Amygdalar Atrophy in Early Alzheimer’s Disease
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Yanica Klein-Koerkamp1, Rolf A. Heckemann2,3,4, Kylee T. Ramdeen3, Olivier Moreaud1,6, Sandrine Keignart6, Alexandre Krainik7,8,9, Alexander Hammers3,4, Monica Baciu1,10, Pascal Hot5,* and for the Alzheimer’s Disease Neuroimaging Initiative**

INTRODUCTION

The neuropathology of Alzheimer’s disease (AD) is characterized by neuronal loss, first affecting the medial temporal lobe (MTL) [1, 2]. In particular, subregions of the hippocampus [3-6] and entorhinal cortex [7] undergo massive pathological changes, leading to progressive memory impairments [8-11]. Several studies suggest that hippocampal atrophy is the best neuroimaging-derived marker of disease and disease progression. However, hippocampal atrophy is associated with a range of other neurological pathologies [12-15], thus limiting its specificity to AD. With advances in automated volumetric segmentation, structures that were previously difficult to assess are now more reliably segmented and evaluated. This has led to the discovery of other structures that undergo change in the course of dementia, notably, other limbic structures close to the hippocampus. In particular, the amygdala has recently received increased attention. Tables 1 and 2 list studies that focus on amygdalar atrophy in mild to moderate AD (Table 1) and moderate to severe AD (Table 2), along with factors that influence (1) the magnitude of atrophy measured (i.e., participants’ characteristics and segmentation procedures); and (2) the association with clinical features (i.e., information related to correlation analysis between cognitive scores and neuroanatomical volumes). Given our interest in amygdalar volume, we have included only those studies that consider data for this structure. Reduction of amygdalar volume compared to elderly HC was a robust finding in severely affected AD patients compared with elderly HC, as shown in Tables 1 and 2 (Clinical Dementia Rating or CDR scores ranging from 0.5 to 3 [4]; Mini-Mental Score Examination or MMSE ranging from 5 to 21 [21]; MMSE ranging from 2 to 27 [22]; MMSE ranging from 11 to 25 [23]). This observation suggests that the diagnosis of AD may be improved if amygdalar volume is considered in addition to hippocampal volume [24]. However, findings are more contradictory for earlier stages of the disease, such as AD patients...
Table 1. Summary of research on amygdalar atrophy in mild to moderate AD.

<table>
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<tr>
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<th>Regression Analysis of Volumes with Cognitive Score</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>AD = 11; MMSE: HC = 29 (1); AD = 21.5 (5)</td>
<td>Manual. MRI: 1.5T - slice thickness: 5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC = AD (20% loss)</td>
<td>HC &gt; AD (33% loss)</td>
<td>A &gt; H</td>
<td>AD; H and A: no significant correlation with MMSE; AD + HC; A: correlation MMSE</td>
<td></td>
<td>[31]</td>
</tr>
<tr>
<td>Mild</td>
<td>HC = 8; AD = 18. Age: HC = 69.2 (2.7); AD = 72.4 (1.5); MMSE: HC = 28.7 (0.4); AD = 22.3 (0.9; 13-27)</td>
<td>Semi-automatic. MRI: 5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (30% loss)</td>
<td>HC &gt; AD (37% loss)</td>
<td>A = H</td>
<td>AD + HC; H: correlation MMSE, memory (Mattis, Wechsler, Grober Buschke Test, Verbal, Intrusion); A: no significant correlations</td>
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<td>[25]</td>
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<tr>
<td>Mild</td>
<td>HC = 40; AD = 24. Age: HC = 79 (3.5); AD = 78.4 (3.2); MMSE: HC = 28.9 (1); AD = 22.1 (1.9)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>ICV-normalized volumes; age as covariate</td>
<td>HC &gt; AD (18% loss)</td>
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<td>AD + HC; A: correlation MMSE</td>
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<td>[44]</td>
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<tr>
<td>Mild</td>
<td>HC = 7; AD = 8. Age: HC = 70; AD = 72; MMSE: AD = 23.9 (17-29)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>Raw volumes</td>
<td>HC &gt; AD</td>
<td>HC = AD (p=0.06)</td>
<td>A &lt; H</td>
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<td>[84]</td>
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<tr>
<td>Mild</td>
<td>HC = 21; AD = 13. Age: HC = 69.3 (6.8); AD = 71.2 (8.3); MMSE: HC = 29.7 (0.2); 28-30; AD = 23.7 (2.7; 20-28)</td>
<td>Manual. MRI: 1.5T et 0.5T - slice thickness: 5 mm</td>
<td>ICV-normalized volumes; age as covariate</td>
<td>HC &gt; AD (19% loss)</td>
<td>HC &gt; AD (33% loss)</td>
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<td>[32]</td>
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<tr>
<td>Mild</td>
<td>HC = 34; AD = 54. Age: HC = 72 (4); AD = 70 (8); MMSE: HC = 28.4 (1.3); AD = 21.7 (3.7)</td>
<td>Manual. MRI: 1.5T et 0.5T - slice thickness: 5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (21% loss)</td>
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<td>AD; A: no significant correlation with MMSE</td>
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<td>[45]</td>
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<tr>
<td>Mild</td>
<td>HC = 16; AD = 32. Age: HC = 70 (5); MA = 69 (8); MMSE: HC = 28.6 (1.4); AD = 22.8 (3.7)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (38% loss)</td>
<td>HC &gt; AD (16% loss)</td>
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<td>AD; A: no significant correlations (MMSE, verbal memory). H: correlation (MMSE, verbal memory)</td>
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<td>[30]</td>
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<tr>
<td>Mild</td>
<td>HC = 94; AD = 118. Age: HC = 74 (5); AD = 75 (6); MMSE: HC = 29 (1); AD = 21 (5)</td>
<td>Automatic: MRI: 1.5T - slice thickness: 1.3 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD</td>
<td>HC &gt; AD</td>
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<td>[85]</td>
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<tr>
<td>Mild</td>
<td>HC 1 = 87; AD 1 = 90. HC 2 = 195; AD 2 = 174. Age: HC 1 = 77.7 (7.9); AD 1 = 77.2 (6.7); HC 2 = 75.6 (5.1); AD 2 = 75.5 (7.3); MMSE: HC 1 = 28.9 (1.2); AD 1 = 24.6 (3.9); HC 2 = 29.1 (1); AD 2 = 23.3 (2)</td>
<td>Automatic: MRI: 1.5T - slice thickness: 1.1 mm</td>
<td>Normalized volumes</td>
<td>HC &gt; AD (18.3% loss AD 1; 19.1% loss AD 2)</td>
<td>HC &gt; AD (19.3% loss AD 1; 18.5% loss AD 2)</td>
<td>A = H</td>
<td>AD; H and A: correlation with MMSE and CDR; A: no significant correlations</td>
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</table>
with mild to moderate disease (see Table 1, MMSE scores ranging from 13 to 29 or CDR scores ranging from 0.5 to 1) [4, 10, 23, 25-28]. In particular, volume loss in mild to moderate AD patients varies from 15-16% [29, 30] to 33-37% [25, 31, 32].

The variation in findings may result from methodological issues related to amygdalar segmentation. Because of the numerous cortical and subcortical nuclei of the amygdala, its proximity to the hippocampus, and the similarity of neighboring tissues, the boundaries of the amygdala are difficult to distinguish...
Table 2. Summary of research on amygdalar atrophy in moderate to severe AD.

<table>
<thead>
<tr>
<th>Disease Severity</th>
<th>Population Characteristics</th>
<th>Segmentation Characteristics</th>
<th>Volume Data</th>
<th>Hippocampal Volume</th>
<th>Amygdalar Volume</th>
<th>Hippocampal vs. Amygdalar Atrophy</th>
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<th>Regression Analysis of Volumes with Cognitive Score</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Moderate</td>
<td>HC = 42; AD = 56. Age: HC = 73.2 (6.7); AD = 71.2 (8.6); MMSE: HC = 29 (1); AD = 18.3 (4.3)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (17% loss)</td>
<td>HC &gt; AD (23% loss)</td>
<td>A = H</td>
<td>AD; H: no correlation (language, memory, orientation, praxis, MMSE). A: correlation (memory, orientation, MMSE)</td>
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<td>Moderate</td>
<td>HC = 10; AD = 10. Age: HC = 56 (11); AD = 57 (9); MMSE: HC = 29 (1); AD = 15 (6)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (16.4% loss)</td>
<td>HC &gt; AD (15% loss)</td>
<td>A = H</td>
<td>AD; H and A: correlation MMSE</td>
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<td>Moderate</td>
<td>HC = 19; AD = 19. Age: HC = 73.6 (5.5); AD = 76.1 (5.7); MMSE: HC = 28.6 (1.1; 27-30); AD = 13.1 (3.8; 5-21)</td>
<td>Manual. MRI: 3T - slice thickness: 1 mm</td>
<td>ICV-normalized volumes; sex and education as covariate</td>
<td>HC &gt; AD (22% loss)</td>
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<tr>
<td>Moderate</td>
<td>HC = 18; AD = 27; Age: HC = 69.5 (6.4); AD = 71 (7.5); MMSE: HC = 30; AD = 19 (3.6)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (35% loss)</td>
<td>HC &gt; AD (16% loss)</td>
<td>A &lt; H</td>
<td>AD; H and A: correlation MMSE</td>
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<tr>
<td>Moderate</td>
<td>HC = 126; AD = 94. Age: HC = 79 (6.73); AD = 73 (8); MMSE: HC = 28 (1.26); AD = 17.8 (4.94)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.6 mm</td>
<td>Raw volumes; ICV as covariate</td>
<td>HC &gt; AD</td>
<td>HC &gt; AD</td>
<td>A = H</td>
<td>AD; age and A volume as covariate: A: no significant correlation (MMSE, cognitive battery)</td>
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<tr>
<td>Moderate</td>
<td>HC = 22; AD = 31; Age: HC = 67.7 (7.9); AD = 68 (6.8); MMSE: AD: 17.2 (3.2,14-23); HC: 28.8 (1.1, 25-36)</td>
<td>Manual. MRI: 0.5T - slice thickness: 5 mm</td>
<td>Normalized volumes</td>
<td>HC &gt; AD</td>
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<td>[87]</td>
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<tr>
<td>Moderate</td>
<td>HC = 12; AD = 46. Age: HC = 66.2 (4.9); AD = 70.3 (7.1); MMSE: HC = 28 (1); AD = 19.6 (3.5; 12-26)</td>
<td>Manual. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>Normalized volumes</td>
<td>HC &gt; AD (15% loss)</td>
<td>HC &gt; AD (18.5% loss)</td>
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<td>AD; H (right) and A; correlation (Wechsler visuo-spatial memory). A: correlation (Wechsler verbal memory)</td>
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<tr>
<td>Moderate</td>
<td>HC = 27; AD = 36. Age: HC = 72 (4.2); AD = 73 (8.9); MMSE: HC = 28; AD = 17.1 (5.2; 2-27)</td>
<td>Semi-automatic. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (24% loss)</td>
<td>HC &gt; AD (21% loss)</td>
<td></td>
<td>AD; volumes, age, sex, and education as covariates: A correlation (Wechsler visuo-spatial and verbal memory subset). H: no significant correlation</td>
<td>[22]</td>
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Amygdalar Atrophy in AD

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<tbody>
<tr>
<td>Moderate</td>
<td>HC = 20; AD = 20; MMSE: MA = 18.8 (5.7); &lt;26</td>
<td>Manual. MRI: 1.5T - slice thickness: 2 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (20% loss)</td>
<td>HC &gt; AD (33% loss)</td>
<td>AD; volumes, age as covariate: H no significant correlation; A (Right) correlation (ADAS non-cognitive score); AD; cognitive scores, age as covariates: H correlation (ADAS non-cognitive score); A correlation (ADAS-non-cognitive score, MMSE)</td>
<td>[88]</td>
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<tr>
<td>Moderate to severe</td>
<td>HC = 17; AD = 20; Age: HC = 63.6 (10.5); AD = 63.8 (9.1); MMSE: HC = 28.9 (1.3); AD = 20.3 (5.1)</td>
<td>Semi-automatic. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>ICV-normalized volumes</td>
<td>HC &gt; AD (20.5% loss)</td>
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<td>[73]</td>
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<tr>
<td>Moderate to severe</td>
<td>HC = 57; Moderate: 79; Severe: 34; Age: HC= 66.1 (8.3); Moderate AD = 73.4 (8.6); Severe AD = 71.3 (9.6); MMSE: HC = 29 (28-30); Moderate AD = 19 (16-22); Severe AD = 12.5 (11-18)</td>
<td>Automatic. MRI: 1.5T - slice thickness: 1.5 mm</td>
<td>Raw volumes - sex, education, and ICV as covariate</td>
<td>HC &gt; moderate &gt; severe</td>
<td>HC &gt; moderate &gt; severe</td>
<td>AD + HC; H and A: correlation (attention, language, visuospatial, memory, executive functions, dementia scores)</td>
<td>AD + HC; volumes, age, sex, education, and ICV as covariate: H correlation (memory, visuospatial, and executive functions, dementia scores). A: no significant correlations</td>
<td>[23]</td>
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</table>

Note: Disease severity was coded as follows: Mean MMSE between 13 and 20: Moderate. Mean MMSE between 3 and 12: Severe. AD = HC means that volumes between both groups are equivalent. HC > AD means that volumes of HC are higher than AD patients. H: hippocampal volume; A: amygdalar volume; HC: healthy control individuals; AD: Alzheimer’s disease patients.

to identify [33, 34]. Various approaches have been implemented to assess MTL atrophy in AD. One of these is based on voxel-based morphometry, which investigates amygdala volume change in AD within the MTL [35, 36]. While this technique allows group comparisons between AD and HC, it cannot provide the absolute volume of the structure at the individual level. The second technique uses manual segmentation of the amygdala on magnetic resonance imaging (MRI) [28, 31, 37, 38]. Although it remains the gold standard, this approach is impractical for cohorts beyond a certain size, because it requires a large amount of expert interaction for each image [33]. The third technique consists of semi-automatic (18) or automatic segmentation of the amygdala and hippocampus [26] on the basis of single-subject digital atlases. A limitation of these techniques is that single-subject atlases do not sufficiently take into account the neuroanatomical variation between subjects. This problem affects amygdala segmentation in particular, because the size and shape of the amygdala varies substantially even within demographically homogeneous groups of healthy subjects. This limitation can be addressed by probabilistic seeding followed by region-growing techniques [39] or by using multiple atlases [40, 41]. In combination with tissue probability maps to enhance registration, multi-atlas approaches are particularly suitable for subjects with neurodegenerative disease [42, 43]. We therefore used multi-atlas propagation with enhanced registration (MAPER) [42] to achieve the first objective of this study, which was to perform amygdalar volumetry in patients with mild to moderate AD.

The relation between amygdalar atrophy and the severity of the disease is still a matter of debate (see Tables 1 and 2). A correlation between amygdalar atrophy and cognitive impairment in AD has been found in some studies [5, 26, 27, 37, 38], but not in others [25, 30, 31]. In most studies, the clinical significance of amygdalar atrophy in AD was estimated without controlling for hippocampal atrophy [25, 30, 31, 37, 38, 44, 45], which adds to the difficulty of interpreting the results. Considering the strong functional connectivity between the hippocampus and the amygdala, specifically in the context of memory [46, 47], it seems especially important to correct for hippocampal atrophy when assessing the specificity of the relationship between amygdalar atrophy and memory decline in AD. Furthermore, the majority of prior studies combined AD and HC groups for correlation.
Control subjects were selected from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). The inclusion criteria for normal subjects were as follows:

1. No memory complaints aside from those common to other normal subjects of that age range
2. Normal memory function documented by scoring above specific cutoffs on the Logical Memory II subscale (delayed paragraph recall) from the Wechsler Memory Scale—Revised (the maximum score is 25). The cutoffs were as follows:
   a. 9 or higher for 16 or more years of education
   b. 5 or higher for 8-15 years of education,
   c. 3 or higher for 0-7 years of education
3. MMSE score between 24 and 30 (inclusive)
4. Clinical Dementia Rating and Memory Box scores of zero
5. Absence of significant impairment of cognitive functions and activities of daily living
6. Absence of depression (scores lower than 6 on Geriatric Depression Scale)

Subjects were excluded if they were on current medication, except for vitamin E, estrogen, and estrogen-like compounds if the dose had been stable for at least four weeks before screening. Eighty-two ADNI control subjects (mean age 76.2 years; SD = 5.1) were compared with the study group. Control subjects and patients did not differ in terms of age and gender composition.

AD Neuropsychological Examination

The neuropsychological examination of AD patients included tests that were standardized for French native speakers and that assessed (1) global cognitive functions (MMSE [51], French version [52]); (2) short-term memory (digit span forward and backward of the Wechsler Adult Intelligence Scale [53]); (3) long-term memory (RL/RI16 [54], a word learning test based on the selective reminding procedure developed by Grober and Buschke [55]; BEM 144 – figure learning, part of the Memory Performance Battery of the BEM-144 [56]; Doors and People Test [57]); (4) language abilities (Famous Faces Test, an unpublished test de-

MATERIAL AND METHODS

Participants

We recruited patients with memory complaints who consulted the Memory Center of the Grenoble University Hospital between October 2010 and February 2012. The diagnosis of probable AD was made according to the National Institute of Neurological, Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [50]. All patients were diagnosed after extensive clinical evaluation, including detailed history, physical and neurological examination, and extensive neuropsychological tests. Only patients who were more than 60 years old (mean age of the AD group: 75.3 years; SD = 5.7) and who were new referrals for evaluation of memory complaints were included. Exclusion criteria were current or past diagnosis of somatic, psychiatric, or neurological disorders such as stroke, head trauma, brain tumor, Parkinson’s disease, or temporal lobe epilepsy. In addition, subjects with findings suggesting another neurodegenerative disease, such as primary progressive aphasia, fronto-temporal dementia, Lewy body dementia, or mixed forms, were excluded. A total of 48 subjects were selected for our study (33 women and 15 men).
signed to assess recognition and naming of 30 monochrome pictures of famous faces; Bachy-Langedock test, in which subjects have to name 36 black-and-white drawings of ordinary objects; Irregular word writing, in which subjects have to write French words with unusual spelling); and (5) executive functions (Literal and category fluency – letter “p” and animals [58]; Trail making test, part A & B [59]).

MRI Data Acquisition

Atlas data as required for MAPER consisted of 30 T1-weighted 3D image volumes acquired from healthy young adult volunteers at the National Society for Epilepsy at Chalfont, United Kingdom at 1.5 T. Detailed demographics, acquisition, and anatomical protocol information is available in Gousias et al., Hammers et al., and Niemann et al. [48, 49, 60]. Hand-drawn segmentations of 83 structures had been prepared according to the protocols described in these reports [48, 49, 60]. Segmentation protocols are also available at http://www.brain-development.org. We focused on four regions of interest, the left and right hippocampus, and the left and right amygdala.

For the matched control subjects, we selected the ADNI 1.5 T screening image and downloaded the pre-processed version (B1 non-uniformity correction, geometric distortion correction, bias field correction, and phantom scaling) as recommended by ADNI. The acquisition parameters for the various centers are available in Jack et al. [61].

MR images of patients were acquired on a 1.5 T Achieva MR scanner (Philips Healthcare) at Grenoble University Hospital, using a whole-body RF transmit coil and eight-channel head receive coils. Anatomical studies consisted of a 3D gradient recalled echo T1-weighted image (TR: 8.1 ms, TE: 3.8 ms, 1 × 1 × 1.3 mm voxel matrix, 256 mm field of view, 100 contiguous slices).

Pre-Processing

Additional pre-processing of MR images was carried out to determine the intracranial volume (ICV) and to obtain tissue-class probability maps. ICV masks were determined for the control group using the procedure described in Heckemann et al. [42]. As this procedure relies on a semi-automatically generated white-and-grey matter mask, which was not available for the study group, we implemented [42] a multi-atlas label propagation procedure to generate ICV masks: a given study group (target) image was paired with each ADNI image and registered using non-rigid image registration. The resulting masks were added in the space of the target and thresholded at 50% to obtain an intracranial label for the target. The labels were visually reviewed for accuracy, and the threshold value modified to improve the ICV label if necessary. Probabilistic tissue-class maps were obtained using FSL FAST [62].

Segmentation

The MAPER procedure has been described and validated previously in AD populations [42, 43]. Each target is paired with each of the 30 atlases to generate an individual atlas-based segmentation. This results in 30 segmentations for each target image, which are subsequently combined by using vote-rule decision fusion [41, 63].

Masking Based on Tissue Class

We masked both regions (hippocampus and amygdala) by multiplication with a binary grey matter label generated as a maximum probability label with FSL FAST [62]. The analysis results reported in this work are based on the masked label sets.

Visual and Statistical Analysis

Masked hippocampal and amygdalar volumes in each target subject were visually checked by an expert (RAH; see example in Fig. 1). AD and HC were compared in terms of demographic and neuropsychological scores by using chi-square tests (categorical variables) or Student’s t tests (continuous variables). Volumetric comparisons between groups were based on statistical analyses, including volumes normalized by ICV. The normalized volume was calculated for each individual and each structure, and expressed as a fraction of the total ICV, scaled by an arbitrary factor of 10^3 [(masked volume/ICV) × 10^3]. Analysis of volumetric differences between AD and HC groups (between-subject factor, the “Group” variable) was performed by using two within-subject factors: “Structure Volume” (ICV-normalized hippocampal and amygdalar volumes) and “Laterality” (right: R and left: L). Analysis of covariance (ANCOVA; General Linear Model [GLM]) was then used with ICV-normalized volumes as a dependent variable; “Group”, “Structure Volume”, and “Laterality” as independent variables, and “age”, “gender”, and “education” as covariates. Since previous work revealed substantial differences in the magnitude of the amygdalar and hippocampal volume loss (see Tables 1 and 2), we additionally calculated an atrophy index for these structures, as follows: [1-(AD subject ICV-normalized volume / HC mean ICV-normalized volume)]

To assess the relationship between AD patients’ volumes and their cognitive scores (assessing memory, language abilities, and executive functions), we first analyzed whether the predictor variables (both hippocampal and amygdalar ICV-normalized volumes and age, sex, and education) were correlated with cognitive scores by using bivariate correlations (Pearson’s r). Subsequently, we used multiple linear regressions to determine whether AD patients’ volumes predict cognitive scores (dependent variables), for each cognitive score separately, considering the contribution of all predictor variables (i.e., age, gender, and education, as well as left and right hippocampal and amygdalar normalized volumes, all as independent variables). Multiple linear regression analysis then takes into account each variable of interest in the same analysis, and so controls the influence of both neuroanatomical structures.

RESULTS

AD and HC groups did not differ significantly in age (AD range: 64-87; HC range: 65-87, t(1,128) = -0.92, p =

3 Multiple regression analysis without the contribution of the demographic data age, gender, and education was also tested; the significance of the predicted hippocampus and amygdala volumes on cognitive scores remained the same whether they were considered or not. To control for demographic modulation, we decided to keep this factor in the statistical analysis.
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0.3), gender \( \chi^2: 1.67, df = 1, p = 0.19 \), or total ICV (AD range: 1.2 – 1.6 l; HC range: 1.1 – 1.7 l, \( t(1,128) = -0.61, p = 0.54 \)). Compared with HC, patients had lower MMSE scores (AD range: 15-29; HC range: 26-30, \( t(1,128) = -14.5, p < 0.001 \)) and a lower level of education (\( t(1,128) = -10.6, p < 0.001 \). MMSE scores of AD patients ranged from 15 to 29. Thirty-two patients (of 48) had mild AD severity (mean ± SD MMSE score 24.9 ± 2.3, range 21-29), and 16 of 48 had moderate AD severity (MMSE 18.1 ± 1.7, range 15-20). Subject characteristics are shown in Table 3.

Fig. (1). Example of brain segmentations from a subject with AD.

Table 3. Healthy control and AD patient group characteristics.

<table>
<thead>
<tr>
<th></th>
<th>HC (n=82)</th>
<th>AD (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± SD)</td>
<td>76.2 ± 5.1</td>
<td>75.3 ± 5.7</td>
</tr>
<tr>
<td>Gender (W:M)</td>
<td>47:35</td>
<td>33:15</td>
</tr>
<tr>
<td>Education (years; mean ± SD)</td>
<td>15.6 ± 3.2</td>
<td>8.1 ± 4.7</td>
</tr>
<tr>
<td>MMSE (score; mean ± SD)</td>
<td>29.1 ± 0.9</td>
<td>22.6 ± 3.9</td>
</tr>
<tr>
<td>ICV (L; mean ± SD)</td>
<td>1.36 ± 1.2</td>
<td>1.35 ± 1.19</td>
</tr>
</tbody>
</table>

Note. W = women, M = men, MMSE = Mini-Mental State Examination, ICV = total intracranial volume, n = number of subjects, SD = standard deviation, HC = healthy controls, AD = Alzheimer’s disease.

Amygdalar and Hippocampal ICV-Normalized Volumes

A significant main effect of Structure Volume \( F(1,125) = 21.67; p < 0.001 \) and Group \( F(1,125) = 131.7; p < 0.001 \) was observed. Moreover, we observed a significant interaction of Structure Volume \( \times \) Group \( F(1,125) = 16.01; p < 0.001 \). Planned comparisons demonstrated a significant effect of the hippocampus \( F(1,125) = 93.4; p < 0.001 \) and the amygdala \( F(1,125) = 113.3; p < 0.001 \) (Fig. 2), indicating that, compared with HC, AD patients showed significantly smaller volumes of both hippocampus and amygdala. The effect of age \( F(1,125) = 16.96; p < 0.001 \) and gender \( F(1,125) = 5.41; p = 0.02 \) suggests that the amygdalar and hippocampal volumes decrease with age and are larger in women than in men. Education level \( F(1,125) = 1.33; p = 0.24 \) had no significant effect on structure volumes.

Fig. (2). Box plot of the normalized hippocampal and amygdalar volumes in AD patients and HC. The center line shows the median, boxes capture the 25%-75% quantile range, and whiskers indicate 1.5 interquartile ranges. The ring denotes an outlier. Normalized volumes represent gray-matter masked volumes in mm^3, divided by total intracranial volume and scaled (arbitrarily) by 10^4. AD = Alzheimer’s disease, HC = healthy controls. The volumes differed significantly between AD and HC subjects (p < 0.05).

No main effect of Laterality \( F(1,125) = 1.84; p = 0.17 \), Laterality \( \times \) Group \( AD \) patients’ right hemisphere volumes: mean ± SD 9.43 ± 2.84; AD patients’ left hemisphere volumes: mean ± SD 8.95 ± 2.15; HC right hemisphere volumes: mean ± SD 12.36 ± 3.09; HC left hemisphere volumes: mean ± SD 11.85 ± 2.32; \( F(1,125) = 1.15; p = 0.69 \), and Structure Volume \( \times \) Laterality \( \times \) Group \( F(1,128) = 0.56; p = 0.45 \) was observed in the full model (the physiological R>L asymmetry (e.g., [60]) was observed when age was removed from the model). For subsequent analyses, left and right volumes of each structure (i.e., hippocampus and amygdala) were averaged.

To assess whether the magnitude of the atrophy was similar across structures in AD patients, we included the magnitude index of (R+L) hippocampal and (R+L) amygdalar volumes into the ANCOVA analysis, controlling for age, gender and education. Results revealed no significant effect of the magnitude of the volume loss (amygdalar volume loss: mean ± SD 25.4 ± 11.5 %; hippocampal volume loss mean ± SD 23.2 ± 14.2 %; \( F(1,44) = 2.29; p = 0.13 \), representation as a percentage in Fig. 3). The volume loss for both structures was thus similar in AD patients. The effect of age \( F(1,44) = 5.27; p=0.02 \) was statistically significant, but that of gender \( F(1,44) = 1.92; p = 0.17 \) or of education level \( F(1,44) = 1.13; p = 0.71 \) was not.
Amygdalar Atrophy in AD

Correlations Between ICV-Normalized Volumes and Neuropsychology Scores

Bivariate correlations indicated that the ICV-normalized hippocampal and amygdalar volumes in AD patients were significantly and positively correlated. In addition, the ICV-normalized hippocampal volume of AD patients was negatively correlated with age (Table 4). MMSE scores were strongly correlated with gender and level of education, indicating that higher MMSE scores were related to a higher level of education and that women had higher scores than men. Significant correlations were found between memory scores and amygdalar volume, whereas language abilities (naming of famous faces and irregular word writing tests) were correlated with hippocampal volume (Table 4).

Multiple linear regression analysis (Table 5) was performed to determine whether volumes in AD patients were predictive of cognitive decline. Age, gender, and education level were additionally included into the regression model as covariates. The amygdalar volume was correlated with memory performance (Grober & Buschke memory test) after we controlled for demographic variables and, of importance, after we controlled for hippocampal volume, indicating that amygdalar atrophy contributes to memory decline independently from hippocampal volume loss. Hippocampus or amygdala volumes did not predict scores for executive functions. The amygdala volume was predictive of naming scores (Bachy-Langedock object naming test).

DISCUSSION

Hippocampal atrophy is considered the hallmark of AD. However, hippocampal volume loss is associated with many other diseases, including Parkinson’s disease [12], frontotemporal lobar degeneration [64], vascular dementia [13, 14], and schizophrenia [15]. Moreover, researchers have detected substantial hippocampal volume loss in cognitively intact individuals [65]. Although hippocampal atrophy has strong sensitivity for AD, several studies showed that its specificity is low. To attempt to improve the identification of this disease by using neuroimaging markers, we evaluated the volume of two limbic structures, the hippocampus and the amygdala. We were also interested in evaluating the relationship between their volumes and neuropsychological test scores.

A critical factor for sensitively in detecting the correlations described in this work was the accuracy of the volumetry method used. Manual image segmentation, while considered the gold standard, is impractical for studies of this size, and the quality of the segmentations depends on many methodological details, e.g., slice thickness [33, 66, 67]. Conventional automatic segmentation approaches frequently fail when the target image is affected by changes typical of neurodegenerative disease, such as ventriculomegaly [43]. We used MAPER [42] and the largest single-investigator atlas database currently available, consisting of 30 manually segmented atlases [48, 49]. This combination eliminates idiosyncrasies typically associated with single-atlas methods and entails high accuracy as well as robustness toward atrophy-related segmentation failures. MAPER has been extensively validated [42] and used [43] for target images acquired from patients with AD.

Amygdalar atrophy was previously regarded as typical for moderate and late AD stages. We report here that it also occurs in early stages. The amount of volume loss is comparable to that seen in the hippocampus. In addition, we observed that amygdala volume predicts cognitive scores even after hippocampal volume is controlled for. This suggests that as a biomarker for early AD, amygdalar volume may be at least as important as hippocampal volume.

A crucial question is whether regional atrophy predicts memory impairment. Contrary to recent findings [26, 37, 38], our results have not revealed any significant correlation between a global index of clinical severity assessed with MMSE and the amygdalar and hippocampal volume. This may be due to the average MMSE score in our study being higher than in previous studies [37, 38]. For early disease, the MMSE scale may not be sensitive enough to reflect a relationship with atrophic regions [25, 31]. In addition, even when strong correlations between volumes and MMSE scores have been found [23, 26, 68] they did not remain statistically significant after factoring out other structure volumes in a linear regression model. In this respect, our results are in agreement with other studies [25, 27, 30, 31, 45].

While significant correlations with MMSE were absent, more specific clinical indices of the disease, such as declarative memory performance (Grober & Buschke test), did correlate with amygdalar volume. Other authors have suggested that such a correlation is explained by the strong correlation between the amygdala and the hippocampus [46, 47]. We performed multiple regression analysis to evaluate amygdalar atrophy and cognitive scores independently of variations of hippocampal volume. We therefore posit that the amygdala has stronger relevance than previously acknowledged.

These results could also reflect an increased sensitivity owing to the accuracy of the MAPER method. Mori et al. [5] stressed that the correlation between hippocampal volume...
Table 4. Bivariate correlational analysis of the associations between predictor variables (demographic data and normalized volumes of the hippocampus and amygdala) and neuropsychological tests in patients with mild to moderate Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Variables</th>
<th>1 – Age</th>
<th>2 – Sex</th>
<th>3 – Education</th>
<th>4 – Normalized Hippocampus Volume</th>
<th>5 – Normalized Amygdala Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s $r$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – Age</td>
<td></td>
<td>-0.14</td>
<td>0.30*</td>
<td>-0.37**</td>
<td>-0.25</td>
</tr>
<tr>
<td>2 – Sex</td>
<td>-0.14</td>
<td></td>
<td>-0.24</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>3 – Education</td>
<td>0.30*</td>
<td>-0.24</td>
<td></td>
<td>-0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>4 - Normalized hippocampus volume</td>
<td>-0.37**</td>
<td>0.21</td>
<td>-0.18</td>
<td>_</td>
<td>0.69**</td>
</tr>
<tr>
<td>5 - Normalized amygdala volume</td>
<td>-0.25</td>
<td>0.22</td>
<td>0.03</td>
<td>0.69**</td>
<td>_</td>
</tr>
<tr>
<td>MMSE</td>
<td>-0.04</td>
<td>-0.28*</td>
<td>0.49**</td>
<td>-0.01</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

Memory

- WAIS : Digit span forward 0.16 -0.21 0.20 -0.22 -0.21
- WAIS : Digit span backward 0.10 -0.3 0.29 -0.27 -0.22
- RL-RI 16: Immediate recall 0.22 -0.25 -0.18 0.08 0.12
- RL-RI 16: Free recall 0.08 -0.03 -0.22 0.22 0.36*
- RL-RI 16: Total recall 0.03 0.10 -0.32 0.30 0.39*
- RL-RI 16: Delayed free recall 0.02 0.01 -0.26 0.28 0.44**
- RL-RI 16: Delayed total recall 0.10 -0.01 -0.34* 0.29 0.35*
- BEM 144: Immediate recall -0.18 -0.3 0.01 0.27 0.24
- BEM 144: Delayed recall -0.15 -0.32 0.00 0.28 0.33*
- Doors and People Tests (set A+ B) -0.31 -0.08 -0.12 0.25 0.09

Language abilities

- Naming of famous faces 0.04 -0.02 0.02 0.50* 0.38
- Bachy-Langedock test 0.14 -0.04 0.15 -0.08 -0.03
- Irregular words writing 0.14 0.03 0.32 0.41* -0.23

Executive functions

- VF: Literal 0.06 0.18 0.39* -0.1 0.09
- VF: Category -0.2 0.09 0.11 0.2 0.2
- TMT: A 0.01 0.07 -0.18 0.17 0.26
- TMT: B 0.37* 0.03 -0.15 0.06 0.03

Note: MMSE = Mini-Mental State Examination, WAIS = Wechsler Adult Intelligence Scale, RL-RI 16 = Rappel libre – Rappel Indicé of the Gröber & Buschke test, BEM 144 = Batterie d'Éfficience Mémorique (Memory Performance Battery), VF = Verbal Fluency, TMT = Trail Making Test. Positive correlation for the sex variable means that females have higher scores than males. Negative correlation for the sex variable means that males have higher scores than females. Significant correlations are marked with asterisks: * $p<0.05$; ** $p<0.01$.

and memory scores depends on whether the hippocampal segmentation encompassed the subiculum region. Critically, AD pathology affects numerous structures that surround the hippocampus, such as the subiculum, parahippocampal gyrus, and entorhinal cortex [69]. Anatomical connections between the subiculum and the hippocampus are particularly important in supporting memory functions [70]. Therefore, additional MTL structures not considered in our study (e.g., subiculum, entorhinal, perirhinal, or parahippocampal cortices) may explain memory impairment beyond the hippocampus. Consistent with this possibility in this framework is that the hippocampus shows evidence for plasticity in dementia. In fact, Dickerson et al. [71] demonstrated that compensatory hippocampal mechanisms are activated during the earliest stages of the atrophy process. Moreover, Jin et al. [72] showed that neurogenesis in the hippocampus occurs even in the context of AD. Taken together, these studies support the view that correlations between hippocampal volume, hippocampal function, and memory impairment in AD are highly non-linear.

Overall, our results demonstrate that MAPER-based amygdalar volumetry can show major neuroanatomical
Table 5. Multiple linear regression analyses of the associations between volumes of the hippocampus and amygdala and neuropsychological tests in patients with mild to moderate Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Neuropsychological Variable</th>
<th>Hippocampus</th>
<th>Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAIS: Digit span forward</td>
<td>5.3 (0.9)</td>
<td>(3-7)</td>
</tr>
<tr>
<td>WAIS: Digit span backward</td>
<td>4.1 (0.7)</td>
<td>(3-5)</td>
</tr>
<tr>
<td>RL-RI 16: Immediate recall</td>
<td>13.4 (2.2)</td>
<td>(8-16)</td>
</tr>
<tr>
<td>RL-RI 16: Total recall</td>
<td>31.1 (11)</td>
<td>(3-48)</td>
</tr>
<tr>
<td>RL-RI 16: Delayed free recall</td>
<td>3.6 (3.6)</td>
<td>(0-12)</td>
</tr>
<tr>
<td>RL-RI 16: Delayed total recall</td>
<td>10.1 (4.1)</td>
<td>(1-16)</td>
</tr>
<tr>
<td>BEM 144: Immediate recall</td>
<td>5.2 (2.1)</td>
<td>(1-5)</td>
</tr>
<tr>
<td>BEM 144: Delayed recall</td>
<td>4.4 (2.7)</td>
<td>(0-10.5)</td>
</tr>
<tr>
<td>Doors and People Tests (set A+ B)</td>
<td>12 (5.3)</td>
<td>(1-22)</td>
</tr>
<tr>
<td>Language abilities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naming of famous faces</td>
<td>55.9 (23)</td>
<td>(12-93)</td>
</tr>
<tr>
<td>Bachy-Langedock test</td>
<td>34.1 (1.6)</td>
<td>(30-36)</td>
</tr>
<tr>
<td>Irregular words writing</td>
<td>9.4 (0.7)</td>
<td>(8-10)</td>
</tr>
<tr>
<td>Executive functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VF: Literal</td>
<td>17 (6.4)</td>
<td>(5-28)</td>
</tr>
<tr>
<td>VF: Category</td>
<td>16.8 (5.9)</td>
<td>(4-27)</td>
</tr>
<tr>
<td>TMT: A</td>
<td>81.4 (58.2)</td>
<td>(26-310)</td>
</tr>
<tr>
<td>TMT: B</td>
<td>202.8 (120.4)</td>
<td>(62-538)</td>
</tr>
</tbody>
</table>

Note: Cognitive tests were entered as dependent variables; hippocampal volumes, amygdalar volumes, age, sex, and education were entered as independent variables. MMSE = Mini-Mental State Examination, WAIS = Wechsler Adult Intelligence Scale, RL-RI 16 = Rappel libre – Rappel Indiqué of the Gröber & Buschke test, BEM 144 = Batterie d’Efficience Mémoire (Memory Performance Battery),VF = Verbal Fluency, TMT = Trail Making Test, SD = Standard Deviation, df = degrees of freedom. The reported mean, standard deviation, and range were calculated after correcting for outliers (Stevens, 1984). The original data were transformed to the logarithmic function. Two outliers on the Cook criterion were excluded. One outlier on the Cook criterion was excluded. Three outliers were excluded on the SDR criterion. Significant associations are marked with asterisks: * p<0.05. ** p<0.01.


The differences between AD patients and HC. Amygdalar atrophy and its association with memory decline in the early stages of AD may be a useful marker for clinical diagnosis. However, additional studies are needed to assess in more detail the clinical relevance of investigating this structure. In particular, amygdalar atrophy patterns in AD could be compared with those occurring in other types of neurodegenerative disease. One study reported more severe amygdalar atrophy in fronto-temporal dementia than in AD [73], but further work is needed to develop differential diagnostic criteria. Future studies should also investigate whether substantial amygdalar volume change occurs in more cognitively intact persons, including patients with mild cognitive impairment (MCI). To clarify the potential role of the amygdala as an early AD biomarker, it may be relevant to assess whether the association between cognitive abilities and amygdalar volume in cognitively intact persons is dissimilar to that obtained in AD patients. In addition, correlations reported between amygdalar volume and cognitive scores are not proof of any mechanism of causation. The fact that the level of volume reduction in this structure can predict several types of cognitive decline, even after hippocampal volume is controlled for, may appear surprising, since the amygdala’s primary role is emotion processing and not memory or language processing. However, the amygdala is intricately connected with the four lobes of the brain (i.e., occipital, temporal, parietal, frontal) [74], leading to its indirect implication in a vast range of cognitive functions, including perception, attention, and declarative memory [75]. Regarding memory function, the amygdala is able to modulate the encoding and consolidation of information when it pertains to emotional stimuli [76-79]. In a recent functional MRI study on MCI and AD [80], authors found that functional connectivity was notably decreased (according to disease severity) between the amygdala and structures of the default mode.
network (e.g., hippocampus, parahippocampal gyrus, super-
ior frontal gyrus, medial frontal gyrus), an ensemble of
regions implicated in an array of cognitive functions, nota-
bly episodic memory [80]. This decrease in functional con-
nectivity between the amygdala and structures of the de-
fault mode network is suggested to underlie the memory
deficits observed in MCI and AD. Through its various con-
nections, the amygdala may have a role in modulating nu-
erous cognitive functions, including those that are af-
ected in dementia. Taken together, these studies show that
the amygdala is not only an emotion-processing structure,
but rather an essential part of a large network of structures
that is able to adapt its activity as changes occur throughout
the network. Further research is needed to assess the spe-
cific amygdalar compensatory mechanisms that take place
throughout the course of dementia. If amygdalar volume
loss is indeed predictive of cognitive decline and dementia,
then affective disorders will most likely ensue, as the
amygdala remains the key structure in processing emotions.
Affective disorders have been previously observed in MCI
and mild AD [81-83], yet whether these disturbances occur
systematically and in parallel with amygdalar atrophy re-
mains unknown.

CONFLICT OF INTEREST
The author(s) confirm that this article content has no con-
flicts of interest.

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ume reduction in subcortical regions according to severity of Alz-


