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Medial temporal atrophy in early and late-onset Alzheimer's disease

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Abstract

Late-onset and early-onset Alzheimer's disease (LOAD, EOAD) affect different neural systems and may be separate nosographic entities. The most striking differences is in the medial temporal lobe, severely affected in LOAD and relatively spared in EOAD. We assessed amygdalar morphology and volume in 18 LOAD and 18 EOAD patients and 36 aged-matched controls, and explored their relationship with hippocampal volume. 3D amygdalar shape was reconstructed with the Radial Atrophy Mapping technique, hippocampal volume was measured using a manual method. Atrophy was greater in LOAD than EOAD: 25% versus 17% in the amygdala and 20% versus 13% in the hippocampus. In the amygdala, LOAD showed significantly greater tissue loss

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than EOAD in the right dorsal central, lateral, and basolateral nuclei (20-30% loss, $p < 0.03$), all known to be connected to limbic regions. In LOAD but not EOAD, greater hippocampal atrophy was associated with amygdalar atrophy in the left dorsal central and medial nuclei ($r = 0.6$, $p < 0.05$), also part of the limbic system. These findings support the notion that limbic involvement is a prominent feature of LOAD but not EOAD.

Keywords

AD; age at onset; morphology; amygdala; hippocampus; MRI

1. Introduction

Alzheimer's Disease (AD) patients are commonly classified into early- and late-onset forms according to the age at onset of symptoms. Early-onset AD (EOAD) includes those patients meeting the clinical criteria for AD (McKhann et al., 2011) with symptoms appearing before age 65. Clinically, EOAD patients often present a non-memory phenotype, of which apraxia and visuospatial dysfunction are the most commonly presenting symptoms (Koedam et al., 2010, Sa et al., 2012). Conversely, late-onset AD (LOAD) patients show symptoms onset after age 65 and they tend to show a more 'typical' clinical profile, with poorer performance in memory and orientation. The pathological underpinnings of these differences, however, are largely unknown. Genetic factors such as APOE genotype might influence EOAD and LOAD forms. The presence of the APOE $\epsilon 4$ allele is a risk factor for LOAD (Blackner and Tanzi, 1998, Meyer et al., 1998), and has been suggested to modulate the disease onset and expression (van der Flier et al., 2011, Wolk et al., 2010). In EOAD, conversely, other genes such as APP, PSEN1 and PSEN2 (Raux et al., 2005, Rovelet-Lecrux et al., 2006) could be involved. Recent evidence from molecular studies suggests possible differences in amyloid production, EOAD but not LOAD forms being linked to abnormal A β 42/40 ratios (Bali et al., 2012). Anatomically, this profile suggests a somewhat more localized disease affecting the medial temporal lobe (MTL) regions (Sa et al., 2012, Smits et al., 2012), (Reid et al., 1996), while the clinical profile of EOAD suggests broader neocortical involvement. These clinical observations are supported by in vivo magnetic resonance imaging (MRI) studies (Frisoni et al., 2005), (Frisoni et al., 2007), (Anonymous, Cho et al., 2013, Ishii et al., 2005, Karas et al., 2007, Moller et al., 2013, Shiino et al., 2006, Shiino et al., 2008, van de Pol et al., 2006, van der Vlies et al., 2013) and by recent pathological evidence (Whitwell et al., 2012). These findings support the view that MTL damage is a key neurological substrate in LOAD. Evidence for this hypothesis comes mainly from studies of the hippocampus (Moller et al., 2013, Shiino et al., 2006, Shiino et al., 2008), and we know less about whether – and to what extent – other MTL structures are involved. Indeed, the MTL system is made up of the hippocampus and amygdala, but the amygdala has never been assessed in both LOAD and EOAD. The amygdala is a part of the limbic system that plays a major role in processing and memorizing emotional reactions (Schafe et al., 2005). Amygdala lesions produce declarative memory impairment in humans mainly related to autobiographical events, olfactory and emotional stimuli (Adolphs et al., 2000, Buchanan et al., 2003, Buchanan et al., 2006). The effects appear early in AD, and contributes to the memory and functional deficits. Post-mortem studies of AD patients have largely shown that amyloid

plaques, neurofibrillary tangles, and neuronal loss occur in the amygdala of AD cases (Arriagada et al., 1992, Tsuchiya and Kosaka, 1990)(Herzog and Kemper, 1980), and that this neurodegenerative process starts in the Braak and Braak stages II-III, i.e. concomitantly with hippocampal neurodegeneration. Similarly, in vivo MRI studies show reduced amygdala volumes in AD patients (Barnes et al., 2006, Cavedo et al., 2011, Cavedo et al., 2011, Cuenod et al., 1993, Heun et al., 1997, Martinez-Castillo et al., 2001, Prestia et al., 2011). Moreover, studies of amygdala morphology reported a significant tissue loss in AD patients in the dorsal medial and central nuclei, and in the ventral basolateral complex (Cavedo et al., 2011, Qiu et al., 2009), in line with neuropathology. In spite of this evidence, no studies have yet investigated amygdala morphology and its relationship with the hippocampus in EOAD and LOAD.

Here we aimed to assess local tissue loss in the amygdala of EOAD and LOAD patients using a parametric surface mesh modelling method. In addition, we investigated the relationship between amygdalar and hippocampal atrophy within AD sub-groups. According to the hypothesis of a greater MTL system neuropathology in LOAD, we expected to find a specific amygdalar tissue loss in those nuclei connected to the hippocampal formation in LOAD but not in EOAD.

2. Material and methods

2.1 Participants and assessment

Patients were selected from those assessed at the IRCCS Centro San Giovanni di Dio Fatebenefratelli (National Center for Alzheimer's Disease), in Brescia, Italy, in the context of a project aimed to detect structural brain changes in the neurodegenerative dementias with advanced neuroimaging techniques (Frisoni et al., 2007, Pievani et al., 2013). Each patient underwent: (i) history taking, (ii) laboratory exams, (iii) physical and neurological examination, (iv) neuropsychological assessment, and (v) MRI scanning. History was taken with a structured interview from patients' relatives (usually spouses) and age at onset was estimated from the caregiver's report of memory disturbances exceeding the episodic forgetfulness that might be regarded as usual for the patient or report of other progressive disturbances (language, praxis, orientation, visuospatial skills) (Frisoni et al., 1996). Laboratory exams included complete blood count, chemistry profile, thyroid function, B12 and folic acid and EKG. Physical examination was performed by a geriatrician and neurological examination by a neurologist. Neuropsychological assessment was performed by a psychologist and assessed: (i) global cognitive functioning with the Mini Mental State Examination (Folstein et al., 1975), (ii) language with letter and category fluency (Novelli et al., 1986) and token tests (DE RENZI and VIGNOLO, 1962)(Spinnler and Tognoni, 1987), (iii) visuospatial functions with the Rey figure copy test (Caffarra et al., 2002), (iv) frontal-executive functions with the Trail Making Test (Amodio et al., 2002), and (v) learning with Rey's word list immediate and delayed recall (Carlesimo et al., 1996) and Rey figure delayed recall tests (Caffarra et al., 2002).

Participants were eligible if they had a diagnosis of AD according to core clinical criteria for probable AD (McKhann et al., 2011). Exclusion criteria were: (i) evidence of depression or dysthymia; (ii) abnormal laboratory tests; (iii) other major systemic, psychiatric or

neurological illnesses; (iv) other causes of focal or diffuse brain damage on their MRI scan (e.g., lacunae and extensive cerebrovascular disorders). A total of 18 patients with an early disease onset (65 years or younger; EOAD) were eligible for the study. Then, 18 patients who were 65 years old or older at disease onset (LOAD) were 1:1 matched to the EOAD patients by dementia severity (as measured by the Clinical Dementia Rating Scale, (Hughes et al., 1982)). When more than one matching LOAD patient was available, the one with the closest matching MMSE was chosen. None had a family history suggestive of autosomal dominant AD. All patients with cerebrospinal fluid data available (n=9, 25%) showed abnormal amyloid beta(1-42) levels according to the established cut-off (normal levels >500 pg/mL; (Sjogren et al., 2001)).

Healthy controls were selected from participants enrolled in a study collecting MRI and clinical data from the normal population, described in detail elsewhere (Galluzzi et al., 2009). Exclusion criteria were the presence of clinical, psychiatric, neurological, or neuropsychological impairment, and of cerebrovascular disease or positive finding at neuroradiological evaluation of the MR. Healthy participants were matched 1:1 to the EOAD or LOAD groups based on their age and sex, to obtain two different groups of healthy controls: the Young Control (n=18, YC) and the Older Adults Control (n=18, OAC) groups. Written informed consent was obtained from patients and controls. No compensation was provided for study participation. The study was approved by the local ethics committee.

2.2 Genetic Analysis

Genomic DNA was extracted from whole-blood samples of participants according to standard procedures. APOE genotyping was carried out by PCR amplification and HhaI restriction enzyme digestion. The genotype was resolved on 4% Metaphor Gel (BioSpa, Italy) and visualized by ethidium bromide staining (Hixson and Vernier, 1990).

2.3 MR imaging

MRI scans were acquired with a Philips Gyroscan 1.0 T at the Neuroradiology Unit of the Citta' di Brescia Hospital in Brescia. High-resolution gradient echo sagittal 3D sequences (TR=20 ms, TE=5 ms, flip angle=30°, field of view=220 mm, acquisition matrix=256×256 and slice thickness=1.3 mm) were used for amygdalar and hippocampal volume measurements (Frisoni et al., 2007), axial dual echo (TR=2000 ms, TE=8.8/110 ms, flip angle=90°, field of view=230 mm, acquisition matrix= 256×256, slice thickness=5 mm) and FLAIR sequences (TR=5000 ms, TE=100 ms, flip angle=90°, field of view=230 mm, acquisition matrix=256×256, slice thickness=5mm) were used to assess subcortical cerebrovascular disease with the age-related white matter changes scale (Wahlund et al., 2001) (total score ranging from 0 to 30).

2.4 Image processing

Images were reoriented along the AC-PC line, all voxels below the cerebellum were removed with MRIcro (<http://www.sph.sc.edu/comd/rorden/mricro.html>), and the origin of the spatial coordinates was manually set to the anterior commissure. Images were normalized with the Statistical Parametric Mapping (SPM2) software

(www.fil.ion.ucl.ac.uk/spm) to a customized template created from the scans of all patients and all controls with a linear (12 parameter) transformation to preserve local shape differences in anatomy across participants, so that they could be quantified in standard space.

2.5 Radial atrophy mapping

Three-dimensional parametric surface mesh models were generated from the manually segmented amygdala tracings according to a standardized protocol (Pruessner et al., 2000). Each amygdala contour was separated into dorsal and ventral components and re-parametrized by normalizing the spatial frequency of surface points between and within slices. A medial curve was automatically defined as the 3-dimensional curve traced out by the centroid of the amygdalar boundary in each image slice. For each boundary point, the radial 3-dimensional distance from the surface points to the medial curve of the amygdala was measured automatically and defined as the radial size. Shorter radial distances were used as an index of amygdala contraction (Thompson et al., 1997), (Thompson et al., 2004). This procedure allows us to perform statistical comparisons between groups at corresponding surface locations. The reconstruction of the anatomic amygdalar nuclei was based on the localization of the right amygdalar nuclei in histologic sections from a human atlas (Mai et al., 1997) (figure 1A). The contour of each nucleus was outlined in a digital 3-dimensional template and their boundaries were projected onto the surface of the 3-dimensional mesh model obtained with the radial atrophy mapping technique (figure 1B).

2.6 Amygdala Volume

The amygdalae were manually segmented on the reoriented and normalized images by a single tracer (E.C.), blind to diagnosis, on contiguous coronal 1.0-mm-thick sections following a standardized and validated protocol (Pruessner et al., 2000) and using an interactive software developed at the Laboratory of NeuroImaging of the University of California at Los Angeles (<http://www.loni.ucla.edu/Software/moreinfo.php?package=MultiTracer>). The amygdala is located in the superior medial part of the temporal lobe, partly superior and anterior to the hippocampus. In the coronal plane, the starting point for amygdala segmentation was at the level where it is separated from the entorhinal cortex by the intrarhinal sulcus, or tentorial indentation, which forms a marked indent at the site of the inferior border of the amygdala. The amygdalostriatal transition area located between the lateral amygdalar nucleus and the ventral putamen was taken as the posterior-lateral border. The posterior end of the amygdala was defined as the point where gray matter started to appear superior to the alveus and laterally to the hippocampus. If the alveus was not visible, the inferior horn of the lateral ventricle was used as a border (Pruessner et al., 2000). Test-retest reliability was first conducted on an independent sample of 20 patients and controls, the expert tracer (EC) reached intraclass correlation coefficients for the right amygdala volume of 0.80 and 0.94 for the left one. Since amygdala manual tracings were carried out on the normalized images, no normalization for total intracranial volume (TIV) was needed.

2.7 Hippocampal Volume

MR images were processed using a combination of scripts written in Perl (<http://www.perl.com>) based on software developed at the McConnell Brain Imaging Centre

(Montreal Neurological Institute, McGill University, Montreal, Canada (<http://www.bic.mni.mcgill.ca/software>)). This processing includes correction for magnetic field non-uniformities, intensity normalization, and brain-to-brain linear registration to a standard template in the stereotactic space (ICBM152) based on the Talairach atlas. Each registered image was visually compared with the target template using REGISTER and, when automatic registration failed (mainly due to high scalp brightness), the intensity normalized image was manually registered to the template. Manual registration was based on 11 anatomical landmark points, distributed over the cerebrum and brain stem (the most anterior point of the temporal poles, most posterior aspect of the occipital lobe, most anterior point of the frontal lobe, central sulcus, inferior ventral aspect of the pons-midbrain cleft, genu and splenium of the corpus callosum, interthalamus adhesion, and eyes). The hippocampi were manually traced by an expert tracer (LB) that reached intraclass correlation coefficients of 0.96 for the right and 0.94 for the left hippocampus on an independent set of training images of 20 patients and controls with the software program DISPLAY (McGill University, Montreal, Canada) on contiguous coronal images, simultaneously checking tracing accuracy on the sagittal and axial planes. The anatomical starting point was the hippocampal head when it first appears below the amygdala, the alveus defining the superior and anterior border of the hippocampus. The fimbria was included in the hippocampal body, while the GM rostral to the fimbria was excluded. The hippocampal tail was traced until it was visible as an oval shape located caudally and medially to the trigone of the lateral ventricles (Pruessner et al., 2000). To obtain the original hippocampal volume (i.e., in the native scanner space), the brain with the traced region of interest was back-transformed from the stereotaxic to the native space. Since the hippocampal volumes were computed in native space, normalization for TIV was needed. The total intracranial volume (TIV) was obtained by manually tracing with the software DISPLAY the entire intracranial cavity (the lower boundary being the foramen magnum) on 7mm sections. Percentage hippocampal reduction was computed as the ratio between the hippocampal volume of each individual Alzheimer's disease patient and the mean of the pertinent control group. Details on hippocampal segmentation and data acquisition are previously described (Frisoni et al., 2007).

2.8 Statistical analysis

Clinical, sociodemographic, neuropsychological, amygdalar and hippocampal volumetric data were analysed using Mann-Whitney U-test for continuous variables and the chi-square test for dichotomous variables. The significance of the interaction between the two main effects, i.e., diagnosis (AD versus controls) and age at onset (<65 versus >65), on neuropsychological tests and amygdala and hippocampal volumes was tested in a full factorial model with the analysis of variance (ANOVA).

The percent difference in mean radial distances between patients and controls and the associated *p* value describing the significance of group differences were plotted onto the model surface at each point of the amygdala using a color code to produce statistical maps. Corrected *p* values were computed for the overall maps of the dorsal, ventral left and right amygdala using a permutation testing approach. Permutation methods measure the distribution of features in statistical maps that would be observed by accident if the participants were randomly assigned to groups (Thompson et al., 1997). The proportion of

the amygdala surface with p values lower than 0.05 was stored and compared to the distribution of this suprathreshold area that arose by chance when the diagnostic group labelling of the data was repeatedly scrambled. By comparing the supra-threshold area to that seen by chance in null data, this provides an appropriate overall p value for the observed effects that is corrected for multiple spatial comparisons. Shape and volumes differences were investigated between: EOAD vs YC; LOAD vs EC and EOAD vs LOAD.

Pearson's correlation was assessed between the amygdala morphology/volume and the percentage hippocampal volume loss within each AD sub-group. Finally, the difference between the slopes for the two patient groups was assessed using an analysis of variance model.

Statistical analyses were conducted using SPSS version 13.0 (SPSS, Chicago, IL).

3. Results

3.1 Clinical and neuropsychological features

The two patient groups were well matched to their respective groups of controls, as expected due to deliberate matching for age and sex (Table 1). EOAD and LOAD groups were identical for clinical dementia rating distribution due to the participants matching and, in addition, showed comparable Mini Mental State Examination score and disease duration. No significant differences were found in the prevalence of hypertension, diabetes, heart disease and total Hachinski Ischemic Score between the two patient groups (Table 1). White matter lesions load was higher in LOAD than EOAD (Table 1; $p < 0.004$). No statistical difference was found in the white matter lesions load between younger and older controls ($p = 0.1$). LOAD patients had a significantly lower educational level than their controls ($p = 0.01$), while no significant difference was found between EOAD and LOAD. The APOE e4 allele frequency was higher in the two patient groups than their controls, but no significant difference was found between patient groups.

Neuropsychological testing showed that the EOAD group performed more poorly than the LOAD on frontal-executive and non verbal memory tests ($p < 0.001$; $p = 0.01$ and $p = 0.03$ respectively; Table 2), while the LOAD group showed a trend for poorer performance, although not significantly poorer, for the Letter Fluency and Verbal Learning Test ($p = 0.056$ and $p = 0.086$ respectively, Table 2).

3.2 Amygdalar and Hippocampal Volume in EOAD and LOAD

Amygdala volume loss in EOAD patients compared to YC was, on average, 17% ($p = 0.005$), while in LOAD patients relative to EC, the deficit was 25% ($p = 0.001$, Table 1). Hippocampal volume loss in EOAD and LOAD compared to their aged matched controls was 13% and 20% respectively. No significant hippocampal and amygdala volume difference was found between EOAD and LOAD ($p > 0.088$ and $p > 0.236$ respectively), nor between younger and older controls ($p > 0.812$ and $p > 0.562$ respectively).

3.3 Amygdalar Morphology in EOAD and LOAD

Surface-based analysis revealed a significant tissue loss in the dorsal and ventral amygdala in both EOAD and LOAD compared to their age-matched controls ($p < 0.05$ on permutation test, Figure 1). In particular, EOAD showed a tissue reduction in the left Anterior and Posterior cortical nuclei (20%, $p < 0.001$, Aco, Pco), the Anterior Amigdaloid Area (20%, $p < 0.001$, AAA) and the Lateral and Central nuclei (20%, $p < 0.001$, La and Ce) bilaterally. LOAD patients versus EC showed a tissue loss up to 30% ($p < 0.001$) in the same nuclei involved in EOAD patients (Aco, Pco, AAA, Ce and La). Moreover, additional tissue loss in LOAD patients mapped to the Basolateral (BL), basolateral ventral medial (BLVM) and Medial nuclei (Me) (25%, $p < 0.01$). All the maps survived after correction for multiple comparisons (corrected p-values are shown below each map in Figure 1). The direct comparison between the two patient groups showed a significant amygdala tissue reduction (up to 20%) in the right dorsal La, BL and Ce nuclei ($p = 0.03$ on permutation test, Figure 1).

3.4 Relationship between amygdala and hippocampal atrophy in EOAD and LOAD

Considering the entire group of AD patients, we found a significant correlation between percentage hippocampal loss and amygdalar volume in both hemispheres (right: $r = -0.371$, $p = 0.028$; left: $r = -0.430$, $p = 0.009$). When the sample was stratified according to the age of onset, a significant correlation was found in LOAD but not in EOAD patients (Figure 2). The difference between the slopes was significant for the left hemisphere (Figure 2, $p = 0.04$). Correlation values were also higher for the left than for the right hemisphere in LOAD although no significance differences were found between r values ($z = 0.92$, $p > 0.05$). Considering maps of morphology the hippocampal atrophy was significantly correlated with amygdalar morphology in LOAD patients only (Figure 3, results not reported for EOAD group due to $p > 0.05$ on permutation test). A greater left hippocampal atrophy was significantly related to tissue reduction in the Me and Ce nuclei and in the AAA region of the left dorsal amygdala ($p = 0.05$, $r = 0.6$, on permutation test; Figure 3, *top row*). The correlation did not survive a permutation test in the right hemisphere (Figure 3, *bottom row*).

4. Discussion

In this study, for the first time to our knowledge, we investigated amygdalar morphology in AD patients stratified by age of onset and in relation to their hippocampal volume. Our findings revealed a similar degree of hippocampal and amygdalar atrophy, and that amygdala shape differences are detectable in both patient groups, but with a more pronounced involvement in LOAD than EOAD patients. Moreover, amygdala atrophy was correlated with hippocampal atrophy in LOAD but not in EOAD, and more strongly in the left hemisphere. Overall, these findings support the view that LOAD forms are more associated than EOAD with involvement of MTL structures.

Although our patients were matched by Clinical Dementia Rating Scale and global cognition, we found a significant difference in the executive function performances between patient groups. This is in line with previous evidence showing greater neocortical impairment in EOAD than LOAD (Sa et al., 2012, Smits et al., 2012)(Reid et al., 1996).

Contrary to expectation, however, we did not observe a worse memory performance in LOAD than EOAD. A possible explanation might be that the tests used to assess memory are also dependent on neocortical functions, and this might have significantly affected the learning performance in EOAD. Alternatively, the observed differences might relate to a more aggressive disease in EOAD than LOAD, albeit evidence to this regard is conflicting (Gomez-Isla et al., 1999, Ho et al., 2002, Jacobs et al., 1994, Kono et al., 1994). Moreover, although white matter lesion load resulted significantly higher in late than in early onset patients, the former patients did not have worse performance in any neuropsychological tests. This is in line with the concept that white matter lesions have an influence on cognition only if they are of severe entity, with a diffuse involvement of white matter (Frisoni et al., 2007), that is not the case of our late onset patients. Finally, both patient groups did not show differences in vascular risk factors, and the mean Hachinski Ischemic Score was below 4 in both groups denoting the absence of vascular disease .

In line with earlier studies (Cho et al., 2013)(Shiino et al., 2008, van de Pol et al., 2006, Whitwell et al., 2012), we found a more severe hippocampal and amygdala atrophy in LOAD than EOAD when compared to their controls. Local distribution of amygdala tissue reduction in EOAD mapped to the Ce, Aco and Pco nuclei. The former is connected to the basal forebrain cholinergic system, the subiculum and the entorhinal cortex (Sah et al., 2003), while the latter are connected to the orbitofrontal and sensory cortices (Price, 2003). LOAD revealed a significant tissue reduction in the basolateral complex (La, BL nuclei) and in the Me nucleus. The La and BL nuclei are connected to CA1 and CA3 sectors of hippocampus and the anterior cingulate cortex (Pikkarainen et al., 1999, Stefanacci and Amaral, 2000), while the Medial Nucleus is connected to the Olfactory bulb (LeDoux, 2007, Price, 2003). The direct comparison between the two patient groups illustrated a greater tissue loss in the Ce, La and BL dorsal right amygdala nuclei connected to the previously described limbic regions.

The topography of amygdala atrophy in LOAD overall mirrors that observed in typical, sporadic AD cases. Indeed, results in LOAD were similar to our own previous findings on an independent sample of AD patients who were not classified according to age of onset; they showed amygdalar tissue loss in the basolateral complex (Cavedo et al., 2011). The results in LOAD also agree with post-mortem studies showing a selective volume loss in the dorsal portion of the BL nucleus in typical sporadic AD patients (Scott et al., 1991). Moreover, LOAD showed a greater tissue loss also in the Ce nucleus receiving afferents from the subiculum and entorhinal cortex (Sah et al., 2003) and involved in modulatory circuits such as the lateral division of the capsular cholinergic pathway (Selden et al., 1998).

Although the structures of the MTL are closely interconnected, prior studies have not directly assessed the relationship between amygdala and hippocampal atrophy in EOAD and LOAD. Our correlation analysis clearly showed that damage to the hippocampus was significantly associated with damage to the amygdala in LOAD but not EOAD. This difference cannot be explained by confounders or different sample size, as samples were well matched. We may interpret this result as reflecting a rather direct spreading of neurofibrillary pathology along the limbic circuit in LOAD patients, specifically along afferents from the subiculum/entorhinal cortex and the olfactory bulb to the dorsal Ce and

Medial amygdala nuclei, as suggested by limbic system anatomy (Sah et al., 2003) (Catani et al., 2013, LeDoux, 2007). Conversely, in EOAD atrophy of the amygdala and hippocampus might not be closely interconnected and may instead result from the spread of neuropathology along distinct connections, e.g., along neocortical-subcortical afferents. Finally, we observed that the hippocampal-amygdala correlation in LOAD was found in the left hemisphere only, in line with a prior clinical investigation denoting a greater temporal/left hemisphere involvement in LOAD (Basso et al., 2006, Krasuski et al., 1998)(Suribhatla et al., 2004). Nevertheless, we cannot exclude that the lack of a significant relationship between the hippocampus and amygdala in EOAD, was due to the high variability in volumetric measures. However, this explanation is unlikely since our EOAD and LOAD groups had similar sample size and the spread of amygdala and hippocampal volumes was similar between groups, as indicated by their standard deviation.

The greater tissue loss of in MTL regions in LOAD supports prior clinical findings in these patients of a clinical profile involving memory, orientation deficits and olfactory dysfunctions (Koedam et al., 2010, Sa et al., 2012, Suribhatla et al., 2004), (Koss et al., 1996).

4.1 Caveats

Some caveats should be noted. First, the sample sizes were small and replications on larger groups are needed to understand whether amygdalar volume and shape measures may consistently distinguish EOAD from LOAD patients. Second, we used a scanner with a marginally lower field strength (1.0T) than that used in most clinical settings (1.5T). This might result in scans with lower contrast to noise for tissue segmentation, although less prone to some artifacts commonly encountered at higher fields (e.g., RF field inhomogeneity, susceptibility gradients near the sinuses). Third, our mapping of the local tissue differences to inferred neuroanatomic regions is indirect, as it was based on 3-dimensional reconstruction of the amygdala. Our inferences about specific nuclei involved in EOAD and LOAD need therefore to be confirmed in independent studies and by neuropathological examination. Finally, differences in regional vulnerability may not be explained by genetic factors such as apolipoprotein E. Further studies should investigate how genetic risk factors affect hippocampal and amygdala morphology in EOAD and LOAD.

4.2 Conclusions

This is the first study investigating amygdala morphology in early- and late-onset AD patients. We found greater amygdala tissue loss in LOAD than EOAD patients in nuclei connected to limbic regions, corroborating the hypothesis that limbic involvement is a prominent feature of LOAD but not EOAD neuropathology.

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Figure 1.

LOAD compared to EOAD patients revealed a significant tissue loss in the right dorsal central, lateral, and basolateral nuclei (20-30% loss, $p < 0.03$) strictly connected to medial temporal lobe regions such as hippocampus and entorhinal cortex. A) 3D reconstruction of the dorsal and ventral amygdala, and its major sub-nuclei, as traced out from the Atlas of Human brain (Mai et al., 1997). B) Contour outline of the amygdalar surface model as traced out from our sample (R=right, L=left). C) Statistical p-value maps, D) percent tissue difference in EOAD and LOAD patients *versus* age- and sex-matched healthy controls (OAC=Older Adults Controls; YC=Young Controls). P-values for multiple comparisons on permutation test are reported for the dorsal and ventral sections.

Figure 2.

The correlation analysis between amygdala volumes and percentage hippocampal atrophy revealed that hippocampal damage was significantly associated with amygdala damage in LOAD (dashed lines) but not EOAD patients (dotted lines). Correlation values were also higher for the left than for the right hemisphere in LOAD although no significance differences were found between r values ($z=0.92$ $p > 0.05$). Significance difference between LOAD and EOAD slopes was found only in the left hemisphere. r and p denotes the Pearson's correlation coefficient and significance. p indicates significance of the difference between slopes.

Figure 3.

Maps of correlation between amygdala morphology and left (top row) and right (bottom row) percentage hippocampal atrophy in LOAD patients. A specific tissue loss in the left dorsal central and medial amygdalar nuclei was significantly correlated to left hippocampal atrophy. Maps show the significance p values and the correlation coefficient (denoted by r values). p-values corrected for multiple comparisons on permutation test are reported for the dorsal and ventral sections.

Table 1

Sociodemographic, clinical, genetic and volumetric features of 18 early onset (EOAD), 18 late onset (LOAD) Alzheimer's disease patients matched by age and sex to 18 young (YC) and 18 older adults control (OAC) participants .

	EOAD	YC	p	LOAD	OAC	p	p LOADvsEOAD
N	18	18		18	18		1
Age, years	62.5±4.4	62.8±4.7	0.849	77.5±5	75.1±3.4	0.606	<0.001
Sex, females (%)	13 (72%)	13 (72%)	1	15 (83%)	15 (83%)	1	0.42
Education, years	7.7±5.3	8.3±3.5	0.171	5±2.5	8.5±4	0.004	0.058
Clinical dementia rating 0.5/1/2	4/13/1	---	---	4/13/1	---	---	1
Mini-Mental State Exam	20.1±3.8	28.7±0.7	<0.001	21.1±3.9	27.8±1.4	0.001	0.42
Disease duration, years	3.17±1.4	---	---	3.6±1.7	---	---	0.34
Hypertension	6 (50%)	---	---	7 (63%)	---	---	1
Diabetes	0	---	---	3/18	---	---	0.229
Heart Disease	0	---	---	4/18	---	---	0.104
Hachinski Ischaemic Score	3.2±1.8	---	---	3.5±2.7	---	---	.719
Age at onset, years	59.4±4	---	---	73.8±6	---	---	<0.001
APOE e4 allele	9 (50%)	2 (11%)	0.01	8 (44%)	1 (5.6%)	0.02	0.73
White matter Disease	1.3±1.4	2.4±4.4	0.342	5.7±3.8	3.7±4.1	0.146	<0.001
P of interaction on ANOVA							
Left Amygdalar Volume	1563±285	1912±226	0.005	1505±264	1986±407	0.001	0.361
Right Amygdalar Volume	1636±309	1956±290	0.020	1442±341	1940±322	0.001	0.236
Left Hippocampal Volume (mm ³)	2396±235	2639±343	0.030	2151±329	2702±378	<0.001	0.088
Right Hippocampal Volume (mm ³)	2438±268	2843±377	0.001	2222±431	2881±528	<0.001	0.564
TIV (cm ³)	1386±138	1482±144	0.036	1345±116	1445±122	0.023	0.949

Values denote mean±SD or N (%). White matter disease was assessed with the Age-Related White Matter Changes scale, ranging from 0 to 30 (Wahlund et al., 2001). P values denote the significance on Mann-Whitney U-test for continuous variable and chi-squared test for dichotomous variables. mm³=cubic millimetres; cm³= cubic centimetres TIV= Total Intracranial Volume.

Table 2

Neuropsychological features of EOAD and LOAD patients and controls. The significant interaction term indicates greater frontal-executive and non verbal learning impairment in EOAD than LOAD.

	EOAD	YC	EOADvsYC	LOAD	OAC	LOADvsEC	P of interaction on ANOVA
N	18	18	18	18	18		
Language	Letter Fluency	22.9±9.6	29.3±8.5	16±9.7	30±11.77	0.002	0.056
	Category Fluency	20.2±9	37±6	14.5±3.9	32.9±8.7	<0.001	0.536
	Token Test	25.3±7.7	32.7±1.6	.009	27.6±4.5	0.013	0.717
Visuo-spatial	Rey Figure Copy	14±13	31.3±6.2	<0.001	14.7±11.5	<0.001	0.715
Frontal-executive	Trail Making Test A	146.4±90.9	46.2±14.2	<0.001	126±63.8	<0.001	0.230
	Trail Making Test B	373.5±219.8	134.7±83.5	.008	268±100	0.036	0.001
	Trail Making Test B-A	252.3±140.1	88.4±71.5	<0.001	168.1±80	0.059	0.016
Learning	Rey list immediate recall	17±8.7	44.2±7.2	<0.001	19.3±6.5	<0.001	0.801
	Rey list delayed recall	3.6±2	9.2±3.1	0.002	2.1±2.2	<0.001	0.086
	Rey figure recall	2.6±1.3	13.6±6.3	<0.001	4.8±2.1	0.016	0.034

Values denote mean±standard deviation. P denotes significant on Mann-Whitney U-test. P of interaction on ANOVA denotes significance of the age × diagnosis interaction term. Raw test scores reported.