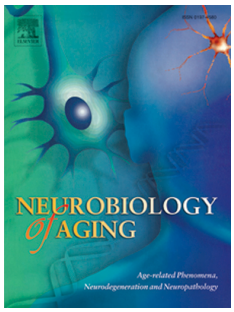

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PII: S0197-4580(20)30034-8

DOI: https://doi.org/10.1016/j.neurobiolaging.2020.02.007

Reference: NBA 10771

To appear in: *Neurobiology of Aging*

Received Date: 27 October 2019

Revised Date: 10 February 2020

Accepted Date: 10 February 2020

Please cite this article as: Coughlan, G., Zhukovsky, P., Puthusseryppady, V., Gillings, R., Minihane, A.-M., Cameron, D., Hornberger, M., Functional connectivity between the entorhinal and posterior cingulate cortices underpins navigation discrepancies in at-risk Alzheimer's disease, *Neurobiology of Aging* (2020), doi: https://doi.org/10.1016/j.neurobiolaging.2020.02.007.

Functional connectivity between the entorhinal and posterior cingulate cortices underpins navigation discrepancies in at-risk Alzheimer's disease

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Summary count: 150

Main text character length: 27,296 (excluding spaces)

Tables: 2 Figures: 4

Abbreviations: AD = Alzheimer's disease; VST = Virtual Supermarket task, APOE = Apolipoprotein Epsilon; EC = Entorhinal Cortex; MCI = Mild Cognitive Impairment; CCI = Cognitive Change Index; PCC = Posterior Cingulate Cortex

ABSTRACT

Navigation processes selectively mediated by functional activity of the entorhinal cortex (EC) may be a marker of preclinical Alzheimer's disease (AD). Here, we tested if a short path integration paradigm can detect the strongest genetic-risk phenotype of AD in large sample of APOE genotyped individuals. We also examined the associations between APOE mediated navigation process, subjective cognitive decline and rest-stating network connectivity. Navigation discrepancies classified 77% the APOE genotyped cohort into their respective low-risk ε3ε3 and high-risk ε3ε4 categories. When connectivity strength between entorhinal and the posterior cingulate cortices (also a functional correlate of strongest APOE-dependant behavioural characteristics) was considered, this classification accuracy increased to 85%. Our findings present a whole picture of at-genetic-risk AD, including select impairment in path integration, self-report cognitive decline, and altered network activity that is reminiscent of the pathological spread of preclinical AD disease. These findings may have important implications for the early detection of AD.

Keywords: spatial navigation; path integration; preclinical Alzheimer's disease; APOE genotype; functional connectivity

1. Introduction

Late-onset Alzheimer's disease (AD) is one of the biggest burdens to modern society with up to 50 million people living with the disease worldwide and no curative therapies to treat the underlying cause of the disease pathology (Nichols et al., 2019). As current evidence shows that gold standard episodic memory tests fail to capture the first symptomatic manifestation of AD (Coughlan et al., 2018b; Jessen et al., 2014; Zimmermann et al., 2019; Zimmermann and Butler, 2018), alternative diagnostic tools are urgently required in order advance cognitive diagnostics for incipient AD. Spatial orientation is a promising preclinical marker and is already established as a critical diagnostic tool in the late clinical and early prodromal stages of disease (Hort et al., 2014, 2007; Howett et al., 2019; Laczó et al., 2009; Lester et al., 2017; Mokrisova et al., 2016; Pai and Jacobs, 2004; Serino and Riva, 2013; Tu et al., 2015; Vlček, K., & Laczó, 2014). For example, spatial navigation tasks, such as the Virtual Supermarket Test (VST) (Tu et al., 2015) distinguish AD from other dementias and have been implemented in high-profile clinical trials due to their high sensitivity and specificity of AD pathophysiology (Ritchie et al., 2016; Ritchie and Ritchie, 2012).

AD patients typically sustain widespread navigation deficits, with severe difficulty storing and retrieving an allocentric representation (or a cognitive map) of the environment (DeIpolyi et al., 2007; Jheng and Pai, 2009; Serino et al., 2015). This is due to significant neuronal loss in the hippocampus where place cells are located and mediate the human and rodent cognitive map (Bird and Burgess, 2008; O'Keefe, John & Nadel, 1978). AD patients also experience loss of egocentric self-reference navigation strategy due to abnormal structural and cellular changes in the retrosplenial cortex, the posterior cingulate cortex (PCC) and parietal cortex of the AD brain (Mokrisova et al., 2016; Pai and Yang, 2013; Pengas et al., 2010; Serino et al., 2015). More recent developments in immersive virtual reality path integration (or self-motion) tests show that even individuals in the earlier stage of the disease spectrum characterised by 'mild cognitive impairment', suffer significant navigational errors during path integration, which has been directly associated with volumetric loss in the entorhinal cortex (Howett et al., 2019). This is important, because in the earlier asymptomatic stage of disease known as ''preclinical AD'', neuropathology is relatively localised to the EC, suggesting that path integration tests may be sensitive to the subtle AD related preclinical changes in navigation performance (Jack et al., 2018; Reisa A. Sperling, Paul S. Aisen, Laurel A. Beckett, David A. Bennet, 2011).

On a cellular level, reduced grid cell representations in the entorhinal cortex correlate path integration deficits in healthy at-genetic-risk apolipoprotein E (APOE) ε4 carriers, who are three to four times more likely to develop AD compared to non ε4 carriers (Corder et al., 1993; Kunz et al., 2015b). The same pattern of navigational discrepancies was replicated on the Sea Hero Quest game, which was then shown to discriminated ε4 carriers from non-carriers with a classification accuracy of 72%, although no MRI data was available to pinpoint the neural changes that gave rise to ε4-related

path integration deficits (Coughlan et al., 2019). Adding further support to the hypothesis that EC mediated navigation changes may represent an early cognitive marker for preclinical AD, transgenic rodent models show spatial memory deficits measured on the Morris water maze occur just before mature tau tangles spread beyond the entorhinal cortex (Fu et al., 2017).

While the field is largely focused on EC-mediate navigation impairments for the early detection of preclinical AD, functional connectivity (FC) changes also occur other brain regions such as the PCC and the precuneus in the preclinical stages of disease (Badhwar et al., 2017; Hanseeuw et al., 2017; Minoshima et al., 1997; Pengas et al., 2010; Reisa A. Sperling, Paul S. Aisen, Laurel A. Beckett, David A. Bennet, 2011). These more partial based changes are understood to be functional responses to early AD pathology within medial temporal lobe (Badhwar et al., 2017; Braak and Del Tredici, 2015; Chase, 2014). Despite this, resting-state FC within the spatial network that connects the EC, the PCC and the precuneus have not yet been examined in the context of navigation impairments in preclinical AD. To address this gap, we examined navigation performance and resting-state FC in APOE genotyped ε4 carriers and non-carriers. We first tested four major navigation process using a short path integration paradigm called the VST, and then examined the relationship between navigation performance and FC in a sub-set of the study sample; with a specific focus on the connectivity strength between the EC, the hippocampus, the PCC and the precuneus. We hypothesised that if ε4 navigation impairment was captured on the path integration test, impairment would correlate with reduced edge strength of the EC in the proposed neural network. As an additional measure, we assessed if subjective cognitive impairment, often considered a first symptomatic manifestation of disease (Jessen et al., 2014), accompanies navigation impairment and/or altered connectivity strength in the neural network.

2. Materials and methods

2.1 Participants

We recruited 150 participants between 50 and 75 years to participate in a research study at the University of East Anglia. Written consent was obtained from all participants and ethical approval was obtained from Faculty of Medicine and Health Sciences Ethics Committee at the University of East Anglia, Reference FMH/2016/2017–11. All 150 participants were pre-screened over the phone for exclusion criteria including psychiatric or neurological disease, substance dependence disorder and clinical depression and/or anxiety. Individuals with medicated cholesterol or blood pressure were included in the study, but medication intake was recorded to ensure there were not differences between genetic groups. Moreover, only participants with normal or corrected-to-normal vision were retained due to the nature of the VR task. Family history of AD was not included in the analysis, as there was considerable uncertainty when participants were asked the number of parents (0, 1 or 2)

with dementia and in particular the type of dementia. Finally, saliva samples were collected via buccal swab from participants who passed this screening and APOE genotype status was determined. Please refer to (Coughlan et al., 2019), for a description on the APOE genotyping method used on the study cohort.

All identified APOE ε4 allele carriers who represent 25% of the population (23% APOE ε3ε4, 2% APOE ε4ε4; Corbo and Scacchi, 1999; Liu *et al.*, 2013), were matched with a subset of the ε3ε3 carriers, which is the population wild-type genotype (60%) for age and sex (see Table S1 for group background characteristics). Homozygous APOE-ε4 carriers (2% of the population) and APOE ε2 carriers (13% of the UK population) were excluded. This yielded a final sample size of 64 (including 32 ε3ε3 carriers and 32 ε3ε4 carriers) all of whom underwent cognitive testing. Mean age of the ε3ε3 group and the ε3ε4 group was 62.28 ± 6.04 and 62.19 ± 6.64 respectively. Twenty ε3ε3 carriers and 20 ε3ε4 carriers also underwent structural and functional MRI. One ε3ε3 participant did not complete the scan due to distress and their data were excluded from the analysis. Two additional participants (two ε3ε4 carriers) who completed the MRI stage of the study were removed due to a software error that led to severe artefacts in the resting-state fMRI data. After these exclusions, MRI data on 37 out of 64 (58%) sample size was used for analysis, reaching an acceptable fMRI sample size (Pajula and Tohka, 2016). Among the genetic groups who underwent neuroimaging the mean age of the e3e3 was 63.21 ± 5.23 (including 11 male 8 females) and the mean age of the e3e4 group was 62.01 ± 6.92 (including 8 male 10 females). Please see SI Figure 1 for a summary of the sample size breakdown across study stages.

2.2 Paradigm overview

The VST is a sensitive and specific measure for differentiating AD from other dementia types (Coughlan et al., 2018a; Tu et al., 2017, 2015) . It includes a path integration test and measures i) egocentric orientation; ii) short-term spatial memory; iii) heading direction and iv) central (vs boundary) based navigation preferences. In brief, an iPad 9.7 (Apple Inc,) is used to show participants 7-14-second video clips of a moving shopping trolley in a virtual reality supermarket from the first-person perspective (Figure 1 A-C). The absence of landmarks in the supermarket aims to ensure the test taps into EC-grid cell dependent strategies rather than striatal-mediated landmark-based navigation. Once the video clip stops, participants indicate in real-life the direction of their starting point (egocentric orientation; Figure 1 D). In a second step, participants indicate their finishing location (short-term spatial memory; Figure 1 E) and heading direction on a VST map (grey arrow). We extended our VST paradigm to a fourth spatial measure based on evidence of an entorhinal-mediated bias or tendency to navigate towards environmental boundaries during path integration in at-genetic-risk AD. Specifically, we recorded the number of responses in the centre and boundary space

of the supermarket map to produce a central navigation preference measure (Figure 1 F; see supplementary text for more information on the creation of the central navigation preference measure).

2.3 Neuropsychological assessment

The aim of the current study was to assess the impact of APOE genotype independent of, and prior to, AD symptomology. The Addenbrooke's cognitive examination (ACE-III) was used to detect cognitive impairment associated with AD (Matias-Guiu et al., 2017). Only participants who scored in the normal range (ACE-III>88) were retained. The Rey–Osterrieth Complex Figure Test (RCFT; with 3-min delayed recall) and the Four Mountains test were used as secondary screening measures to assess any non-verbal episodic memory or spatial memory differences between genetic groups (Chan et al., 2016; Shin et al., 2006).

2.4 Subjective cognitive change assessment

Subjective cognitive decline was evaluated to identify decline in self-perceived episodic memory and executive function over the 5 years before testing. In prior work, subjective memory concerns have been identified in asymptomatic familial AD carriers, and concerns are seemly predictive of faster rates of memory decline (Samieri et al., 2014; Weston et al., 2018). The presence of subjective cognitive concerns is also related to abnormal changes in Aβ and tau biomarkers in APOE ε4 carriers (Risacher et al., 2015) and is thus considered important for early detection. Here, we measure this using the Cognitive Change Index (CCI; Rattanabannakit et al., 2016) that consists of 20 questions relating to the perceived decline. Responses are given on a five-point scale ranging from 1 = "normal ability" to 5 = "severe problem", with higher scores indicating larger concerns.

2.5 Functional MRI acquisition

Structural and functional MRI data for 40 participants (20 ε3ε3 carriers and 20 ε3ε4 carriers) was obtained using a 3 tesla Discovery 750w widebore system (GE Healthcare, Milwaukee, WI, USA) with a 12-channel phased-array head coil for signal reception. After localisers, T_1-weighted (T_1w) structural data was acquired using a whole-head 3D inversion-recovery fast spoiled gradient recalled echo (IR-FSPGR) sequence with the following parameters: repetition time = 7.7 ms; echo time = 3.1 ms; inversion time = 400 ms; field-of-view = 256 mm; acquired matrix = 256 × 256; 200 sagittal sections of 1 mm thickness ; flip angle = 11°; and an ASSET acceleration factor of 2 in the phase-encoding direction. Furthermore, a 3D T_2-weighted fluid attenuated inversion recovery (T_2w FLAIR) sequence was prescribed as follows: repetition time = 4,800 ms; echo time = 129 ms; inversion time =

1,462 ms; field-of-view = 256 mm; acquired matrix = 256 × 256; 182 sagittal sections of 1 mm thickness ; flip angle = 90°; an ARC acceleration factor of 2 in the phase-encoding direction; and a 'HyperSense' compressed sensing subsampling factor of 2.

Functional images were acquired using a gradient echo echo-planar imaging sequence with the following parameters: repetition time = 3,500 ms; echo time = 30 ms; field-of-view = 240 mm; acquired matrix = 96 × 96, reconstructed to 128 × 128; 42 axial slices of 3.5 mm thickness; flip angle = 80°; and an ASSET acceleration factor of 2 in the phase-encoding direction. The fMRI time series consisted of 200 images, and the total acquisition time was 11 minutes 54 seconds. During functional runs, subjects were required to not fall asleep and keep alert with their eyes closed for 10 min. To avoid the effect of participants employing specific strategies to maintain alertness (e.g. reminiscing or counting scan number), participants were instructed not to think about anything in particular. Prior to analyses, all participant scans were visually inspected for significant head movements and artefacts. Please see supplementary information (SI) for pre-processing structural and functional MR images.

2.6 Statistical analysis

Statistical analysis was performed using SPSS (v25.0), FSL (v6.0.0), MATLAB (MathWorks, R2018a), Octave (v4.4.1) and FreeSurfer for SI ROI morphometry analysis (v11.4.2).
An ANCOVA adjusted for age and sex was used to examine APOE differences on the neuropsychological assessment. Pearson's Chi square assessed differences on secondary characteristics between APOE groups including, marital status, educational attainment, occupation, and medically controlled cholesterol and blood pressure. All group comparisons on VST spatial performance and CCI were conducted using the same general linear model including APOE, as main predictor of interest, Despite recruiting age and sex matched groups, we included age and sex as covariates given their strong effect on brain function and volume, navigation performance and vulnerability to AD (Coutrot et al., 2018; Ferretti et al., 2018; Lester et al., 2017; Neu et al., 2017). We thus aimed to exclude any variance in the outcome measures that could be explained by these variables in order to determine an unbiased effect of APOE. Associations between VST and CCI measured were tested using partial Pearson correlation in SPSS adjusted for age and sex.

Voxel-based morphometry (VBM) was conducted on whole-brain T_1weighed scans, using the VBM toolbox in FSL to confirm no grey matter structural differences between the genetic groups (Douaud et al., 2007; Good et al., 2001). FreeSurfer was used to segment and parcellate whole-brain T_1-weighed images and generate volumetric measures for anatomical ROIs. FC between pairs of the six ROIs were analysed by extracting the first eigenvector from the BOLD timeseries for each ROI, and each single participant, using 'fslmeants'. If two brain regions show similarities in their BOLD timeseries, they are functionally connected (Haneef et al., 2014). A total of 195 of the 200 functional timepoints for each ROI were retained for analysis. All functional network modelling with timecourse

data was carried out using FSLNets v0.6 so that the functional connectivity results were family wise error (FWE) corrected for multiple comparisons. After computing the subject-specific $6^{nodes} \times 6^{nodes}$ connectivity matrix, direct and ridge regularised partial correlations were calculated between all pairs of ROIs, where direct correlations are correlations between two ROIs, controlling for the effect of all other ROI-ROI correlations. The resulting Pearson correlation coefficients were converted to z scores via Fisher's transformation to test the significance of any functional connectivity differences between the genetic groups (Smith et al., 2011). All functional analyses were carried out in MNI standard space. Significance testing for functional MRI differences was conducted using voxel-wise general linear modelling by employing the threshold-free cluster enhancement (TFCE) method (Smith and Nichols, 2009). The TFCE produces voxel-wise P-values via 5,000 permutation-based non-parametric testing (Nichols and Holmes, 2001). All scripts ran in the aforementioned software packages are available from authors.

3. Results

3.1 Neuropsychological assessment

As expected, no differences between the two genetic groups evident for on the neuropsychological assessment (Table 1) or on secondary characteristics (see SI Table 1), which confirmed that the impact of APOE genotype prior to clinically detectable MCI/AD symptomology could be measured.

3.2 Spatial navigation assessment

Heading direction (F=.799., P = .375, SI Figure 1B) and short-term spatial memory (F=.014, P = .907) were unaffected by genotype and thus, we concluded group differences on other VST sub-measures could not be accounted for by differences in short-term spatial memory ability. Egocentric orientation was significantly different between genetic groups, with ε3ε4 participants making fewer correct responses, compared with ε3ε3 participants (F = 4.21; P = .042). Central navigation preference was also significantly different between the groups (F = 12.45, P < 0.005), with ε3ε3 participants favouring more central responses and ε3ε4 carriers favouring more boundary responses (see Figure 2A-C; Table 2 for mean values).

3.3 Subjective cognitive change assessment

Next, we examined the significance of any differences on self-reported cognitive decline (within the last 5 years) between the genetic groups. ε3ε3 participants reported less decline on both episodic memory (F=5.24 p=.026) and executive function (F=5.92 P=.018; SI Figure. 1 D-E). Thus, we then

sought to test associations between navigation performance on the VST and CCI scores. Heading orientation, short-term spatial memory and central navigation preference were not significantly associated with CCI scores. Egocentric orientation was associated with self-reported decline in executive function (r=-.347, *p*=.008; i.e. better egocentric performance is related to less subjective decline).

3.4 Volumetric and/or functional connectivity

Having clarified the behavioural characteristics of ε4-related navigation impairment on the VST, we sought to investigate 1) the statistical significance of volumetric differences and/or functional connectivity changes between genetic groups and 2) if a neural correlate(s) for e4-related navigation impairment on the VST could be identified. No significant grey matter volumetric differences between the groups (p = 0.18) were present. As a secondary measure, we tested the mean ROI network volumes (right/left hippocampus, right/left EC, PCC, Precuneus; see Figure 3A). No difference was found between the genetic groups (see SI Table 2 for mean ROI volumetric values between groups).

Next, we examined FC between the ROIs to investigate potential differences in connectivity strength between the genetic groups. Full and partial correlations were tested in FSLNets to correct for multiple comparisons, meaning only effects withstanding familywise error correction were reported as significant. Right EC and PCC FC was significantly lower in ε3ε4s relative to ε3ε3s (t=-2.608; uncorrected p=.01; corrected p=.03; 95%CI [-.426 -.053]; rs = .171, F=6.80, p=098), even after multiple comparison correction (P_{FWE} = 0.027) at a partial level (i.e. not controlling for all other ROI-ROI correlations). When controlling for all other ROI-ROI correlations (i.e. direct), the effect of APOE on right EC and PCC FC was significant at the uncorrected (P =0.017), but not at the corrected level (P_{FWE} = 0.157). Trend differences in the opposite direction were observed between the precuneus and the PCC, with higher FC between these regions in ε3ε4s compared to ε3ε3s (t=-2.225; uncorrected p=.03; corrected p=.06; 95%CI=[.009 .214]; ; rs = .228; P=035; Figure 3B for group comparison connectivity matrix). Finally, to localise PCC connectivity differences in the EC, we used dual regression to test PCC connectivity in the whole brain which revealed reduced connectivity was localised to the dorsomedial subregion of the right EC (MNI [x y z] coordinates, [24 -6 -32], tfce corrected P<0.05). Please see SI for the independent ε3ε3 and ε3ε4 connectivity matrices (SI Figure 2).

3.5 Functional connectivity and ε4 sensitive navigation processes.

Having determined altered FC changes in the EC, PCC and precuneus between genetic groups, FC strength between each ROI pair was correlated the ε4-sensitive VST measures: egocentric orientation and central navigation preference. We expected that right-EC-PCC FC would correlate with at least one of the ε4-related behavioural characteristics. Right EC-PCC connectivity strength negatively correlated with central navigation preference (t=2.45, r=0.40, corrected P_{FWE} =0.018) when direct (but not partial) correlations were used as a connectivity metric (Figure 3C). No correlate in the pre-defined neural network for egocentric orientation was present. See SI for investigations on the functional neural correlates of the two additional VST measures.

3.6 ε4-related functional connectivity and subjective cognitive decline

Based on the ε4-related changes on self-reported cognitive decline, we then measured associations between EC-PCC FC with CCI scores. Connectivity strength between the right EC – PCC was negatively correlated with subjective decline in episodic memory (t=-3.01, r=-.407, uncorrected *P*=.005, corrected P_{FWE}=.017), but not with executive function (t = -2.02, r=-.341, uncorrected P=.052; Figure 3C).

3.7 Classifying genetic groups based on VST and functional connectivity

Although no neuro-functional correlate was identified for egocentric orientation or subjective executive function decline, the neuro-functional correlate of the two strongest e4 behavioural characteristics, central navigation preference and subjective episodic memory decline, overlapped. Thus, as a final step, we tested its clinical utility to classify at-genetic-risk AD. In the first instance, we did not include functional connectivity and subjective decline measures, as our primary aim was to test the diagnostic value of the VST for at-genetic-risk AD. Thus, the first logistic regression model entered aimed to classify ε3ε3 and ε3ε4 carriers based on central navigation preference and egocentric orientation measure. This model was statistically significant (X^2(2) 20.22, $P < .001$) and correctly classified 77.4% of the overall cohort (n=64). The percentage of classification was equal across ε4 carriers and non-carriers (Figure 4 A). We then included the right EC –PCC measure to weigh the utility of including a neuro-functional correlate to improve the classification. Note the sample size dropped to 37 with the inclusion of MRI measures. As expected, the regression model was statistically significant, x^2(3) 16.85, $P < .001$) and classification accuracy shifted from 77.4% to 85%. Specifically, the model correctly classified 82.3% of ε3ε3 carriers and 88.3% of the ε3ε4 carriers (Figure 4 A). The log odds units presented are the values for the logistic regression equation for predicting APOE status from the three independent variables. The prediction equation is:

$$\log\left(\frac{p}{1}-p\right) = 6.86 - 7.33 * central\ navigation\ preference\ - .28 * egocentric\ orientation - 1.47 * [right\ EC - PCC])$$

ROC curves were computed with these three predictors. Area under the curve (AUC) values indicated right EC-PCC connectivity (AUC .702, SE .092) and the egocentric task (AUC .659, SE .098) had a similar level of diagnostic accuracy. Central navigation preference showed the best accuracy of the three predictors (AUC .810, SE .073; Figure 4 B).

4. Discussion

APOE ε4 is the strongest genetic risk factor for late-onset AD. Yet, whether preclinical stage cognitive changes are detectable on a short clinically feasible task is unknown. Our results show that the classification accuracy of the path integration test, coupled with intrinsic FC strength between the EC - PCC reaches 85% providing a springboard for the development of a simple multimodal framework for at-genetic-risk AD. Extending the existing literature, we show that i) navigation discrepancies following path integration co-exist with subjective cognitive concern in adults at-genetic risk of AD and ii) reduced network connectivity between the right EC and the PCC correlate with navigation discrepancies and subjective episodic memory concerns which characterise the at-genetic-risk behavioural phenotype.

Significant differences between the APOE genetic groups were found in two out of four of the VST spatial sub-measures: Egocentric orientation and central navigation preference. Egocentric orientation requires participants to form an accurate representation of the supermarket environment during self-motion, and then integrate this representation at the finishing location to produce an accurate directional representation of the starting point. ε4 carriers demonstrated significantly more difficulty identifying their starting point, suggesting ε4-related problems integrating allocentric-egocentric frames. Although short-term forgetting could explain this effect, the ε4 group showed no impairments on the spatial memory control measures, compared to the non-carrier group (i.e. the VST short-term spatial memory task and the four mountains task), making a memory-based causation unlikely. The central navigation preference adopted for this study measures the number of allocentric location responses in the centre vs the boundary area of the virtual supermarket following path integration. This measure then provides a means of dissociating between central vs the boundary responses preferences (or biases). The most striking ε4 behavioural discrepancy appeared here, as ε4 carriers exhibited a strong response biases towards the boundary, compared to non-carriers.

Behaviourally, the ε4-related bias for reduced central navigation preferences is consistent with entorhinal-mediated navigation pattern changes *during* path integration observed on two other experimental navigation tasks (Kunz et al., 2015; please see Hardcastle et al, 2015 for discussion on

border-cell mediated error correction in response to dysfunctional grid-cell activity in the EC). This is the first time ε4-related border biases were found *following* path integration, however, and although no neural FC correlate emerged for ε4-related egocentric orientation deficit, reduced FC between the right EC - PCC emerged as a significant neural substrate for border preferences in the at-genetic-risk group. Right EC – PCC FC also predicted the degree of episodic memory decline, as reported on the CCI (see Contreras et al., 2017 for more information on the CCI).

To the best of our knowledge, this is the first study to show APOE ε4 carriers with self-assessed episodic memory and executive function decline also show navigation discrepancies, and that border navigation preferences and perceived episodic memory decline are mediated be the functional connectivity strength of neural pathways between the EC and PCC. Considering that subjective episodic memory decline is believed to be predictive of early Aβ accumulation (Contreras et al., 2017; Jessen et al., 2014; Mulder et al., 2010) and co-insides subtle navigation deficits as shown here, we conclude that subjective complaints may well contribute to a more sensitive and specific diagnosis of preclinical AD, although the relevance of subjective concerns for clinical practice is outside the boundaries of this study.

The role of reduced EC – PCC functional connectivity in preclinical may not be surprising, as typically intracellular tau projects from the EC and surrounding areas, to the PCC in the first stages of disease (Belloy et al., 2019; Hanseeuw et al., 2019; Jacobs et al., 2018), consistent with animal models that show in amyloid positive rodents, tau pathology propagation begins in the EC before spreading to the parietal cortex (Ahmed et al., 2014; Khan et al., 2013). This pattern of projection may explain the reduced functional connectivity in the at-genetic-risk group, and potentially the impeded translation of the allocentric or egocentric coordination system, given that the allocentric system relies on entorhinal-hippocampal axis and the egocentric system relies on parietal/PCC regions. In opposition of this theory, the egocentric orientation measure did not correlate with the FC strength between the EC and PCC or any other ROI-ROI correlates. It may be the that egocentric orientation changes are underpinned by functional changes between regions not examined, for example in prefrontal lobe areas where extra-cellular deposition of Aβ plaques are also found early in disease (Braak and Del Tredici, 2015). This is certainly possible, given the shared variance between egocentric orientation and frontal lobe-mediated executive function measured on the CCI, which was found here and elsewhere (Moffat et al., 2007). Finally, increased PCC-precuneus connectivity in the genetic-risk group was also found and may be understood in the context of animal models that demonstrate moderate levels of Aβ in the brain can enhance FC due to compensatory brain mechanisms, explaining why increased connectivity strength in intrinsic brain networks is commonly found in ε4 cohorts (Badhwar et al., 2017; Chase, 2014; Machulda et al., 2011).

Despite our results largely supporting and extending current theories of preclinical AD models, the study has limitations. Firstly, the sample size fell from sixty-four to thirty-seven when investigation the neural correlates of ε4-related navigation impairment which prevents generalization.

We also cannot rule out the possibility that boundary-driven navigation behavioural is caused be another neural mechanism and/or the fact that boundary landmarks, although intentionally hidden in the VST, may exert an influence toward to border in the ε4 group. Of course, longitudinally tracking these participants to confirm whether multimodal framework presented here is indeed predictive of future development of MCI or clinical AD is desired but will take up to a decade to achieve. We thus recommend replication of the results in biomarker positive individuals; using flortaucipir and Pittsburgh compound B positron emission tomography tracers to assess tau and Aβ pathology, respectively. Moreover, as we cannot say if navigation changes precede subjective cognitive deficits or vice versa, this ought to be followed up in future investigations. Future studies should also consider using a PCC-mediated memory consolidation task in a similar cohort (such as that presented in Bird et al., 2015), to examine if this process is compromised in preclinical AD. This will add further insight into whether the field should consider PCC-mediated behavioural discrepancies as a marker for preclinical AD, as the current focus is primarily on EC-mediated tasks.

In conclusion, we have shown a distinct association between navigational deficits and altered FC in three key nodes of the spatial navigation network. Our results provide important insight into the navigational discrepancies sustained by the presence of ε4 genotype and the underlying neurofunctional entities that appear to be consistent with the topographical spread of preclinical disease from the EC to the PCC. As recent clinical trials of disease-modifying agents in Alzheimer's disease have failed (Sevigny et al., 2016), the addition of simple multimodal diagnostic models for at-risk AD should facilitate earlier and targeted intervention to those 'at-risk'. This would allow neuroprotective compounds a higher opportunity of success, with intervention prior to macroscopic neuronal loss (Dubois et al., 2014; Reiman et al., 2015). Although further work is required to recommend VST as means of enrolling individuals in future clinical trials, the present study aims to stimulate the integration of navigational testing for consideration in upcoming preclinical AD-screening practices.

Data Availability

Link will be provided https://osf.io

Acknowledgements

We would like to thank Dr Sicong Tu who co-developed the virtual supermarket test. We also wish to extend a thank you to all participants who devoted their time to this research.

Author Contributions

G.C. and R.G conducted the experiments; G.C and P.Z analysed the data; M.H, G.C. and AM.M designed the experiments; G.C. wrote the paper, M.H, AM.M, V.P and P.Z contributed towards the finalisation of the text.

Funding

This research was funded by the Faculty of Medicine and Health Sciences, University of East Anglia and by the Biotechnology and Biological Sciences Research Council (BBSRC) UK.

Competing interests

None

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Figure Legends

Figure 1 | Spatial orientation was assessed using an ecological virtual supermarket environment. (A): The layout of the virtual environment did not include any notable landmarks. (B): An iPad 9.7 (Apple Inc., etc) was used to show participants 7-14-second video clips of a moving shopping trolley. All trials began at the same location in the supermarket but followed different routes to reach a different end point in each trial. Videos were presented from a first-person perspective and participants were taken to a set location while making a series of 90-degree turn. (C): Once the video clip stopped, (D):

participants indicate the real-life direction of their starting point. (E): Immediately following, participants indicate their finishing location (short-term spatial memory) and heading direction on a VST map. (F): Number of place responses made in the center space and boundary spaces were recorded.

Figure 2 | (A): The effect of genotype on short-term spatial memory, (B): egocentric orientation and (C) central navigation preference.

Figure 3 | (A): APOE-dependent correlations in functional connectivity between selected ROIs: *right/left hippocampus, right/left EC, PCC, Precuneus*. (B): The 6node × 6node network matrix of correlation coefficients represents connectivity strength between nodal pairs in a dual regression to test two-group subject difference on subject specific nodal pair connectivity. Right EC and PCC connectivity was significantly lower in the ε3ε4 group than in the ε3ε3 group. Trend differences in the opposite direction were observed between the precuneus and the PCC, with higher functional connectivity between these regions the ε3ε4 group than in the ε3ε3 group. (C): Significant association between the right entorhinal and posterior cingulate cortices connectivity and i) central navigation preference ii) cognitive change index – episodic memory but not iii) cognitive change index – executive function.

Figure 4 | Logistic regression and ROC curves for right EC– PCC functional connectivity strength (green line) and VST cognitive measures central preference (blue) and egocentric orientation (red) predicting variants of the APOE genotype. (A): Logistic regression indicated that the regression model based on function connectivity and VST cognitive predictors was statistically significant. (B): Area under the curve (AUC) values indicated EC-PCC and egocentric orientation had a similar level of diagnostic accuracy, while central preference had the best accuracy of the three predictors.

Table 1 **Primary demographic and neuropsychological profile**

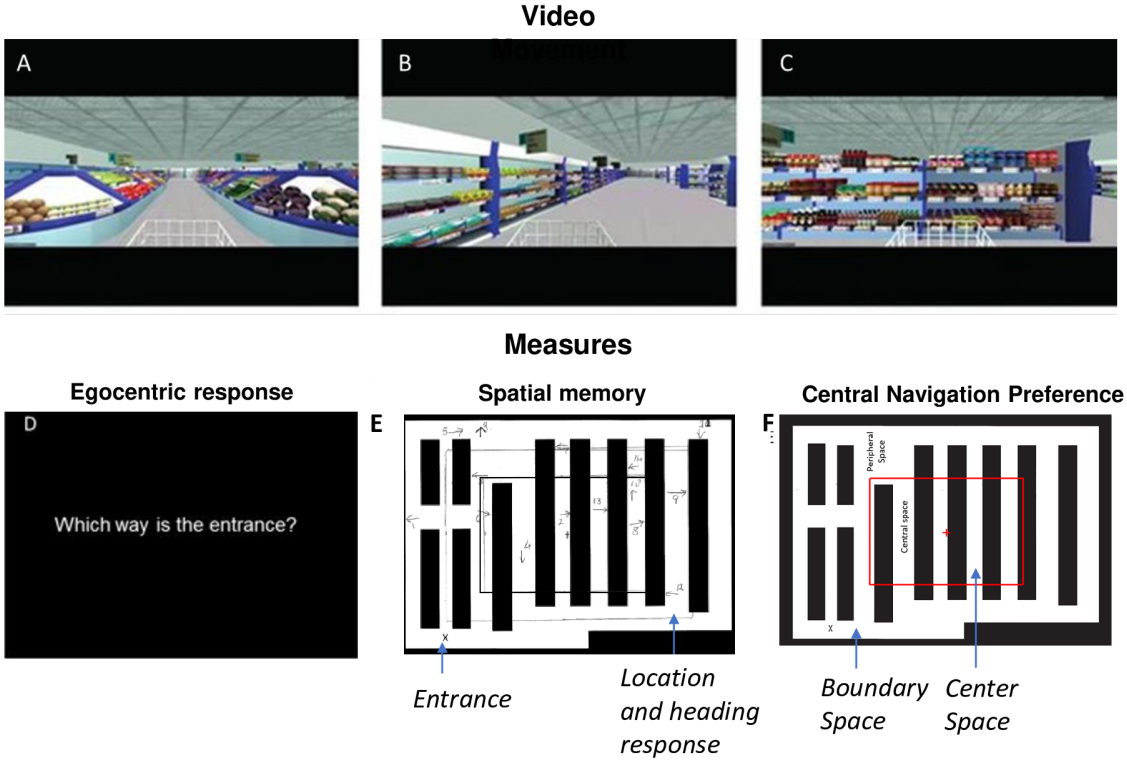
	ε3ε3 carrier (n=32)	*ε3ε4 carrier (n=32)*	*P value*
Age (years)			
Mean (SD)	62.24 (5.32)	62.19 (5.58)	-
Sex			
Male	17	22	-
Female	15	10	-
ACE	94.47 (3.83)	92.88 (3.78)	.12 (F=2.49)
FMT	10.22 (2.91)	9.47 (1.23)	.49 (F=.484)
ROCT			
Recall	22.92 (2.77)	17.66 (4.95)	.06 (F=2.061)
Copy	33.77 (6.36)	32.12 (2.67)	.57 (F=1.287)

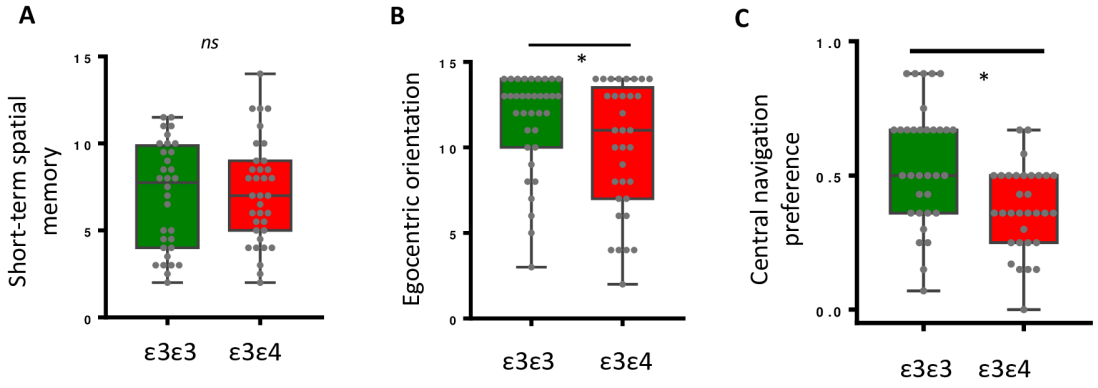
Primary demographic and neuropsychological characteristics of the genetic groups (Independent sample t-test, two-tailed). ACE= Addenbrooke's cognitive examination. FMT = Four mountains test. ROCT = The Rey–Osterrieth complex figure. Recall administered three minutes after copy.

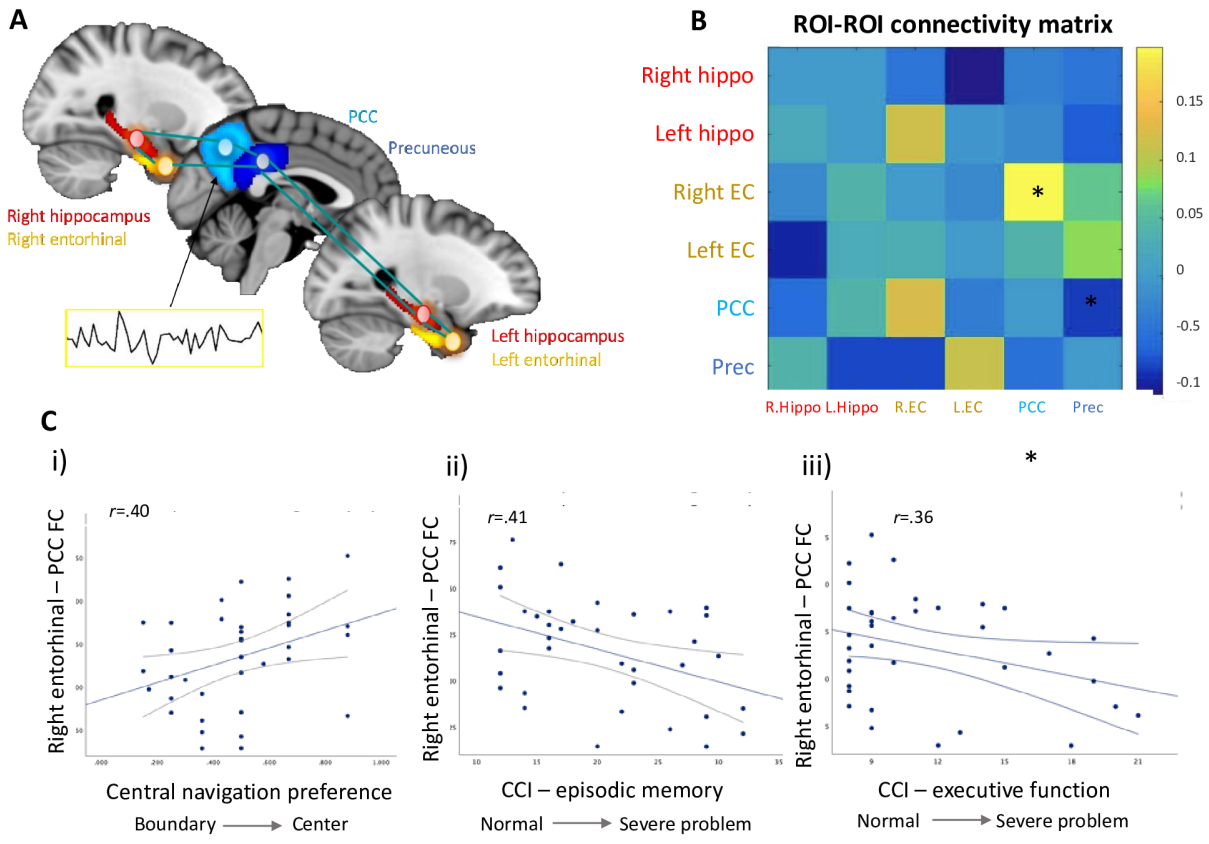
Table 2 **Effect of genotype on the VST spatial orientation paradigm**

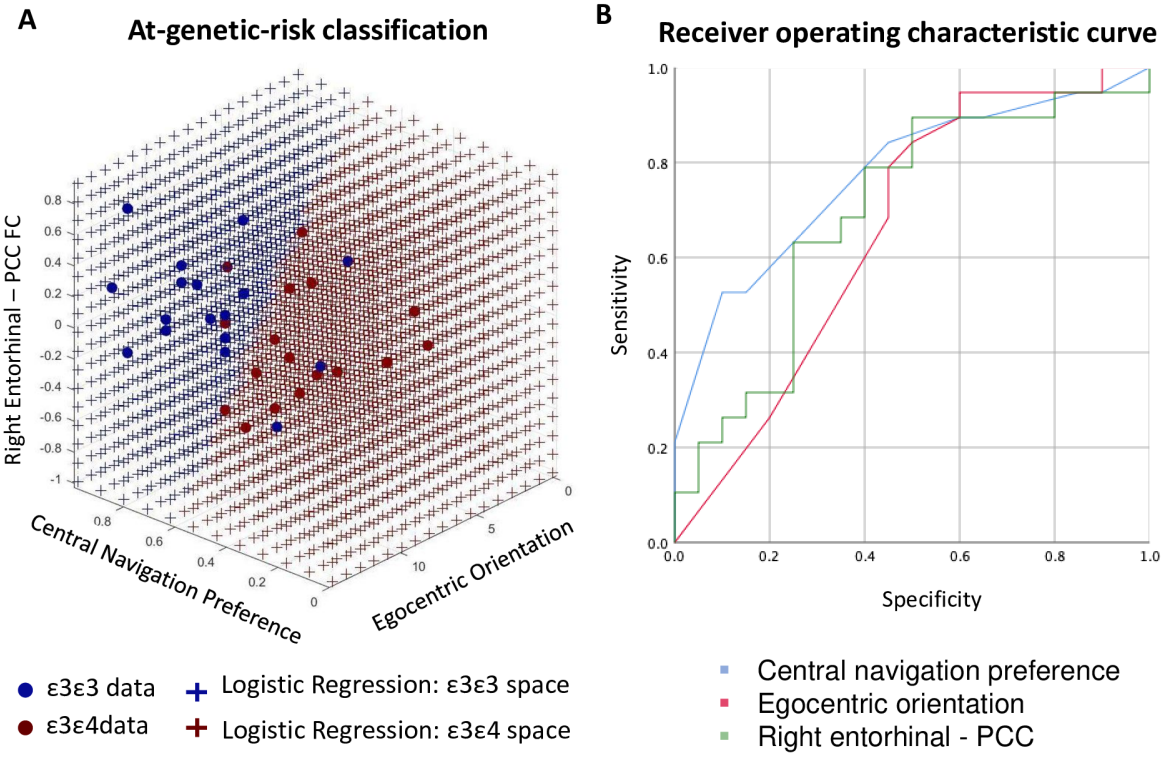
	Mean (SD)	*F*	*P value*
Egocentric orientation			
ε3ε3	12.01 (2.3)	4.21	.042
ε3ε4	10.94 (3.7)		
Heading direction			
ε3ε3	11.92 (2.7)	.799	.375
ε3ε4	11.31 (3.0)		
Spatial memory			
ε3ε3	7.43 (2.7)	.014	.907
ε3ε4	7.34 (3.0)		
Central vs boundary preference			
ε3ε3	.57 (.21)	12.45	< 0.005
ε3ε4	.38 (.14)		

ANCOVA with age and sex as covariates testing the difference of egocentric orientation, heading orientation, short-term spatial memory and central navigation preference.


Video
Movement
A
B
C
Measures
Egocentric response
Spatial memory
Central Navigation Preference
D
Which way is the entrance?
E
F
Peripheral Space
Central space
Entrance
Location and heading response
Boundary Space
Center Space


A
ns
Short-term spatial memory
15
10
5
0
ε3ε3
ε3ε4
B
*
Egocentric orientation
15
10
5
0
ε3ε3
ε3ε4
C
*
Central navigation preference
1.0
0.5
0.0
ε3ε3
ε3ε4


A
PCC
Precuneous
Right hippocampus
Right entorhinal
Left hippocampus
Left entorhinal
B
ROI-ROI connectivity matrix
Right hippo
Left hippo
Right EC
Left EC
PCC
Prec
R.Hippo L.Hippo R.EC L.EC PCC Prec
0.15
0.1
0.05
0
-0.5
-0.1
C
i)
r=.40
Right entorhinal – PCC FC
Central navigation preference
Boundary → Center
ii)
r=.41
Right entorhinal – PCC FC
CCI – episodic memory
Normal → Severe problem
iii)
*
r=.36
Right entorhinal – PCC FC
CCI – executive function
Normal → Severe problem


A
At-genetic-risk classification
Right Entorhinal – PCC FC
Central Navigation Preference
Egocentric Orientation
ε3ε3 data
ε3ε4data
Logistic Regression: ε3ε3 space
Logistic Regression: ε3ε4 space
B
Receiver operating characteristic curve
Sensitivity
Specificity
Central navigation preference
Egocentric orientation
Right entorhinal - PCC

Highlights

- Navigation deficits differentiate high-risk and low-risk AD groups
- Navigation deficits onset approximately the same time as subjective concerns
- Connectivity between the EC and the PCC underpins the phenotype of the high-risk group

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Gillian Coughlan, Rachel Gillings and Michael Hornberger: Data curation, Writing- Original draft preparation.
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Michael Hornberger: Supervision.
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Gillian Coughlan, Peter Zhukovsky, Vaisahk Puthusseryppady, Anne-Marie Minihane, Michael Hornberger: Writing- Reviewing and Editing,