

RESEARCH ARTICLE

Brain Information Sharing During Visual Short-Term Memory Binding Yields a Memory Biomarker for Familial Alzheimer's Disease

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Abstract: Background: Alzheimer's disease (AD) as a disconnection syndrome which disrupts both brain information sharing and memory binding functions. The extent to which these two phenotypic expressions are shared their pathophysiological mechanisms remain unknown.

Objective: To unveil the electrophysiological correlates of integrative memory impairments in AD towards new memory biomarkers for its prodromal stages.

Methods: Patients with 100% risk of familial AD (FAD) and healthy controls underwent assessment with the VSTM binding test (VSTMBT) while we recorded their EEG. We applied a novel brain connectivity method (Weighted Symbolic Mutual Information) to EEG data.

Results: Patients showed significant deficits during the VSTMBT. A reduction of brain connectivity was observed during resting as well as during correct VSTM binding, particularly over frontal and posterior regions. An increase of connectivity was found during VSTM binding performance over central regions. While decreased connectivity was found in cases in more advanced stages of FAD, increased brain connectivity appeared in cases in earlier stages. Such altered patterns of task-related connectivity were found in 89% of the assessed patients.

Conclusions: VSTM binding in the prodromal stages of FAD are associated to altered patterns of brain connectivity thus confirming the link between integrative memory deficits and impaired brain information sharing in prodromal FAD. While significant loss of brain connectivity seems to be a feature of the advanced stages of FAD increased brain connectivity characterizes its earlier stages. These findings are discussed in the light of recent proposals about the earliest pathophysiological mechanisms of AD and their clinical expression.

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1. INTRODUCTION

There is a need for affordable and reliable biomarkers for Alzheimer's disease (AD) [1, 2]. Electrophysiological tools are particularly appealing due to their low cost and growing robustness [3-5]. The present study investigates whether the impairment in visual short-term memory (VSTM) binding previously reported in familial AD (FAD) [6] is linked to abnormal brain information sharing mechanisms as informed

by electrophysiological methods. We focused on brain connectivity analysis to unveil electrophysiological signatures of AD in the prodromal stages of the familial variant caused by the single mutation E280A of PSEN1 gene [7]. Such evidence would refine the theory about mechanisms of memory impairment in AD and would strengthen the reliability of this novel cognitive marker, making a case for a novel combined behavioural-electrophysiological neurocognitive biomarker for AD.

Both traditional and novel neuropsychological tasks assess context-rich memory functions linked to structures within the medial temporal lobe such as the hippocampus [8-

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12]. Memory functions relying on the functional integrity of the hippocampus decline early in the course of AD [13-15]. However, such functions also decline rapidly during healthy ageing [16]. This undermines the specificity of such tasks and may delay the early detection of memory impairments in people who are likely developing AD. There is growing consensus that structures of the medial temporal lobe other than the hippocampus (*i.e.*, entorhinal and perirhinal cortex), which support context-free memory functions, decline in early stages of AD recently described as sub-hippocampal stages [17, 18]. Such regions have been found to be preserved throughout healthy ageing in both humans [19] and animal models [20].

The VSTM binding task complies with the definition of tests of the sub-hippocampal stages of AD [17]. VSTM binding has been found to be insensitive to healthy ageing [21-23] and very sensitive to AD [6, 24, 25]. Recent neuroimaging and neuropsychological studies confirmed that such a cognitive function can be carried out with a damaged hippocampus [26-29]. Moreover, VSTM binding declines in young asymptomatic carriers of the mutation E280A-PS1, which inevitably leads to the development of early-onset FAD [7], even when they still perform tasks of hippocampal functions without difficulties [6, 30]. This evidence has led to the proposal that VSTM binding is an early cognitive marker for AD.

Previous electrophysiological studies in presymptomatic carriers of the E280A-PS1 mutation have reported task-related activations which clearly distinguish between mutation carriers and non-mutation carriers [31, 32]. This evidence warrants similar investigation of VSTM binding, a function which has proved behaviourally sensitive to the otherwise asymptomatic stages of E280A-PS1 FAD [6, 30]. Since the memory binding functions responsible for integrating visual features into unified objects require effective communication between specialised brain regions (*e.g.*, colour and shape processing areas), and AD is known to disrupt information transfer between brain regions, such investigation would benefit from a functional network connectivity approach [33]. Typically, in the VSTM binding task participants are asked to remember the link between objects' features such as shapes and colours which are known to be processed in different brain regions. To form memory representations of such complex objects accurately, the brain regions involved in feature processing (*e.g.*, shape and colour areas) should be effectively connected [34]. This task therefore offers the opportunity to explore, from an electrophysiological perspective, patterns of brain connectivity associated to memory binding. The analysis of brain connectivity opens up new opportunities to discover phenotypes of pre-clinical AD [35]. The two main approaches used to investigate patterns of brain connectivity, or lack thereof, in patients with AD are the analysis of resting-state (*e.g.*, Default Mode Network –DMN) and task-related connectivity. Both have proved informative in the presymptomatic and prodromal stages of AD (*e.g.*, [36-38]). Nevertheless, there remain some challenges to be met in order to enable a wider and more reliable use of this novel methodology. First, methods aimed at investigating brain connectivity in AD ought to have both sensitivity and specificity for this type of demen-

tia. Second, such methods should prove robust when applied to EEG.

The present study addressed the hypothesis that VSTM binding deficits in the prodromal stages of FAD are linked to measurable electrophysiological changes which can add reliability to this methodology in the early identification of the AD pathology.

2. METHODS

2.1. Participants

Ten patients in the Mild Cognitive Impairment (MCI) stages of FAD (MCI-FAD) and ten healthy controls entered the study. They were recruited in large kindred from the Colombian province of Antioquia in South America. The MCI-FAD patients carry a gene mutation (*i.e.*, E280A in the PSEN-1 gene) that leads to early-onset familial Alzheimer's disease (E-FAD) in 100% of carriers [7]. Healthy controls were recruited from the general population. At the time of assessment all the participants were having an active working life, were living independently and were off medication. Patients with MCI-FAD met the definition proposed by Acosta-Baena *et al* [39]. They were randomly selected from the database of the Neuroscience Group of Antioquia and had undergone a comprehensive clinical and neuropsychological assessment no longer than 6 months prior to EEG assessment. MCI-FAD patients and controls did not significantly differ in age or years of education (see Table 1). All participants gave informed consent to take part in the study which was approved by the relevant Ethics Committees.

2.2. Assessment

2.2.1. Neuropsychological Battery

The Neuropsychological Battery comprised of Mini Mental State Examination (MMSE) [40], Memory Impairment Screen [10] and Memory Capacity Test [41] which have been validated in this population [42], the brief version of the Boston Naming Test [43], Word List Learning from CERAD [44], Spanish version of Verbal Fluency Test (Category Animals, adapted from [45], the Copy and Recall of the Complex Figure of Rey-Osterrieth [46, 47], Part-A of the Trail Making Test (TMT) [48]. We also collected data from Instrumental Activities of Daily Living (IADL, [49]) and basic Activities of Daily Living (Barthel Index, [50]).

2.2.2. The VSTM Binding Task

The VSTM binding task was reported by Parra *et al.* [6]. The task assessed VSTM for arrays of stimuli presented on a computer screen (Fig. 1). Stimuli were randomly selected from a set of eight shapes and a set of eight colours and were presented either independently (*i.e.*, VSTM for single features) or combined (*i.e.*, VSTM binding). Each type of stimuli was presented in a separate condition. During the task, participants were presented with arrays of three items. The trial design for each condition of the VSTM task is shown in Fig. (1). The task was based on a change detection paradigm. At the beginning, there was a fixation screen for 1000 msec. This was followed by the study display which was presented for 500 msec. The study display presented three items as explained above. After the study display there was an un-

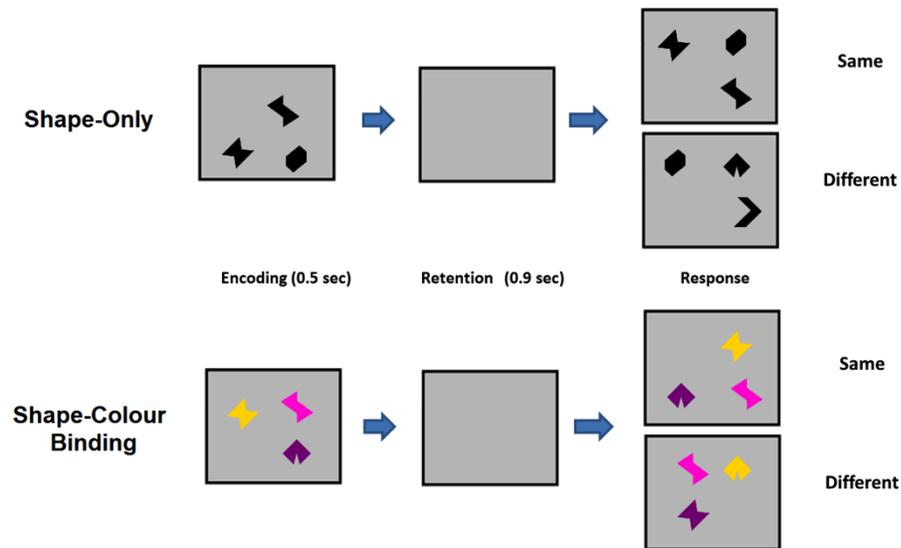


Fig. (1). The VSTM task (see description in text).

filled retention interval of 900 msec which was followed by the test display. The participants had to recognise if the items presented in the test display were the same or different from those presented at study. In 50% of the trials the items were the same in both displays (*i.e.*, “same trials”). In the other 50%, two items in the test display were different (*i.e.*, “different trials”).

One condition assessed VSTM for shapes and one assessed VSTM for shape-colour bindings. In the Shape Only condition, arrays of shapes were presented in the study display. In the test display for the “different trials”, two shapes from the study array were replaced with two new shapes which were randomly drawn from the set of shapes. Hence, only VSTM for individual shapes was required to detect a change. In the Shape-Colour Binding condition, combinations of shapes and colours were presented in the study display. In the test display for the “different trials” two shapes swapped the colours in which they had been shown in the study display. Hence, memory for bindings of shape and colour in the study display was required in order to detect this change. No shape or colour was repeated within a given array. Each condition consisted of a brief practice session using flashcards followed by 100 test trials per experimental condition (200 trials in total). The task for the participants was to detect when a change had occurred and to report orally ‘same’ or ‘different’ as appropriate. Participants were instructed to respond when the experimenter requested such responses. This procedure allowed the isolation of the artefact induced by verbal responses. The experimenter then entered participants’ responses using the keyboard. Trials were fully randomized across participants and conditions were delivered in a counterbalanced order.

For the present study we chose to use Shape Only as the baseline condition instead of both Shape and Colour Only as reported in earlier studies [6, 24]. Previous research has shown that memory for bindings is consistently defined by memory for the more challenging feature [26, 51, 52]. It is therefore revealing when this relationship between memory for bindings and memory for shapes is lost in AD. Colour,

by contrast, has not been seen to constrain memory for bindings as consistently as shape has. Therefore, the shape-binding comparison presents a conservative and reliable indicator of an impairment that is out with task difficulty, thus our use of it in the present study.

2.2.3. EEG Recording

EEG recording was carried out while participants performed the VSTM Binding task (Fig. 1). EEG activity was collected with SynAmps 2.5 ® system from Neuroscan. A 64-channel Quick-Cap was used to acquire the signal with linked mastoids as the reference. EOG signal was collected with 4 electrodes (HEOR, HEOL, VEOL, and VEOU) to eliminate oculomotor artefacts. Impedances were kept below 10 K Ω . EEG signal was sampled at 500 Hz and filtered with a bandwidth of 1 -200 Hz. A 50Hz notch filter was also used. The recording session consisted of approximately 30 minutes corresponding to the VSTM Binding task and 10 minutes of eyes-closed resting state.

2.3. Analysis

2.3.1. Behavioural

For the VSTM task we calculated corrected recognition as the proportion of hits minus false alarms. These data were compared across the two groups using non-parametric Mann-Whitney U test (see also Supplementary Fig. 1). Only patients underwent full neuropsychological assessment. However, controls also received the Mini Mental State Examination (MMSE) to ascertain normality in this group. The patients’ demographic and neuropsychological data were compared to the control group or to available norms [53, 54] using independent sample or one sample *t*-Tests, respectively. For each pairwise comparison we calculated the effect size (Cohen-*d*: 0.2 small, 0.5 medium and 0.8 large).

2.3.2. Brain Connectivity

Given that VSTM binding requires effective communication between regions of the brain responsible for integrating stimulus features, we used a new measure of integration and

global information sharing across distant cortical regions, called Weighted Symbolic Mutual Information (wSMI) [55], adapted for active tasks assessment [56] (see Fig. 2). wSMI assesses the extent to which two signals present non-random joint fluctuations (sharing information) providing (a) a fast and robust estimation of the signals' entropies, (b) detection of nonlinear coupling and (c) discarding spurious correlations between EEG signals arising from common sources (See [55]). WSMI involves a symbolic transformation which depends on temporal separation (here, $\tau=1=2$ ms, $2=4$ ms, $3=8$ ms, $4=16$ ms or $5=32$ ms) each of which is sensitive to information sharing at specific frequency bands [56]. wSMI was computed as follows [55]. First, EEG signals from each channel were transformed in a series of discrete symbols that were defined by the ordering of the amplitude values of k time samples with a temporal separation of τ . Then, the joint probability of each pair of symbols was estimated and the joint probability matrix was multiplied by binary weights. The weights were set to zero for identical or opposite symbols, as they could reflect common sources, on the same or opposite sides of a single electric dipole, respectively. Formally, wSMI was computed as:

$$wSMI(\hat{X}, \hat{Y}) = \frac{1}{\log(k!)} \sum_{\hat{x} \in \hat{X}} \sum_{\hat{y} \in \hat{Y}} w(\hat{x}, \hat{y}) p(\hat{x}, \hat{y}) \log \frac{p(\hat{x}, \hat{y})}{p(\hat{x})p(\hat{y})}$$

where, x and \hat{y} are the symbols present in signals X and \hat{Y} respectively; $p(x)$ and $p(\hat{y})$ are the probabilities of the symbols in each signal; $p(x, \hat{y})$ the joint probability of co-occurrence of symbols x and \hat{y} in signals X and \hat{Y} respectively; k the number of symbols and $w(x, \hat{y})$ the binary weight.

The wSMI frequency specificity was estimated by King *et al.* [55] based on simulations, which were restricted to taus 4, 8, 16 and 32 ms. We have implemented the measure also adding $\tau = 2$ ms but without following estimation procedures. We were interested in extending our analysis to faster frequency domains. We calculated the median wSMI across all channel pairs for each τ .

This measure (*i.e.*, wSMI) was applied to both resting-state and task-related EEG. The two approaches have proved differentially informative about of the impact of early pathological changes in the course of AD. For example Koch *et al.* [38] reported reduced connectivity in patients with prodromal AD relative to healthy controls over parietal regions during resting MRI but increased connectivity over the same region during an attention-demanding task. As the patients assessed here were also in the prodromal stages of FAD, we anticipated that a pattern similar to that previously found [38] would be observed in the present study. Regarding task-related connectivity, we performed analyses during the whole VSTM task (from encoding to retrieval). However, as AD is known to disrupt memory encoding more severely than memory retrieval we ran a second analysis for the encoding phase. Discrepancies between these analyses would suggest that the locus of connectivity impairments in MCI-FAD may reside either beyond memory encoding or expand across memory processes. Alternatively, new insights would be obtained into the encoding deficits hypothesis of AD [57] from a connectivity perspective. T-tests were carried out with the significance threshold set at 0.01 for within-group comparisons and at 0.05 for between-group comparisons. In order to identify the most reliable differences, only the last quartile of the significant differences was reported and plotted for each tail of the t -Test distribution (see Fig. 2).

To better quantify the differences in short- and long-distance connections between groups, we used a Euclidian distance connectivity function among channels [55], and applied it to the different conditions of the VSTM task. These were obtained with the following equation.

$$d_{i,j} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}$$

Where x , y and z are the Cartesian spatial coordinates which define the Euclidean distance of each pair of electrodes. Thus, the function d describes in a linear correlation how the connectivity varies with increasing distance. The correlation function analysis allowed us to investigate the interaction between electrodes which are at a distance d (for all possible values of d), assessing connectivity variations at different levels: at the small spatial level, we analysed electrodes in close proximity; at the large spatial level, we studied distant regional connectivity. This correlation function was calculated for each subject (and for all pairs of electrodes) and averaged within groups. To analyse the difference between conditions, we used non-parametric bootstrapping.

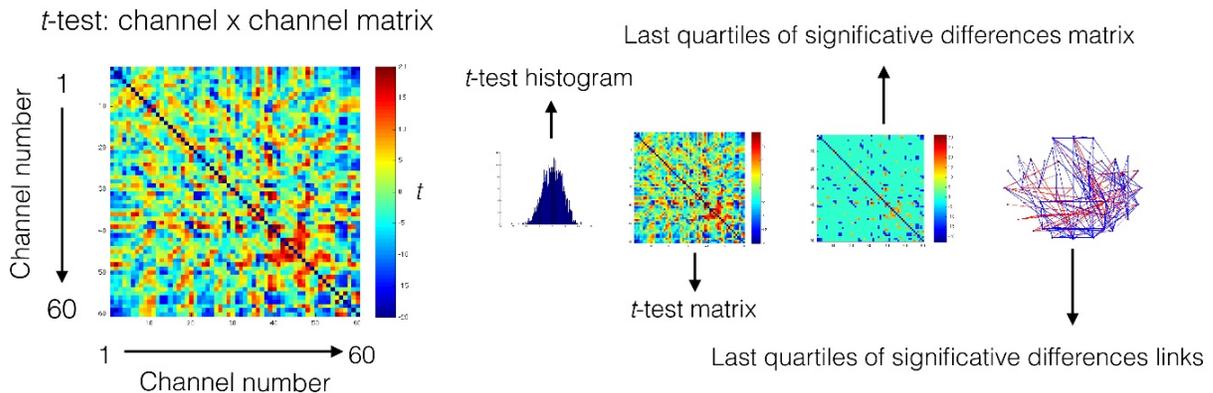


Fig. (2). Algorithm used to build the brain connectivity maps using wSMI data (see text for more information about this methodology).

In addition, in order to obtain an individual-based characterization of connectivity patterns [58], mean wMSI values from each patient were compared with a control group using a modified *t*-Test [59-63]. For these analyses we calculated connectivity inter-ROI and intra-ROI. The motivation for selecting the ROIs was explained above. For inter-ROI comparisons, we calculated the average wMSI which included each individual electrode of one to-be-contrasted ROI with all the electrodes of the other ROI. These individual averages (*i.e.*, per electrode) were then averaged out to calculate the inter-ROI wMSI. In the case of intra-ROI comparisons, it was the average connectivity between all the electrodes in that given ROI. Multiple single-case statistics allows assessment of significant differences between multiple individual's test scores and normative values derived from a small sample (*i.e.*, 10 controls). This modified test is more robust for non-normal distributions and generates few type-I errors than other single case comparisons [59-63].

3. RESULTS

3.1. Neuropsychological Data

Mean data from the neuropsychological assessment is presented in Table 1. MCI-FAD Patients presented with an amnesic multiple-domain profile [39].

3.2. Behavioural Data from the VSTM Task

FAD in its prodromal stages affected VSTM for bindings (Mann-Whitney U: 22.5, $Z = -2.08$, $p = 0.035$, $d=0.83$) more pronouncedly than VSTM for single features (Mann-Whitney U: 25.0, $Z = -1.98$, $p = 0.063$, $d=0.85$) (see Supplementary Fig. 1 for more information on the behavioural data).

3.3. EEG-Brain Connectivity

3.3.1. Resting State Connectivity

Nine participants per group had their resting EEG recorded. As we aimed at investigating connectivity differences at the group and individual level in resting and task related EEG, we decided to include in this analysis only participants with both recordings ($n=18$). A pattern of higher connectivity was observed for the control group mainly in the 0-20Hz frequency band ($\tau = 4 - 16$ ms and $5 - 32$ ms, see Fig. 3A), both when considering pairs of electrodes and each electrode relative to all the others electrodes. In the latter case, the differences were more evident over centro-parietal and occipital electrodes (Fig. 3B). Interestingly, Euclidian distance analysis showed that the significance of the differences found between patients and controls increases as a function of the distance (Fig. 3C). For both groups wMSI values increased from 2 cm to around 8 cm and then stabilized.

In order to verify whether abnormal connectivity is identifiable at a single subject level we ran a multiple case-based statistics using the Crawford *t*-Tests across 6 ROIs (Fig. 4) (see methods for motivation to choose these ROIs and Supplementary Fig. 2 for the full set of ROIs). This analysis revealed that reduced brain connectivity as assessed by wMSI

characterizes all the patients that entered this analysis (Fig. 4 A and B).

3.3.1.1. Task-related Connectivity

The analysis of the whole task yielded two main findings. The first and predicted one was a reduced brain connectivity associated to task performance in MCI-FAD cases relative to controls. This was noticeable in all the frequency bands ($\tau = 2-32$ ms) in both conditions of the VSTM task (Shape Only and Shape-Color Binding). However, as compared to healthy controls, MCI-FAD patients showed higher connectivity density during the Shape Only condition than during Shape-Color Binding condition (Fig. 5). This reduction of brain connectivity in FAD-MCI was apparent over anterior and posterior brain regions. Of note, reduced network activity over anterior regions was rather unspecific as it characterized both Shape Only and Shape-Color Binding, whereas over posterior regions it was more specific to Shape-Color Binding. Decreased information transfer over frontal lobes characterized 3 out of the 9 patients who entered this analysis (see Supplementary Table 1). These patients were in more advanced stages of the disease as indicated by the MMSE (mean of patients with decreased connectivity: 21.0 ± 4.4 vs. 26.8 ± 3.7 for the remaining sample) and the cued recall task of MCT (5.7 ± 1.5 vs. 12.0 ± 2.8). The results from the analysis of the whole task mirrored those from the analysis involving the encoding phase only (Supplementary Fig. 3), thus suggesting that impaired connectivity in MCI-FAD patients may originate during the early stages of memory processing (*i.e.*, encoding).

Table 1. Mean scores from MCI-FAD patients on the demographics, clinical and neuropsychological variables and results from the contrast carried out against controls or normative values.

	Norms/Controls (n=10)		MCI-FAD (n=10)		t-Test	Effect Size
	Mean	SD	Mean	SD	t, p	Cohen's d
Age ^a	44.3	5.6	44.4	3.2	0.05, n.s.	0.02
Education ^a	11.3	13.9	7.3	4.1	0.87, n.s.	0.38
MMSE ^a	29.1	1.1	25.2	4.5	2.75, 0.023	0.86
IADL (Lawton) out of 8 ^b			7.2	1.0		
ADL (Barthel) out of 50 ^b			50.0	0.0		
MIS (A+B) ^c	7.2	1.2	3.8	2.5	4.04, 0.004	1.28
MCT Cued List 1 ^c	14.9	1.4	10.9	3.5	3.23, 0.014	1.10
MCT Cued List 2 ^c	12.9	2.7	5.5	3.4	6.19, <0.001	1.91
MCT Cued List 1-2 List1 ^c	14.2	1.9	9.9	3.8	3.22, 0.015	1.07
MCT Cued List 1-2 List2 ^c	13.2	2.7	5.9	3.6	5.75, 0.001	1.80
Verbal Fluency (Animals) ^c	21.4	4.8	15.3	5.0	3.66, 0.006	1.02
Boston Naming Test ^c	14.1	1.3	11.1	3.2	2.79, 0.024	0.90
Word List Total Recall ^c	17.6	3.7	13.1	5.4	2.51, 0.037	0.75
Word Delayed Recall ^c	6.68	1.64	3.89	2.76	3.03, 0.016	0.93
Word List Recognition ^c	9.65	0.61	7.22	3.56	2.04, n.s.	0.68
TMT ^c	73.67	26.44	87.75	38.30	1.04, n.s.	0.33
ROF Copy ^c	26.38	4.99	21.89	5.03	2.68, 0.028	0.73
ROF Recall ^c	14.32	5.18	7.33	4.89	4.29, 0.003	1.14

^a Scores derived from the control group; ^b Abnormalities are considered based on performance drop relative to the total scores. ^c Values from norms; IADL: Instrumental Activities of Daily Living; ADL: Activities of Daily Living; MCT: Memory Capacity Test; MIS: Memory Impairment Screen; TMT: Trail Making Test; ROF: Rey-Osterrieth Figure.

The second and unpredicted finding was increased brain connectivity around central regions during the Shape-Color Binding condition (Fig. 6A-B). Patients' brain connectivity between neighboring regions within this large ROI was higher than that of controls. Of note, increased information transfer (*i.e.*, wSMI) over such regions characterized 7 out of 9 patients whereas decreased information transfer within the same regions only characterized 1 patient (Fig. 6C). In line with the first finding described above, brain connectivity decreased as the distance between regions increased and this was more noticeable during the Shape-Color Binding condition (Fig. 6D). The pattern of brain connectivity between adjacent regions was higher in patients than in controls whereas for distant regions the opposite was observed particularly during the Shape-Colour Binding condition.

4. DISCUSSION

The present study was set out to investigate whether the well documented deficits in VSTM binding found in patients with sporadic [24, 64] and familial AD [6, 30] could be accounted for by altered brain information sharing mechanisms as revealed by the analysis of brain connectivity. We pre-

dicted that if such an association existed and was detectable *via* such a combined approach (*i.e.*, task performance and EEG recording), this methodology would yield a novel memory biomarker for AD. We found that patients with the mutation E280A-PSEN-1 who showed a profile compatible with amnesic MCI based on a standard clinical and neuropsychological assessments, showed significant impairments in the VSTM Binding task. Due to sample size limitations, we could not rely on a mixed ANOVA model to test the hypothesis of selective binding deficits which have been previously reported in samples of sporadic and familial AD patients [6, 24]. However, recent studies suggest that in MCI patients, such a dissociation (*i.e.*, VSTM binding deficits >> VSTM deficits during shape processing) may be contingent upon memory load [65]. Patients with MCI show the well documented pattern of selective VSTM binding deficits when they are assessed with visual arrays 2 items [65] but not with visual arrays of 3 items [65-67]. Taken together these earlier findings and our current data, we feel confident to suggest that MCI patients who are already embarked on the neurodegenerative course of FAD, such as those assessed here, show a pattern of binding impairments akin to that seen

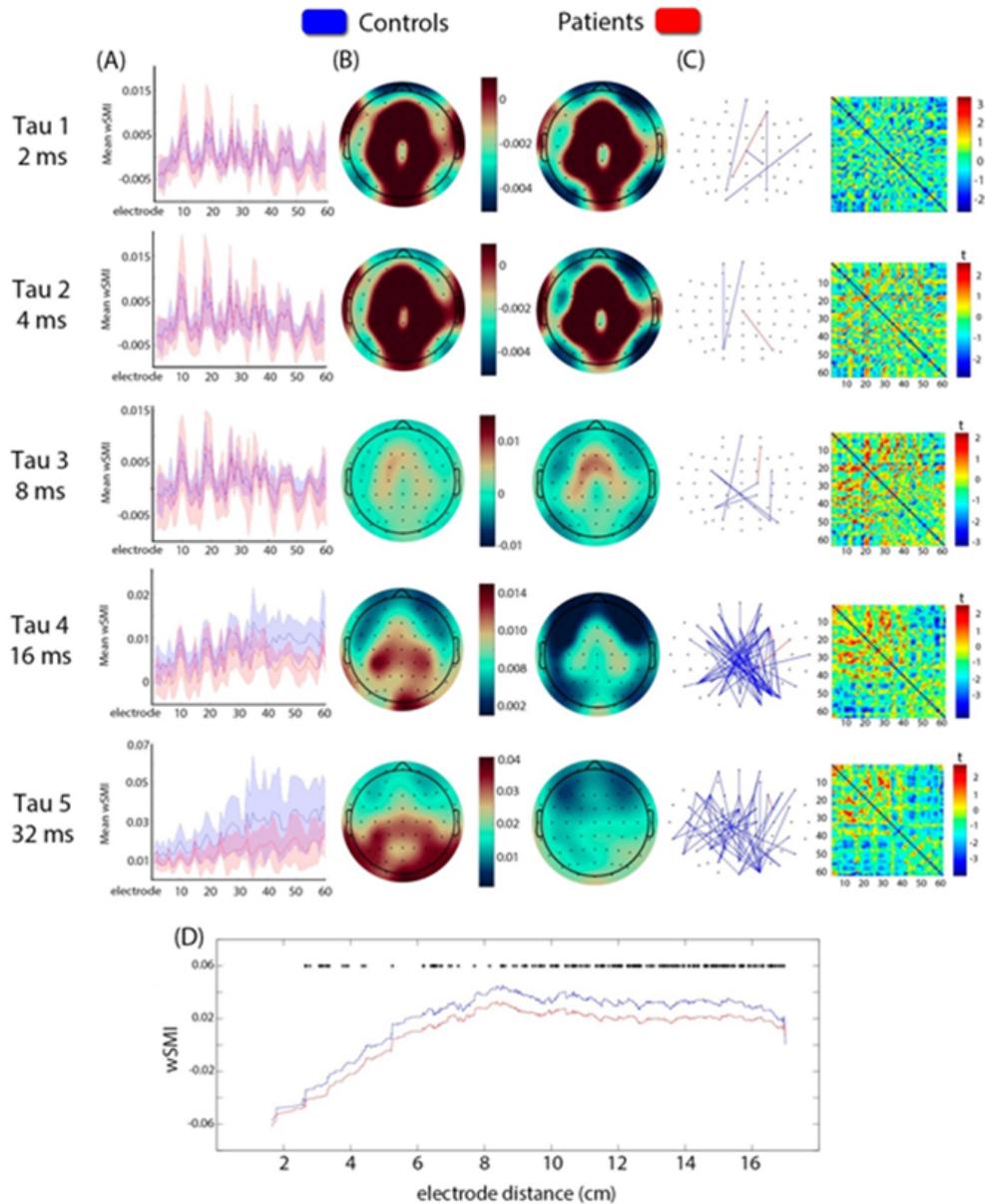


Fig. (3). (A) wSMI for each τ across the whole scalp. Graphs show mean wSMI (Y axis) for each group plotted across the entire array of electrodes (X axis). From left to right electrodes correspond to frontal, central, and posterior regions. Shaded area represents standard deviation across subjects. (B) Connectivity topography. Mean wSMI for each channel (left: control subjects, right: patients). (C) Connectivity differences. Left: line plots showing the last quartile of differences meeting the significance threshold. Right: matrix of t values. The graphs illustrate stronger connectivity in controls than in MCI-FAD patients in slow frequency bands ($\tau = 4 - 16$ ms and $5 - 32$ ms) over central-parietal and posterior channels. (D) Functional connectivity as a function of distance between electrodes. Patients' connectivity is remarkably lower between distant than neighbouring regions (* denotes contrasts reaching the significance threshold $p < 0.001$).

in patients with mild AD. Future studies should consider assessing them with the task presenting visual arrays of 2 items (see also Supplementary Fig. 1 for more information on these recent findings).

Brain connectivity analyses confirmed that such cognitive impairments originated mainly from abnormal brain activity in the low-frequency bands. Resting state analysis showed a significant decrease in brain connectivity which was found at the single subject level in the entire sample of patients. Task related connectivity (*i.e.*, VSTM Binding task)

yielded mixed findings. Significantly reduced connectivity was found in patients relative to controls over frontal and posterior regions but increased connectivity was observed over central regions during correct trials. Abnormal task-related connectivity over central regions was found in 89% of the assessed patients.

Previous studies in asymptomatic carriers of the mutation E280A-PSEN-1 have reported deficits in VSTM binding in the absence of other memory impairments including associative memory [6, 30]. The mutation carriers assessed in the

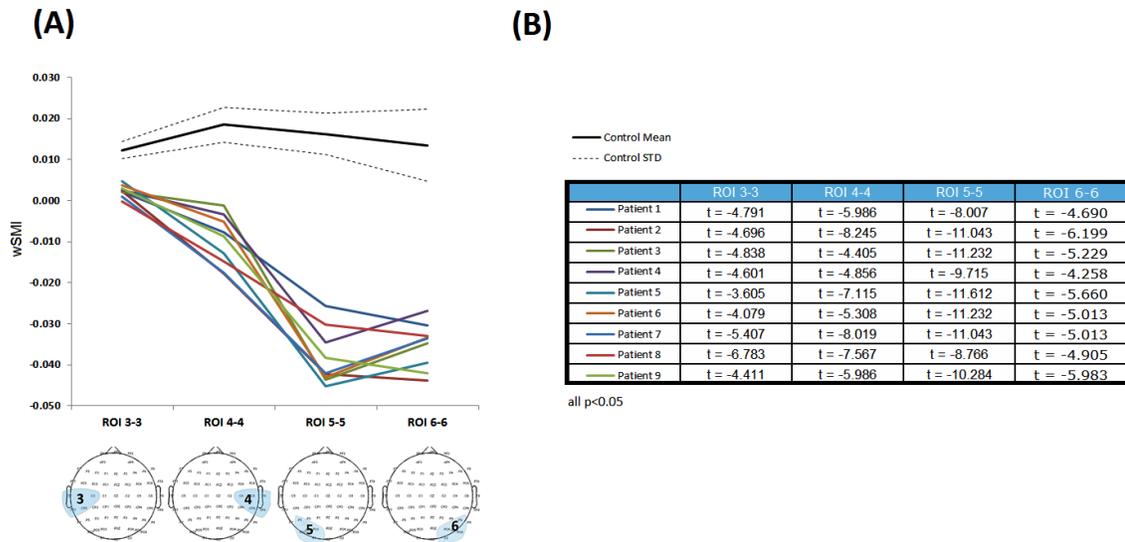


Fig. (4). (A) Individual lines plots confirming patients’ reduced connectivity (*i.e.*, wSMI) in the selected ROI, more remarkably over parietal-occipital electrodes [3-3 vs 4-4: $t = 0.35$, $p=n.s.$; 5-5 vs 6-6: $t = -1.506$, $p=n.s.$; 3-3 vs 5-5: $t = 16.092$, $p<0.001$; 3-3 vs 6-6: $t = 18.375$, $p<0.001$]. Such a decrease in functional connectivity accurately discriminated between MCI-FAD and healthy controls. (B) Outcomes from the statistics confirming that all the patients were correctly classified with high confidence.

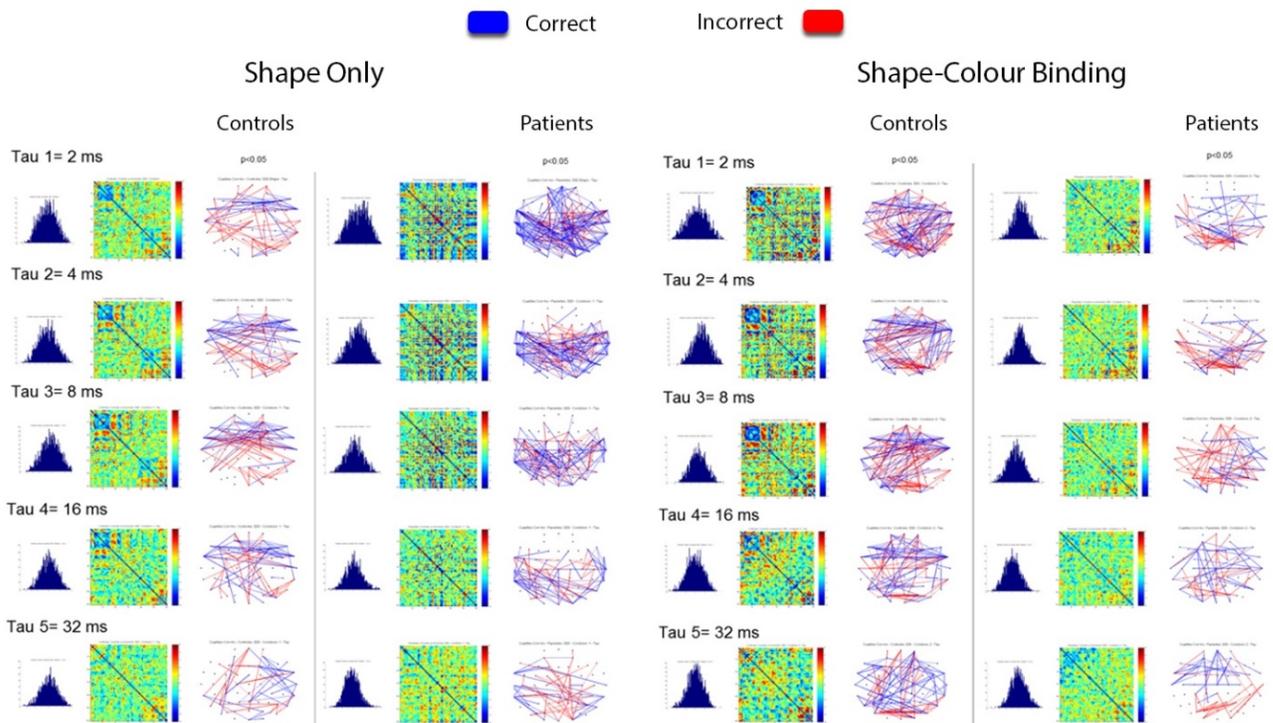


Fig. (5). Connectivity analysis during the two conditions of the VSTM task including the whole task (*i.e.*, from encoding to test) and the two groups. Histogram and matrix of the t-test. Line plots showing the last quartile of differences meeting the significance threshold.

present study were in more advanced stages of the disease process [38] than those assessed in earlier studies. In the current sample, VSTM binding deficits were accompanied by associative memory impairments (*i.e.*, MIS, MCT). Recent studies have reported on the usefulness of associative memory task such as the MCT in the early detection of AD

[68]. However, taken together the results from previous studies and the present study, we can argue that VSTM binding deficits anticipate associative memory impairments. This evidence seems to fit well recent hypotheses about the sequence of pathological changes in the course of AD [17]. Didic *et al.* [17] hypothesised that AD first undergoes a sub-

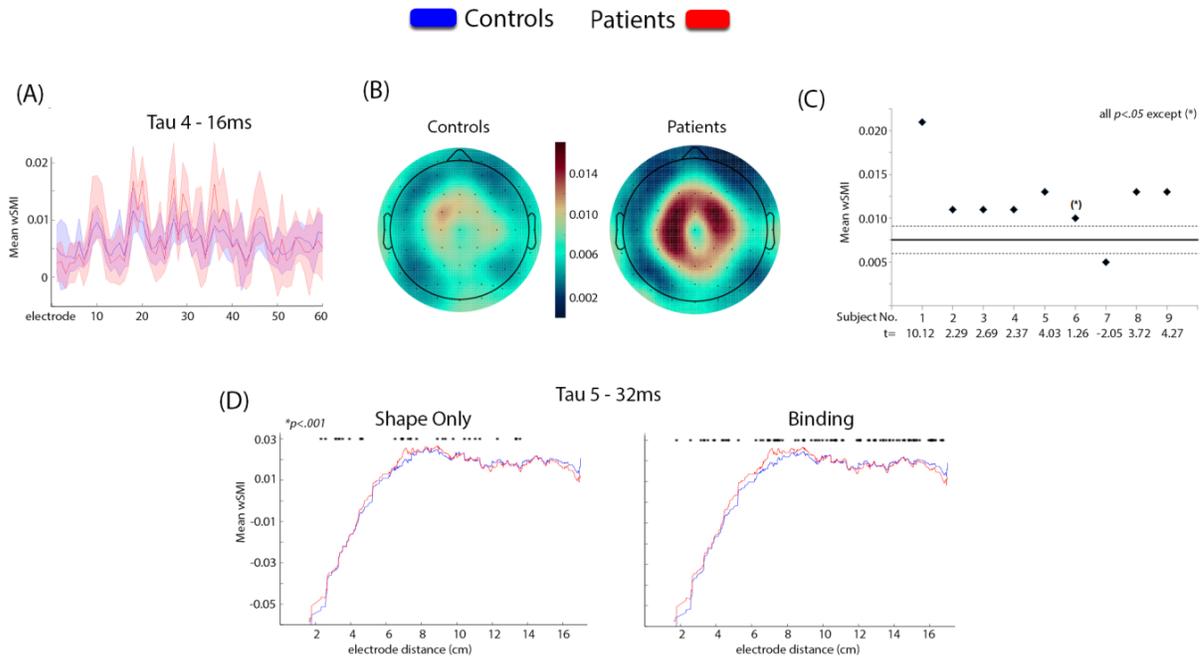


Fig. (6). (A) wSMI data corresponding to the Shape-Colour Binding condition of the VSTM task (correct trials) across the two groups distributed over the scalp. MCI-FAD patients show values lower than controls in frontal and occipital electrodes but higher than controls in more central electrodes. (B) Electrodes showing increased brain connectivity in MCI-FAD patients relative to controls. (C) Case-based statistics [63] confirming that all but one patient (case 6 who was marginal) was correctly classified when either increased or decreased brain connectivity was considered. (D) Functional connectivity as a function of the distance between electrodes. Patients' connectivity between adjacent regions was higher than that of controls whereas the opposite pattern was found between distant regions particularly during the Shape-Colour Binding condition (* denotes contrasts reaching the significance threshold $p < 0.001$).

hippocampal stage during which context-free memory functions (*e.g.*, familiarity based recognition, object recognition) supported by extra-hippocampal regions (*e.g.*, perirhinal and entorhinal cortex) are affected. This is followed by the hippocampal stage during which context-rich memory functions such as associative memory start to decline reflecting the impact of AD on the hippocampus. The hippocampal stage of AD appears to correspond to the Braak stage III which clinically expresses as MCI [17].

A recent fMRI study with healthy young volunteers showed that the network supporting performance during the VSTM task involves occipito-temporal regions seemingly involved in feature processing (*i.e.*, shapes and colours) and posterior parietal regions specifically supporting feature binding [26]. This earlier study failed to find hippocampal related activity during feature binding either during whole-brain or ROI-based analysis.

As it was predicted in the current study, poor VSTM binding functions in MCI-FAD are the result of impaired connectivity between relevant brain regions. Brain connectivity analysis during resting state and task performance confirmed that parietal-occipital regions are a locus of early pathological changes in the course of E280A-FAD. The EEG literature reporting reduced brain connectivity in AD during resting state is growing rapidly [69-71]. In the present study we adapted a new measure of integration and global information sharing across distant cortical regions (*i.e.*, wSMI) [55]. This method proved robust to discriminate between patients with different states of consciousness in an earlier study and

then was adapted for successfully tracking short-time connectivity during active tasks [56]. The current study is the first one reporting the use of wSMI in FAD. Of note, this method is particularly suited to address question about the binding problem as it allows investigation of information sharing across distant brain regions, a cornerstone of cognitive binding [72, 73]. By applying this method to resting state EEG, we were able to find abnormal patterns of brain connectivity in all the cases that entered the analysis. Previous studies investigating resting state connectivity in AD have achieved sensitivity values of 72–85% and specificity of 77–80% [74-76], perhaps reflecting the heterogeneity of non-genetic variants. Our results suggest that during resting state, connectivity mediated by long tracts is the most discriminative between MCI-FAD patients and Controls. Earlier studies which analysed cerebral coherence during resting state reported an association between reduced long-tract connectivity and clinical progression of AD [77] (see also [78, 79]). However, similar findings were reported by King *et al.* [55] in patients with different levels of consciousness, thus suggesting that connectivity problems during resting state may be sensitive but not be specific for clinical purposes (see also [80]).

A potential solution to this caveat is to identify the pattern of brain connectivity associated to performance on the VSTM Binding task which has proved both sensitive and specific to AD [64, 81]. Such an analysis revealed a network which comprises frontal, central, and parietal-occipital regions. The pattern of connectivity shown by these regions

during the different conditions of the VSTM Binding task suggests that they may serve different functions. In healthy controls, the frontal regions showed increased connectivity during correct recognition of both Shape Only and Shape-Colour Binding. However, the parietal-occipital regions showed a pattern of connectivity specific to Shape-Colour Binding. Whereas the frontal regions appear to form part of the network subserving general working memory functions, the parietal-occipital regions are seemingly serving as the feature binding hub [82-84]. This evidence closely follows previous fMRI studies with healthy young volunteers which found binding-specific activation over parietal-occipital regions ([26] see also [84-86]). Such a network organization observed in healthy controls was missing in MCI-FAD patients, particularly in the slow frequency bands ($\tau = 5 - 32\text{ms}$). MCI-FAD patients showed a pattern of connectivity more compatible with a random network (see [87] for similar results using fMRI).

In contrast to the reduced functional connectivity observed in MCI-FAD patients in anterior and posterior nodes of the task-related network, increased connectivity was found in this group relative to healthy controls over central regions (see Fig. 6). Interestingly, a better classification power in patients was achieved by increased connectivity than by decreased connectivity (see Supplementary Table 1). It is worth noting that compared to other brain pathologies (e.g., stroke), neurodegeneration can trigger atrophy-related hyperconnectivity [88]. This has been previously confirmed in AD [89, 90]. These results add to the array of evidence confirming functional reorganization in E280A-PSEN1 mutation carriers at stages of the disease prior to diagnosis of dementia [31, 32, 91, 92]. Such compensatory changes have also been observed in the course of late-onset sporadic AD in patients who are either in the Subjective Cognitive Deficits – SCD [93, 94], in the MCI [95-98], or in the early stages of dementia ([99]; see also [100] for a recent meta-analysis). They have been interpreted as a maladaptive reorganization of brain functions which detrimentally contributes to cognitive functioning. Compensatory changes are also found in the normal course of ageing (i.e., Scaffolding Theory of Cognitive Ageing) [101-104]. Therefore, in populations at risk for late-onset sporadic AD it would be difficult to tease apart the contributions of normal and abnormal ageing to such compensatory changes. However, confounding factors such as age and comorbidities are not present in the population investigated here. This grants us more confidence that the functional reorganization found in this and in earlier studies involving this population more genuinely reflects AD related mechanisms. Such a reorganization was representative of the majority of patients investigated here, indicating that this is a rather phenotypic feature of MCI in the course of FAD. In fact, recent studies carried out in the same population confirmed that the functional reorganization in asymptomatic mutation carriers can be observed as early as a mean age of 13.7 ± 2.6 [36]. Taken together the results from these studies suggest that functional network reorganization, as indicated by increased connectivity, may reflect the early stages of the disease process. However, decreased connectivity may reveal more advanced stages.

In the light of the results reported here one may argue that just relying on resting state connectivity would suffice to

achieve a correct identification of MCI-FAD cases (see for example [105]). Resting state connectivity alterations in AD have been interpreted as Default Mode Network (DMN) abnormalities [75, 106]. Dysfunction of the DMN has been observed in a wide variety of neurological and psychiatry disorders [107]. From this perspective, DMN connectivity in the context of dementia assessment may resemble the MMSE, both are sensitive but non-specific. However, the increased task-related connectivity found in this study indicates the presence of impaired-compensated networks which have been associated to the $A\beta$ -pathology in the prodromal stages of AD [38]. This impaired connectivity is linked to a memory impairment (i.e., binding) known to be sensitive and also specific to both sporadic and familial AD. One might question the extent to which the results presented here are representative of AD more generally or just of this rare genetic variant due to the mutation E280A-PSEN1. We have recently demonstrated that cases of prodromal sporadic and familial AD share a common behavioral and electrophysiological phenotype when it comes to VSTM binding [66]. Earlier behavioral studies compared cases diagnosed with dementia due to sporadic and familial AD using a visual VSTM Binding test [30]. The authors reported that based on performance they could not distinguish between the two variants of AD even though the familial cases were younger patients from Colombia who had low educational level and the sporadic cases were older adults from the UK with high educational attainment [30]. This evidenced grants us confidence to suggest that the deficits we are observing here characterize AD generally regardless of its clinical variant (i.e., familial or sporadic).

A limitation of the present study is the small sample size. To reduce the impact of such limitation we adopted a statistical approach (i.e., multiple single-case statistics) that allowed us to confirm effects at the individual level. This approach evaluated in a small sample of patients with 100% probability of developing AD grants reliability to our findings. Future studies should confirm if the findings reported here hold with larger samples of MCI-FAD patients. Another limitation is that the low and high frequency ranges covered by the τ values calculated in our study are too widespread. In fact, such bands are far too broad to detect standard functional brain oscillations within specific frequencies and their link to physiological states. Nevertheless, abnormal oscillatory activity in AD has been reported for a wide range of frequencies ranging from low to high bands [90]. Thus, the decision of using a measure of information sharing which relies on a broad-band approach covering low ($\tau = 32\text{ms}$) and high ($\tau = 4\text{ms}$) EEG frequencies may be considered a more feasible methodology to characterize aberrant oscillations in the prodromal course of FAD (see also ([91])).

CONCLUSION

By applying the analysis of a novel connectivity method to EEG data collected during VSTM binding performance from carriers of the mutation E280A-PSEN1 who inevitably develop FAD we have identified electrophysiological signatures of the impairment found in this memory function. This is the first study confirming the role of brain information sharing in VSTM binding functions and the disruption of such mechanisms as the underlying physiopathology of inte-

grative memory deficits found in FAD. Such a disruption may be informed either by patterns of increased or decreased connectivity. They appear to account for poor task performance at different stages of the disease process. This biological evidence further strengthens the theory supporting this novel memory task and confirms its potential value as a memory biomarker for AD.

ABBREVIATIONS

AD	=	Alzheimer's disease
E280A-PSEN1	=	Mutation E280A in the Presenilin 1 gene
EEG	=	Electroencephalogram
FAD	=	familial Alzheimer's disease
MCI	=	Mild Cognitive Impairment
MCI-FAD	=	Mild Cognitive Impairment due to familial Alzheimer's disease
VSTM	=	Visual Short-Term Memory.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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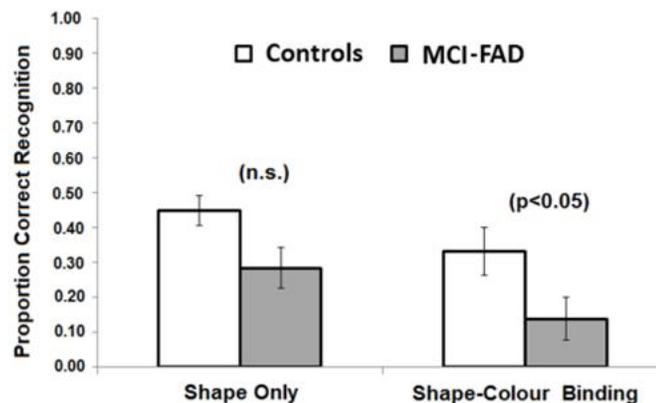
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Supplementary Material

Brain Information Sharing During Visual Short-Term Memory Binding Yields a Memory Biomarker for Familial Alzheimer's Disease

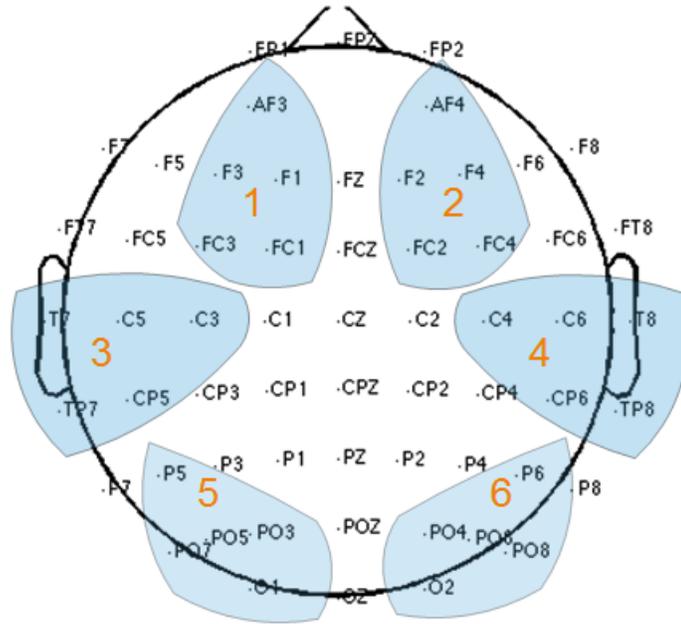
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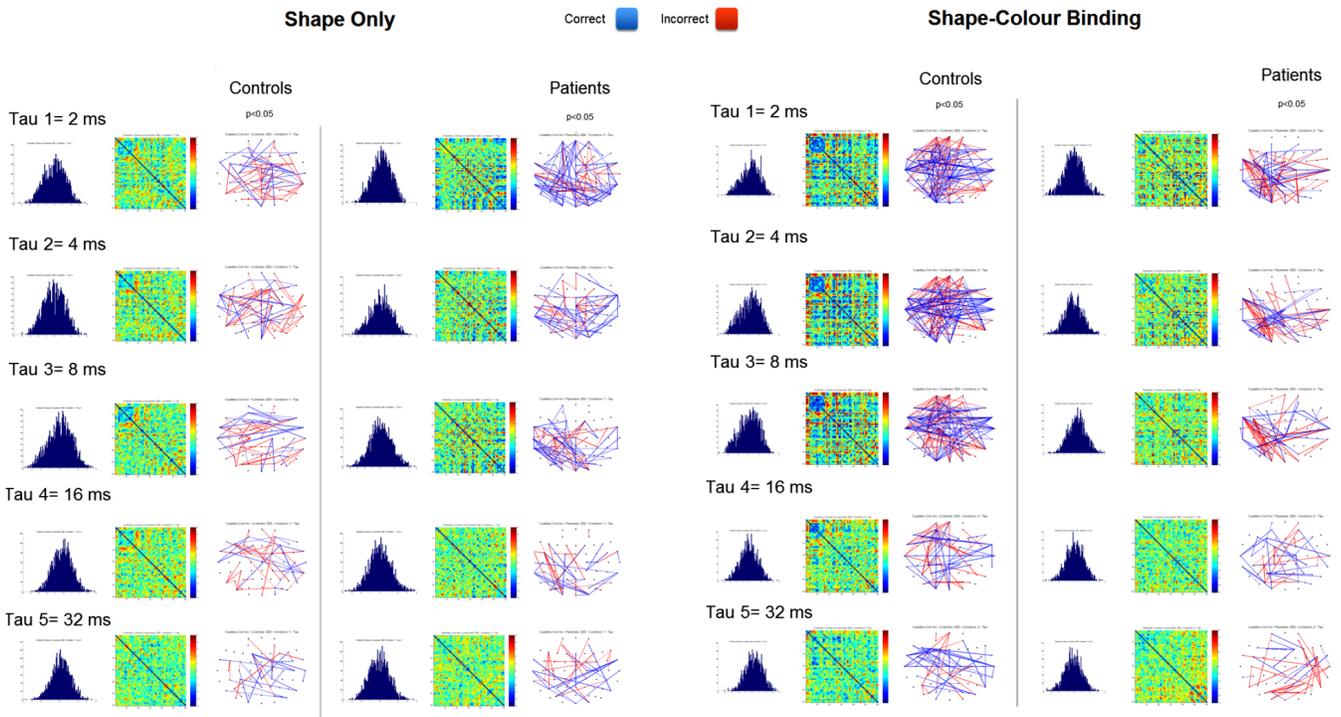


Supplementary Figure 1. Mean performance during the VSTM task.

Due to the small sample size we found it risky to rely on a mixed ANOVA model to test the hypothesis of selective binding deficits as a null finding would be difficult to interpret (*i.e.*, limited power). Our most recent work suggests that the selectivity of binding impairments in patients with MCI might be contingent upon memory load [1]. Following the results reported by [2], who observed selective binding deficit in patients with Subjective Cognitive Decline (SCD) but not in patients with MCI, we have demonstrated that with high memory load, the latter group will show poor performance on both conditions of the VSTM task, shape only and shape-colour binding [1]. Previous studies reporting on the sensitivity and specificity of VSTM binding for AD, we used titration procedures [3-6]. That is, patients who met criteria for AD dementia were assessed with a version of the task that presented 2 items and controls or asymptomatic carriers of a mutation leading to familial AD [4] were tested with a version of the task that presented 3 items. Under these testing conditions, patients' and controls' performance on single feature conditions (*i.e.*, shape only) was kept at the same level, and a selective impairment in binding was revealed in patients. Titration is difficult to undertake in clinical settings. We have started to assess patients and controls with the same task ([1] see also [7]). Our most recent work suggests that 3 items may already overload VSTM in MCI patients masking the selectivity of STM binding. As the data presented here suggest, binding performance is near ceiling while shape performance drops relative to controls, but is still off-ceiling. Pietto *et al.* [8] have recently reported very similar findings. Together, these results suggest that when such a selectivity is a desired outcome, patients with MCI should be tested with a smaller set size (*i.e.*, 2 items version of the task).



Supplementary Fig. (2). Topology of the ROIs explored during the resting state connectivity analysis.



Supplementary Fig. (3). Connectivity analysis during the encoding phase of the two conditions of the VSTM task in the two groups. Histogram and matrix of the t-test. Line plots showing the last quartile of differences meeting the significance threshold. These results mirror those found during the whole task analysis (see manuscript for a full description of the results).

Supplementary Table 1. Case-based statistics [9-12] contrasting mean wSMI from ROI showing reduced connectivity in patients relative to controls over frontal regions in the two frequency bands (tau) which showed the best classification power. The outcomes identified 3 cases with a significant decrease in brain connectivity (wSMI). Such cases were among those with the lowest scores on the MMSE and on the cued recall of the Memory Capacity Test (MCT, [13]), thus suggesting more advanced stages of the disease process.

		P1	P2	P3	P4	P5	P6	P7	P8	P9
TAU 4	t	4.051	-3.698	0.581	-0.661	0.108	-0.790	-0.156	1.414	2.887
	p	0.998	0.003	0.711	0.264	0.542	0.226	0.440	0.902	0.990
TAU 5	t	2.415	-2.393	1.481	2.111	-2.393	-1.756	-0.412	-0.188	-1.910
	p	0.979	0.022	0.912	0.966	0.022	0.059	0.346	0.428	0.046
MMSE		29	23	24	21	16	30	27	30	24
MCT-Cued Recall List 1		14	4	8	-	7	14	10	14	6

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