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Failure to Recover from Proactive Semantic Interference and Abnormal Limbic Connectivity in Asymptomatic, Middle-Aged Offspring of Patients with Late-Onset Alzheimer's Disease

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Abstract.

Background: We have obtained previous evidence of limbic dysfunction in middle-aged, asymptomatic offspring of late-onset Alzheimer's disease (LOAD) patients, and failure to recover from proactive semantic interference has been shown to be a sensitive cognitive test in other groups at risk for LOAD.

Objective: To assess the effects of specific proactive semantic interference deficits as they relate to functional magnetic resonance imaging (fMRI) neocortical and limbic functional connectivity in middle aged offspring of individuals with LOAD (O-LOAD) and age-equivalent controls.

Methods: We examined 21 O-LOAD and 20 controls without family history of neurodegenerative disorders (CS) on traditional measures of cognitive functioning and the LASSI-L, a novel semantic interference test uniquely sensitive to the failure to recover from proactive interference (frPSI). Cognitive tests then were correlated to fMRI connectivity of seeds located in entorhinal cortex and anterodorsal thalamic nuclei among O-LOAD and CS participants.

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Results: Relative to CS, O-LOAD participants evidenced lower connectivity between entorhinal cortex and orbitofrontal, anterior cingulate, and anterior temporal cortex. In the offspring of LOAD patients, LASSI-L measures of frPSI were inversely associated with connectivity between anterodorsal thalamus and contralateral posterior cingulate. Intrusions on the task related to frPSI were inversely correlated with a widespread connectivity network involving hippocampal, insular, posterior cingulate, and dorsolateral prefrontal cortices, along with precunei and anterior thalamus in this group. Different patterns of connectivity associated with frPSI were observed among controls.

Conclusion: The present results suggest that both semantic interference deficits and connectivity abnormalities might reflect limbic circuit dysfunction as a very early clinical signature of LOAD pathology, as previously demonstrated for other limbic phenotypes, such as sleep and circadian alterations.

Keywords: Entorhinal cortex, functional connectivity, late-onset Alzheimer's disease, limbic, proactive semantic interference, thalamus

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes up to 80% of all dementia cases worldwide [1]. It is characterized by the coexistence of two neuropathological hallmarks, namely extracellular plaques of amyloid-β (Aβ) and intracellular neurofibrillary tangles made up of hyperphosphorylated tau protein. Severe neurodegeneration and widespread neuroinflammation are ubiquitous, albeit less specific neuropathological features of the disorder. Over 99% of AD cases are late-onset (LOAD), i.e., initial cognitive symptoms appear after 65 years of age. Familial or early-onset AD (EOAD) cases account for less than 1% of cases, including the patient of the original description of the disorder [2]. The Amyloid Cascade Hypothesis (ACH) posits that AD begins with the deposition of Aβ, resulting from increased production, reduced clearance, or a combination or both processes [3]. The ACH has been supported by evidence that familial forms of AD were due to coding mutations affecting enzymes that participate in the metabolism of amyloid. Down's syndrome (resulting in an extra copy of the gene coding for the amyloid precursor protein located on chromosome 21), is associated with increased incidence and earlier onset of AD, lending further support to the ACH. The primary heuristic problem with this hypothesis is the lack of correlation between Aβ deposits and cognitive changes, as well as the absence of a clear and orderly pattern of anatomical progression as the disease advances. Further, significant *in vivo* Aβ deposition is present in a substantial number of cognitively normal elderly individuals who may never experience clinical symptoms of AD in their lifetimes. This lack of correlation between Aβ deposition and cognitive symptoms may, in part, explain the failure of several trials of multiple pharmacological agents targeting Aβ deposition, whose development was inspired by the ACH [4]. An additional issue underlying these therapeutic failures (regardless of the pathophysiological processes being targeted), is that most trials have been carried out mostly with symptomatic individuals (i.e., those in whom the pathology is so advanced it is likely to be irreversible). Hence, there is a critical need to detect AD-related changes as early as possible, in the hope that targeted therapeutic agents can be applied before multi-system degeneration has occurred [1]. Early neuropathological studies of AD-characteristic changes in the general population have indicated that tau pathology predates Aβ accumulation [5]. Tau pathology in AD follows a highly predictable progression, has a widely accepted staging system [5], and relates to cognitive symptom development [6, 7].

Those studies show that changes characteristic of AD occur already in the 3rd and 4th decades of life, involving phosphorylated tau accumulation within selected neurons, including those in the locus coeruleus, the anterior thalamic nuclei, and the vicinity of the entorhinal cortex, long before involvement of the hippocampus and neocortex [5]. Further, tau-affected neurons survive for decades, during which time intracellular tau pathology has been shown to cause a number of alterations in the normal functioning of the neuron [7]. Thus, early tau (but also extracellular Aβ) pathology is often detectable before symptoms and the emergence of neurodegeneration/neuronal loss. Dendritic excitability has a key role in synaptic plasticity, and aberrant dendritic morphology and ion channel activity contribute to hyperexcitability signaling and disruption of neuronal circuits [7]. Such changes occur long before neuronal death, and are probably unlikely to be detected by current clinical biomarkers of AD pathology, such as regional brain atrophy on structural MRI or, presumably, elevated tau levels in the CSF.

In contrast, fMRI could detect the consequences of neuronal dysregulation caused by hyperphosphorylated tau accumulation long before volumetric loss or cortical thinning due to neurodegeneration has occurred. Indeed, there is evidence that a family history of LOAD affects resting state connectivity in asymptomatic individuals [8] and that LOAD alterations in resting state connectivity are different from those found in EOAD patients [9].

There is also an emerging literature that novel cognitive stress tests such as the Loewenstein-Acevedo Scales for Semantic Interference and Learning (LASSI-L) taps semantic interference and may be more sensitive to early changes in AD before impairment is observed on traditional cognitive measures. The strength of this testing paradigm is the use of controlled learning and cued recall as a means to maximize encoding and subsequent storage of semantic information while limiting the effect of different learning strategies and compensatory mechanisms. Subjects are then presented with a second, competing list of semantically similar items that generates proactive semantic interference (PSI), a phenomenon where old learning interferes with new learning. A unique feature of the LASSI-L is a second learning trial which allows the assessment of brain plasticity and the ability to recover from PSI [10].

Cognitive stress tests such as the LASSI-L are therefore better equipped to detect subtler cognitive impairments and have repeatedly shown significant discriminatory power in preclinical and early forms of AD [11–14]. Because the design of the LASSI-L cued recall condition magnifies semantic interference effects, it has shown to have greater sensitivity and specificity to detect very early and subtle cognitive impairment among asymptomatic older adults with apparently normal cognition [11–14].

In the present study, we tested the hypothesis that clinically asymptomatic middle aged adults with a parental history of LOAD versus those without parental history of LOAD would exhibit deficits in semantic interference and that such impairments would be associated with alterations in the functional connectivity of critical regions of the limbic system. We specifically probed functional connectivity of two brain regions originally described to bear the earliest tau-related pathological changes in the encephalon, namely the entorhinal cortex in the vicinity of the transentorhinal area, and the anterodorsal thalamic nuclei [5]. Whereas the entorhinal cortex is a critical part of limbic circuits participating in episodic memory, anterodorsal thalamic nuclei are not directly involved in this function [15], and thus we predicted connectivity of this area would be less related to cognitive function test results in the present study.

METHODS

Design and sample

A cross-sectional study was performed to compare cognitive measures and brain connectivity data between a sample of 21 offspring of late onset Alzheimer's disease (O-LOAD) and 20 control subjects with no family history of AD (CS). Both groups were comparable in age, gender, education level, and depressive symptoms (Table 1). All participants provided their written informed consent for the study as approved by the local bioethics committee and in accordance with the Declaration of Helsinki.

The inclusion criteria for O-LOAD were as follows: 1) having at least one parent diagnosed with probable LOAD according to the DSM-5 criteria [14]; 2) participants were 40–65 years old at the time of recruitment; 3) having seven or more years of formal education to complete study instruments; 4) Mini-Mental State Examination (MMSE) score >26; 5) no evidence of current progressive neurologic disease or medical conditions likely to impair cognitive function; 6) no history of substance abuse (alcohol, marijuana, stimulants, benzodiazepines, or other drugs); and 7) Hachinski score <4 to screen out subjects with vascular-derived cognitive impairment.

All participants were asked to fill in names, dates of birth, age at death, cause of death, and clinical information of all affected family members. The information was confirmed with other family members by interview with the examining neurologist, discussing the parents' symptomatology and progression of disease. Only individuals whose parents had lived to age $\geq$65 were included. For individuals who had received no treatment at FLENI Foundation ($n = 5$), the parents' diagnosis of LOAD was clinician certified. In addition to clinical definition of LOAD in the parents, structural MRIs were available to confirm atrophy changes suggestive of AD and absence of significant vascular disease in the parents of 15 participants. Of these, 3 had a positive PET-PiB test.

The 20 participants in the CS group had the same inclusion criteria outlined above and were required to have no family history of any type of neurodegenerative disease.

Table 1
Clinical and demographic data

	Group				
	CS ($n = 20$)		O-LOAD ($n = 21$)		p
	Mean or frequency	SD or %	Mean or frequency	SD or %	
Female	16	80	13	61.9	0.203
Age (y)	51.80	8.70	54.86	7.30	0.232
Education (y)	17.74	3.18	17.52	3.47	0.841
CRQ	17.80	2.88	16.24	2.74	0.083
MMSE	29.55	0.76	28.95	1.15	0.059
BDI-II	8.18	8.02	9.22	5.82	0.664
LASSI-L					
2A Cued Recall	14.11	1.05	13.67	1.11	0.207
1B Cued Recall	8.05	3.10	7.48	2.46	0.522
1B Cued Intrusions	0.79	0.98	2.19	2.58	0.029
2B Cued Recall	12.21	1.78	11.33	1.49	0.102
2B Cued Intrusions	0.58	0.51	1.62	1.63	**0.010**
2B >1 Intrusions	0	0%	10	47.6%	**<0.001**
Delayed List A	12.16	1.61	10.52	2.56	**0.020**
Total Delayed Recall	22.05	3.15	20.19	3.57	0.088
Delayed Intrusions	0.21	0.42	0.76	0.70	**0.004**
Cognitive tests					
RAVLT Learning Curve	47.05	9.89	42.84	6.38	0.129
RAVLT Delayed Recall	10.89	2.21	8.21	2.72	**0.002**
RAVLT Recognition	14.00	1.25	12.68	2.16	0.029
TMT A (s)	31.80	10.66	30.95	7.61	0.773
TMT B (s)	64.65	19.60	67.53	14.33	0.603
Semantic Fluency (items)	22.44	5.34	20.95	4.10	0.341
Phonological Fluency (items)	18.83	3.37	18.33	3.86	0.668

CRQ, Cognitive Reserve Questionnaire; MMSE, Mini-Mental State Examination; BDI-II, Beck Depression Inventory, Second Version; LASSI-L, Loewenstein-Acevedo Scales of Semantic Interference and Learning; RAVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test. p values surviving FDR correction for multiple comparisons are marked in bold.

Cognitive assessment

The neuropsychological tests used in this study were selected based on their frequent and widespread use in AD testing. The battery included the MMSE as a screening test of cognitive function [17], Trail making Test A (TMT A) to assess simple visual scanning ability and sustained attention, TMT B, a more complex visual scanning measure that assesses cognitive flexibility (an executive function measure) [18], phonological fluency (letter "P") to assess verbal productivity (an executive function measure) [19], semantic fluency ("animals" category) to measure semantic memory [20], and Rey Auditory Learning Test (RAVLT) to assess verbal episodic memory [21, 22].

LASSI-L cognitive stress test

For this study, a novel cognitive stress test was also incorporated, the Spanish version of the LASSI-L [23], which has proved very effective in discriminating between AD, mild cognitive impairment, and healthy subjects, and was highly related to amyloid load among older adults who scored within normal limits on traditional neuropsychological measures [11]. This test assesses the effects of proactive and retroactive interference (PSI and RSI, respectively) and failure to recover from PSI (frPSI) after controlled cued learning and recall of two different word lists that share the same semantic categories [12]. We focused on LASSI-L cued recall and intrusion measures that have shown the highest degree of discriminability and relationship to volumetric reductions within the brain in previous studies [10, 13]. These included List A2 cued recall (maximum learning and retrieval), List B1 cued recall and intrusions (susceptible to proactive interference), List B2 cued recall and intrusions (susceptible to failure to recover from proactive interference; frPSI) as well as delayed recall and intrusions.

Finally, two questionnaires were administered to all subjects: the Beck Depression Inventory-II to screen for presence and severity of depressive symptoms that may impact cognitive performance; and the Cognitive Reserve Questionnaire, which is used to assess in a quick fashion the most relevant elements

associated to the cognitive reserve [24]. All tests were administered and scored by a trained neuropsychologist. The traditional neuropsychological assessment was performed in one 60-min session. The LASSI-L was administered in a separate 30-min session on another day to avoid interference with the RAVLT since both tests consist on memorizing 15 word lists.

All participants were cognitively asymptomatic, neuropsychological performance was within normal limits, and none of the individuals met criteria for mild cognitive impairment or dementia.

fMRI image processing

The imaging data was analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (MathWorks Inc., Natick, MA). Images were subjected to temporal alignment and the time series of volumes were corrected for movement using a six-parameter automated algorithm. The realigned volumes were spatially normalized to the stereotaxic space of Talairach and Tournoux [25] using Montreal Neurological Institute reference brain. The normalized volumes consisting of $2 \times 2 \times 2\,mm^3$ voxels were spatially smoothed with an 8-mm FWHM isotropic Gaussian kernel. Cardiac-, respiratory-, linear trend-, and motion-induced noises were regressed out from the signal using REST software (http://resting-fmri.sourceforge.net/). Data were band pass-filtered in the range of 0.01–0.08 Hz using a sixth-order Butterworth filter.

Voxel-wise functional connectivity

A seed-based approach was used to calculate the connectivity maps between the whole brain and the entorhinal seed or the anterodorsal thalamic nucleus seed in each hemisphere. The seed regions were extracted from the Free Surfer parcellation (https://surfer.nmr.mgh.harvard.edu/). For this end, we calculated for each subject, the pairwise Pearson correlation between the mean signal from the seeds and the time series of each voxel. The individual maps were then used in a second level analysis consisting in: 1) random effect analysis for each group, and 2) two-sample *t*-test between both groups, implemented in SPM8.

ROI-wise functional connectivity

Volumes were regionally parcellated using the Automatic Anatomical Labeling (AAL) Atlas [26] from we selected the following regions of interest (ROIs) in each hemisphere (see next section): posterior cingulate cortex, middle frontal gyrus, hippocampus, insula, and precuneus. We also used the entorhinal cortex from Desikan Killiany Atlas [27] and the antero dorsal thalamus from Morel Thalamus Atlas [28]. For each ROI, the mean time series was estimated by averaging the fMRI time series over all voxels within each region. Then, we performed the pairwise Pearson correlation between the mean temporal series of either entorhinal cortex or anterodorsal thalamus with the other ROIs. The statistical test was performed using the statistical toolbox of Matlab. For Fig. 3, we used the BrainNet Viewer [29] toolbox of Matlab.

Statistical analyses

Intergroup comparisons of cognitive variables in CS and O-LOAD were performed with an independent-samples *t*-test or χ^2 tests as indicated. In order to explore the relationship between LASSI and ROI-Wise connectivity, we performed a Pearson correlation analysis between LASSI-L 2B cued recall or 2B cued intrusions and connectivity between either entorhinal cortex or anterodorsal thalamus and a series of ROIs known to be anatomically related to them, and related to cognitive performance [30–32], namely posterior cingulate gyrus, precuneus, insular cortex, hippocampus, and middle frontal gyrus. We report uncorrected *p* value and inform those comparisons surviving Benjamini-Hochberg's False Discovery Rate correction for multiple comparisons (http://www.sdmproject.com/utilities/?show=FDR). In addition, comparisons of cognitive variables were controlled for age and cognitive reserve with an ANCOVA. We controlled for the same variables running partial correlation analyses between connectivity measurements and LASSI-L 2B cued recall or 2B cued intrusions, as indicated above. Statistical significance was assumed at an $\alpha = 0.05$. All analyses were two-tailed and were performed using the SPSSv18.

RESULTS

Table 1 shows the clinical and demographic characteristics of the sample as well as connectivity between anatomical regions relevant to LOAD neuropathology and cognition. Samples of the middle aged children of LOAD patients and controls without

family history were equivalent with regards to gender age, years of education, and reported depressive symptoms. Offspring of LOAD patients displayed lower delayed recall in the RAVLT as previously described [33], but also had lower scores on delayed recall of List A targets on the LASSI-L as well as intrusions for A and B List targets combined. In addition, children of parents with LOAD had greater difficulties with intrusion errors during 1B Cued Recall and 2B Cued Recall suggesting difficulties with PSI and frPSI. Remarkably, 10 of the 21 offspring of LOAD patients had more than 1 intrusion error on List 2B recall while 0 of 20 control participants had more than 1 intrusion error ($\chi^2 = 12.06$, df = 40, $p < 0.001$).

In addition, controlling for age and cognitive reserve resulted in significant differences between groups for 2B Cued Intrusions (F = 5.724, $p = 0.022$), Delayed List A (F = 4.923, $p = 0.033$), Delayed Intrusions (F = 7.654, $p = 0.009$), and RAVLT Delayed Recall (F = 9.264, $p = 0.005$).

Figures 1 and 2 depict functional connectivity patterns of both entorhinal cortex and anterior thalamic nuclei respectively, in asymptomatic offspring of LOAD patients and controls without family history of the disorder. Controls displayed strong connectivity between each entorhinal area with the contralateral area as well as temporal structures such as the fusiform cortex, and bilateral parahippocampi (Fig. 1A, left panels). Posterior cingulate and pontine tegmentum also display significant connectivity with entorhinal cortices. Offspring of LOAD patients, show robust connectivity of the entorhinal cortex with posterior cingulate and precunei, greater than that observed in individuals without family history of LOAD (Fig. 1A, right panels). Overall connectivity of the entorhinal cortex was greater in controls, specifically in anterior cingulated, medial orbitofrontal cortex, and anterior temporal lobes (Fig. 1B). Figure 2 shows the connectivity of anterodorsal thalamic nuclei. Both groups displayed similar patterns of connectivity with structures participating in cortico-subcortical limbic circuits including hypothalamus, bilateral insular cortex, hippocampi, parahippocampi, and cingulate cortex (Fig. 2A). As shown in Fig. 2B, there were no significant differences between groups regarding connectivity of anterodorsal thalamic nuclei. Figure 3 depicts correlations between LASSI-L B2 cued recall or B2 cued intrusions, which are both sensitive to the failure to recover from proactive interference (frRSI), and resting state connectivity in persons without family history of LOAD (Fig. 3A) or asymptomatic offspring of LOAD patients (Fig. 3B). These are important contrasts in that frPSI has been shown to be one of the earliest cognitive markers of AD [10, 11]. In controls, greater performance in B2 cued recall was associated with less connectivity between right entorhinal cortex and ipsilateral insula (Fig. 3A). In offspring of LOAD patients, greater B2 cued recall was inversely associated with connectivity between anterodorsal thalamus and contralateral posterior cingulate, whereas 2B cued intrusions were inversely correlated with a widespread connectivity network involving hippocampal, insular, posterior cingulate, and dorsolateral prefrontal cortices, along with precunei and anterior thalamus (Fig. 3B). When controlling for age and cognitive reserve, correlation between LASSI-L 2B intrusions and connectivity of left entorhinal cortex with both right hippocampus ($r = -0.555$, $p = 0.014$) and right middle frontal gyrus ($r = -0.478$, $p = 0.038$).

Among controls, RAVLT measures showed no relationships with connectivity patterns. In offspring of LOAD patients, RAVLT delayed recall was correlated with connectivity between left middle frontal gyrus and bilateral anterodorsal thalamic nuclei (not shown).

DISCUSSION

The main findings of the present study are that 1) LASSI-L measures tapping the failure to recover from proactive interference (frPSI) differentiate between clinically asymptomatic, middle-aged individuals with and without family history of LOAD and are associated with different patterns of functional connectivity in limbic and neocortical regions; 2) patterns of connectivity of the entorhinal cortex, but not anterodorsal thalamus are affected by a family history of LOAD; 3) overall functional connectivity of entorhinal cortex, previously described as relating to early tau-related lesions in LOAD, was found to be lower in asymptomatic offspring of LOAD patients; and 4) the offspring of LOAD patients show a pattern of connectivity in relation to frPSI characterized by poor involvement of several bilateral limbic circuit structures and dorsolateral prefrontal cortex.

Unexpectedly, offspring of patients with LOAD displayed increased connectivity between entorhinal cortex and precuneus/posterior cingulate or pons, i.e., areas known to be structurally affected very early in LOAD, and in at-risk individuals at an early age

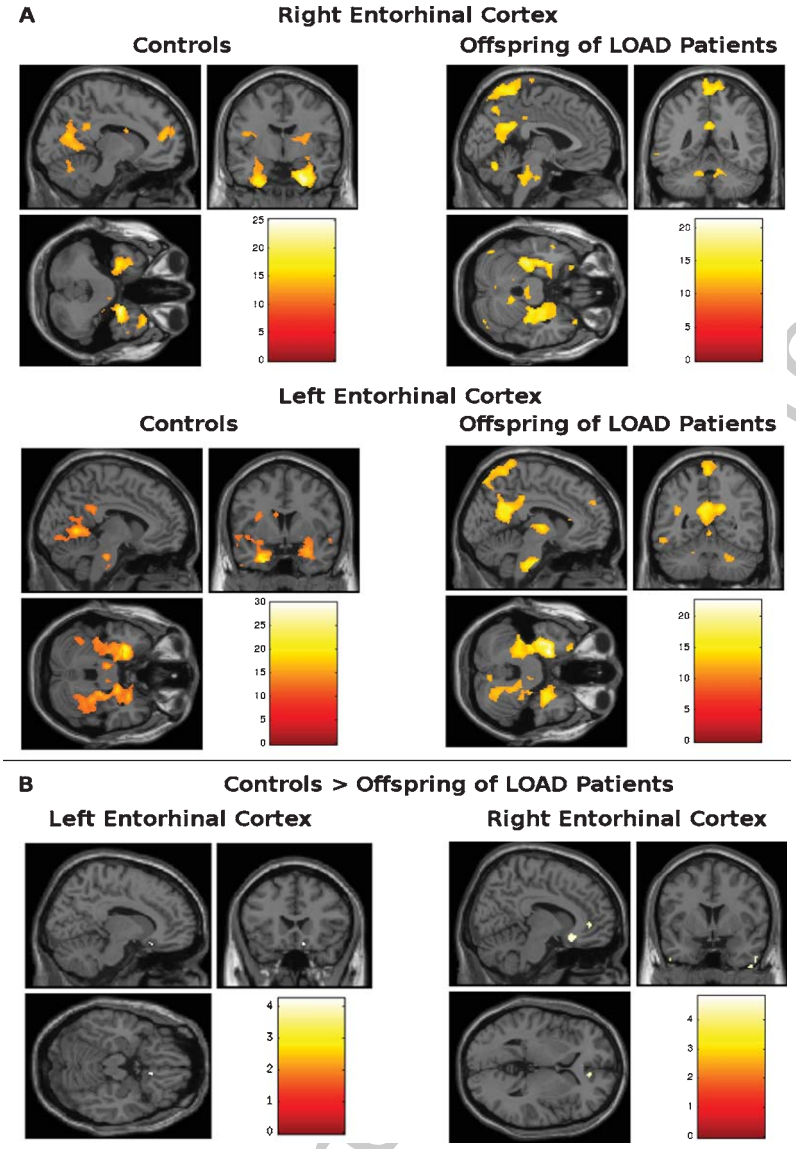

Fig. 1. A) Connectivity maps with a seed in the entorhinal cortex at right (top panel) and at left (bottom panel) for controls (left column) and offspring of LOAD patients (right column). MNI coordinates are: (–9, –5, –38) up-left, (–2, –47, –26) up-right, (–6, 3, –28) bottom-left, (–5, –53, –32) bottom-right ($p < 2*10^{-10}$). B) Differences of connectivity maps between controls and offspring of LOAD patients using the left (left panel) and right (right panel) entorhinal cortices as seed. MNI coordinates are (12, 22, –18) left panel, (10, 3, 1) right panel ($p < 0.0001$).

[34]. This is in contrast with observations in patients with established LOAD [9], who displayed decreased connectivity of posterior cingulate and precuneus compared with healthy controls. It is also in contrast with the main finding of the study by Wang et al. [8], i.e., that resting state fMRI connectivity between medial temporal structures and posterior cingulate cortex is decreased among at-risk individuals compared to controls without family history.

Given that our subjects were not individuals with established LOAD, and on average 10 to 20 years younger than the mentioned sample of at-risk individuals probing functional connectivity [8, 35], our findings may actually reflect compensatory processes associated with an earlier stage of disease. Interestingly, this phenomenon has been observed previously by Bassett et al. [35] who found that, in response to an episodic learning task, individuals with at least one parent with LOAD showed greater areas of activation than controls without family history of the disease. Thus, increased connectivity might result from a compensatory increase in activity of brain regions

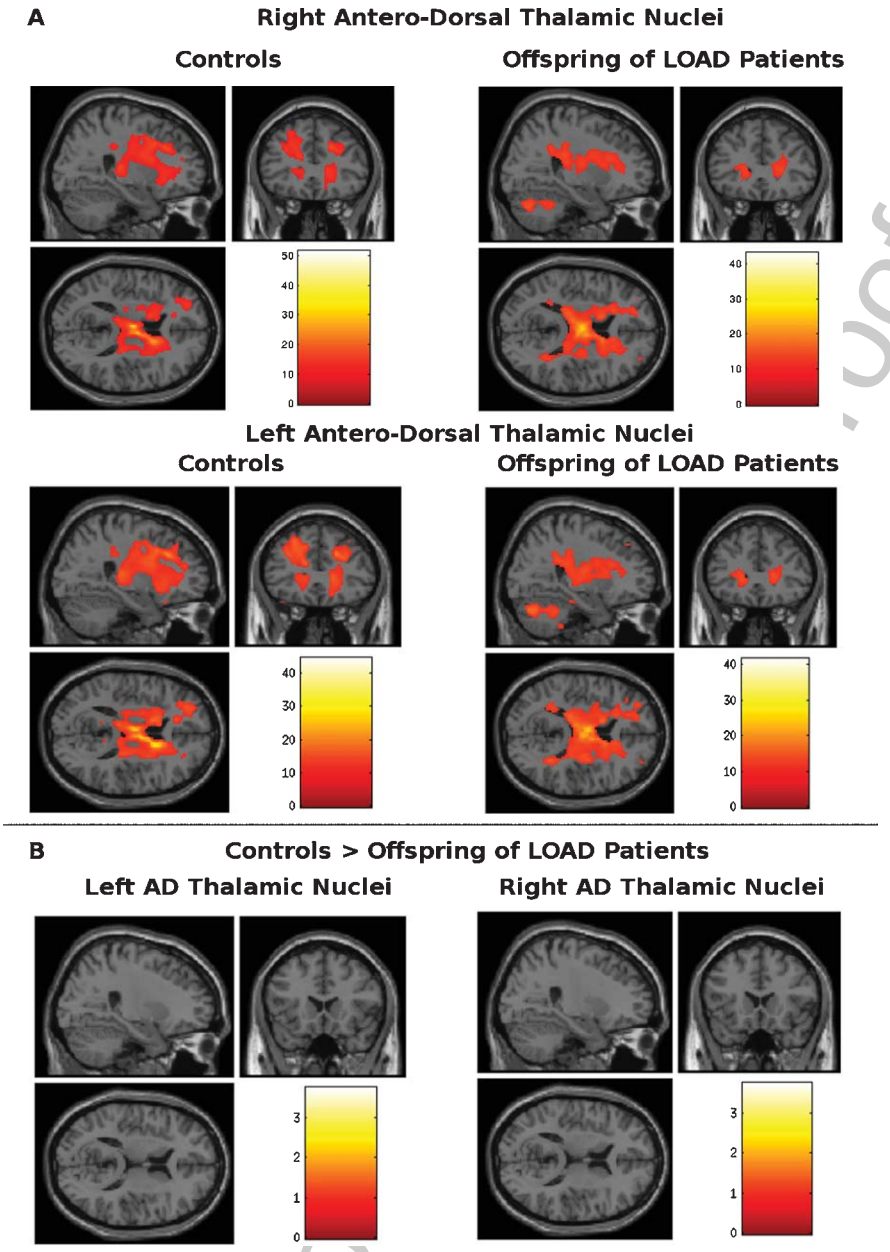

Fig. 2. A) Connectivity maps with a seed in the anterior thalamic nuclei at right (top panel) and at left (bottom panel) for controls (left column) and offspring of LOAD patients (right column). MNI coordinates are: (26, 29, 14) in all panels. ($p < 2*10^{-10}$). B) There are no differences of connectivity maps between controls and offspring of LOAD patients using the left (left panel) and right (right panel) anterior thalamic nuclei as seed. ($p < 0.0001$).

with early neurodegenerative (or neurodevelopmental) alterations. However, intergroup comparison only revealed greater connectivity between entorhinal area and orbitofrontal cortex, anterior cingulate, and anterior temporal areas, suggesting early dysfunction of entorhinal cortex in at-risk subjects before neurodegeneration is evident.

As expected, and described in clinical and preclinical samples of LOAD patients, deficits on LASSI-L measures tapping frPSI differentiated children of LOAD versus those without a family history of LOAD, and the delayed recall of LASSI-L list A also differentiated between groups. More importantly, LASSI-L measures associated with frPSI in

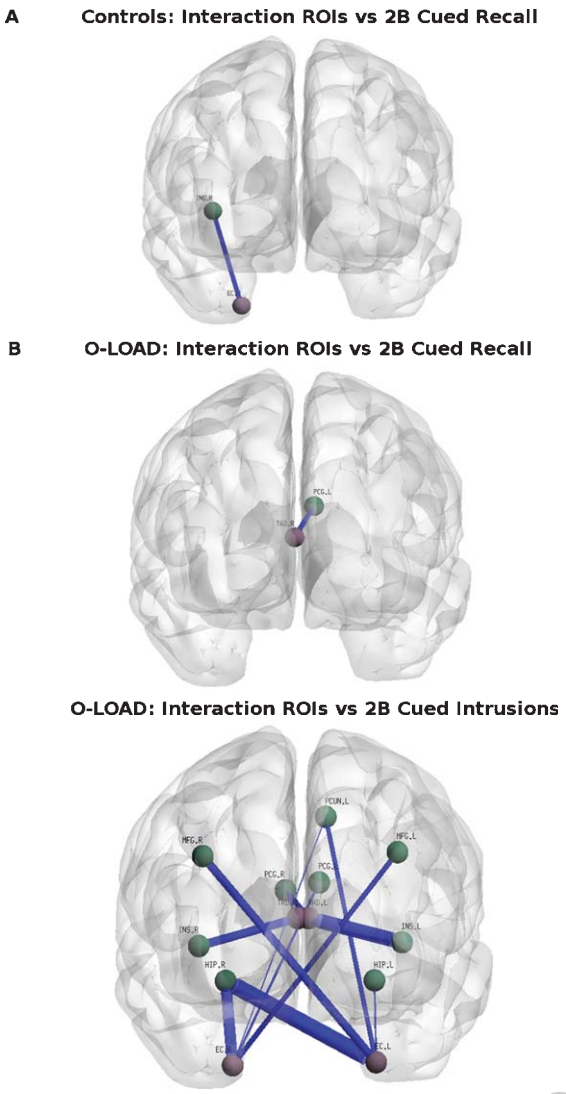

Fig. 3. A) Correlations between LASSI-L 2B cued recall in controls. B) Correlations between 2B cued recall (top panel) and 2B cued intrusions (bottom panel) in O-LOAD. In all cases the blue links represent negative values of correlation. Regions of interest from AAL Atlas (green nodes): posterior cingulum cortex (PCG), middle frontal gyrus (MFG), hippocampus (HIP), insula (INS), precuneus (PCUN). Region of interest from Desikan-Killiany Atlas (purple nodes): entorhinal cortex (EC). Region of interest from Morel Thalamus Atlas (purple nodes): antero dorsal thalamus (TAD).

the present study were related to connectivity in limbic and neocortical brain regions relevant to LOAD pathophysiology [5, 8]. In O-LOAD, poor performance on LASSI-L measures sensitive to frPSI was associated with lower connectivity in a fairly extensive network involving both subcortical/allocortical limbic structures and prefrontal neocortex. Interestingly, Loewenstein et al. [11] showed that frPSI was related to both total and regional amyloid load in cognitively normal community-dwelling elders, however the frPSI effect may have well been mediated by underlying tau pathology. One possibility is that tau-mediated alterations in the entorrhinal area, limbic thalamic nuclei, and presumably abnormal brainstem limbic projections, as described in early neuropathological studies of unselected general population samples [5], underlie the current findings. Although as stated above, the present findings presumably capture the functional consequences of early *intracellular* tau accumulation, the proposed hypothesis could plausibly be confirmed with *in vivo* evidence of tau deposition in these AD-sensitive areas with newly available tau radioligands. If thus confirmed, the present results could be antecedent to establish early, widely available clinical and fMRI measurements sensitive enough to capture LOAD-related changes many years before the expected onset of clinical symptoms. This would help to satisfy a major need of the field, in light of the repeated failures of therapeutic trials addressing AD etiology, as well as the fact that EOAD findings might not be analogous to the neuropathology and clinical characteristics of LOAD (e.g., [9, 36, 37]). LOAD research might thus necessitate a definition of therapeutic targets other than amyloid metabolism [4, 9].

The present results add to increasing evidence of early limbic dysfunction in persons at risk for LOAD. Our group has previously found a relationship between limbic phenotypes related to the sleep wake cycle and cognitive performance in middle-aged, asymptomatic children of patients with LOAD [33]. In this study, children of LOAD patients displayed subtle but significant deficits in verbal episodic memory and language compared to control individuals without a family history of AD, even though all participants had cognitive results that were clearly within the clinically normal range. Most importantly, children of LOAD showed a phase-delayed rhythm of body temperature, and a series of cognitive variables in this group were associated with cardiac autonomic sleep-wake variables. Specifically, indicators of greater sympathetic activity at night were related to poorer cognition. We interpreted these preliminary results as an early pathophysiological manifestation of underlying dysfunction in brainstem and limbic circuits [33]. The present results are concordant with these observations in that they demonstrate an altered functional connectivity of limbic structures presumably affected early on in the process of LOAD. In both

studies, whereas results suggest an increased risk of early AD phenotypes by virtue of having a parent with the disorder, we should emphasize that in contrast with EOAD (which in many cases is associated with an identifiable autosomic dominant mutation), association with genetic variants is less robust in the case of LOAD.

The present investigation has several limitations. First, there were modest numbers of subjects in each group and the present findings need to be replicated with a larger number of subjects. Unfortunately, we were unable to employ tau imaging to confirm the hypothesis that connectivity and semantic interference abnormalities described herein are due to cellular dysfunction induced by actual tau deposition. Last, the samples of children of LOAD patients belong to a single geographical location and ethnicity, thus limiting generalizability of the findings.

In conclusion, we demonstrated functional neuroimaging evidence of early limbic alterations in middle-aged, cognitively asymptomatic individuals by virtue of their family history of LOAD. If confirmed in other samples, the present results suggest that both LASSI-L (particularly measures sensitive to frPSI) and functional connectivity abnormalities are noninvasive techniques sensitive to detect preclinical changes related to LOAD. The neuropathological basis for these alterations, and whether they can be applied to other samples of at-risk individuals remains open to further investigations.

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