right individual subfields of each subject were averaged. Chi-square test for binomial variables and one-way analyses of variance (ANOVAs) for discrete variables were used to compare the demographic characteristics of the three groups of participants. One-way analyses of covariance (ANCOVAs) with years of formal education as a covariate were used to compare participants' performance scores on the MMSE and memory tests and on hippocampal volumes. When the group factor was significant, least significant difference post hoc tests were performed to qualify the main effect. A two-way ANCOVA with the group (AD vs. MCI) as between factor and hippocampal subfield (CA1 vs. CA2-3 vs. CA4-DG vs. presubiculum vs. subiculum) as within factor with years of formal education as a covariate was performed to directly compare the rate of volume decrease of individual hippocampal subfields among patients. Because the absolute volume of the hippocampal subfields differed greatly, standardized z values (calculated over the mean and standard deviation of the HC group) were used as dependent variable. To evaluate the accuracy of hippocampal subfield volumes and of the overall hippocampal volume in assigning participants to their own group, receiver operating characteristic (ROC) analyses were performed. Finally, a number of partial correlation coefficients were calculated between hippocampal volumes and memory measures, controlling for years of formal education separately for the three groups of participants.

3. Results

3.1. Demographic and clinical characteristics

As can be seen in Table 1, the three groups (AD, MCI, and HC) did not differ for sex distribution or age. Instead, they differed for years of formal education, with AD patients significantly less educated than MCI and HC participants. The three groups also differed for the mean MMSE adjusted score. Indeed, AD patients scored worse than the other two groups and patients with MCI scored worse than HCs. Finally, as for *APOE* genotypes, the ε4 allele was present in 17% of AD patients and in 38% of MCI patients who underwent the genetic investigation.

3.2. Memory performance

Average performance scores of the three groups of participants on the 15-word learning list test with relative statistical comparisons are reported in Table 1. The three groups differed on both the immediate and delayed recall test, with AD patients scoring worse than MCI patients who, in turn, scored worse than HCs.

3.3. Hippocampal subfields

Table 2 reports volumes of different hippocampal subfields and of the overall hippocampus in the three groups of participants with relative group comparisons. One-way ANOVAs documented that, with the exception of CA1, the volume of all the other subfields, as well as of the entire hippocampus, significantly differed among groups. Post hoc analyses documented that although both groups of patients significantly differed from HCs for volumes of the CA2-3,

Table 2
Mean volumes (and SD) of hippocampal subfields in the three groups of participants

Groups	HC (n = 38), volume (mm^3)	MCI (n = 41), volume (mm^3)	AD (n = 30), volume (mm^3)	$F_{2,106}$ (df)	*P*
CA1	305.2 (41.4)	288.0 (52.4)	283.6 (60.5)	1.4	.25
CA2-3	841.1 (125.8)	773.7 (160.4)*	723.3 (164.8)**	4.6	.01
CA4-DG	478.6 (69.5)	430.5 (93.4)*	393.7 (93.8)**	7.4	.0009
Presubiculum	411.4 (59.2)	355.3 (73.9)**	316.2 (70.1)**,***	15.5	.0001
Subiculum	534.0 (67.1)	472.3 (100.0)**	425.1 (100.5)**,***	11.5	.0001
Overall hippocampus	2980.2 (390.4)	2686.8 (528.6)*	2475.2 (535.5)**	8.3	.0004

Abbreviations: SD, standard deviation; HC, healthy control; MCI, mild cognitive impairment; AD, Alzheimer's disease.
*$P < .05$ with respect to HCs.
**$P < .01$ with respect to HCs.
***$P < .05$ with respect to MCI patients.

CA4-DG, presubiculum, subiculum, and overall hippocampus, patients with AD had reduced volumes of the presubiculum and subiculum with respect to MCI patients.

Fig. 1 shows standardized *z* values of hippocampal subfield volumes in the two groups of patients. The two-way ANOVA documented a nonsignificant main effect of the group ($F = 2.0$; $P = .17$) but highly significant effects of hippocampal subfield ($F = 35.0$; $P < .001$) and of the group × hippocampal subfield interaction ($F = 4.2$; $P = .002$). In particular, the significant effect of the hippocampal subfield was due to a volume reduction (with respect to HCs) that was minimal in the CA1 subfield, progressively larger in the CA2-3 and CA4-DG subfields, and maximal in the presubiculum and subiculum (all comparisons highly significant at the least significant difference test, with the exception of the presubiculum vs. subiculum, $P = .94$). Instead, the significant group × hippocampal subfield interaction reflected the nonhomogeneous volume decrease in the AD with respect to the MCI group across the different subfields. Indeed, planned comparisons confirmed a nonsignificant difference in volume reduction in the CA1 ($P = .74$), CA2-3 ($P = .20$), and CA4-DG ($P = .11$) subfields but a significantly larger volume decrease in the AD than in the MCI group in the presubiculum ($P = .02$) and subiculum ($P = .05$).

3.4. Discriminative power of hippocampal subfield volume

Table 3 reports the results of the ROC analyses for each hippocampal subfield volume and for the overall hippocampal volume discriminating the three groups of participants. In all cases, the presubiculum produced the largest area under the curve and the highest diagnostic sensitivity/specificity values, followed by the subiculum, the overall hippocampal volume, the CA4-DG, CA3-DG, and finally, the CA1 subfield. Volumes of all subfields and total hippocampal volume discriminated AD patients and HCs at a significant level; overall hippocampal volume and volume of all subfields, with the exception of CA1, discriminated significantly between MCI patients and HCs; only the presubiculum and subiculum subfield volumes significantly discriminated AD from MCI patients.

3.5. Correlation of hippocampal subfield volumes with memory performance

Table 4 reports the correlation matrices of the hippocampal subfield volumes with performance scores on memory tests separately for patients with AD, patients with MCI, and HCs.

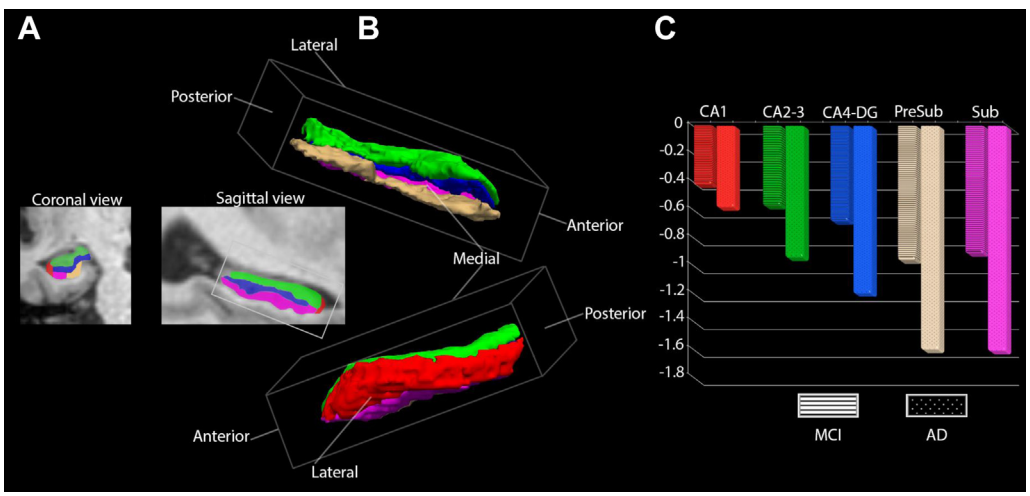

Fig. 1. Subfield volumes in the MCI and AD samples. The figure shows a representative subfield segmentation of the left hippocampus of one subject (A), its 3D reconstruction (B), and the graph showing standardized *z* values of subfields volumes in the two samples (C). Sub, subiculum; PreSub, presubiculum; MCI, mild cognitive impairment; AD, Alzheimer's disease.

Table 3
Results of the ROC analyses discriminating the group membership of participating individuals

	HC vs. MCI			MCI vs. AD			HC vs. AD		
Groups	AUC	Sens.	Spec.	AUC	Sens.	Spec.	AUC	Sens.	Spec.
CA1	0.60	0.61	0.59	0.54	0.70	0.50	0.65*	0.76	0.60
CA2-3	0.64*	0.66	0.61	0.59	0.59	0.55	0.72**	0.74	0.70
CA4-DG	0.68**	0.82	0.56	0.60	0.70	0.50	0.75**	0.71	0.73
Presubiculum	0.73**	0.79	0.61	0.65*	0.71	0.60	0.84**	0.82	0.83
Subiculum	0.71**	0.79	0.59	0.64*	0.68	0.57	0.81**	0.79	0.73
Overall hippocampus	0.69**	0.74	0.61	0.62	0.71	0.47	0.77**	0.79	0.70

Abbreviations: ROC, receiver operating characteristic; HC, healthy control; MCI, mild cognitive impairment; AD, Alzheimer's disease; AUC, area under the curve; Sens., diagnostic sensitivity; Spec., diagnostic specificity.
*$P < .05$.
**$P < .01$.

Although in the HC group several correlation coefficients were negative, in the AD and MCI groups, all correlation coefficients were positive, indicating that in these patients, reduced anatomical volumes were consistently associated with reduced memory performance. In the HC group, no correlation coefficient was significant. Conversely, in the AD group, several subfields and the overall hippocampus correlated with memory scores. Notably, the only subfield that did not show any significant correlation with verbal memory measures was the CA1. In the MCI group, the only significant correlations involved the presubiculum, subiculum, and overall hippocampus.

4. Discussion

Results of the present study document progressive hippocampal atrophy in the different phases of AD that is not homogeneous across the various subfields. The CA1 subfield was the one least involved in the AD-related atrophy. Its size did not significantly differ in HCs, patients in the initial phases of the Alzheimer's degeneration, and patients with fully developed AD. The CA2-3 and CA4-DG subfields presented atrophic changes that remained substantially stable during the progression of AD pathology. Indeed, CA2 and CA3 volumes were significantly smaller in the AD and MCI groups with respect to the HC group but did not significantly differ between the patients' groups. Finally, atrophic changes in the presubiculum and subiculum progressively worsened passing from the MCI to the AD group. As a matter of fact, not only did the volumes of these two structures present the most remarkable reduction in the MCI and AD groups with respect to the HC group, but they were also the only ones that showed a significantly larger reduction in the AD than in the MCI group.

Supporting the view that the progression of atrophy was not homogeneous across the various hippocampal subfields, the volumes of the presubiculum and subiculum best discriminated the correct group membership of the individual participants, followed by the CA4-DG, CA2-3, and finally, the CA1 subfield. This was true both when we considered HCs with respect to individuals in the two patients' groups and when we contrasted MCI versus AD patients.

The correlation pattern between anatomical volumes and memory scores depended greatly on the pattern of hippocampal atrophy. Indeed, hippocampal subfield volumes were significantly associated with performance scores on the verbal episodic memory tests to the extent that they were significantly reduced. Therefore, no significant association between anatomical volumes and memory performance was found in the HC group, whereas in the MCI group, only the volumes of the presubiculum and subiculum were positively associated with performance on the memory tests; finally, in AD patients, the volumes of all hippocampal subfields (with the notable exception of the CA1)

Table 4
Correlation matrices (Pearson's r) of anatomical volumes and performance scores on memory tests in patients with AD, MCI, and healthy participants

Groups	CA1	CA2-CA3	CA4-DG	Presubiculum	Subiculum	Overall hippocampus
AD patients						
Word-list immediate recall	0.25	0.41*	0.42*	0.30	0.37*	0.40*
Word-list delayed recall	0.34	0.55**	0.58**	0.45*	0.53**	0.55**
MCI patients						
Word-list immediate recall	0.15	0.25	0.30	0.32*	0.35*	0.33*
Word-list delayed recall	0.08	0.15	0.21	0.28	0.26	0.25
Healthy participants						
Word-list immediate recall	−0.09	−0.04	0.00	0.16	−0.01	0.03
Word-list delayed recall	−0.19	−0.06	−0.07	0.05	−0.13	−0.06

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment.
*$P < .05$
**$P < .01$.

were significantly and positively related to memory scores. In other words, regional hippocampal atrophy seems to be a reliable indicator of memory functioning.

Our finding of a particularly severe volume reduction of the subicular complex in AD is consistent with the neuropathological observations made by Mizutani and Kasahara [15,16]. These authors investigating an autoptical series of AD patients reported disproportionate atrophy in the subicular complex with respect to Ammon's horn fields (CA1, 2, 3, and 4) and the dentate gyrus. They attributed the particularly severe volume loss of these structures to isomorphic fibrillary gliosis resulting from early and severe degeneration of the perforant pathway while penetrating the hippocampus through the subicular field, in its course from the entorhinal cortex to the dentate gyrus. Mizutani and Kasahara [15] also noted that the atrophic subiculum showed marked loss of pyramidal neurons, whereas the hippocampus proper showed only a slight loss of neurons. Both degeneration of the perforant pathway and loss of pyramidal neurons would account for our finding that subicular volume predicts the severity of the verbal memory deficit in AD patients. Indeed, according to Hyman et al. [29,30], the episodic memory deficit in AD patients results from the anatomical and functional segregation of the hippocampus from associative sensory inputs due to degeneration of the perforant pathway. On the other side, the neuronal loss in the subicular field [13,16] further compromises the anatomical and functional communication of the hippocampus with extrahippocampal connected regions.

Previous neuroimaging studies obtained contrasting data regarding the pattern of hippocampal subfield atrophy in AD patients. As for patients with fully developed AD, all studies agree that the subiculum and the presubiculum show atrophic changes but differ as to the pattern of the volume reduction in Ammon's horn subfields. Normal volume of the CA1 subfield was reported by Lim et al. [31], who used the same automated segmentation protocol used in the present study, and by Kerchner et al. [32], who inferred volume by measuring the thickness of the CA1 subfield; both of the aforementioned studies included patients whose disease severity was similar to that of our patients (as indicated by MMSE and CDR scores). Instead, the CA1 subfield was found significantly atrophic in a study that used the same automated method as ours and included patients with similar dementia severity [33] and in studies that used a procedure involving manual segmentation of hippocampal subfields [34,35] or based on 3D surface reconstruction and shape analysis of the hippocampi [36,37], irrespective of the dementia severity of the included patients. Conversely, the volume of the CA3 and CA4 subfields was found in the normal range in most of the studies that used manual segmentation [34,35] and 3D surface reconstruction and shape analysis [36] but was invariably atrophic in studies based on automated segmentation procedures [31,33]. Analogous literature on individuals with MCI is still more controversial, with discrepant data resulting from adoption of the same subfield segmentation procedure. Thus, for example, the volume of the subiculum was reported to be normal in MCI in a study that relied on manual segmentation [35] and in another study that used an automated segmentation method [38] but was significantly reduced in two other studies that also used a method of manual [39] or automated segmentation [40].

There are several complementary explanations of these contrasting data (see, for a detailed discussion, [33,35]). Probably, the most critical issues regard the diagnostic criteria adopted in sample selection and the reliability of the segmentation techniques used for discriminating between individual hippocampal subfields. Regarding the first issue, inconsistencies might be due to the dementia severity in the group of AD patients and the criteria adopted for defining MCI. It seems quite obvious that both the dementia severity in AD patients and the pattern of cognitive deficits in MCI patients (only amnestic, amnestic plus other domains, or not amnestic at all [41]) have a great impact on the expected pattern of hippocampal regional atrophy. We selected relatively large groups of patients with AD and MCI with the aim of investigating the progression of regional atrophy across different stages of the disease. All the AD patients included here were in the mild phase of the disease, and in recruiting MCI patients, we adhered closely to diagnostic criteria for single-domain amnestic MCI [18]. The fact that the MCI patients exhibited a regional pattern of hippocampal atrophy that was qualitatively similar (even though less severe) to that of fully developed AD patients is consistent with the hypothesis that the single-domain amnestic profile of MCI is the one most likely to evolve to AD [42].

As for the second point of concern, controversial data were obtained particularly in studies that relied on manual versus automated segmentation methods. Automated methods used geometric rules as the criteria for defining subfields, whereas manual segmentation and 3D surface reconstruction methods used anatomical points derived from central nervous system atlases (e.g., [8]). Indeed, there were differences in defining the CA1 and subiculum in previous studies. For example, as acknowledged by Mueller et al. [34], the border used in that study was chosen "because it could be easily and reliably identified although by doing so, parts of the presubiculum and subiculum proper were counted towards CA1" (p. 45). The choice of these repere points determined whether a larger or a smaller portion of the hippocampal formation would be included in the CA1 or in the contiguous subfields and the probability of finding a significant volumetric difference between HCs and AD patients. We chose to rely on an automated technique to segment the hippocampal subfields even in order to evaluate the transferability of the present data to the clinical context for diagnostic and possibly therapeutic (e.g., drug effectiveness) purposes. Indeed, although the high-field MRI technology needed for delineating hippocampal subfields has increased in recent years, imaging studies have been limited by the fact that manual work was required to outline the subfield borders. Although automatic procedures offer comparable results to manual techniques, they are bias

free, tracer independent, and allow processing a large amount of data within a relatively brief period of time (i.e., 8–16 subjects per day, depending on the computational power of available computers).

Before conclusion, some limitations have to be discussed. First, a critical aspect is the difference in resolution between our images (1 × 1 × 1 mm) and those originally used by van Leemput et al. [28] (0.38 × 0.38 × 0.8). Although this difference exists, it must be noted that one of the steps of the segmentation procedure is the interpolation of input images to the reference ones at 0.38 × 0.38 × 0.8 mm. Thus, the segmentation is carried out with more resolved images, and the discrepancy between the two resolutions is minimized. Although we are aware that this step could introduce some biases, it should be outlined that the original MRI sequence used by van Leemput et al. [28] was about 35 minutes long. This makes it useless in clinical settings, especially in the framework of degenerative diseases, given its unfitness for patients and its proneness to movement artifacts.

Second, the choice to include exclusively amnestic MCI deserves some comments. In fact, on one side, it was aimed at constituting a sample with characteristics as homogeneous as possible, thus avoiding blurring factors due to symptomatic heterogeneity. On the other side, the aim of the present study was to investigate progressive hippocampal atrophy in the different phases of AD, including the preclinical stages of the illness, that is, MCI. Most of works support the hypothesis that amnestic subtype of MCI is more likely to subsequently convert to AD [43], although this is still a debated issue [44]. In addition to this, to further verify the high risk for the amnestic MCI patients of our sample to be in the preclinical stage of the illness, we investigated the *APOE* polymorphisms in a clinical subsample. In fact, among individuals that manifest late-onset AD, the ε4 allele is present at a two- to threefold higher rate compared to the general population, and some studies indicate up to 65% of clinically diagnosed cases carry at least one ε4 allele [45]. However, not all ε4 carriers develop AD, and not all AD patients are ε4 carriers; rather, the ε2 allele seems to play a protective role [45,46]. To note, the risk to male family members with *APOE* ε3/ε4 is similar to that for the *APOE* ε3/ε3 carriers. As illustrated in Table 1, most of the AD and MCI subjects of our subsample were ε3/ε3 (83% and 59%, respectively) and, to a lesser extent, ε3/ε4 carriers (17% and 38%, respectively), whereas the ε2 allele is absent but in a subject. These data are quite reassuring about the fact that the MCI patients included in our study are in the preclinical stage of AD. Future longitudinal studies will be definitely informative in supporting or not our results.

In conclusion, our data point to a nonhomogeneous pattern of atrophy in the hippocampus of AD patients, with prevalent involvement of the presubicular-subicular complex from the very early phases of the disease that is still evident in the most advanced phases. This finding is consistent with neuropathological observations in AD patients [15,16], and it is likely the macroscopic expression of the severe degeneration of fibers in the perforant pathway. An obvious clinical application of the presently reported data would be to evaluate the effectiveness of this pattern of regional hippocampal atrophy in predicting the conversion from MCI to fully developed clinical AD. To resolve this issue, further research is needed in larger groups of patients followed longitudinally for an adequate number of years.

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RESEARCH IN CONTEXT

1. Systematic review: There is no consensus about which hippocampal subfields become atrophic earliest in the course of Alzheimer's disease (AD) or which neuropathological change is primarily implicated in volume reduction.
2. Interpretation: Our data point to a prevalent atrophy of the presubicular-subicular complex from the early phases of AD. This could represent the morphological counterpart of the functional isolation of the hippocampus from both the afferent and efferent neural inputs that underlie the declarative memory loss in individuals with AD.
3. Future directions: An obvious clinical application of the presently reported data would be to evaluate the effectiveness of this pattern of regional hippocampal atrophy in predicting the conversion from MCI to fully developed clinical AD. To resolve this issue, further research is needed in larger groups of patients followed longitudinally for an adequate number of years.

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