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Gaël Chételat, Marine Fouquet, Grégoria Kalpouzos, Isabelle Denghien, Vincent de La Sayette, Fausto Viader, Florence Mézenge, Brigitte Landeau, Jean-Claude Baron, Francis Eustache, et al.

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Chételat G [1], Fouquet M [1], Kalpouzos G [1], Denghien I [2], De la Sayette V [1,3], Viader F [1,3], Mézenge F [1], Landeau B [1], Baron JC [4], Eustache F [1], Desgranges B [1]

[1] Inserm - EPHE - Université de Caen Basse-Normandie, Unité E0218, GIP Cyceron, CHU Côte de Nacre, Caen, France

[2] IFR49, Neurospin, CEA, Gif sur Yvette, France

[3] Département de Neurologie, CHU Côte de Nacre, Caen, France

[4] Dept of Neurology, University of Cambridge, UK

Correspondence and reprint request:

Gaël Chételat, Inserm - EPHE - Université de Caen Basse-Normandie, Unité E0218, Centre Cyceron, Bd H. Becquerel, BP 5229, 14074 Caen cedex, France.

Tel: +33 (0)2 31 47 01 07; Fax: +33 (0)2 31 47 02 75, E-mail: chetelat@cyceron.fr

Abstract

The hippocampus is the brain structure of highest and earliest structural alteration in Alzheimer's disease (AD). New developments in neuroimaging methods recently made it possible to assess the respective involvement of the different hippocampal subfields by mapping atrophy on a 3-D hippocampal surface view. In this longitudinal study on patients with mild cognitive impairment (MCI), we used such an approach to map the profile of hippocampal atrophy and its progression over an 18-month follow-up period in rapid converters to AD and 'non converters' compared to age-matched controls. For the sake of comparison, we also assessed the profile of hippocampal atrophy associated with AD and with increasing age in a healthy control population ranging from young adult to elderly. We found major involvement of the lateral part of the superior hippocampus mainly corresponding to the CA1 subfield in MCI and AD while increasing age was mainly associated with subiculum atrophy in the healthy population. Moreover, the CA1 subfield also showed highest atrophy rates during follow-up, in both rapid converters and 'non converters' although increased effects were observed in the former group. This study emphasizes the differences between normal aging and AD processes leading to hippocampal atrophy, pointing to a specific AD-related CA1 involvement while subiculum atrophy would represent a normal aging process. Our findings also suggest that the degree of hippocampal atrophy, more than its spatial localization, predicts rapid conversion to AD in patients with MCI.

Keywords: Alzheimer's Disease, Mild Cognitive Impairment; Normal Aging; Structural MRI; Hippocampus; Voxel-Based Morphometry; Atrophy; Longitudinal Imaging; Hippocampal Subfields.

Introduction

In Alzheimer's disease (AD), atrophy has been assessed in numerous studies in a priori regions of interest, such as in medial temporal lobe structures, or throughout the whole brain using voxel-based morphometry (VBM) (Baron et al., 2001; Chételat and Baron, 2003, for review). These studies have consistently reported early and marked atrophy in the medial temporal region, including the parahippocampal gyrus, hippocampus proper and amygdala, then extending to the temporal neocortex before involving temporo-parietal areas and the cingulate gyrus.

Several studies have assessed patients with amnestic mild cognitive impairment (MCI) to investigate the very early stage of AD. Indeed, these patients have isolated memory impairments without dementia, and up to 20% of them (the "converters") will convert to AD each year. They showed a very similar pattern of atrophy, although slightly less extended and less pronounced (Chételat et al., 2002; 2003), consistent with the fact that most MCI patients are in the pre-dementia stage of AD. It is to note that this pattern of atrophy in patients with MCI and AD, as measured in vivo from structural MRI data, is very consistent with the well described pattern of neurofibrillary tangles (NFT) progression in the course of AD evidenced post-mortem (Braak and Braak, 1991; Duykaerts et al. 1997; Delacourte et al., 1999). This parallel distribution between atrophy and NFT suggests that they should be related, although this relationship is not fully elucidated.

As for AD, the hippocampal region is the most significantly atrophied in MCI. The degree of hippocampal atrophy was found to be associated with subsequent conversion to AD, but with limited accuracy, and to progress from healthy aging to MCI and then to AD where it correlates with Braak and Braak stage (Jack et al., 2002). Rate of hippocampal atrophy is also higher in MCI converters compared to non converters, although individual values overlap.

New developments in neuroimaging data analyses and mapping techniques recently made it possible to get a more detail insight on hippocampal atrophy, allowing the distinction of its different subfields onto its three-dimensional (3-D) surface (Csernansky et al., 2000; 2005; Wang et al., 2003; 2006; Thompson et al., 2004; Frisoni et al., 2006; Apostolova et al., 2006a; 2006b). Since the hippocampal subfields were found to be differentially affected by neurofibrillary tangles and neuronal loss (West et al., 1994; 2004; Rössler et al, 2002; Bobinski et al., 1998; Schönheit et al., 2004), with highest involvement of the CA1 region (and subiculum in some studies), it seems thus of particular interest to assess whether such differences could be recovered in vivo assessing atrophy from structural MRI.

Csernansky et al. (2000) were the first to apply such an approach in AD allowing MRI-based segregation of hippocampal subfields. The authors showed that hippocampal inward deformation in the head and the lateral surface mainly corresponding to CA1 subfield was the highest change in patients with CDR=0.5, i.e. with very early AD, compared to control subjects with CDR=0 (Csernansky et al., 2000; Wang et al., 2006), and the only significant predictor of conversion to CDR=0.5 in subjects with CDR=0 (Csernansky et al., 2005). These changes were found to accentuate and spread with AD progression (Wang et al., 2003). Thompson et al. (2004) have developed another approach allowing to map 3D hippocampal surface atrophy, and showed that major inward deformations occurred in the CA1 and subiculum subfields in AD, while CA 2 to 4 subfields and gyrus dentatus were mostly preserved (Thompson et al., 2004; Frisoni et al., 2006). Moreover, CA1 atrophy was found to be associated with future conversion to AD in patients with MCI (Apostolova et al., 2006a) and additional involvement of CA-2 and 3 subfields was found in patients with AD compared to MCI (Apostolova et al., 2006b).

In this longitudinal study on patients with MCI, we used a more classical voxel-based morphometry (VBM) approach commonly used in the neuroimaging community, associated

with projection of atrophy maps onto a 3-D surface view of the hippocampus. Our purpose was to map the differential profile of hippocampal atrophy in rapid converters and non converters compared to age-matched controls, and to map the progression of hippocampal change over an 18-month follow-up period in each group. For the sake of comparison with normal aging and Alzheimer's disease, we also assessed the profile of hippocampal atrophy in AD compared to age-matched controls, and that associated with increasing age in a healthy control population ranging from young adult to elderly.

Methods

Subjects

Healthy controls. Fifty-nine healthy controls from 20 to 84 years old were included in the present study (see demographics on Table 1). The whole sample was used to assess the profile of hippocampal atrophy associated with increasing age over the entire adult life, while a sub-group of this sample, consisting in 15 healthy elderly aged more than 60 years old (see Table 1), was used as controls when assessing atrophy in MCI or AD. Inclusion and exclusion criteria were the same for all control subjects. They all lacked abnormality of clinical, MRI, and neuropsychological examinations, as demonstrated by: 1) normal somatic examination, 2) body weight in the normal range, 3) no known vascular risk factor and smoking <10 cigarettes per day, 4) no alcohol or coffee abuse, according to DSM4 criteria, 5) blood pressure within normal limits or corrected to, 6) no history or clinical evidence of sensorineural loss, dementia, or psychiatric disorder (a formal psychiatric interview was not performed), 7) no current use of medication (except birth control pills, oestrogen replacement therapy, anti-hypertensive drugs), and especially no use of centrally acting drugs (sleeping pills, antidepressant drugs) for at least 6 weeks, 8) normal standard T1-, T2- and/or FLAIR-

weighted MRI, and notably no significant white matter T2- FLAIR-weighted hyperintensities. The Mattis dementia rating scale (Mattis, 1976) was used for subjects over 50 years to exclude subjects with scores below the normal range for age indicating potential underlying neurodegenerative pathology. They also underwent cognitive tasks assessing episodic memory, semantic memory and working memory. There was no evidence of significant cognitive decline beyond that expected for normal aging in any subject, and no subject complained about his/her memory.

MCI patients. Seventeen patients with amnestic MCI (Petersen *et al.*, 2001) were prospectively studied (see demographics on Table 1). They were all recruited through a memory clinic, and all complained of memory impairment. They underwent medical, neurological, neuropsychological, and neuroradiological examinations, and were selected according to the following stringent criteria: i) lack of present or historical evidence of significant neurological, psychiatric or any other medical disease, use of medication that could affect brain functioning or structure, and depression or substance abuse; ii) modified Hachinski ischemic score ≤ 2 (Loeb and Gandolfo, 1983); iii) age over 55 years; iv) objective memory impairment, as defined by performance 1.5 SD below the normal mean for age-matched controls in at least one subscore of the Grober and Buschke test (Grober and Buschke, 1987) or at the Rey's figure delayed recall test (Rey, 1959); and v) NINCDS-ADRDA criteria for probable AD (McKhann *et al.*, 1984) not met, as assessed by an extensive neuropsychological examination evidencing normal scores (no more than 1.5 SD below the normal means according to age and education when available) in general intellectual function, i.e. MMSE (Folstein *et al.*, 1975) and Mattis dementia rating scale (Mattis, 1976), and in cognitive functions other than episodic memory, including executive (Stroop test; Golden, 1978), visuospatial (copy of Rey's figure; Rey, 1959), limb praxis (imitation of four meaningless gestures, production of four symbolic gestures and four object

utilization gestures), language (writing of 12 irregular words under dictation) and image naming (DO80; Deloche and Hannequin, 1997) functions. Independence in daily living activities was verified during the clinical interviews, and the final decision regarding patient inclusion corresponds to a consensus between the neurologist, the neuropsychologist, and the researcher in charge of the project.

Using the same neuropsychological battery, all subjects were evaluated every 6 months over an 18-month period to assess conversion, i.e. whether they met NINCDS-ADRDA criteria of probable Alzheimer's disease or not. Patients were declared as "rapid converters" if they had impaired performances (more than 1.5 SD below the normal means according to age and education when available) in at least one of general intellectual function scales as well as in at least two areas of cognition including memory, leading to impaired daily activities as judged by the clinicians from the consultation interview. At the end of the follow-up period, 7 MCI patients had converted to AD (the "rapid converters") while 10 were still classified as MCI (and they were termed as "non-converters" in what follows). A second MRI scan was obtained in each MCI patients at the end of the follow-up period, i.e. 18 months after the first MRI scan.

AD patients. Seventeen patients with probable AD were also studied (see demographics on Table 1). At the time of the study, none of the patients was being or had been treated with specific medication such as anti-acetylcholinesterase agents since they were recruited into this study immediately following clinical diagnosis and were started on cholinesterase inhibitors only after their baseline investigation. All were prospectively selected using standard NINCDS-ADRDA diagnostic criteria for probable AD (McKhann et al, 1984). The diagnosis of probable AD was based on an extensive neuropsychological examination which included the mini mental status examination (MMSE; Folstein et al., 1975), Mattis dementia scale (Mattis, 1976), Wechsler's Memory scale (Wechsler, 1969), Story and Figure recall tests from

Signoret`s battery (Signoret, 1991), verbal span (forward and backward; Signoret, 1991), verbal working memory (Brown-Peterson paradigm; Peterson and Peterson, 1959), verbal fluency (letter and category; Cardebat et al., 1990), and copy of Rey's figure (Rey, 1959). We purposely selected patients with mild dementia, based on a MMSE score of 20 or higher.

All the subjects included gave written informed consent to participate in the study which was done in line with the Declaration of Helsinki and approved by the Regional Ethics Committee. All subjects in this study were right handed and had at least 7 years of education. The three samples of AD, MCI and controls partly overlap with those of our previous publications (Baron et al., 2001; Desgranges et al., 2002; Eustache et al., 2004; Chételat et al., 2002; 2003; 2005; Kalpouzos et al., 2007).

MRI data acquisition

Within an interval of two months at most from inclusion for the controls and a few days for MCI and AD patients, each subject underwent a high-resolution T1-weighted volume MRI scans at baseline and MCI patients underwent a second MRI scan 18 months apart. Acquisitions consisted of a set of 128 adjacent axial cuts parallel to the Anterior Commissure-Posterior Commissure line and with slice thickness 1.5 mm and pixel size 1 × 1 mm, using the SPGR gradient echo sequence (TR = 10.3 ms; TE = 2.1 ms; FOV = 24 x 18; matrix = 256 x 192). All the MRI data sets were acquired on the same scanner (1.5 T Signa Advantage echospeed; General Electric).

MRI data handling and transformations

All MRI data were analyzed using SPM5 and the VBM protocol, described in details elsewhere (Good et al., 2001), and already used in our laboratory (Chételat et al.,

2003a; 2005). Briefly, the procedure included segmentation and normalization of original MRI data using the default MNI template of SPM5 as priors. Indeed, the use of a customized template is not recommended while using SPM5 on limited – i.e. less than hundred(s) of subjects – sample size. To blur individual variations in gyral anatomy and to increase the signal-to-noise ratio, the spatially normalized GM data sets were smoothed using a Gaussian kernel of 8mm for cross-sectional assessments (i.e. age and disease effects) and of 6mm for longitudinal assessments (i.e. atrophy progression). Low smoothing values were chosen to limit spatial resolution degradation (note that a more classical 12mm Gaussian kernel was also tested and led to similar conclusions; data not shown), and a lower value was used for longitudinal assessments since each individual's first scan was used as reference for the follow-up scan (see also Chételat et al., 2005). The resulting smoothed and spatially normalized GM data sets were used in the following analyses and processing.

Age effects: correlation coefficient SPM-R maps

Using SPM5, negative correlations were performed between GM density and age in the whole control sample (59 healthy subjects). The resultant SPM-T map was then converted to correlation coefficient R-map using the following formulae: $R = 1 / \sqrt{((n-2)/T^2+1)}$.

Disease effect: Z-score maps

Z-score maps were computed from the resultant smoothed and normalized GM data sets ([patient individual data - control sample mean] / control standard deviation), for each patient at each time point (see Kawachi et al., 2006 for a similar approach). Individual Z-score maps were then averaged across MCI patients according to six conditions (baseline or follow-up data of the whole MCI sample, or only rapid converters, or non converters), and across AD patients.

Atrophy progression in MCI: GM and percent annual loss (PAC) maps

In addition, maps of absolute GM loss over the 18-month follow-up period (baseline – follow-up GM density) and percent annual loss (PAC) maps (i.e. ([baseline GM density - follow-up GM density] / baseline GM density) x [12 / 18] x 100), were also created for each MCI patient to specifically address the issue of atrophy evolution. Individual maps were then averaged across the whole MCI sample and according to their clinical evolution (rapid converters or non converters), resulting in three mean GM loss and three mean PAC maps (MCI, rapid converters and non converters).

Result display

Anatomical localization was based on the superimposition of mean z-score maps, GM loss and PAC maps, as well as SPM-R maps onto the surface of a hippocampal 3D view, using the publicly available "Anatomist/BrainVISA" software (www.brainvisa.info). The hippocampal ROI used to obtain this 3D view (hippocampal mesh) was delimited on the right and left hemispheres on coronal slices of a whole-brain customized template of the healthy aged sample (created by averaging the normalized MRI of the 15 healthy elderly). Delineation of hippocampal subfields on 3-D surface superior and inferior views were schematized (see Figure 1A) from those provided in previous publications (Csernansky et al., 2005; Wang et al., 2003; Frisoni et al., 2006; Apostolova et al., 2006a).

Results

*Age effects: correlation coefficient SPM-R maps (**Figure 1B**)*

The SPM-R map of correlation between increasing age and decreasing GM density in the whole control sample (aged 20-84 years old) projected on the hippocampal 3-D surface is

illustrated in **Figure 1B**, and the plot of the correlation in the hippocampal peak of highest statistical significance is illustrated in **Figure 2**. Highest R-values (>0.5) were found on the most medial part of the superior view, and on the medial half of the inferior view. Based from hippocampal maps of subfields delineation provided in previous publications and schematized in **Figure 1A**, regions of highest R values mainly corresponded to the subiculum, while CA1 and other subfields were relatively preserved during normal aging.

*Disease effect: Z-score maps (**Figure 1C, D, E** and **Figure 3**)*

The mean Z-score maps of GM loss in AD, MCI at baseline, and MCI at follow-up, as compared to the healthy elderly control group and projected on the hippocampal 3-D surface are illustrated in **Figure 1C**, **D**, and **E**, respectively. Lowest Z-score values (<-2) in patients with Alzheimer's disease were found to concern the lateral half of the superior view and the most lateral part of the inferior view, with a right-sided predominance. These areas mainly corresponded to the CA1 subfield (see **Figure 1A**), also slightly encroaching the other subfields. At baseline, MCI patients demonstrated highest gray-matter loss (Z-score<-2) in an anterior part of the right hippocampus only, which fell onto the CA1 zone (**Figure 1D**), while eighteen months later, the profile of hippocampal atrophy was highly similar to that found in the AD group, mainly involving the CA1 subfield, and extending to the other subfields, with a right predominance (**Figure 1E**).

The mean Z-score maps of gray matter loss in rapid converters and non converters, at baseline and at follow-up, as compared to the healthy elderly control group and projected on the hippocampal 3-D surface are illustrated in **Figure 3**. Z-score values were found to be much lower in rapid converters than in non converters (see color scales ranging from 0 to -2 for non converters and from 0 to -5 for rapid converters). In non converters, the middle part of the hippocampus was preserved, main effects being found in the anterior and posterior parts,

while in rapid converters atrophy predominated in the anterior half. Apart from these differences, atrophy was found to be located in areas mainly corresponding to the CA1 subfield in both rapid converters and non converters (also slightly encroaching the other subfields for the former), highest effects being found in the anterior lateral portion of the superior view. There was a decrease of Z-score values in both groups from baseline to follow-up: in non converters, all z-score values were >-1.75 at baseline while there was an area with z-score values <-1.75 on the right anterior part of the hippocampal superior view (located in CA1) at follow-up. In rapid converters, lowest z-score values (< -3) were found bilaterally in the anterior lateral part of the superior view at baseline, while they extended bilaterally to the posterior part and to the most lateral portion of the inferior part 18 months later, with lowest z-scores dropping from -5.4 at baseline to -7.4 at follow-up.

*Atrophy progression in MCI: PAC maps (**Figure 4**)*

The mean absolute GM loss and PAC maps from baseline to follow-up in MCI and in rapid converters and non converters separately, as projected onto the hippocampal 3-D surface view, are illustrated in **Figure 4**. Highest PAC values (>2%) in patients with MCI were found to concern the lateral half of the superior view only. In rapid converters and in non converters, very similar profiles were observed, with highest hippocampal PAC values concentrated in the CA1 subfield, including the anterior portion of the superior part and extending laterally to the posterior hippocampus, while the inferior part was relatively preserved. However, rapid converters showed a more extensive pattern slightly encroaching the other subfields. Moreover, effects were mainly symmetric and quantitatively much higher in rapid converters than in non converters, with PAC values rising up to 5.6% in the former while they did not exceed 3.8% in the later. The profiles of absolute GM loss from baseline to follow-up were

highly consistent with those of PAC and showed again more strongly how regions of highest GM loss matched with the CA1 subfield.

Discussion

In this study, we showed for the first time the profiles of hippocampal atrophy in patients with amnestic MCI according to their clinical evolution, and its progression over time, compared to the profile of gray matter loss across normal aging. We found a marked atrophy in the lateral part of both hippocampi corresponding to the CA1 subfield, which was higher in rapid converters than non converters, and very similar to that observed in AD. Atrophy is much more significant in rapid converters than in non converters, and this effect is more marked at follow-up. Finally, the evolution maps showed that higher hippocampal atrophy rates in the following 18 months were observed in the same superior lateral part corresponding to the CA1 subfield, in both rapid converters and non converters. By contrast, GM loss with increasing age mainly concerned the inferior part of the hippocampus corresponding to the subiculum.

Two approaches have previously been proposed to assess the differential effect of atrophy according to hippocampal subfields. Csernansky et al. (2000) first proposed a method termed as “high-dimensional brain mapping (HDBM)”, and based upon the evaluation of hippocampal shape deformation from a template hippocampus traced on an arbitrary selected healthy subject (i.e. scoring 0 at the CDR scale). Thompson et al. (2004) also proposed a very refined hippocampal 3-D radial atrophy mapping approach, where hippocampal surface deformation is estimated in each subject after individual hippocampal tracing. In the present study, we used a classical VBM approach commonly used in the neuroimaging community to map structural changes in the whole brain, associated with a 3D surface mapping thanks to the

publicly available Brainvisa software, to assess the spatial extent of hippocampal change throughout this structure. Theoretically, the present method has both some advantages and disadvantages compared to both previous ones. Thus, it uses a group of healthy controls as reference so that details of neuroanatomical structure that might be peculiar to any single subject would not influence the results, while the HDBM technique depends on the choice of an individual subject presumed to be representative of the populations being compared. Moreover, the present method doesn't necessitate individual hippocampal tracing required in the radial mapping approach, which is a gain in term of time and expertise dependency. One would yet expect the meticulous individual tracing to improve sensitivity and accuracy of hippocampal change measurements by contrast to the spatial normalization process required here. However, over and above these theoretical differences between different approaches of 3-D hippocampal atrophy mapping, it is to note that only a study designed to compare the different methodological approaches from a same data sample, and ideally associated with direct pathological validation, would allow to state upon their relative advantages and disadvantages notably in terms of sensitivity, which was not the aim of the present study. Note also that hippocampal subfield localisation was not specifically assessed in the present study, but relies on delineation provided in previous publications. However, both previous methods lead to similar subfield localisation onto hippocampal surface in terms of inferior versus superior and medial versus lateral axes, which can thus be used as provisory atlas pending post-mortem confirmation.

Interestingly, despite the use of very different methodologies, findings were highly consistent among studies. Thus, we found most significant and highest changes in the CA1 subfield in both AD and MCI patients compared to healthy elderly, which is consistent with previous reports in early AD or MCI (Csernansky et al., 2000; Wang et al., 2003). The

involvement of the subiculum was also reported together with the CA1 subfield in the study by Frisoni et al. (2006) assessing mild to moderate AD patients, suggesting that AD-associated pathological process may first involve CA1 and then extends to the subiculum (see below). In the present study, we also reported higher degree of atrophy in rapid converters than in non converters, more specifically in the lateral hippocampal portion corresponding to CA1. This finding was also consistent with Apostolova et al. (2006a) reporting significant differences between converters and non converters in the CA1 subfield, and Csernansky et al. (2005) showing that the lateral zone of the left hippocampus was the only significant predictor of conversion to CDR=0.5 in subjects initially scoring 0 over and above hippocampal volume measures. Finally, assessing the progression of atrophy in MCI, and specifically in rapid converters and non converters, we also found that major changes over time concerned the CA1 subfield, in accordance with Wang et al. (2003) where atrophy increased and extended over two years follow-up in very early AD, but still mainly involved the CA1 subfield. Thus, at this very early stage of the disease (i.e. in patients with MCI or very early AD), the CA1 subfield is the most atrophied part of the hippocampus compared to age-matched controls, and this region continues to show accelerated atrophy while the disease progresses over the following 18/24 months. In patients with probable AD (including rapid converters at follow-up), atrophy extended to some portion of the other subfields in the present study, which is also consistent with the report by Apostolova et al. (2006b) of greater atrophy in CA1, 2 and 3 hippocampal subfields in AD relative to MCI.

More surprisingly, we found similar pattern of hippocampal atrophy in both rapid converters and non converters, i.e., hippocampal areas of higher atrophy compared to controls, and accelerated atrophy over time, were found to concern the CA1 subfield in both groups, although rapid converters showed increased effects. Previous classical ROI studies

have consistently reported lower hippocampal volume and higher rate of hippocampal atrophy in converters compared to non converters (see Introduction and Chételat and Baron, 2003, for review), and the VBM approach has led to the same conclusion regarding the hippocampus (Chételat et al., 2005; Bozzali et al., 2006). We thus expected to find higher effects in converters compared to non converters. Regarding hippocampal specific localisation, two previous studies directly comparing converters to non converters both pointed to the CA1 subfield as the region of higher atrophy in converters (Apostolova et al., 2006a; Csernansky et al., 2005), and we thus assumed that non converters would present a different profile from the CA1 involvement characterizing converters (and AD). The present study instead indicates that both groups differ more quantitatively than qualitatively, i.e. what identifies patients that will rapidly convert to AD in the following 18 months is a higher degree and rate of hippocampal (CA1) atrophy, and, may be also, atrophy in other cortical regions (Chételat et al., 2005; Bozzali et al., 2006), instead of a particular spatial profile of hippocampal atrophy. Non converters showed hippocampal atrophy limited to a small anterior CA1 area at baseline, which progressed specifically in this region during the following 18 months to include a large part of CA1 at the end of the follow-up period, while they still had isolated memory deficits. This pattern of hippocampal alteration was different from that observed during normal aging, suggesting that some pathological processes should occur even in non converters. These MCI sub-sample may include patients that will later convert to AD because they were at an earlier stage at inclusion or because they progressed more slowly, patients with another slowly progressing pathology, and healthy subjects with particularly low memory performance due to non-pathological factors such as environment, education or genetic.

It should be kept in mind that the use of a binary approach to classify MCI patients according to short-term clinical outcome (i.e. rapid conversion to AD or not over an 18-month

period), has some limitations (even though the follow-up was the same for all subjects). The choice of a relatively short period here was somewhat arbitrary (e.g. some non-converters might have converted two months later). Our study therefore highlights the differences between those MCI patients that progress to AD rapidly versus slowly (or not at all). It does however have clear clinical relevance, since rapid vs. slow or non conversion does reflect distinct cognitive evolution, while the identification of rapid converters will be critical in therapeutic studies testing disease-modifying agents (Chetelat et al., 2005). The relatively high conversion rate (about 27% per year) observed in the present study presumably reflects the use of very strict inclusion criteria for amnestic MCI, i.e. statistically significant and isolated memory deficit, as compared to less stringent criteria (e.g. inclusion of patients with deficits in other areas of cognition or the use of subjective global scales such as the CDR as sole criteria; see Chetelat and Baron, 2003, for further discussion) which entail lower conversion rates.

The right hippocampus generally appeared more affected than the left counterpart in the present study, although this visual difference was not assessed statistically. Asymmetry is a characteristic feature of the brain pattern of AD-related alterations, but the preferentially affected hemisphere diverges among studies. More specifically regarding the hippocampus, some previous ROI studies have found higher atrophy in the right hippocampus, but the opposite finding has also been reported (see Chetelat and Baron, 2003, for a review). In studies using hippocampal atrophy surface mapping, Thompson et al. (2004) found higher involvement of the left hippocampus in early AD and the left CA1 subfield was found to be the only significant predictor of conversion to AD (Csernansky et al., 2005), but right more than left inward deformation was found when comparing patients with early AD to matched controls in Csernansky et al. (2000). In addition, we found a preferential involvement of the

anterior hippocampus in MCI and AD, as well as while assessing atrophy progression, consistent with previous reports suggesting that atrophy progressively spreads to the hippocampal head (Wang et al., 2003; Csernansky et al., 2000). This finding is also consistent with the fact that CA1 is more represented in the head than in the body or the tail of the hippocampus.

By contrast, we found a clearly different pattern of GM loss with increasing age, with a major involvement of the subiculum contrasting with a relative preservation of the CA1 subfield. Only two previous hippocampal surface mapping studies have assessed changes associated with non pathological aging, and they reported diverging findings (Csernansky et al., 2000; Wang et al., 2003). In the first one, authors reported a general flattening of the hippocampus, with inward deformations in the head and the tail but outward changes in the body (Csernansky et al., 2000), while Wang et al. (2003) found a spread of hippocampal deformation in small areas of the hippocampal head, subiculum, and lateral aspect of the hippocampal tail. However, these differences compared to our findings are very likely to be due to the use of different approaches than that used in the present study, the first one comparing old to young controls (Csernansky et al., 2000), and the second one reporting atrophy progression over two years in old controls, while we assessed correlation with age across a large age range from 20 to 84 years old. It is to note that the cross-sectional approach used here is suboptimal to assess the effect of age, because of a potential bias related to cohort effects as well as potential underestimation of non linear effects. Moreover, the very old subjects are under-represented in our study (6 subjects > 70 years), which is related to the use of very strict criteria for optimal health. However, it provides an estimate of the linear relationships that may exist between hippocampal size and age from 20 to 84 years, pointing to the subiculum as the hippocampal area of highest atrophy. Further longitudinal studies in

healthy controls would be needed to optimally describe hippocampal changes over the entire life span.

Atrophy is thought to reflect neuronal loss itself related, at least party, to NFT (Rössler et al., 2002; Kril et al., 2002; Price et al., 2001; von Gunten et al., 2006; Zarow et al., 2005). Consistently, our overall findings of CA1 involvement in MCI and AD and highest change in the subiculum in the course of normal aging are in line with the preferential repartition of neuronal loss and NFT reported by most post-mortem studies. Thus, presence of NFT and major decrease in neuron number in the CA1 subfield of the hippocampus in AD compared to age-matched controls is a constant finding (Zarow et al., 2005; Kril et al., 2002; Price et al., 2001; von Gunten et al., 2006). When the other hippocampal sub-regions were also assessed, lesions in the CA1 were found to be the earliest and most distinctive feature of AD, reflecting a disease-specific process not related to age *per se* (West et al., 1994; Rössler et al, 2002; Bobinski et al., 1998; West et al., 2004; Schönheit et al., 2004). By contrast, correlations between age and neuron number in control populations ranging from young to elderly subjects have revealed strong relationships in the subiculum, while CA1 and other subfields remained relatively preserved (West et al., 1993; 1994). Some studies also reported the involvement of the subiculum in AD (Davies et al., 1992; Hyman et al., 1984; see Scher et al., 2007, for review). However, given that this sub-region should already be atrophied with normal aging, and probably affected by AD-related pathological process at a more advanced stage (see also above), together explain why we did not find significant change in this hippocampal part when comparing mild AD to elderly controls in the present study. Indeed, by comparing MCI (or AD) to healthy elderly, we map the effect of the disease with the effect of normal aging subtracted out. Thus, showing in AD the involvement of the CA1 subfield only doesn’t mean

that the subiculum is unchanged, but instead that it is not significantly more atrophied in AD than in the normal elderly.

Overall, we found a striking divergence in the hippocampal repartition of normal aging and AD-related GM loss, respectively involving the subiculum and the CA1 subfield, which may constitute a specific distinctive feature of potential clinical interest pending confirmation on the individual profile of the patients and controls.

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Table 1: Demographics of each control and patient group.

	Healthy controls	Healthy elderly	MCI	Rapid Converters	Non Converters	AD
Number	59	15	17	7	10	17
(women / men)	(30 / 29)	(8 / 7)	(11 / 6)	(5 / 2)	(6 / 4)	(13 / 4)
Baseline age:						
mean ± SD	48.4 ± 18.1	68.3 ± 7.1	71.4 ± 8.6	73.3 ± 4.3	70.4 ± 11.2	69.4 ± 5.4
(range)	(20-84)	(60-84)	(55-87)	(73-80)	(55-87)	(62-82)
MMSE :						
mean ± SD	-	-	27.5 ± 1.2	26.7 ± 1	28.1 ± 1	23.9 ± 2.2
(range)	-	-	(25-29)	(25-28)	(26-29)	20-28

Figure 1.

A: Delineation of hippocampal subfields on 3-D surface superior and inferior views as schematized from hippocampal maps provided in previous publications (Csernansky et al., 2005; Wang et al., 2003; Frisoni et al., 2006; Apostolova et al., 2006a) ;

B: Hippocampal profile of GM atrophy across normal aging as represented by the superimposition of the SPM-R map of correlation between increasing age and decreasing GM density in the whole control sample (aged 20-84 years old) onto the hippocampal 3-D surface view.

C, D, E: Hippocampal profile of GM atrophy in AD (**C**) and MCI at both baseline (**D**) and follow-up (**E**), as represented by the superimposition of the mean Z-score maps of GM loss in each group compared to the healthy elderly control group, onto the hippocampal 3-D surface view.

Figure 2. Plot of the correlation between increasing age and decreasing GM density in the hippocampal voxel of highest statistical significance located in an inferior medial part of the hippocampus corresponding to the subiculum (MNI coordinates x y z = 18 -34 -6; $p< 10^{-10}$; t=-8.9; r=-0.763; r^2=0.58).

Figure 3. Hippocampal profile of GM atrophy in MCI non converters (**A**) and rapid converters (**B**) at both baseline (left) and follow-up (right) relative to the healthy elderly control group, as represented by the superimposition of the mean Z-score maps of GM loss onto the hippocampal 3-D surface view.

Figure 4. Hippocampal profile of atrophy progression from baseline to follow-up in patients with MCI (**A**), and in non converters (**B**) and rapid converters (**C**) separately, as represented

by the superimposition of the mean percent annual change (PAC; left) and absolute GM loss (right) maps onto the hippocampal 3-D surface view (see Methods for details).