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ApoE ε4 allele related alterations in hippocampal connectivity in early Alzheimer's disease support memory performance

Matteo De Marco[a,b], Annamaria Vallelunga[c], Francesca Meneghello[b], Susheel Varma[d], Alejandro Frangi[d,e,f], Annalena Venneri[a,b], for the Alzheimer's Disease Neuroimaging Initiative*

[a] University of Sheffield, Sheffield Teaching Hospital NHS Foundation Trust, Medical School, Beech Hill Road, S10 2RX, Sheffield, UK;

[b] IRCCS Fondazione Ospedale San Camillo, via Alberoni 70, 30126, Lido, Venice, Italy;

[c] Neuroscience Section, Department of Medicine and Surgery, Center for Neurodegenerative Diseases (CEMAND), University of Salerno, Salerno, Italy;

[d] Centre for Computational Imaging & Simulation Technologies in Biomedicine (CISTIB), Department of Electronic and Electrical Engineering, University of Sheffield, Sheffield, UK

[e] Insigneo Institute for in silico Medicine, University of Sheffield, Sheffield, UK

[f] Department of Electronic and Electrical Engineering, University of Sheffield, Sheffield, UK

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A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Address for correspondence:

Prof. Annalena Venneri

Department of Neuroscience - Medical School, University of Sheffield

Beech Hill Road, Royal Hallamshire Hospital, N floor, room N130

Sheffield - S10 2RX - United Kingdom

a.venneri@sheffield.ac.uk

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Abstract

Background. Whether the presence of the Apolipoprotein E ε4 allele modulates hippocampal connectivity networks in abnormal ageing has yet to be fully clarified.
Objective. Allele-dependent differences in this pattern of functional connectivity were investigated in patients with very mild neurodegeneration of the Alzheimer's type, carriers and non-carriers of the ε4 allele.
Method. A seed-based connectivity approach was used. The two groups were similar in demographics, volumetric measures of brain-structure, and cognitive profiles.
Results. ε4 carriers had increased connectivity between the seed area in the left hippocampus and 1) a left insular/lateral prefrontal region and 2) the contralateral right parietal cortex. Moreover, hippocampus-to-parietal connectivity in the group of ε4 carriers was positively associated with memory performance, indicating that the between-group difference reflects compensatory processes. Retrospective analyses of functional connectivity based on patients from the ADNI initiative confirmed this pattern.
Conclusion. We suggest that increased connectivity with extra-DMN areas reflects both compensatory recruitment of additional areas, and pathological interwining between the DMN and the salience network as part of a global ε4-dependent circuital disruption. These differences indicate that the ε4 allele is associated with a more profound degree of DMN network breakdown even in the prodromal stage of neurodegeneration.

Keywords

Functional connectivity; Disinhibition hypothesis; Posterior cingulate; Hippocampus; Alzheimer's disease; Network disruption;

Running Title

Hippocampal connectivity in ApoE ε4-positive early-AD

1. Introduction

The brain default-mode network (DMN) is a functional resting-state circuit that normally activates while a person does not engage in any explicit mental task [1]. It includes midline kernels localised in the posterior cingulate and medial prefrontal cortices, which are connected functionally with the inferior parietal lobule, the lateral temporal cortex, and the hippocampal formation [2]. Alzheimer's disease (AD) causes a global disruption of functional connectivity within the DMN [3]. Further segmentation of the circuit into sub-components suggests that AD down-regulates connectivity within the structures located in the posterior portion of the DMN and up-regulates connectivity of the prefrontal hubs [4-5]. This process begins in prodromal AD, when the disease is characterised by a transitional phase of Mild Cognitive Impairment (MCI) [6-7]. Within the set of regions included within the DMN, the hippocampus plays a distinguishing role. In fact, hippocampal subfields are subjected to a well-established volumetric loss along the timeline of AD [8], and this shrinkage is predictive of conversion from MCI to the dementia stage [9]. Morevoer, when compared with healthy controls, patients diagnosed with amnestic MCI (thus suggestive of potential AD) show disrupted connectivity, with pathological up-regulation of connectivity within the hippocampal formation and between the hippocampus and the posterior-cingulate/precuneus region [10]. Modifications of memory-associated patterns of hippocampal activation have been also reported in MCI [11], and this aspect was found to be associated with cortical thinning of "signature regions" of AD including frontal, temporal and parietal cortices [12].

The ε_4 isoform of the Apolipoprotein E (ApoE) gene is a well-established risk factor for the development of the sporadic late-onset forms of AD [13-14], and is associated with a younger age of disease onset [15]. The homonymous peptide coded by the gene plays a crucial role in lipoprotein metabolism and neurobiology [16]. As for the latter set of functions, the ApoE ε_4 allele appears responsible for a large number of detrimental effects on neuronal and synaptic function, in comparison with the "standard" ε_3 isoform [17], and appears to exert its impact on cell biology both either in the presence or in the absence of neurodegeneration [18]. Following the Imaging Genetics model, the direct effect of the various ApoE isoforms on cellular mechanisms translates into indirect, yet consequent, effects of the genotype on the development of brain structure and brain function [19]. Within this latter category of variables, a large number of studies on healthy adults have found that the ε_4 isoform is associated with alterations of the DMN both in healthy ageing and young adulthood [20-28], in correspondence or even prior to the initiation of the neuropathological cascade seen in AD. Conversely, the study of the impact of the ε_4 allele on functional connectivity in the symptomatic stages of AD has been scarce. A few studies investigated electro- and magneto-

encephalographic connectivity in samples of MCI and mildly-to-moderately demented AD patients. These revealed that the ε_4 isoform is associated with decreased levels of functional connectivity as measured by various proxies of connectivity such as signal coherence, synchronisation likelihood, and ROI-based lagged-phase synchronisation [29-32]. Aside from well-established limits in spatial resolution, however, these techniques do not allow a specific focus on all major DMN hubs affected by AD, as these are located in regions that are not easily capturable by measurements obtained at the scalp level. Overcoming these methodological limitations, two very recent rest-fMRI studies found that the DMN of ε_4 patients diagnosed with early-stage AD is significantly down-regulated [33-34]. Despite the limited number of studies, this body of evidence indicates that the presence of the ε_4 allele impacts negatively on DMN integrity even after the onset of a clinically-established symptomatology of dementia. Nevertheless, it is still undetermined whether variability for the ApoE genotype is associated with a distinctive signature of disruption of hippocampal functional connectivity in the prodromal phases of the disease, when the person still retains their daily life independence. The hippocampus, harshly affected in AD, is of particular interest in this early phase of the disease. In fact, this stage is crucial because it represents the earliest moment of subjective/objective awareness of the presence of a possible neurodegenerative disease. In addition, published studies suggest that this clinical stage is associated with a high degree of retained mechanisms of neuroplasticity, sufficient to induce remarkable changes in brain functioning [e.g. 35-36]. It is thus of paramount importance to characterise and interpret appropriately allele-dependent differences in the connectivity of a corel region like the hippocampus during such a clinically relevant stage, as qualitative differences in the functional architecture of the brain might translate into qualitative differences in the efficacy of treatments between carriers and non-carriers (e.g. a memory-enhancing training).

In this study we investigated the fMRI network signature of the ApoE ε_4 allele in hippocampal connectivity among patients suffering from very mild AD. To do so, we implemented seed-based connectivity methods in a sample of patients ε_4 carriers and in a sample of ε_4 non-carriers. We hypothesised that allele-dependent differences would exist between the two groups, and that the presence of the ε_4 allele would be associated with a signature of connectivity involving associative areas which sustain high-order cognitive processing.

2. Material and methods

2.1. Participants

Sixty-one patients were referred to neurological examination between 2011 and 2014 because of suspected incipient cognitive decline. On that occasion, all patients agreed on completing cognitive assessment and an MRI procedures (detailed below). A proportion of these patients was diagnosed with very mild dementia of the AD type [37], while a proportion received a diagnosis of MCI [38]. This latter group was followed-up over time, and progression of disease and conversion were monitored until early 2015. Based on this longitudinal neurological monitoring, only patients who showed evidence of a clinically-established progression towards AD dementia at follow up were included in this study.

A neurological examination served to rule out the presence of major exclusion criteria, which were set as follows: a significant disease at clinical level, history of transient ischemic attacks, a diagnosis of vascular brain disease of clinical severity (e.g. the presence of chronic cerebrovascular disease as main aetiology), a structural MRI revealing a different diagnostic entity which could otherwise explain the presence of cognitive symptoms, presence/diagnosis of uncontrolled seizures, peptic ulcer, cardiovascular disease, sick sinus syndrome, neuropathy with conduction difficulties, significant disabilities, proof of abnormal baseline levels of folates, vitamin B12 or thyroid-stimulating hormone, a significant psychiatric condition, consumption of drugs for research purposes or with toxic effects to internal organs.

After genetic assays, all AD patients carrying at least one copy of the ε_4 allele (n = 15) were enrolled. None of these had a $\varepsilon_4\varepsilon_4$ or a $\varepsilon_2\varepsilon_4$ genotype. A group of non-carriers ($\varepsilon_3\varepsilon_3$ only) was then selected from the pool of remaining patients to match the two groups as closely as possible for demographic characteristics. Other non ε_4 genotypes were not included in the control sample to avoid contamination of protective factors such as in the case of the ε_2 genotype, and in an attempt to minimise variance in the control patient group. All participants were Caucasian and were inhabitants of one of the islands within the Venetian lagoon. This study was carried out according to the Declaration of Helsinki and was approved by the Institutional Review Board of the IRCCS Fondazione Ospedale San Camillo (Venice, Italy). Written informed consent was obtained from each study participant.

An extensive battery of neuropsychological tests was administered to each patient as part of the initial diagnostic classification procedures. This included tests assessing short- and long-term verbal and non-verbal memory, attention, naming by confrontation, logical abstract reasoning, verbal fluency and visuoconstructional abilities (see Table 1 for details).

2.2. MRI Acquisition, Preprocessing and Analysis

A structural 3D T1-weighted brain scan and two resting-state fMRI runs were acquired on a 1.5 T Philips Achieva system, and preprocessed and analysed using Statistical Parametric Mapping (SPM) 8 software (Wellcome Trust Centre for Neuroimaging, London, UK) running in Matlab R2011b (Mathworks Inc., UK). T2-weighted and FLAIR-weighted sequences were also included in the protocol to verify neuro-anatomical exclusion criteria and suitability for inclusion in the study. A senior neuroradiologist reviewed each anatomical scan to ascertain study compatibility. Participants were asked to remain as still as possible for the full duration of the scan. No stimuli were presented.

Preprocessing of T1-weighted images was carried out using a standard Voxel-Based Morphometry approach [39]. Native-space volumes of grey matter, white matter, and cerebrospinal fluid were obtained to calculate individual brain parenchymal volume, total intracranial volume, and tissue-class ratios (grey-matter, white-matter, and brain parenchymal fraction). Modulated and normalised tissue-class maps were then smoothed with a 8 mm full-width at half maximum gaussian kernel. Additionally, native-space T1-weighted images were also segmented to extract the hippocampal maps for further sample characterisation. For this purpose, the STEPS algorithm was implemented [40]. Briefly, this methodology allows an automatic and precise segmentation of the hippocampus by registering each scan to the most appropriate image among a series of available templates. Absolute and ratio-based volumetric properties of left and right hippocampus were thus extracted.

Resting-state fMRI acquisitions were preceded by 20-seconds of dummy scans to allow the scanner to reach a state of electro-magnetic equilibrium. Each run included 120 volumes of T2* weighted echo planar images (repetition time = 2 s, echo delay time = 50 ms, flip angle 90◦, voxel dimensions $3.28 \times 3.28 \times 6.00$ mm^3, field of view 230 mm). Each volume included 20 contiguous axial slices, acquired in ascending order. Slice-timing was carried out first. Volumes in each run were then realigned and resliced independently. Concurrently, linear and rotational parameters of head motion were estimated by the use of 4th Degree B-Spline interpolation. Volumes were subsequently

normalised using the first realigned volume as source image to match the SPM 8 echoplanar template, and voxel size was re-dimensioned to $2.0 \times 2.0 \times 2.0$ mm^3. Images were then band-pass filtered at 0.008 – 0.1 Hz using the REST toolbox [41]. Finally, volume smoothing was carried out with a $6.0 \times 6.0 \times 6.0$ mm^3 full-width at half maximum isotropic gaussian kernel.

Hippocampal connectivity was computed by means of seed-based first-level models. Seed regions were devised based on the IBA16 atlas implemented in the WFU-Pickatlas toolbox [42]. Both left and right seeds were loaded on a structural template to ascertain the absence of major spatial misplacements over the temporal horn of the ventricles. By doing so, miscalculations of average seed signal due to the presence of cerebrospinal liquid were minimised. Signal extraction from the two seed regions was carried out using the MarsBaR toolbox [43]. Two additional vectors were extracted from the map of white matter and from that of cerebrospinal fluid. First-level analyses were carried out to obtain individual maps of seed-based connectivity, regressing out the signal from white matter and cerebrospinal fluid, and controlling for in-scanner motion vectors. For inferential analyses, a *p* value of 0.01 (uncorrected) was set. Age, education levels, MMSE scores, and grey-matter fraction were used as covariates. MMSE scores were included in the model to account for variability of disease severity, as the mechanisms of AD affect the connectivity of the hippocampus [44-45], while grey-matter fraction served as proxy of brain reserve. Of all the output clusters only peaks surviving Family-Wise Error (FWE)-correction at a cluster level were reported as significant to minimise chances for Type I Errors. Peak coordinates were converted into Talairach stereotaxic space thanks to a non-linear transform (http://imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mni2tal-m) and interpreted using the Talairach Daemon client (www.talairach.org/client.htm), single-point coordinate search [46-47].

3. Results

There was no significant difference in age, level of education and male/female proportion between the two groups. Group comparisons revealed also no differences in absolute/proportional properties of brain structure. Although global difference in white-matter ratio survived correction for multiple statistical comparisons (with $\varepsilon_4\varepsilon_3$ patients having a significantly higher ratio of white matter), the voxel-based analyses revealed no between-group differences neither in the regional maps of grey matter nor in those of white matter. Moreover, all *p* values indicated that in our sample the two hippocampal regions were comparable between the two groups, as both absolute and relative

(fractional) volumetric values did not differ between ε_4 carriers and non-carriers. No between-group difference was found in any of the raw scores obtained over the set of neuropsychological tests, not even when age and education were included in the analyses. No significant differences were found also when age- and/or education corrected scores based on published norms were compared between the two groups. Both groups of patients had had an amnestic onset as established by their neuropsychological profile. Verbal declarative memory was, in fact, the cognitive domain in which both carriers and non-carriers showed performance levels below cut-off. All these between-group comparisons are reported in Table 1.

- Insert Table 1 about here -

Hippocampal connectivity findings are reported in Table 2 and illustrated in **Fig.1**. ε_4 carriers showed enhanced connectivity between the left hippocampus and two clusters, one located in the left insula extending to the inferior frontal cortex, and one located in the right inferior parietal lobule, with a peripheral peak located in somatosensory areas. No differences were found in the functional connectivity of the right hippocampus.

- Insert **Fig.1** and Table 2 about here –

In order to clarify whether this allele-dependent difference in the pattern of functional connectivity between the hippocampus and the inferior parietal lobule was beneficial or not, this latter cluster was binarised, and signal extraction was carried out from this region as originally carried out for the two seeds. A seed-to-target index of connectivity was then computed. A coefficient of partial correlation was calculated between seed and target vectors, controlling for the same regressors as with the voxel-based analyses, and a *Fischer's r to z* transformation was then applied. Since the recruited sample was prevalently characterised by amnestic problems, a composite index of memory performance was computed by transforming the corrected scores of four tests investigating various aspects of verbal and visuospatial memory (Rey Complex Figure Test – Recall, Visual Supraspan Test, Prose Memory Test - Global Recall, and Paired Associates Test) into *z-scores*, which were then averaged for each patient. This variable distributed normally (Shapiro-Wilk test of

normality $p = 0.920$). A linear-regression model was then designed for both groups. To test the hypothesis by which this pathway of connectivity predicted memory performance, two blocks were created. The Mini-Mental State Examination score and the fractional volume of the left hippocampus were inputted in the first block to control for potential cognitive and neurostructural confounds, and the *z-index* of connectivity was included in the second block. In the group of non-carriers, the slope associated with the predictor was not significant ($b = 0.131$; r^2-*change* statistic = 0.001; $p = 0.905$), whereas a significant slope was found in the group of carriers ($b = 0.759$; r^2-*change* statistic = 0.211; $p = 0.029$; **Fig.2**). This allele-dependent association did not generalise to other cognitive functions. In fact, the strength of this specific pathway of connectivity did not predict executive performance (average of *z*-transformed corrected scores in the Stroop Test – Time and the Letter Fluency Test) in neither of the two groups.

- Insert **Fig.2** about here -

3.1 Validation of the results in the ADNI cohort

To verify this pattern of group difference, an additional sample of 65 MCI patients was identified (30 with an $\varepsilon_4\varepsilon_3$ or $\varepsilon_4\varepsilon_4$ genotype, and 35 with an $\varepsilon_3\varepsilon_3$ genotype) (see Table 3 for details). These were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). ROI-based analyses were carried out to compute the pattern of functional connectivity between the hippocampus and Brodmann areas. Univariate *ANOVA*s were then run between the two groups controlling for age, education levels, MMSE and ventricle size. Increased functional connectivity was found in the group of ε4 carriers in the supramarginal gyrus ($p < 0.029$) and in the prefrontal cortex ($p < 0.039$), paralleling our original findings.

- Insert Table 3 about here -

4. Discussion

In this study, differences within the maps of hippocampal connectivity were investigated between a group of early stage AD patients carrying a copy of the ApoE ε_4 allele, and a group of patients with a $\varepsilon_3\varepsilon_3$ genotype. The sample was extensively tested with statistical procedures in order to maximise comparability along the most relevant axes of demographic, neurostructural and cognitive variability.

The findings show that carriers have increased connectivity between the left hippocampus and two clusters, the first of which was centred in the right inferior parietal lobule. Being the hippocampus and the inferior parietal lobule both hubs of the DMN [2], this piece of evidence suggests that the presence of the ε_4 allele might be associated with a more preserved DMN in prodromal AD. This trend, however, goes in the opposite direction as that emerged from the studies characterising the DMN when AD is more severe, in which ε_4 carriers were instead found to show reduced connectivity within aspects of the DMN [34], and, specifically, between the hippocampus and prefrontal, parietal, and temporal regions [33]. As a consequence, we suggest that the ε_4 allele might influence the patterns of hippocampal connectivity with a quadratic tendency. This trend would not come as a novelty. In fact, it follows the longitudinal progression of hippocampal function as reported by a study of task fMRI, in which activation of this region is increased in MCI patients during memory processes, but reduced in AD dementia [11]. Furthermore, recent evidence indicates that there is an inverse association between hippocampal function (as measured by FDG PET metabolism) and DMN connectivity (as estimated by hippocampus-to-precuneus BOLD-signal correlation) in patients with AD dementia, but such association is not visible in MCI [48]. These pieces of evidence indicate that the progressive disruption of DMN and hippocampal connectivity are not linear along the timeline of AD progression. We hereby suggest that the enhanced hippocampus-parietal connectivity seen in the group of ε_4 carriers reflects an intensification of this "naturally-occurring" phenomenon. Up-regulation of connectivity between hippocampus and left lateral prefrontal cortex was seen in the group of ε_4 carriers. The involvement of the frontal lobe as ApoE-dependent between-group difference has already been reported in studies of healthy individuals, in which augmented connectivity was observed in ε_4 carriers between hubs of the DMN and extra areas that normally are not part of this circuit [e.g. 22, 24]. This was accounted for by the

hypothesis of compensatory mechanisms taking over from the AD-dependent disruption of "standard" patterns of connectivity. The idea that a functional reorganisation of regional connectivity occurs in healthy adults with a risk factor for AD is also supported by studies of task-associated fMRI, in which evidence of computational differences has been repeatedly reported, albeit with no constant pattern [49]. Our results suggest that compensatory mechanisms may be triggered in ε_4 carriers even after the possible onset of neurodegeneration, or it might be the outcome of the brain over-time coping with the subtle negative effects of this genetic risk factor.

At a first glance, the up-regulated connectivity between the hippocampus and the parietal lobe might also be compensatory in nature, as it consists of an intensification of a pattern of connectivity which is normally visible by default. Despite this straight-forward interpretative remark, there is another potential explanations that needs to be taken into account, which suggests that the evidence of "hippocampal hyperconnectivity" is not necessarily index of compensation. A recent randomised trial found that amnestic MCI patients receiving mild antiepileptic medication showed a significant improvement in memory performance, which associated with significantly reduced hippocampal activation. As a consequence the enhanced magnitude of hippocampal function seen at baseline was interpreted by the authors as a dysfunctional trait [50]. Based on this same interpretational paradigm (albeit transposed, in a speculative way, to the construct of connectivity), it might be suspected that the increased connectivity seen in the DMN of ε_4 carriers might be maladaptive in nature, despite being located in a functional pathway where, at least in heathy adults, "more would be better". Along this plausible line, a study found increased task-based connectivity of hippocampal seeds in amnestic MCI patients compared with healthy adults in a set of regions including the prefrontal, temporal, parietal and limbic lobe [51]. Additionally, many of these pathways of connectivity were inversely associated with cognitive performance, as an indication of the dysfunctional nature of this excessive connectivity [51]. To test the conflicting hypotheses of compensation vs. maladaptative rewiring, we carried out post-hoc analyses to explore the association between the strength of the functional connectivity between the hippocampus and the inferior parietal lobule, and an index of memory performance. While no association was found in the group of non-carriers, a positive association was found in the group of carriers, indicating that, compatibly with a compensatory mechanism, the more connected the two regions, the more productive the memory processing.

There is evidence that increases in functional connectivity seen in AD neurodegeneration might be the result of a pathological interconnection of distinct network patterns. This was reported by Wang and colleagues [34], who, using a graph-theory approach, found decreased levels of inter-network

connectivity among AD patients carriers of the ε4 isoform. Conversely, Seeley and colleagues [52] came up with the same interpretational avenue to explain the spreading (and, therefore, increase) of functional connectivity of the DMN to extra-DMN structures. Neural cerebral cortex circuitry is a biological system characterised by balanced involvement of parallel networks. The DMN is negatively correlated with the salience network, a resting-state circuit whose activation is associated with the integration of sensory processing and internal autonomic-visceral processing [53]. This anticorrelation would reflect an inter-network balance based on mutual inhibition between the two circuits. When the DMN is damaged by AD, this harmonic equilibrium would collapse, and, as a consequence, the anticorrelated network would no longer be inhibited. This dihinibition would trigger enhanced connectivity within the anticorrelated network and reorganisation of connectivity patterns to induce between-network interactions [52]. Published evidence provides additional support for this hypothesis as a potential signature ot the ε4 allele. In a study of healthy older individuals, ε4 carriers showed more connectivity within the salience network than non-carriers [21]. Another study of network differences associated with the presence of the ε4 isoform in middle-aged adults found diminished connectivity within structures of the DMN in carriers, including the left hippocampal/parahippocampal complex, the left anterior temporal pole and the dorsomedial prefrontal cortex bilaterally. At the same time decreased anticorrelated connectivity was observed between the posterior cingulate and regions in the salience network. Complementarily, seed-based connectivity devised to estimate the SN revealed increased connectivity with DMN areas in the same group [54]. Consistently with the disinhibition hypothesis, we interpret the enhanced functional connectivity seen in ε4 carriers between the seed in the left hippocampus (part of the DMN) and the left insula, that is one of the main hubs of the salience network, as maladaptive.

These findings support the idea that allele-dependent diversity in resting-state circuitry is detectable even after conversion from healthy ageing to the first symptomatic phases of AD. The various interpretations of differences found between the two groups converge in indicating that patients carriers of the ApoE ε4 allele have additional circuital damage in comparison with non-carrier individuals. This additional network disruption is supported by the necessity to rely on a higher magnitude of compensation and by the dysfunctional nature of some of the circuital rewiring. It is noteworthy to highlight that no cognitive differences and no neurostructural discrepancies existed between the two groups of patients. Nonetheless, circuital breakdown was more profound in the group of ε4 carriers. This may have important implications in the therapeutical management of these patients. Two individuals with comparable cognitive phenotype might have a diverse degree

of hidden circuital deterioration/dysfunction that is genotype-dependent. A more profoundly damaged neural architecture might be associated to less capacity for neuroplastic changes, and some forms of therapeutic intervention might not be as beneficial as expected.

These results support the view that, despite its non-linear tendency (increase of DMN connectivity seen in the prodromal phases, and decrease of DMN connectivity documented in the later dementia phases), the ε_4-associated network disruption might be independent of disease stage. Recent findings suggest that the neural representations of at least some aspects of certain cognitive functions are subjected to a comprehensive re-organisation in association with the development of MCI [55-56]. This insidious functional "re-moulding" could potentially nullify or at least minimise the detrimental impact of the ε_4 allele, which would be superseded by the impact of pathology. Our study concludes that this does not occur, as the impact of genetic variability for the ApoE gene keeps expressing in the form of more profound condition of network disruption even after the onset of prodromal pathological processes. Other findings are in line with this conclusion. There is a strong body of evidence suggesting that ε_4 carriers with MCI tend to have smaller hippocampi and amygdalae than patients not carrying the ε_4 allele but having similar demographic characteristics [57-60]. Other studies have instead reported volumetric loss in ε_4 carriers extending to other cortical and subcortical areas [61-64], although some have suggested that non-carriers can cope with a much more pronounced and extensive brain volume loss before manifesting the same level of cognitive disruption [64]. In addition, there is evidence that MCI patients carrying the ε_4 isoform have reduced cortical metabolism bilaterally in the precuneus, the superior temporal gyrus and the inferior parietal lobule [65]. Albeit being in line with our findings, all these studies were based on a localisation-based approach. This type of approach is not as suitable as a connectivity-based framework to characterise cellular and synaptic disruption, and its association to the underlying cognitive functions [66]. Moreover, it is worth noting that evidence emerging from connectivity analyses should not be taken for granted as the natural consequence of the aforementioned localisation-based differences reported in the literature. Indeed, the parallel study of the impact of the ε_4 allele on structural connectivity of AD patients (which describes different, yet theoretically contiguous aspects of signal propagation) has led to incongruous findings [67-68].

Although a "classical" view indicates that the left hippocampus would be mainly involved in verbal memory processes (as opposed to the right hippocampus, inolved instead mainly in visuospatial memory processes), recent evidence found instead no evidence of such lateralisation [69]. On this note, the creation of a memory composite score based on both verbal and visuospatial memory

performance would be a better modality-independent estimate of real mnemonic capacities, as it is a value obtained averaging four and not simply two values.

This study is not free from limitations. First, this is a cross-sectional study. Longitudinal investigations need to be planned to clarify the impact of the ApoE genotype along the axis of disease progression. Second, no specific control was carried out on other relevant risk factors involved in AD. Third, albeit these findings, surviving a Family-Wise Error-corrected cluster-level $p < 0.05$, show a degree of robustness against the Type I Error, larger samples are necessary to control further for more variables, and possibly, to investigate the impact of two copies of the ε_4 allele, since genotype effects might be actenuated in individuals carrying only one copy of the gene, and dose-dependent effects may exist. Fourth, no information on amyloid pathology was available for these patients. Although this would have not affected the diagnosis in these patients (as this had been reached after planned follow-up assessments over an extended period), it would have allowed us to understand whether the differences in connectivity shown by ε_4 carriers are associated with regional difference in amyloid deposition.

Despite these limitations, this study highlights the role of the ε4 allele as a modifying-factor of neural pathways that are relevant for AD neurodegeneration. Although these findings shed some additional light on the role of the ε4 allele in AD, the overall context in which these results have to be inserted remains extremely complicated. The clinical role of the hippocampus is not confined to the sole AD, but is involved in other conditions of neurological relevance. For example, alterations of resting-state hippocampal blood perfusion has been described in individuals at high risk of psychotic symptoms [70], and abnormal increases of hippocampal connectivity are visible as a consequence of post-traumatic brain disorders [71]. At the same time, the ε4 allele was found to influence the activity and network properties of other, non-hippocampal regions, such as the prefrontal cortex [72] or midline structures and insula [73]. Finally, it is still unclear how the interplay of ApoE genotype and disease mechanisms influence not only resting-state by also task-based functional connectivity, especially during memory retrieval processes [51].

Conclusion

In summary, we found evidence of network spatial discrepancies between prodromal AD patients carrying one copy of the ApoE ε_4 allele and patients homozygous for the ApoE ε_3 allele showing no differences in demographic, neurostructural, and neuropsychological characteristics. Increased

connectivity was seen in carriers between the left hippocampus and 1) parietal areas, 2) prefrontal regions, and 3) the insular cortex. Although this pattern may be partially seen as the result of compensatory mechanisms (especially with regard to the increased connectivity seen in the frontal lobe), there are two interpretational avenues that identify these differences as maladaptive. First the "hyperfunctional hippocampus" hypothesis suggests that the excessive seed-to-parietal connectivity might be dysfunctional as negatively associated with cognitive performance [48-49]. This eventuality was ruled out by post-hoc analyses, which confirmed that the magnitude of connectivity along this pathway was positively associated with memory performance (characterising it as compensatory). Second, the "dishinibition hypothesis" indicates that excessive hippocampus-to-insula connectivity might be result of maladaprive rewiring of a portion of the DMN with a portion of the salience network [50, 52]. As a consequence, the presence of maladaptive processes and the necessity to rely on extra compensation indicate that ε_4 carriers show an accentuated degree of network involvement independent of the diagnostic group and dependent on the ApoE genotype.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure Captions

Fig.1

Pattern of enhanced functional connectivity of the left hippocampus found in patients who were carriers of the ε_4 allele. Slices in MNI space are as follows: z = -10, z = 46. The seed region is illustrated on the left (slices in MNI space are as follows: x = -26, y = -13, z = -13).

Fig.2

Association between mediotemporal-parietal connectivity and memory function as investigated at post-hoc. On the left, the association found in the group of patients who were homozygotes for the ε_3 allele (r = -0.037; p = 0.905); on the right, the association found in the group of patients with an $\varepsilon_4\varepsilon_3$ genotype (r = 0.602; p = 0.029).

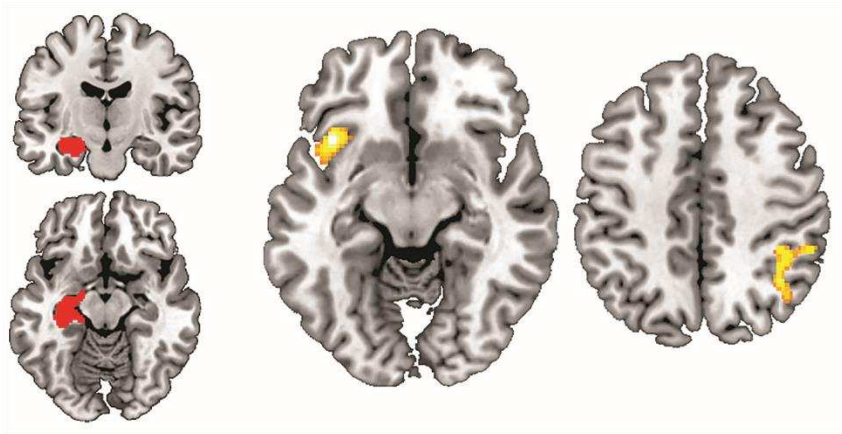

Figure 1

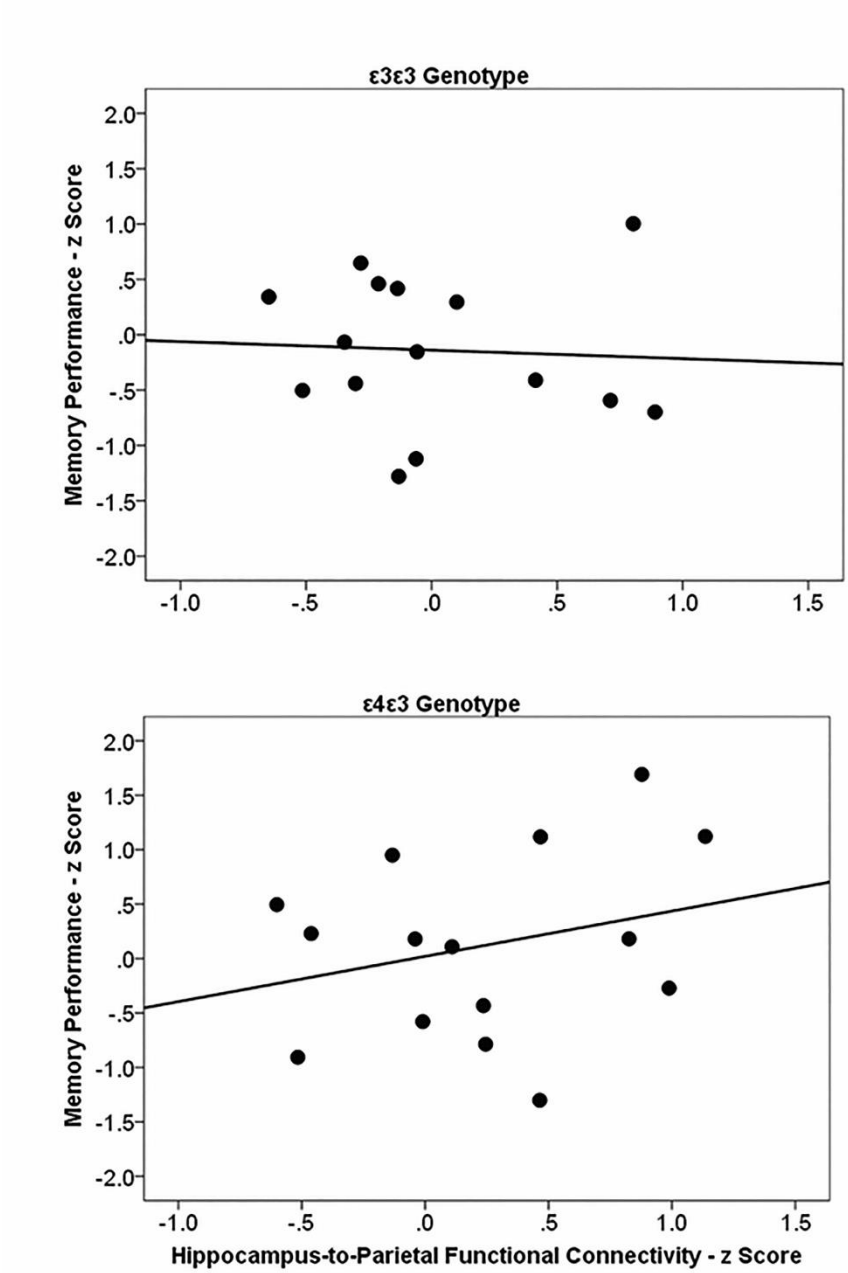
ε3ε3 Genotype
Memory Performance - z Score
2.0
1.5
1.0
.5
.0
-.5
-1.0
-1.5
-2.0
-1.0
-.5
.0
.5
1.0
1.5
ε4ε3 Genotype
Memory Performance - z Score
Hippocampus-to-Parietal Functional Connectivity - z Score

Figure 2

Table 1: Sample Characterisation

Descriptive Variables	ε4ε3	ε3ε3	Between-Group Statistics	
Demographic Characteristics				$p_{t\ test/chi\ square}$
Age at Scan (years)	73.20 (5.85)	73.80 (4.87)		0.762
Education Level (years)	9.20 (4.11)	9.13 (3.56)		0.962
Gender (f/m)	7/8	7/8		0.999
Global Structural Indices				$p_{t\ test}$
Grey-Matter Volume (cl)	538.74 (64.45)	536.66 (59.93)		0.893
White-Matter Volume (cl)	451.09 (65.14)	423.83 (63.69)		0.256
Brain Parenchymal Volume (cl)	990.83 (123.53)	960.49 (114.58)		0.491
Cerebrospinal-Fluid Volume (cl)	697.05 (122.87)	770.58 (112.12)		0.098
Grey-Matter Fraction	0.32 (0.02)	0.31 (0.03)		0.244
White-Matter Fraction	0.27 (0.02)	0.24 (0.02)		**0.002**
Brain Parenchymal Fraction	0.59 (0.03)	0.56 (0.04)		0.012
Total Intracranial Volume (cl)	1687.88 (231.15)	1731.07 (181.37)		0.574
Left Hippocampal Volume (cl)	2.12 (0.37)	2.24 (0.27)		0.322
Right Hippocampal Volume (cl)	2.24 (0.30)	2.30 (0.30)		0.543
Left Hippocampal Fraction	2.15^{-3} (2.95^{-4})	2.35^{-3} (2.85^{-4})		0.065
Right Hippocampal Fraction	2.27^{-3} (2.82^{-4})	2.41^{-3} (2.99^{-4})		0.190
Hippocampal Asymmetry (left/right)	0.95 (0.10)	0.97 (0.04)		0.356
Neuropsychological Raw Scores			$p_{t\ test}$	$P_{Corrected\ ANOVA}$
Mini Mental-State Examination	25.93 (3.81)	26.00 (3.21)	0.959	0.921
Raven Progressive Matrices	26.33 (5.43)	25.00 (6.47)	0.546	0.501
Letter Fluency Test	33.00 (11.93)	27.60 (10.36)	0.196	0.163
Category Fluency Test	27.07 (8.52)	25.47 (6.62)	0.570	0.645
Digit Cancellation Test	48.00 (9.18)	46.67 (9.40)	0.697	0.729
WAIS - Similarities	17.93 (5.59)	17.13 (5.29)	0.690	0.686
Token Test	33.57 (2.00)	33.50 (2.10)	0.930	0.927
Rey Complex Figure Test - Copy	29.23 (3.95)	28.13 (6.36)	0.574	0.601
Rey Complex Figure Test - Recall	8.40 (4.40)	7.87 (3.92)	0.728	0.809
Stroop Time Interference Effect	36.43 (20.32)	40.43 (14.04)	0.536	0.547
Stroop Error Interference Effect	1.83 (2.74)	2.60 (2.83)	0.457	0.515
Digit Span Test - Forward	5.40 (0.74)	5.80 (0.86)	0.183	0.199
Digit Span Test - Backwards	3.80 (0.86)	3.67 (0.62)	0.630	0.547
Corsi Test	4.13 (0.74)	4.13 (0.74)	0.999	0.876
Visual Supraspan Test	14.91 (7.62)	9.83 (7.66)	0.085	0.091
Prose Memory Test - Immediate Recall	5.67 (3.73)	5.93 (3.15)	0.834	0.731
Prose Memory Test - Delayed Recall	6.20 (4.84)	6.53 (3.85)	0.836	0.772
Prose Memory Test - Global Recall	11.87 (8.17)	12.47 (6.60)	0.826	0.739
Paired Associates Test	9.61 (4.27)	7.60 (2.68)	0.139	0.159
Confrontation Naming Test	18.07 (2.12)	18.00 (1.65)	0.924	0.972

Neuropsychological Corrected Scores			$p_{t\ test}$	**Cut-Off**
Raven Progressive Matrices	28.71 (4.28)	28.05 (6.09)	0.736	≤ 18.96
Letter Fluency Test	37.47 (9.81)	31.87 (11.33)	0.159	≤ 16
Category Fluency Test	32.00 (9.14)	31.07 (6.70)	0.752	≤ 24
Digit Cancellation Test	49.22 (7.18)	46.32 (9.25)	0.346	≤ 30
Token Test	31.65 (7.33)	33.68 (1.99)	0.309	≤ 26.25
Rey Complex Figure Test - Copy	30.85 (3.71)	29.23 (6.64)	0.419	≤ 28.87
Rey Complex Figure Test - Recall	11.93 (4.85)	12.20 (4.18)	0.873	≤ 9.46
Stroop Time Interference Effect	26.38 (19.43)	29.48 (14.03)	0.620	≥ 36.92
Stroop Error Interference Effect	1.02 (2.25)	1.52 (2.21)	0.544	≥ 4.24
Digit Span Test - Forward	5.57 (0.69)	6.02 (0.96)	0.151	≤ 3.5
Corsi Test	4.42 (0.74)	4.50 (0.63)	0.742	≤ 3.25
Visual Supraspan Test	16.89 (7.24)	11.48 (7.77)	0.063	≤ 5.5
Prose Memory Test - Global Recall	<u>12.67 (7.29)</u>	<u>13.07 (6.69)</u>	0.877	≤ 15.76
Paired Associates Test	11.14 (3.81)	9.37 (3.01)	0.174	≤ 6

Between-group statistics were run using *chi square* (gender), *one-way ANOVA* (differences in cognitive performance corrected for age and levels of education) and *independent-sample t* (all remaining comparisons) inferential models. Hippocampal asymmetry was calculated based on the raw volumes computed using the STEPS protocol. Brain parenchymal volume was computed as the sum of grey-matter and white-matter volumes. Tissue fractions were calculated dividing tissue class volume by total intracranial volume. Hippocampal fractions were instead calculated dividing hippocampal volumes by brain parenchymal volumes. "*pCorrected ANOVA*" indicates the significance level after covariating for years of age and years of education. In bold the sole between-group difference surviving Bonferroni correction for multiple comparisons (*pGlobal Structural Indices* < 0.0038). The aspects of cognitive functions showing performance below cut-off are instead underlined.

Table 2: Between-group differences in hippocampal connectivity

Cluster Number	Cluster Size (voxels)	Cluster-Level pFWE	Z Value at Local Maximum	Hemisphere	Cerebral Region	Brodmann Area	Talairach Coordinates		
							x	y	z
1	377	0.047	3.72	L	Inferior Frontal Gyrus	47	-38	15	-9
			3.44	L	Insula	13	-46	12	-1
			2.62	L	Inferior Frontal Gyrus	13	-32	13	-14
2	506	0.010	3.25	R	Inferior Parietal Lobule	40	40	-43	43
			3.21	R	Inferior Parietal Lobule	40	38	-52	45
			3.18	R	Postcentral Gyrus	2	59	-29	42

Table 3: Characteristics of the sample of patients extracted from the ADNI cohort

Descriptive Variables	ε4ε3 - ε4ε4	ε3ε3	Between-Group Statistics
Age at Recruitment (years)	71.43 (5.61)	72.43 (7.69)	0.559
Education Level (years)	16.70 (2.88)	15.97 (2.42)	0.272
Gender (f/m)	12/18	17/18	0.488
Ventricular Size (mm^3)	36785.23 (20974.18)	37645.34 (240505.53)	0.879
Mini Mental State Examination	27.50 (1.96)	28.00 (1.61)	0.263
Rey Auditory Verbal Learning Test - Immediate Recall	36.53 (9.53)	34.34 (9.90)	0.604
Rey Auditory Verbal Learning Test - Learning	4.37 (3.18)	4.89 (2.98)	0.318

Between-group statistics were run using *chi square* (gender), *one-way ANOVA* (differences in cognitive performance corrected for age and levels of education) and *independent-sample t* (all remaining comparisons)

inferential models