Behavioural and neural characteristics of navigation impairments in preclinical Alzheimer's disease

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Abstract

Detection of incipient Alzheimer disease (AD) pathophysiology is critical to identify preclinical individuals and target potentially disease-modifying therapies towards them. Cognitive fingerprints for incipient AD are virtually non-existent as diagnostics and outcomes measures are still focused on episodic memory deficits as the gold standard for AD, despite their low sensitivity and specificity for identifying at-risk preclinical individuals. This thesis focuses on spatial navigation deficits, which are increasingly shown to be present in at-risk individuals, because the navigation system in the brain overlaps substantially with the regions affected by AD in both animal models and humans. Experimental chapters 2 and 3, show that a novel test battery captures navigation deficits that precede the onset of verbal and non-verbal episodic memory deficits in preclinical disease and that resting-state functional connectivity between the EC and the PCC underpins such deficits. Evidence for moderate test re-test reliability in the same non-clinical sample is presented in chapter 4. Moving beyond detection of preclinical disease, and towards prevention, in chapter 5 we examined whether marine fish oils help preserve the volume of AD vulnerable brain regions and found that low circulating DHA blood concentration predicts preservation of hippocampal and entorhinal volume in preclinical AD. This is potentially due to increased DHA uptake from the blood to the brain due to preclinical disease. Taken together, the research advances our conceptual understanding of the pathological and compensatory changes that characterise preclinical AD and offers important information toward generating more accurate risk profiles for AD vulnerable adults.

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Supervisor Signature

I, Michael Hornberger (primary supervisor), confirm that any required taught courses have been satisfactorily completed:

_____

Author's declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Parts of this work have been presented at conferences and published in academic journals

Ethical approval

Ethical approval for the work in this thesis has been granted by the Faculty of Medicine and Health Sciences Ethics Committee at the University of East Anglia.

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Oral Presentations arising from this thesis

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Understanding the Clinical Application of Sea Hero Quest. Sea Hero Quest Conference 2018, University College London, Division of Psychology and Language Sciences. Organised by Prof Hugo Spiers. 23 Oct 2018 13:10 – 13:35 UK

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Maps, monsters and mazes in the fight against dementia. Cambridge Science Festival, Wolfson Lecture Theatre, University of Cambridge, Organised by ARUK, Seminar Series on Making Sense of the Brain. 12 March 2018 18:00 – 18:40 UK

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Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid Beta
ACE	Addenbrooke's cognitive examination
APOE	Apolipoprotein E
BBB	Blood brain barrier
CANN	Cognitive Ageing, Nutrition and Neurogenesis
CCI	Cognitive change index
CN	Cognitively normal
CNP	Central navigation preference
CSF	Cerebrospinal fluid
DHA	Docosahexaenoic acid
EC	Entorhinal cortex
FWE	Family-wise Error
FC	Functional connectivity
FSL	fMRIB Software Library
GLM	Generalised linear model
HC	Hippocampus
ICC	Intraclass correlation coefficient
JDR	Join Dementia Research
LPC	Lysophosphatidylcholine
MRI	Magnetic Resonance Imaging
fMRI	Functional Magnetic Resonance Imaging
MCI	Mild cognitive impairment
MTL	Medial temporal lobes
PCC	Posterior cingulate cortex
RCT	Randomised control trails
ROC	Receiver Operating Characteristic
ROCF	Rey-Ossterrich Complex Figure test
ROI	Region of interest
RSC	Retrosplenial cortex
RCT	Randomised control trail

SHQ	Sea Hero Quest
SCC	Subjective cognitive concern
VBM	Voxel-based Morphometry
VST	Virtual supermarket test
4MT	Four mountains test
ω -3 PUFAs	Omega-3 polyunsaturated fatty acids

Chapter 1: General Introduction

Spatial navigation deficits — overlooked cognitive marker for preclinical Alzheimer disease?

Published Paper

Introduction

Alzheimer disease (AD) is the most common form of dementia, with increasing worldwide prevalence(Alzheimer's Association, 2015; Blennow et al., 2006). Accurate early diagnosis is crucial as it provides the chance to intervene at an early stage before substantial neuronal death occurs. This approach is particularly relevant in an era in which research is focused on the efficacy of upcoming pharmacological (Habchi et al., 2016; Sevigny et al., 2016; Yang et al., 2008) and non-pharmacological prevention and treatment strategies (Vauzour et al., 2017), which might allow intervention when neuronal loss is at its minimum to stop or delay the progress of the pathophysiology.

Current 'gold standard' clinical diagnostic and outcome measures for AD are strongly focused on episodic memory (Dubois et al., 2014). Episodic memory loss is one of the most common features of AD and is considered the most sensitive and specific cognitive marker of underlying AD pathophysiology (Rajah et al., 2017). However, it is becoming increasingly clear that decline in memory is so common in healthy ageing that early detection of incipient AD pathophysiology is difficult (Bellassen et al., 2012), which in turn often delays diagnosis as clinicians schedule follow-up appointments in an attempt to confirm a progressive decline in memory performance. The situation is further complicated by the fact that other brain diseases, (Birrer and Vemuri, 2004; Bronnick et al., 2011; Pennington et al., 2011) such as frontotemporal dementia (FTD), can manifest with substantial memory deficits, despite having a different underlying pathology (Flanagan et al., 2016).

Given this limited specificity of episodic memory deficits for incipient underlying AD pathophysiology, a new approach is required. Emerging data reveals that spatial navigation and orientation deficits have higher specificity than episodic memory in distinguishing AD from other dementias, particularly FTD (Tu et al., 2015; Yew et al., 2013). More specifically, in animal studies AD pathophysiology has been shown to affect navigation-specific brain areas before episodic memory areas are affected (Fu et al., 2017). Further, healthy older adults do not experience topographical disorientation in well-known environments, which contrasts starkly with the spatial disorientation seen in early AD (Lithfous et al., 2013; Serino et al., 2015). Finally, analysis of spatial performance allows better translation of animal intervention studies to human clinical trials as conceptualization of episodic memory is difficult to apply to nonhuman species (Templer and Hampton, 2013). Despite these highly promising findings, the utility of such spatial navigation deficits for diagnosis in preclinical individuals with a high genetic risk of AD or with mild cognitive impairment (MCI) remains underexplored (Allison et al., 2016; Kunz et al., 2015b). The Review chapter appraises the available evidence for spatial navigation deficits in preclinical, prodromal and confirmed AD, as well as identifying research gaps and future research priorities.

Alzheimer's disease diagnosis and criteria

Diagnostic criteria

According to the National Institute on Aging (2011), AD diagnostic criteria include a history of worsening amnestic and nonamnestic symptoms in the visuospatial, language and executive function domains that reflect the amyloid-β (Aβ) burden and neurodegeneration in the brain (Jack, Jr et al., 2011). Non-cognitive diagnostic methods include plasma biomarkers (Schindler et al., 2019), cerebrospinal fluid (CSF) biomarkers and PET amyloid imaging, which increase confidence in the clinical diagnosis and predict AD progression from prodromal stages (Medina and Avila, 2014). CSF biomarkers include amyloid-β1– 42, total tau and phosphorylated tau. Thresholds for biomarker positivity are set at approximately amyloid 5550 pg/ml, tau 4375 pg/ml. (Mulder et al., 2010). Aβ can also be measured directly in the brain using amyloid PET imaging scans (Klunk et al., 2004). Importantly, Aβ plaque deposition has been observed in post-mortem evaluations of individuals who were not judged to be symptomatic in their lifetime (Knopman et al., 2003; Nelson et al., 2012), highlighting the complexity of the underlying AD pathology and time lag to clinical manifestation. A

further complication is that non-cognitive biomarkers are not brain region-specific, and do not equate to clinical outcomes or have real-life symptom relevance for patients (Chételat et al., 2013; Morris et al., 2014). Magnetic resonance imaging (MRI) can be used to measure volumetric loss (atrophy), is brain region specific, and may be linked to specific cognitive function (Sperling et al., 2011). Nevertheless, the role of cognitive evaluations is important in the early diagnostic process and has great potential to complement established biomarkers. Given that the amnestic syndrome continues to appear as a 'core' criterion to support diagnosis of the most typical form of AD, we discuss the issues surrounding the use of memory tests in clinical settings below (for atypical AD see elsewhere (Galton et al., 2000)).

Neuropathology over the lifetime

At a biological level, AD clinical symptoms are associated with the accumulation of extracellular Aβ plaques and intracellular tau tangles, leading to neuronal apoptosis. The extracellular deposition of Aβ plaques usually occurs first in the basal temporal neocortex but becomes widespread over the cortex even in healthy ageing. By contrast, the intraneuronal neurofibrillary tangles of tau protein show a highly specific spreading pattern through the brain in AD (Braak and Del Tredici, 2015). Typically, tangles first develop in the most superficial cellular layer of the transentorhinal cortex (Braak & Del Tredici Stage I) advancing to the entorhinal cortex and Ammon's horn in the hippocampus (Stage II), later spreading to the amygdala, the anterodorsal thalamic nucleus and the rest of the hippocampal formation (Stage III). Finally, tau tangles continue to spread to neighbouring regions within the cerebral cortex causing neocortical atrophy[23] (Stages IV–VI). The interaction of Aβ and tau leads to progressive neuronal loss, which in turn is believed to underlie AD symptomology, such as forgetfulness, disorientation and confusion (Galton et al., 2000). The reason for the pronounced directional expansion of the tau pathological process in typical AD as well as the tau– Aβ interplay is still unknown.

Limitations of episodic memory for Alzheimer's disease diagnosis

Given that patients with AD present with profound symptoms of forgetfulness and substantial Aβ load in the medial temporal lobe (MTL), it is not surprising that episodic memory is currently the gold standard for diagnosing probable AD (Dubois et al., 2014). Indeed, patients with substantial memory problems are highly likely to have underlying AD pathology. However, the reliance on episodic memory deficits for diagnosis in the prodromal or even

preclinical stages is problematic, because episodic memory peaks in very early adulthood (Hartshorne and Germine, 2015) and progressively declines with normal ageing. Indeed, diagnosis can be challenging in people >75 years of age, in whom memory and associated MTL structures show considerable age-related changes not due to typical AD pathophysiological processes (Park et al., 2003). As a result, cognitive decline in healthy older people >75 years might have been considerably underestimated by longitudinal studies (Brayne et al., 1999). Moreover, delayed recall ability, one of the current main cognitive diagnostic indicators of AD, progressively declines in healthy adults aged ≥65 years (Brailean et al., 2016), highlighting the potential difficulties with the sensitivity of episodic memory to diagnose and predict AD pathophysiology in an older population. Similarly, as mentioned above, it is becoming increasingly recognised that patients with other forms of dementia can also show significant episodic memory problems. For example, FTD subtypes — such as the behavioural variant of FTD (bvFTD) — often manifest with similar deficits of episodic memory as AD, even for pathologically confirmed cases of bvFTD (Bertoux et al., 2014; Hornberger et al., 2010; Hornberger and Piguet, 2012; Pennington et al., 2011), which can make the differential diagnosis difficult (Bellassen et al., 2012; Flanagan et al., 2016; Wong et al., 2014; Yew et al., 2013). Differential diagnosis is important when determining the underlying pathology and choosing the correct treatment strategy to manage symptoms. An early and differential diagnosis is particularly problematic for patients with AD who exhibit neuropsychiatric symptoms, who can be very difficult to clinically distinguish from patients with bvFTD (Wong et al., 2014).

Unlike episodic memory impairments, spatial navigation or orientation problems are rarely reported in healthy older adults (Cerman et al., 2017) or non-AD dementias, and experimental studies have shown that spatial navigational paradigms that are independent of mnemonic process can differentiate patients with AD from individuals with other dementias and healthy control groups (Tu et al., 2017, 2015). This finding is not surprising given that the neuropathology of AD starts in the entorhinal areas, which are crucial for successful navigation (Fu et al., 2017; Fuhs and Touretzky, 2006; Hafting et al., 2005; Killian and Buffalo, 2018; McNaughton et al., 2006). This evidence raises the question as to whether such symptoms might be more sensitive and specific to underlying AD pathophysiology, even at a preclinical (that is, pre-memory symptom onset) stage. In the following sections, we briefly introduce current knowledge of navigation strategies and their neural correlates before reviewing the evidence in normal ageing and AD.

Spatial navigation

Navigation strategies

Spatial navigation is the process of determining and maintaining a trajectory between different points in our environment. Successful navigation relies on two co-dependent strategies: allocentric and egocentric navigation. These strategies use different types of spatial reference frames but are highly correlated (Boccia et al., 2014) (Figure 1.1).

Egocentric strategies are generally used when the same route is followed over and over again (Hartley et al., 2003; Wolbers et al., 2004). These self-centred navigation frames encode spatial information from the viewpoint of the person navigating to form an internal representation that is based on a sequence of bodily movements. This sequence of movements allows the navigator to maintain their route-goal trajectory relatively free of conscious control. Perceptual processing is required, as available visual input, bodily distance from landmarks, sensorimotor and vestibular knowledge about position in space and self-motion are all utilised as navigational cues. The temporal order in which environmental stimuli are encountered is important and facilitates the landmark-based behavioural responses that are stored in spatial memory (for example, turn left at a supermarket and right at the lights).

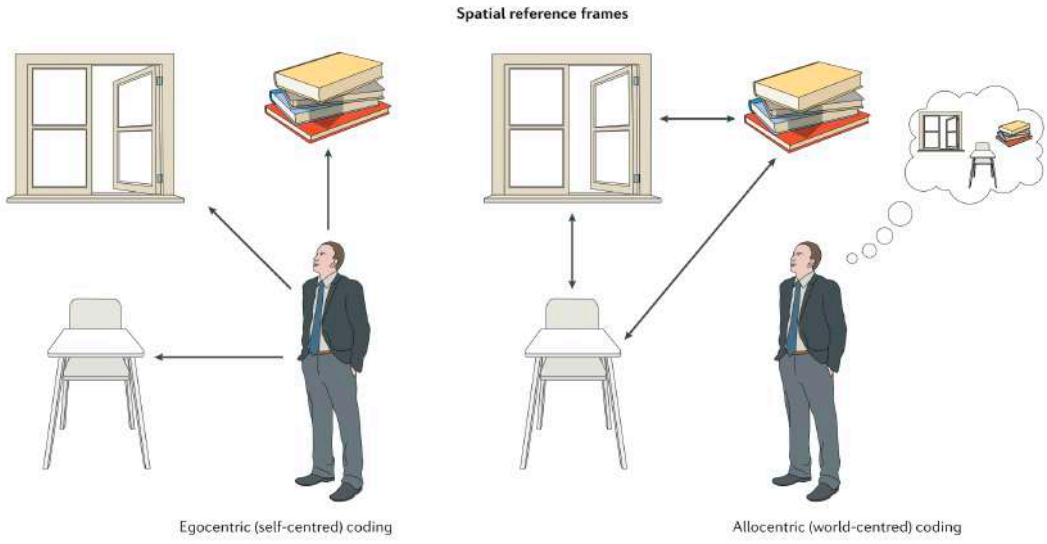


Figure 1.1 Egocentric and allocentric spatial coding. Egocentric self- centred navigation frames encode spatial information from the viewpoint of the navigator and are usually implemented when travelling a familiar route (left). Allocentric strategies are based on the navigator's perception of landmark positions relative to other landmarks and are usually implemented in a novel environment (right)

On the other hand, when traveling a lesser known or novel route, spatial representations of sequential bodily movements are not available, and allocentric, world-centred strategies are employed instead. Allocentric strategies are based on the navigator's perception of landmark positions relative to other landmarks. These positions are memorised and estimated by the navigator, contributing to an internal representation or 'cognitive map' that enables an individual to plan shorter routes regardless of their starting point (Hartley et al., 2003; O'Keefe, John & Nadel, 1978). Allocentric representations of self-location are updated by self-motion on the basis of visual, auditory, vestibular and proprioceptive information (Loomis et al., 1998, 1993), in a process known as path integration. This process has a pivotal role in an individual's ability to successfully maintain movement through the environment (McNaughton et al., 2006; Spiers and Barry, 2015).

The ability to use environmental landmarks to navigate also relies on the translation of egocentric to allocentric information (for example 'I am 20 meters from the church' to 'the supermarket is to the left of the church') and vice versa. For example, when one's location in an environment has been determined, the navigation system calculates subsequent routes on the basis of a combination of egocentric and allocentric information. For instance, self-motion, distance travelled, head direction during the journey (Byrne et al., 2007), and temporal order of observed stimuli are combined across navigation frames. This strategic translation between allocentric and egocentric reference frames is a core determinant of one's navigational ability and might be of particular importance for detecting very early signs of disorientation by clinical examination.

Thus, egocentric and allocentric navigation strategies integrate for optimal performance in daily functioning and are associated with a network of brain regions that operate conjointly but can also be dissociated from each other (Chiu et al., 2012). Indeed, successful navigation can be achieved by employing just one of these navigation processes at a time. For example, employing only egocentric navigation, it is possible to go from one landmark to another without knowing the relationship between landmarks (allocentric information), as the overall path might be stored in a series of visual snapshots or scene memories (Gaffan, 1994; King et al., 2004). Similarly, egocentric navigation is also not required for allocentric navigation. When walking from one's house to the garden, the ability to measure bodily distance from landmarks (egocentric strategy) might not be necessary if a cognitive representation of the

spatial trajectory already exists. Such a dissociation is often employed in experimental navigation tests by asking participants to remember locations on the basis of direction information while background cues are rotated or removed (Feigenbaum and Morris, 2004; King et al., 2004; Parslow et al., 2004). Evidence also suggests inter-individual differences for navigation preference, such that individuals preferentially choose specific strategies or reference frames when attempting to solve spatial tasks (Chiu et al., 2012). Outside the experimental paradigm, however, the human navigation system encourages the natural interaction (or strategic translation) of egocentric and allocentric strategies and, therefore, it is important to identify translational impairment in the clinical setting.

Neural correlates of spatial navigation

Advances in the field have shown that a large network of brain regions, involving MTL regions (hippocampus, entorhinal cortex and parahippocampal cortex (Ekstrom et al., 2003)), parietal lobe regions (posterior cingulate, precuneus (Maguire et al., 1998) and retrosplenial cortex (RSC) (Auger et al., 2012; Auger and Maguire, 2013)), frontal lobe regions (Moffat et al., 2007), and subcortical structures (caudate nucleus (Hartley et al., 2003) and thalamus (Aggleton et al., 2016; Aggleton and Nelson, 2015)) underlie our ability to navigate (Figure 1.2). Electrophysiological recordings in freely moving rodents offered the original insights into spatially tuned neurons that independently code for various aspects of navigation such as place location, head direction, speed and environmental boundaries. Likewise, in humans these sophisticated cells together form the neural architecture that underlies the navigation system.

Allocentric navigation strategies are thought to be represented by highly selective cell ensembles commonly found in the hippocampal CA1 and CA3 regions of the MTL. These so-called ‘place cells’ contribute to the formation of cognitive maps of the environment, providing local information about one’s location within that environment. Both rodent and human models show that place cells become stable and more spatially restricted with repeated exposure to an environment (that is, as one becomes more familiar with the surrounding area). On the other hand, large-scale spatial information is provided by grid cells located primarily in the medial entorhinal cortex, which can encode grid-like representations of distinct positions in space (self-location) and calculate routes between locations (Doeller et al., 2010; Fuhs and Touretzky, 2006; Hafting et al., 2005). Grid cells represent a core

component of the neural system that underlies path integration, as they also seem to measure 'distance travelled' akin to an odometer (Burgess et al., 2007; Doeller et al., 2010).

In addition, head direction cells (which were first identified in the postsubiculum of the rat) encode orientation in space and are activated whenever one is facing a certain direction (the reference direction (Taube et al., 1990) . Since their first discovery in rats, these cells have been found in the posterior parietal cortex, RSC, dorsal presubiculum, postsubiculum and anterior thalamus in humans (Muller et al., 1996; Shine et al., 2016). Boundary vector cells (Lever et al., 2010) and cells coding specifically for self-motion (path integration) (Fuhs and Touretzky, 2006; Mahmood et al., 2009; Spiers and Barry, 2015), complement other spatial representations and together might be used to rapidly form goal-independent maps of the environment.

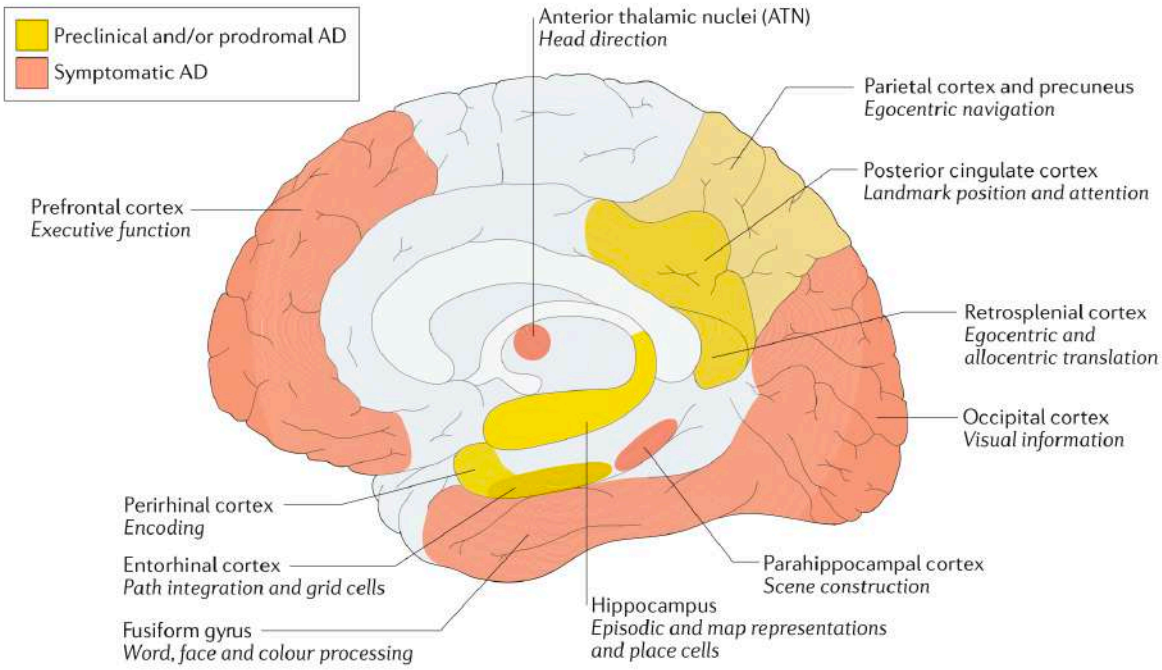


Figure 1.2 Anatomical illustration of AD- related neuropathological changes. Yellow areas are brain areas affected by Alzheimer disease (AD) pathophysiology in preclinical and prodromal stages of the disease; red areas are brain areas affected in symptomatic stages of the disease. Figure reproduced from Vann et al 2015, *Macmillan Publishers Limited.*

Previous work suggests that the posterior cingulate region, RSC and precuneus have major roles in the integration of egocentric and allocentric spatial information streams (Alexander and Nitz, 2015). For example, the rodent posterior cingulate receives dense direct

hippocampal connections from the subiculum and is thus considered an integrative hub for projections from the hippocampus and anterior thalamic nuclei (Czajkowski et al., 2014). Interactions between the egocentric parietal and allocentric MTL systems are mediated by the RSC (Bird et al., 2015; Byrne et al., 2007; Dhindsa et al., 2014; Vass and Epstein, 2013) as it projects to the parahippocampal gyrus and other areas including the entorhinal cortex, presubiculum, thalamus and posterior parietal cortex (Clark et al., 2012; Knight and Hayman, 2014). Moreover, the medial prefrontal cortex has been shown to receive information from the posterior parietal cortex and the hippocampus and may be involved in upstream processing of the spatial information generated (Chersi and Pezzulo, 2012; Sheynikhovich et al., 2009).

Functional imaging studies in rodents and humans have shown that the RSC is a major contributor to navigational performance, especially accurate path integration in darkness (Elduayen and Save, 2014), recognising permanent environmental objects (Auger et al., 2012), binding together multiple cues within the environment (Alexander and Nitz, 2015), and encoding and storing spatial information (Czajkowski et al., 2014). In healthy individuals, the RSC responds selectively to environmental objects with high permanence such as telephone booths or street lights (Auger et al., 2012; Mullally and Maguire, 2011). However, self-reported 'poor navigators' show reduced retrosplenial cortex activation in response to high-permanence landmarks and have more difficulty estimating object permanency compared with self-reported 'good navigators' (Auger and Maguire, 2013). Moreover, the functional connectivity between the RSC and anterior thalamus nuclei is reduced in poor navigators (Auger and Maguire, 2013), consistent with the literature from rodent studies (Aggleton and Nelson, 2015). The ability to recognise permanent objects in the environment is a general skill required to make appropriate decisions related to navigation, thus emphasising the important role, within the wider posterior cingulate region, of the RSC in spatial navigation. Understanding spatial navigation performance in healthy individuals is a crucial starting point before inferences can be drawn about initial sites of functional abnormality in patients with AD or preclinical individuals.

Ageing and spatial navigation

The majority of research on spatial navigation in normal ageing supports a general consensus that human and rodent navigational ability, especially allocentric processing, (Iaria et al.,

2009; Moffat et al., 2002) declines with age (Gazova et al., 2013; Lithfous et al., 2013; Moffat, 2009). Reduced resting-state blood flow (Heo et al., 2010), synaptic dysfunction (Bach et al., 1999), and decreased hippocampal volumes in humans (Driscoll et al., 2009) are some of the mechanisms that underpin this gradual decline. Along with these age-related neural changes, deficits in place learning (Moffat et al., 2002), perception of self-motion, (Lalonde-Parsi and Lamontagne, 2015) and retrieval of spatial memories (Holden and Gilbert, 2012) have been reported in humans. Based on rodent studies, such navigational errors are believed to be a consequence of computational changes within neural circuits of the medial prefrontal cortex and CA1 and CA3 regions of the hippocampus (Lester et al., 2017). These age-related changes give rise to deficits in spatial working memory, as well as difficulties maintaining and retrieving allocentric representations (Carpenter et al., 2016; Lester et al., 2017). Interestingly, reduced allocentric processing, mostly related to spatial memory (that is, encoding and retrieval of route trajectories and environmental maps) is suggested to lead to a compensatory shift toward egocentric or path integration navigational processes, as they do not rely on memory per se (Gazova et al., 2013; Rodgers, K. M., Sindone J. A., 2012). This idea is consistent with the finding that older adults between 60-80 years of age actually outperform younger adults on egocentric (Zheng Bian and George J. Andersen, 2013) and allocentric distance tasks for example, manually adjusting the length of a line until its length matches the distance of a target (Norman et al., 2015). The preservation of these spatial processes in healthy ageing might therefore have important implications for the effective discrimination of age-related and AD-related decline in navigational ability (Gazova et al., 2013), and also has implications for navigational rehabilitation strategies for AD in the future.

Spatial navigation as a diagnostic tool

Early identification — based on navigational difficulties — of individuals who are likely to develop AD is currently complicated by the challenges of measuring different features of spatial navigation in humans. Reliable tests of spatial navigation that are suitable for clinical settings across centres and different patient populations are still in development, as most experimental tests are not feasible for clinical evaluation. Validated, simple visuospatial tests such as the widely used Mental Rotation Test (Vandenberg, S. G. & Kuse, 1978) and the Money Road Map test (Money, J. 1965) have been shown to be poor predictors of

navigational abilities (Mitolo et al., 2015; Schinazi et al., 2013) and cognitive decline (Mapstone et al., 2003). Newly developed virtual reality or real-world tests of spatial cognition have proven more sensitive in identifying spatial navigation deficits in patient populations. In particular, virtual reality testing can be applied as an alternative to real-world reality tests (that are difficult to administer with space constraints in clinical settings) to measure navigational abilities in younger and older age groups (Cushman and Duffy, 2008), patients with MCI and early AD (Cogné et al., 2017). These computer-generated virtual environments provide tightly controlled testing conditions and also enable manipulation of navigational parameters, such as landmark availability and navigation complexity. The adoption of tablet computers by clinical services for cognitive testing will make the testing of spatial navigation deficits more sophisticated and sensitive in everyday clinical practice. Furthermore, extraction of critical features from these virtual reality tests might enable development of further pencil & paper or bedside assessments.

Early Alzheimer's disease

Previous studies using virtual reality techniques have shown that spatial disorientation in patients with AD typically includes both egocentric and allocentric impairments linked to widespread neurodegeneration in medial temporal, parietal and frontal brain regions (Irish et al., 2015; Jheng and Pai, 2009; Pengas et al., 2012; Serino et al., 2015; Serino and Riva, 2013) (Figure 1.2). In accordance with these findings, both types of navigational strategy have been found to be impaired in early AD dementia, alongside impairments in the translation of both reference frames (Serino and Riva, 2013) and the ability to construct novel scenes from spatial and contextual information, which is dependent on posterior parietal regions such as the supramarginal and angular gyrus (Irish et al., 2015). Virtual reality studies found that patients with early AD were unable to store an allocentric viewpoint-independent representation and to synchronize this representation with the allocentric viewpoint dependent representation (e.g., memorize the position of the plant and retrieve the plant's position from a different location) (Serino et al., 2015) probably as a result of reduced hippocampal neuronal density particularly in CA1 and CA3 subregions (Padurariu et al., 2012). Egocentric impairments are also present, mainly as a result of hypometabolism and structural medial parietal changes, which are signature features of AD (Weniger et al., 2011). Surprisingly, these medial parietal changes and associated egocentric impairments have been much less investigated in AD, despite having potentially much higher specificity for AD pathology. Indeed, retrosplenial (Brodmann areas 29 and 30) volumetric changes have been

shown to efficiently distinguish AD from FTD, even in patients with similar hippocampal atrophy (Tan et al., 2013; Tu et al., 2015).

As noted above, patients with AD also experience difficulty translating between allocentric and egocentric reference frames, a function that strongly correlates with RSC and posterior cingulate dysfunction and has been shown to distinguish AD from FTD (Serino and Riva, 2013; Tu et al., 2015) . Given the role of the RSC in integrating different navigational frames, orientation and visual information from the occipital lobes, its dysfunction in early AD is in agreement with deficits in translation between allocentric and egocentric representations that occur at early clinical stages of the disease and are highly specific to underlying AD pathophysiology (Morganti, F., Stefanini, S., & Riva, 2013). For this reason, the RSC is often considered an initial site of functional abnormality in patients with AD or in preclinical individuals.

Prodromal Alzheimer's disease and mild cognitive impairment

Similar to early AD, patients with MCI often show spatial navigation impairments (Hort et al., 2007). Although the exact trajectory from MCI to AD is still under discussion (Jack et al., 2016), a large number of studies show functional and structural changes in the MTL and parietal cortex in patients with MCI (DeIpolyi et al., 2007; Dubois and Albert, 2004; Julkunen et al., 2009; Laczó et al., 2014; Lithfous et al., 2013; Weniger et al., 2011). Investigations of egocentric and allocentric memory in individuals with amnestic MCI (aMCI) indicate that these patients have substantial volume reductions in the hippocampus, right-sided precuneus and inferior parietal cortex, and are severely impaired at learning both allocentric and egocentric tasks (Weniger et al., 2011). Indeed, path integration in spatial navigation tests is substantially impaired among prodromal cohorts and might represent a cognitive marker for AD (Mokrisova et al., 2016). Furthermore, a study employing a human real-life version of the Morris water maze found that patients with AD had problems navigating and using both allocentric and egocentric orientation; aMCI groups were more severely impaired on allocentric trials (Hort et al., 2007), probably due to the stronger emphasis on memory in these trials (Laczó et al., 2009).

Interestingly, genetic vulnerability interacts with aMCI to influence spatial navigation performance. The apolipoprotein E ε4 allele (*APOE ε4*), which is a known genetic risk factor for AD, has high prevalence but moderate penetrance in the population, with a threefold increased risk of developing AD in *APOE ε3*/*ε4* heterozygotes and a tenfold increased risk in

APOE ε4/*ε4* homozygotes compared with *APOE ε4* non-carriers (see genetics section later, and for more details see elsewhere (Genin et al., 2011)). On a computerised human analogue of the Morris water maze test (Hidden Goal Task), aMCI *APOE ε4* homozygous carriers are poorer on all spatial navigation subtasks, including allocentric (hippocampus-dependent) and egocentric subtasks, compared with aMCI *APOE ε4* heterozygous carriers (Laczó et al., 2014).

Despite the strong focus on MTL contributions to navigational deficits in AD, findings suggest an increasingly important role for the posterior cingulate cortex (PCC) and RSC; these areas are affected early in the course of AD (Scahill et al., 2002) and in patients at early MCI stages (Fennema-Notestine et al., 2009; Pengas et al., 2010), especially those who then progress to AD (Hämäläinen et al., 2007; Julkunen et al., 2009; Pengas et al., 2010; Whitwell et al., 2008). However, questions around the contributions of the RSC and associated posterior cingulate areas to spatial navigation deficits in early AD remain unanswered. In addition, whether changes in the PCC reflect a compensatory mechanism that occurs as a result of early AD pathology in the transentorhinal cortex and hippocampus at the microscopic level remains unclear. Such a finding would not be surprising, however, as the entorhinal cortex has a strong anatomical connection with the RSC and PCC via the hippocampal bundle for example. (van Groen and Michael Wyss, 1990)

Preclinical Alzheimer's disease

Although evidence is emerging that spatial navigation is impaired in early AD and MCI, its integrity in preclinical populations is less well understood. Various attempts have been made to determine spatial neural and cognitive biomarkers in individuals as young as 45 years who have an elevated sporadic or genetic risk of progressing to AD (Tan et al., 2014). Preclinical investigations have reported functional MRI abnormalities in the resting-state default mode network, including reduced functional connectivity in PCC and precuneus regions (Patel et al., 2013; Pihlajamaki et al., 2010), both of which underlie egocentric ability and egocentric to allocentric translation.

Studies in sporadic preclinical AD have partially relied on CSF Aβ levels. For example, a study from 2016 (Allison et al., 2016) investigated spatial navigation as a marker for AD, using two non-immersive desktop virtual maze environments for allocentric and egocentric conditions. Individuals were considered preclinical if they had low CSF Aβ levels (<500

pg/ml) (Skoog et al., 2003), with no cognitive deficits. Selective deficits in allocentric strategy among preclinical individuals were reported relative to individuals with a normal Aβ level in the CSF (>500 pg/ml). Despite allocentric acquisition impairments, the preclinical group retained sufficient information to solve the wayfinding task. However, the exact contribution of decreased CSF Aβ levels to impairment of allocentric and egocentric processing is uncertain, as increasing evidence suggests that the tau protein has a critical role in the generation of cognitive deficits (Johnson et al., 2016; Villemagne et al., 2015)[9]. Experiments in aged transgenic mice expressing human tau suggest that the interaction of reduced excitatory grid cell firing in the dorsal medial entorhinal cortex and increased activity of inhibitory cells in response to enhanced theta oscillations results in the spatial memory deficits seen in early AD (Fu et al., 2017). This finding links the destabilization of grid cell fields (which code for route trajectories and update spatial information) with the earliest stage of tau pathology. The significance of this finding in relation to preclinical AD, however, remains to be determined (see Figure 1.3)

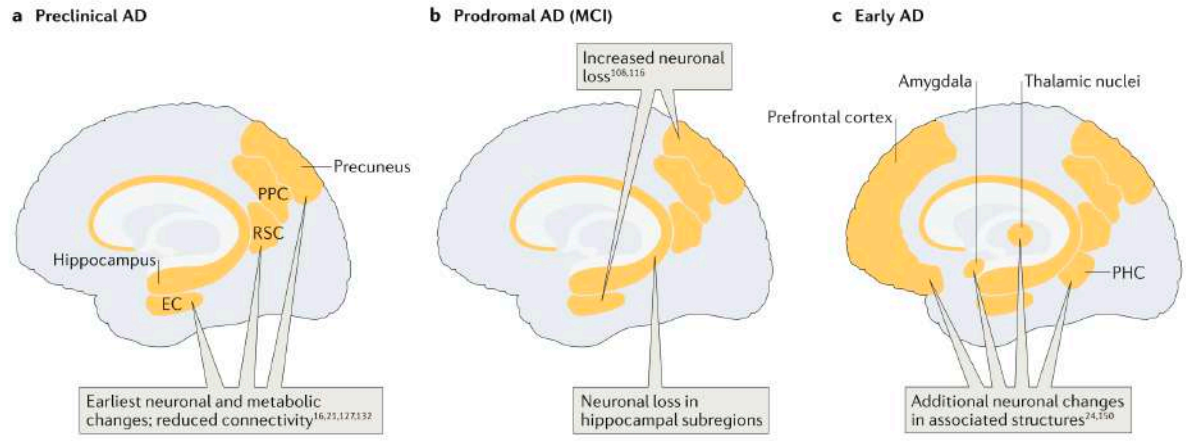


Figure 1.3. The progressive pathophysiological changes that underlie navigational impairment in AD. a | Grid-cell dysfunction leads to an altered navigation pattern at the preclinical stage. Cortical thinning in the egocentric- mediated precuneus and retrosplenial cortex (RSC) is also evident in the preclinical stage. b | Early volumetric decline in the medial temporal lobe (MTL) and parietal lobes gives rise to select allocentric and egocentric disturbances in prodromal Alzheimer disease (AD). c | In early AD, neurodegeneration continues to progress throughout the MTL and frontal lobe regions, rendering the neural navigation system severely impaired at this stage. EC, entorhinal cortex; MCI, mild cognitive impairment; PHC; parahippocampal cortex; PPC, posterior parietal cortex.

In human studies, preclinical individuals carrying mutations in the presenilin 1 and amyloid precursor protein genes show entorhinal and posterior cingulate cortical thickness changes up

to 8 years before disease onset (Weston et al., 2016). However, to our knowledge, spatial navigational ability has not been investigated in these particular preclinical patients. These predictive MRI findings underline the strong need for longitudinal investigations to examine the sensitivity of cortical thickness changes as neural markers for AD and the manifestation of spatial navigation disparities for predicting later conversion to MCI and early AD.

Most navigation studies in cohorts of patients with genetic risk factors for AD have been conducted with *APOE*-genotyped individuals. The association between the APOE ε4 allele and AD risk has spurred a growing number of studies investigating the cognitive and neurophysiological effect of APOE ε4 in younger 18-24 year olds (Bunce et al., 2014; Yasen et al., 2015) middle-aged 40-60 year olds (Evans et al., 2014; Greenwood et al., 2015; Parasuraman et al., 2002; Salvato et al., 2016) and elderly 60-90 year old individuals (Berteau-Pavy et al., 2007) adults, also in relation to spatial performance and hippocampal volume (Laczó et al., 2014).

A study examined a possible link between APOE ε4 and spatial navigation in genetically at-risk young healthy adults. Reduced grid-cell-like representation was observed in APOE ε3ε4 carriers compared with APOE ε3ε3 individuals, suggesting functional (but no structural) differences between young APOE ε4 carriers and non-carriers. Grid-cell representations were temporally unstable in young adult carriers as functional connectivity between the right entorhinal cortex and hippocampus was impaired, leading to a behavioural preference to navigate along the border of the virtual environment. The authors proposed a potential compensatory mechanism of the hippocampus due to neuronal loss in the entorhinal cortex and reduced grid-cell representations which enabled young adult carriers to navigate successfully and complete the task (Bott et al., 2016; Kunz et al., 2015b). Clearly, preclinical cohorts are of great interest for future navigation research in AD pathophysiology, not only for individuals at genetic risk, but also for sporadic high-risk groups.

An alternative approach is to investigate preclinical cognitive forms of AD via healthy elderly participants who have significant cognitive concerns (SCCs) but do not reach cut-offs for objective memory impairment on standard neuropsychological measures. Such SMCs are a potential harbinger of AD pathology (Risacher et al., 2015a). However, it should be emphasised that no gold standard tool currently exists to identify SCCs, and no threshold values to suggest clinically relevant SCCs have been established. Unsurprisingly, few studies

have explored spatial navigation in SCC cohorts (Hort et al., 2007). However, evidence does suggest a positive association between amyloid pathology, genetic risk of AD, entorhinal cortex integrity and SCCs (Amariglio RE, Townsend MK, Grodstein F, Sperling RA, 2011; Amariglio et al., 2012), which rationalises future investigations to examine of SMCs and navigational difficulties are comorbid among genetically at-risk cohorts.

Intervention opportunities

Many early opportunities arise as a result of early detection. However, most people currently living with dementia have not received a formal diagnosis. Even in high income countries, only 20-50% of dementia cases are recognised and documented in primary care. This 'treatment gap' means that there are many potential lifestyle and pharmacological interventions that could be tested in clinical trials if diagnosis is made early in the disease course (Prince et al., 2011) before substantial neural apoptosis. Thus, individuals are far more likely to retain cognitive ability for longer despite having early AD pathology. Research shows that dietary factors such as docosahexaenoic acid (DHA), a form of polyunsaturated fatty acid (PUFA) found in marine fish oils, promotes neurogenesis in the hippocampus during early AD (He et al., 2009). Given that DHA is an accessible resource, it may indeed be an important lifestyle factor to consider for at-risk or genetically vulnerable individuals.

Docosahexaenoic acid as a neuroprotective mechanism

It is becoming increasing clear that neuroinflammation and synaptic dysfunction play a significant role in AD pathogenesis (Heneka et al., 2015; Shankar et al., 2008; Shankar and Walsh, 2009). Neuroinflammatory processes have been linked to plaque formation, APOE4 genotype, synaptic dysfunction and excessive accumulation of tau phosphorylation (Calsolaro and Edison, 2016; Guo et al., 2004; Streit et al., 2004). Intriguingly, ω-3 polyunsaturated fatty acids (PUFAs) or marine oils are associated with decreased neuroinflammation, alongside greater synaptic plasticity and the preservation of functional neuronal membranes that are fundamental to the conservation of healthy cognitive function (Janssen and Taviani 2014, 2014; Vauzour et al., 2017). ω-3 PUFA DHA supplementation over 3 years reportedly reduces amyloid burden in the cortex and increases hippocampal, retrosplenial and prefrontal volumes in preclinical AD transgenic mice (Cutuli et al., 2016). Similarly, a DHA-enriched diet significantly elevates levels of human drebrin, a dendritic

spine protein that plays a role in synaptic plasticity (Iturria-Medina et al., 2016). Major prospective/cross-sectional epidemiological human studies also show that consumption of fish and LCn-3 PUFAs is robustly associated with lower mortality and decreased brain atrophy in an older population (Gu et al., 2015; Y. Zhang et al., 2018).

Human randomised control trials (RCT) suggest that DHA supplementation over a relatively short period (6 months) can improve cognition including memory performance, but many such trials with human subjects have failed to support the beneficial effects of ω-3 PUFAs supplementation on cognitive functioning (Otaegui-Arrazola et al., 2014; for possible limitations). Mazereeuw and collegues (2012) did report an effect of DHA supplementation on attention and processing speed in prodromal patients but not in healthy or demented elderly (Mazereeuw et al., 2012). Clinical studies also report reduced levels of ω-3 PUFAs, namely DHA, in the brains, plasma/serum, and erythrocyte membranes of AD patients (Cunnane et al., 2012; Tully et al., 2003). This provides evidence to support the theory that DHA has neuroprotective qualities and thus, may play an important role in preventing the devastating neurodegenerative effect of AD pathophysiology. Currently, no investigations on DHA blood levels, brain volume and spatial cognition in humans. Given that disease staging may influence the associations between DHA, brain volume and cognition, the modulative effect of APOE on the relationship between DHA and neurocognitive health should be investigated in preclinical and prodromal AD populations.

Conclusions

This review underscores the presence of spatial navigation impairments in early AD and its prodromal and preclinical forms. The evidence reviewed clearly highlights the great potential of spatial navigation and orientation deficits as diagnostic measures and predictors of incipient AD pathophysiology. The findings presented in this review should not be surprising as MTL and posterior parietal regions, which constitute the core network for navigation, are highly susceptible to AD pathophysiology even in the preclinical stages of the disease. An urgent need exists to revisit the notion that episodic memory should be the gold standard for early AD diagnosis and outcome intervention studies. Specifically, the literature indicates that spatial navigation deficits can identify individuals at risk of developing AD, which has obvious

implications for clinical practice and thus, will be the focus of the aforementioned experimental chapters.

Despite clear clinical applications of the research, spatial navigation has several limitations as a diagnostic tool for early AD. One major question is whether spatial deficits occur before episodic memory deficits or whether both deficits manifest concurrently in humans, which we will test in the experimental chapters of this thesis. Navigation and orientation tests can only be considered superior to episodic memory tests if they are shown to be more sensitive and specific for AD pathology and thus, we will examine both memory and spatial navigation in preclinical groups. The aims of the aforementioned work are to help develop a standardized and validated diagnostic spatial test battery that does not rely on topographical memory. If sensitive tests are identified, they might also be used as a clinical diagnostic tool and outcome marker in upcoming treatment efficacy trials, as current navigation tools are limited and not standardized across research centers.

Importantly, spatial navigation studies in AD might be limited in their comparability, as many of the studies predate the publication of robust diagnostic criteria for AD (Dubois et al., 2014). As a result, the potential for mixed or other forms of dementia to confound an established cohort of patients with AD cannot be ruled out. It is also difficult to say with certainty that cut-off points for disease staging (preclinical, early MCI, late MCI) are consistent across studies published before the 2014 guidelines (Dubois et al., 2014). In addition, heterogeneity in the definition of patient cohorts and differences in spatial navigation paradigms and testing procedures have created inconsistencies across studies. Moreover, the current lack of epidemiological data from healthy populations for spatial navigation is a further obstacle. Inter-individual differences in spatial navigation remain elusive, with no population-level data available to rectify conflicting ideas around, for example, sex differences in navigational abilities. One notable exception is the launch of Sea Hero Quest (http://www.seaheroquest.com), an online mobile game to measure spatial navigation. To date (April 2018), Sea Hero Quest has been played by over 4.5 million people, in 193 countries between the ages of 19 and 95 years (Coutrot et al., 2018). Initial results from Sea Hero Quest show that not only age but also gender and cultural background have a substantial effect on navigation behaviour, which clearly needs to be investigated further (Cothi, 2017). There is considerable scope to use the data from the game to create the first population benchmarks for healthy navigation abilities across ages, gender and countries.

Benchmark scores will allow us to develop easy-to-administer, sensitive spatial navigation tools validated against benchmark population data and also to relate it to real-life navigation problems that patients encounter. Taken together, the presented evidence highlights the enormous potential of spatial navigation for AD diagnosis, which in turn could have a major impact on clinicians, patients and their families.

Aims and objectives

The evidence reviewed above clearly suggests that spatial navigation/orientation deficits have great potential as diagnostic and treatment outcome measures for underlying AD pathophysiology. This is not surprising as regions of the medial temporal and parietal lobes, both highly relevant for navigational abilities, are susceptible to AD pathophysiology even in the prodromal stages of the disease. Although spatial orientation and navigation abilities are impaired in individuals with AD and in those genetically at risk to develop AD (Kunz et al., 2016), most spatial assessment tools are highly experimental and have not been translated into diagnostic measures in a clinical setting.

The aims of the aforementioned experimental chapters of this thesis include:

- to understand inter-individual navigation ability and demographic factors that influence navigation performance using population level navigation data from the SHQ game (see Chapter 2)
- investigate the cognitive phenotype of preclinical AD using a novel test battery and its neural correlates using structural and functional neuroimaging techniques (see Chapter 2 and 3)
- investigate the test-re-test reliability of a novel test battery for preclinical at-risk AD over two timepoints (see Chapter 4)
- examine whether dietary factors, namely DHA, predict better neurocognitive outcomes in preclinical and prodromal AD (see Chapter 5)

A consolidation and discussion of the overall findings will be offered in the general discussion, the closing chapter (see Chapter 6). The novel test battery specifically includes Sea Hero Quest, the Virtual Supermarket Test and the Cognitive Change index, which will be

discussed in detail in proceeding chapters two and three. Each experimental chapter will include a set of specific hypotheses. The overarching hypotheses of the research thesis are:

- At-risk or 'preclinical' individuals otherwise not cognitively impaired will display entorhinal-mediated navigation difficulties or changes in navigation strategy
- Navigation changes will be associated with the functional integrity of the MTL/IPL
- A significant positive association between DHA concentration and hippocampal/entorhinal brain volume, influenced by APOE status in preclinical and prodromal AD.

Chapter 2: Towards personalised cognitive diagnostics of at-risk Alzheimer's disease'

Published Paper

Introduction

Spatial navigation is a promising cognitive fingerprint for underlying Alzheimer's disease pathophysiology (Allison et al., 2016; Coughlan et al., 2018b; DiBattista, Amanda M., Nicolette M. Heinsinger, 2016; Kunz et al., 2015b; Lithfous et al., 2013; Serino et al., 2015; Serino and Riva, 2013; Tu et al., 2017) and has been adopted by many high profile clinical trials (such as the European Prevention of Alzheimer's Dementia Consortium) to improve the sensitivity of neurocognitive testing and assess the efficacy of potentially disease-modifying treatments. Recent evidence suggests that abnormal spatial navigation patterns may be present before episodic memory deficits, which are the current gold standard for AD diagnosis (Coughlan et al., 2018b; Dubois et al., 2014; Tu et al., 2015).

A major challenge before using spatial navigation test for early detection however, is to understand how inter-individual and demographic factors affect spatial navigation in order to identify earliest pathological spatial navigation changes in AD (Doody et al., 2014; Ferretti et al., 2018; Husain, 2017; Sevigny et al., 2016). Understanding diversifying factors that influence variability in spatial ability in the *healthy* population and individuals at risk to develop AD will advance the diagnostic power of the spatial tests and support more personalised diagnostics and treatment approaches (Chan et al., 2018; Ferretti et al., 2018; Nelson et al., 2012; Pettigrew et al., 2013; Snyder et al., 2016). Among factors underlying navigation, age is a well-documented predictor of declining spatial abilities, as older adults show a strong bias toward egocentric rather than allocentric strategies (Hartley et al., 2013; O'Keefe, John & Nadel, 1978) leading to suboptimal navigation performance (Lester et al., 2017). Age-related decline in allocentric process are due to changes in coding patterns of place, grid, border and head direction cells that underpin our ability to form cognitive maps of the environment and integrating environmental and self-motion cues to optimise

navigational performance (Byrne et al., 2007; Epstein et al., 2017; Spiers and Barry, 2015). However, decline in other cognitive domains such as general planning and cognitive control abilities (Hartshorne and Germine, 2015) also contribute to spatial deficits in old age, suggesting that like most diagnostic tests, age-range normative cut-off scores are required (Hartshorne and Germine, 2015; Malek-Ahmadi et al., 2015). Similarly, sex differences in navigation behaviour and underlying neuroanatomy have generated arguments for sex-specific clinicopathological AD phenotypes (Driscoll et al., 2005; Ferretti et al., 2018; Kong et al., 2017; Mielke et al., 2014; Mosconi et al., 2017; Snyder et al., 2016). Rodent models of the Morris Water Maze have shown that male rats consistently outperform females (Perrot et al., 1996) and human studies display similar sex differences favouring males (Acevedo et al., 2010; Astur et al., 1998; Berteau-Pavy et al., 2007; Yasen et al., 2015) across 57 countries in both map-dependent allocentric and map-independent egocentric navigational strategies (Coutrot et al., 2018). Therefore, although spatial navigation tools must retain sensitivity and specificity to preclinical AD pathophysiology, it will be critical to develop diagnostic tools that can adjust for underlying sex differences.

Finally, one of the biggest challenges in preclinical AD studies is to identify those who are at-high-risk to develop symptomatic AD in the future. Genetic variation in the apolipoprotein E 4 allele carriers is currently the strongest known genetic risk factor for sporadic AD (Corder et al., 1993; Kunz et al., 2015b; Laczó et al., 2014; Reiman et al., 1996) and recent findings also show that temporal grid-cell like representation in the entorhinal cortex of apoE4 carriers are functionally unstable leading to a boundary-driven error correction during wayfinding (Hardcastle et al., 2015). Taken together, there is increasing evidence that spatial deficits related to wayfinding are present in preclinical AD long before episodic memory symptoms emerge. However, at this stage it is very difficult to employ such knowledge on a clinical level, due to unknown inter-individual variability in navigation behaviour across people, which is vital for sensitive and specific diagnostics on an individual level.

In the current study we address this issue by using Big Data (n=27,308) for navigation behaviour from the Sea Hero Quest App (Morgan, 2016) to: i) determine whether we can replicate previous wayfinding affects in APOE ε3ε4 carriers compared to the Big Data; ii) to further disentangle inter-individual the effects of genetic risk for AD from the effects of sex, age and baseline cognition on spatial discrepancies; and iii) to explore whether AD specific spatial navigation changes can be detected on an individual level, when using the Big Data as

benchmark comparison. We predicted that i) we would replicate previous APOE spatial navigation findings (Kunz et al., 2015b); ii) sex differences would make a significant impact on navigation behaviour; and iii) AD specific navigation changes can be detected in an individual level when using the normative benchmark Big Data of Sea Hero Quest.

Methods

Participants

APOE genotyped cohort.

Between Feb 2017 and June 2017, 150 people between 50 to 75 years of age were recruited to participate in a research study at the University of East Anglia. All 150 participants were pre-screened for a history of psychiatric or neurological disease, history of substance dependence disorder or any significant relevant comorbidity. All participants had normal or corrected-to-normal vision. Family history of AD and history of antidepressant treatment with serotonin reuptake inhibitor (SSRI) drugs was retrospectivity obtained. Saliva samples were collected from those who passed this screening and APOE genotype status was determined.

In total, 64 participants underwent cognitive testing. As just 23% of the population carry APOE ε3/ε4, all participants in our sample who tested positive for the ε3/ε4 genotype completed cognitive testing. We selected a subset of the ε3ε3 carriers that form the majority of the population (75%) to match the ε3ε4 risk group for age and sex (see SI Appendix, Table S1 for group background characteristics). We did not include a third genetic subgroup of homozygous APOE ε4 carriers from the tested cohort, because they were too rare (n=5). E2 carriers were also excluded. During testing, four participants showed signs of distress playing SHQ and their data was excluded from subsequent analyses. The final group sizes (post-exclusion) were: APOE ε3ε3, n=29 and APOE ε3ε4, n=31). 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.

The Benchmark Population.

A unique population level benchmark dataset was generated by extracting a subset of the global Sea Hero Database (Coutrot et al., 2018) that matched the demographic profile of our

lab-based genotype cohort, namely players from the UK aged 50-75 years old. Following extraction, 14,470 British men and 12,710 British women (N = 27,108) remained as a representative normative sample of heathy navigation performance on the basis that epidemiological studies have shown that the majority of the general population (~75%) are non ε4 carriers (Liu et al., 2013). Participants from the benchmark sample were given the option to opt in or opt out of the data collection when they played the game on their personal mobile phone, iPad or tablet. If a participants' response was to opt in, their SHQ data was anonymised and stored securely by the T-Systems' datacentre under the regulation of German data security law. Ethical approval was previously granted by Ethics Research Committee CPB/2013/015. *For more information on the global SHQ database see www.seaheroquest.com

Measures and Materials

Sea Hero Quest (SHQ)

The SHQ app was developed in 2015 by our team and funded by Deutsche Telekom and Alzheimer's Research UK. The app was created to be a reliable and valid measure of spatial navigation performance both in monitored research settings and unmonitored at-home settings (Morgan, 2016). It was made available for free on the App Store and Play Store from May 2016 and since then over 4 million people have downloaded the App worldwide. The game performance is divided into two main domains: goal-oriented wayfinding and path integration.

Goal-orientated wayfinding. In wayfinding levels, players initially see a map featuring a start location and several checkpoints to find in a set order, as illustrated in Figure 2.1. Checkpoints are buoys with flags marking the checkpoint number. Participants study a map of the level for a recorded number of seconds. When participants exit the map view, they are asked to immediately find the checkpoints (or goals) in the order indicated on the map under timed conditions. As participants navigate the boat through the level, they must keep track of their location using self-motion and environmental landscape cues such as water-land separation. The initiation time is zero as the boat accelerates immediately after the map disappears. If the participant takes more than a set time, an arrow appears pointing in the direction along the Euclidean line to the goal to aid navigation. To familiarize themselves

with the virtual environment and game controls, participants started with two easy learning levels 1 and 2. Wayfinding levels generate two measures of interest:

- *Wayfinding distance* travelled to visit all required checkpoints is defined as the Wayfinding distance between all points recorded and is a proxy for navigation efficiency. To navigate efficiently, individuals need to form and retain a cognitive map of the environment (after viewing the map at the start of the level) and then consistently update self-location in that cognitive map based on the visual cues from the SHQ game.
- *Wayfinding duration* is defined as the time in seconds to complete a wayfinding level. While inefficient navigation also results in longer time to visit all checkpoints, increased duration is primarily due to the amount of acceleration that the player used. By "swiping up", one can increase the speed of the boat temporarily, therefore reducing travel time but not changing the distance travelled at all. Since speeding up requires confidence in one's sense of direction, the resulting wayfinding duration score we take duration as less representative of participants' ability to navigate along the shortest path and more representative of non-navigational factors such as confidence or the tendency to sample more cues before speeding up.

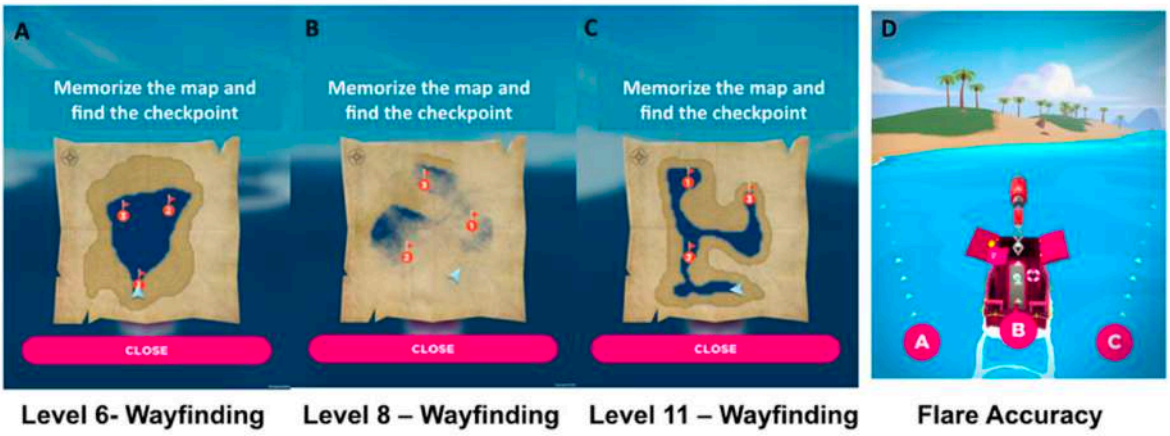


Figure 2.1 SHQ goal-oriented wayfinding levels 6 (A), 8 (B), and 11 (C). In flare accuracy levels (here, levels 9 and 14), participants are not provided with an allocentric map. Instead, they immediately navigated along a river to find a flare gun. Once they find the flare gun at the end of the river, the boat rotates by 180°, and participants are asked to choose one of three possible directions (right, front, and left) that they believe points to the starting point (D)

Flare Accuracy. In path integration levels (in the game this is measured by flare accuracy on levels 9 and 14), participants are not provided with an allocentric map. Instead, they immediately navigated along a river to find a flare gun. Once they find the flare gun at the end of the river, the boat rotates by 180°, and participants are asked to choose one of three possible directions (right, front, left) that they believe points to the starting point. This level requires participants to a) form an accurate representation of the starting point relative to their position and b) integrate this representation with a representation of the direction they are facing after the rotation. (see Tu and colleagues for a similar path integration based experimental design (Tu et al., 2015)). In this case, gaming proficiency was not advantageous because participants simply view navigate a single passage and are then required to choose A,B,C direction as a single response. Depending on their accuracy, players receive either one, two or three stars.

Procedure

Data collection

Spatial navigation data was collected for both the APOE genotyped cohort and benchmark datasets using Sea Hero Quest, a digital game that we pre-designed to measure human navigation ability. Decisions on level selection was made by considering which levels had the most normative data and level type/difficulty (wayfinding or path integration). Level 1 and 2 were included for learning and practice navigating the boat, as well as normalising the data for App interaction with player proficiency. Level 3-5 were excluded as they did not challenge participants' navigation skills and were intended to ease the players into the game. Further, starting with level 14, the sample size of the benchmark population drops substantially. This then left us with three wayfinding levels (6,8,11) and two path integration levels (9 and 14). Participants in the lab based APOE cohorts provided their demographic information during a screening call and were then invited to the UEA to play SHQ. Participants from the benchmark population provided information regarding their sex, age, location and educational attainment (high-school, college, university) demographics in-app before playing SHQ

APOE Genotyping

DNA was collected using a Darcon tip buccal swab (Fisher Scientific, Leicestershire, United Kingdom, LE11 5RG). Buccal swabs were refrigerated at 2-4°C until DNA was extracted

using the QAIGEN QIAamp DNA Mini Kit (QAIGEN, Manchester, United Kingdom, M15 6SH). DNA was quantified by analysing 2 µL aliquots of each extraction on a QUBIT 3.0 Fluorometer (Fisher Scientific, Leicestershire, United Kingdom, LE11 5RG). Successful DNA extractions were confirmed by the presence of a DNA concentration of 1.5µg or higher per 100µg AE buffer as indicated on the QUBIT reading. PCR amplification and plate read analysis was performed using Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Ashford, United Kingdom, TN23 4FD). TaqMan Genotyping Master Mix was mixed with two single nucleotide polymorphisms of APOE (rs429358 at codon 112 and rs7412 at codon 158). These two single nucleotide polymorphisms determine the genotype of APOE2, E3, and E4 (Applied Biosystems, 2007).

Statistical Analysis

The data was analysed using SPSS (Version 23), RStudio (Version 1.0.153) and MATLAB (R2017a). Chi square and simple two tailed t-tests were used to test the significance of any demographic or neuropsychological differences between the genetic groups in our lab cohort. When quantifying the group differences, Cohen's d was used as a measure of effect size. To control for the influence of player proficiency on digital devices, the SHQ data was pre-processed in MATLAB and participant performance on each level within the game was divided by the sum of the two practice levels:

$$level\ N\ normalised = \ln\left(\frac{level\ N}{(level\ 1 + level\ 2)}\right)$$

To assess the fixed effects of genotype and sex, we first compared competing statistical models with the inclusion and exclusion of different demographic factors using the nlme package in R (https://cran.r-project.org/web/packages/nlme/index.html) that allows fitting fixed and random effects to evaluate the most appropriate model for data. In each model, subject-level random effects were included to vary the intercept for each subject and importantly to account for interdependence between repeated measures from playing multiple levels of the game. Three sets of linear models were fitted that included the following outcome variables: a) wayfinding distance and b) wayfinding duration, using scores from SHQ levels 6, 8 and 11 completed by each subject and c) flare accuracy on each of the two path integration levels (9 and 14). Model selection was based on relative goodness of fit and model simplicity (determined using gold standard Bayesian information criterion, BIC)_

Age, sex and genotype, were retained as explanatory variables for the final model for each of the outcome variables. ACE defined by total score on the Addenbrooke's Cognitive Examination-III screening tool (Matias-Guiu et al., 2017), education, occupation, time spent on viewing the wayfinding maps (see Figure 2.1 for maps) and non-verbal episodic memory (defined by three minute delayed recall on Rey–Osterrieth Complex Figure Test; ROCF (Shin et al., 2006)), were tested in the final model but did not exhibit a significant main effect and were excluded to retain the maximum degrees of freedom. Once the best fit model was identified, standardised residuals were extracted and plotted against fitted values to examine underlining assumption of normal distribution and heteroscedasticity. We also tested for an interaction between genotype and sex. All statistical tests are two-tailed, $p<0.05$.

To ensure that the benchmark population reflected the demographic profile of our lab-based cohort, we could only use a sub-population of our global SHQ database. We developed a data extraction method using MATLAB that allowed us to generate the population level database. This data was then pre-processed using the same normalisation procedure as detailed above. Linear mixed models examined the effects of sex and age on a population level benchmark. Finally, logistic regression was used to quantify how well SHQ variables such as distance travelled could classify APOE status using both the lab-based sample and the benchmark population. ROC curves were used as measures of sensitivity and specificity of SHQ as opposed to standard memory tasks such as the ROCF test to detect preclinical AD.

Results

Background Characteristics and Neuropsychology

In the lab-based cohort, the ε3ε3 and ε3ε4 groups did not differ in terms of their demographic characteristics (see Appendices: Supplementary Table 2.1) or their neuropsychological examination (Table 2.1). We examined the relationship between the three SHQ outcome variables (see Figure 2.1): Wayfinding distance travelled and wayfinding duration correlate (Pearson $r = 0.61$, $p < 0.001$); duration and flare accuracy correlate ($r = -0.31$, $p < 0.001$); but wayfinding distance travelled and flare accuracy are not correlated ($r = 0.04$, $p = .795$); suggesting dissociable neural correlates that underlie performance, corroborating current notions that wayfinding distance relies more on grid-cell based navigational processes, and flare accuracy relies more on RSC mediated processes. We consider wayfinding distance as

the primary outcome measure (and the other outcomes are secondary) as early AD is characterised by abnormal changes in the grid cell code of the entorhinal cortex.

Table 2.1: Neuropsychological background for the ε3ε3 carrier group and the ε3ε4 carrier group from the lab-cohort

Measure	**Genotype**	**Mean**	***SD***	***p* value**
Addenbrooke's Cognitive Exam (n=60)	ε3ε3	94.9	3.44	p>0.05
	ε3ε4	92.7	3.77	
ACE Memory (n=60)	ε3ε3	24.9	1.86	p>0.05
	ε3ε4	23.9	1.69	
ACE Visuospatial Ability (n=60)	ε3ε4	15.0	1.36	p>0.05
	ε3ε4	14.7	1.48	
RCFT Immediate Recall (n=59)	ε3ε3	33.1	2.83	p>0.05
	ε3ε4	32.3	2.58	
RCTF 3-minute delay recall (n=59)	ε3ε3	20.8	6.59	*p*=0.10
	ε3ε4	18.5	5.39	

ACE* Addenbrooke's Cognitive Examination used as a measure of general cognitive ability.
RCFT* Rey Complex Figure Task. Recall task was administered three minutes following RCFT copy task

Genotype effects on wayfinding

There was a main effect of genotype (b=0.22; *p*=0.004; Figure 2.2 A) on wayfinding distance, with ε3ε3 carriers (M=3.79, SD=0.63) travelling a shorter distance during wayfinding relative to ε3ε4 carriers (M=4.45, SD=0.94) after controlling for age and sex. The mixed model for wayfinding duration (i.e. time taken to complete wayfinding levels) showed no main effect of genotype between ε3ε3 (M=4.66, SD=2.65) and ε3ε4 carriers (M=4.97, SD=1.36; Figure 2.2 B). See Table 2.2 for group mean values and Table 2.3 for the effects of genotype on wayfinding distance and duration. Please refer to Appendix for results including a small high-risk ε4/ε4 carrier group, which showed an even larger effect for distance travelled (Appendices: Supplementary Figure 2.1).

Table 2.2. Mean Sea Hero Quest performance for the ε3ε3 carrier group, the ε3ε4 carrier group and for the benchmark

Performance Variable	ε3ε3 carriers	ε3ε4 carriers	Benchmark players
n	29	31	27108
Mean Wayfinding Distance	3.791 (0.638)	4.455 (0.946)	3.918 (1.536)
Mean Wayfinding Duration	4.661 (2.652)	4.973 (1.361)	4.744 (2.147)
Mean Flare Accuracy	4.723 (1.162)	4.612(1.542)	4.932 (1.011)

Data are Mean (SD)

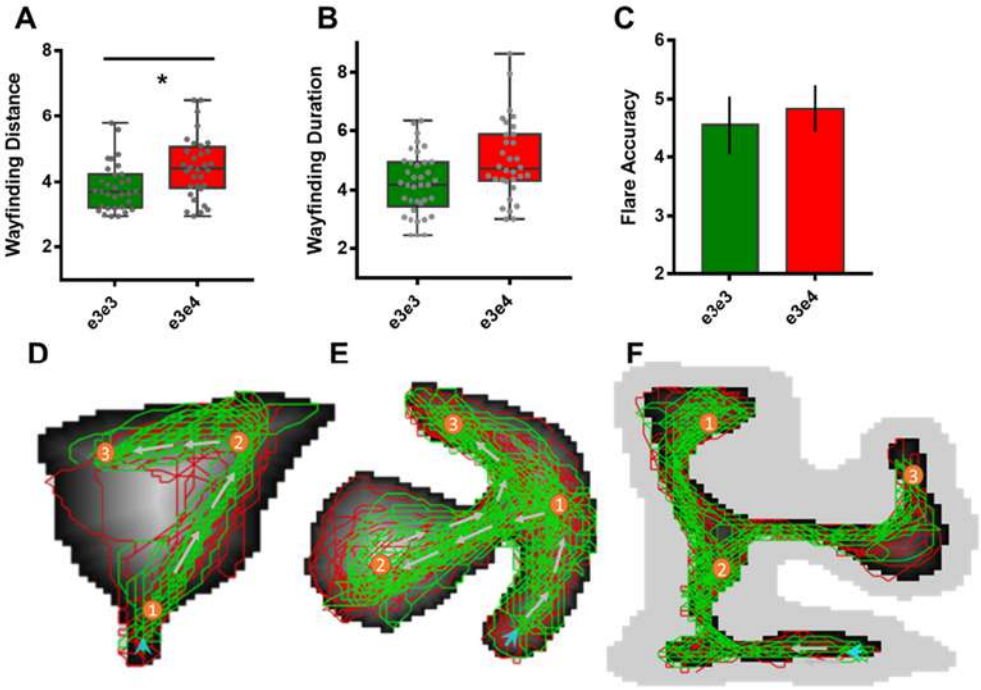


Figure 2.2 (A) main effect of genotype (b = 0.22; P = 0.004) on wayfinding distance; (B) No main effect of genotype on wayfinding duration (i.e., time taken to complete wayfinding levels); both groups used the same boat acceleration during wayfinding. (C) No main effect of genotype on flare accuracy. The spatial trajectory of each participant (colours red and green were used to differentiate the trajectories by the genetic groups) on wayfinding level 6 (D), level 8 (E), and level 11 (F), using x and y coordinates generated during game play.

Table 2.3. Mixed effects of APOE genotype and demographic factors on SHQ performance

Mixed Linear Model Outcome	Fixed Effect	b coefficient	Std. Error	F value	*p* value
SHQ Wayfinding Distance					
	APOE*	**0.22**	**0.07**	**9.30**	**>0.005**
	Sex	0.02	0.084	0.44	0.12
	Age	0.01	0.006	0.18	0.67
SHQ Wayfinding Duration					
	APOE	0.04	0.15	0.07	0.77
	Sex*	**0.39**	**0.17**	**5.45**	**0.02**
	Age	0.01	0.01	0.11	0.74
SHQ Flare Accuracy					
	APOE	0.04	0.01	2.19	0.14
	Sex*	**-0.36**	**0.26**	**3.88**	**0.04**
	Age	-0.02	0.39	1.08	0.30

Prior to the main analysis, competing mixed effect models were tested to examine the best model fit and model simplification based on standard Bayesian information criterion. The final model in the table above (featuring subject-level random effects) was adopted since it demonstrated the best model fit for the data and was retained for the main analysis.

Higher values on Wayfinding distance and wayfinding duration indicate poorer performance, conversely higher values on Flare accuracy indicate better performance. *p<0.05

To further examine the different routes taken by the two genetic groups, we plotted the exact trajectory of each participant on wayfinding level 6, 8 and 11 using (x,y) coordinates generated during gameplay and found that ε3ε4 carriers show a lower average distance to border than their ε3/ε3 counterparts (Figure 2 D-F). On level 6 and 8, *ε3/ε4* carriers deviate from the shortest distance between the checkpoints and travel toward the border of the environment compared to the ε3ε3 carriers, who tend to navigate along the centre of the virtual environment. To check if the increase in wayfinding distance in ε3/ε4 carriers compared to the ε3ε3 group was driven by any specific level, fixed effects linear models were fitted for level 6, 8 and 11 to test if the properties in one specific level captured this effect, or if this effect was an accumulative error over the three wayfinding levels. Using the same explanatory variables as in the final base model, the ε4 allele was found to increase wayfinding distance on level 6 (F_{60}=5.48, p=0.023) and level 8 (F_{60}=4.08, p=0.04) but not on level 11 (see Appendices: Supplementary Figure 2.2).

Genotype and sex effect on wayfinding

No effects of sex were found on wayfinding distance as men (M=4.06, SD=0.87) and women (M=4.22, SD=0.91; b=0.02, p=0.12) took similarly efficient paths, but sex did affect duration taken to complete wayfinding levels, with men (M=4.33, SD=1.09) requiring less time to complete levels than women (M=5.26, SD=2.17; b=0.39, p=0.02; SI Appendix, Figure S3(A)). Importantly, no interactive effects of genotype and sex on wayfinding distance or wayfinding duration were uncovered.

Genotype and sex effects on path integration

We then tested the effects of genotype and sex levels on flare accuracy, a measure of path integration. No main effect of genotype (b=0.04, p=0.14; Figure 2.2 C) and no genotype×sex interactions were found. However, sex had a significant main effect on flare accuracy, with men (M=5.11, SD=1.3) scoring higher than women (M=4.31, SD=1.4; b=-0.36, p=0.04; Appendices: Supplementary Figure 3.3 B).

Memory and spatial navigation as predictors of APOE genotype

The sensitivity and specificity of a traditional memory task to predict APOE genotype compared to spatial navigation on SHQ was done using logistic regression and ROC curves. This was motivated by the prediction that memory deficits would not be detectable on current gold standard episodic memory tasks. Covarying for sex, non-verbal episodic memory (three-minute total recall score for the ROCF) and wayfinding distance in SHQ were used separate predictors in two logistic regression analyses. The regression model for wayfinding distance x^2 (2) = 9.1, p=0.03, was statistically significant and correctly classified 71.3% of the APOE genotyped cohort (75%: ε3ε*3* 63.3%*:* ε3ε4). As predicted, the model for ROCF delayed recall was not significant x^2 (2) = 9.1, p=0.393. A ROC curve was then computed showing both navigation and delayed recall as predictors of APOE genotype (Figure 2.3). Consistent with the above, area under the curve values indicated that wayfinding distance (AUC .714, SE .068, 95% CI .555 - .822; pink curve), but not delayed recall (AUC .541, SE .074, 95% CI .286 - .578; gold curve) has a significant level of diagnostic accuracy.

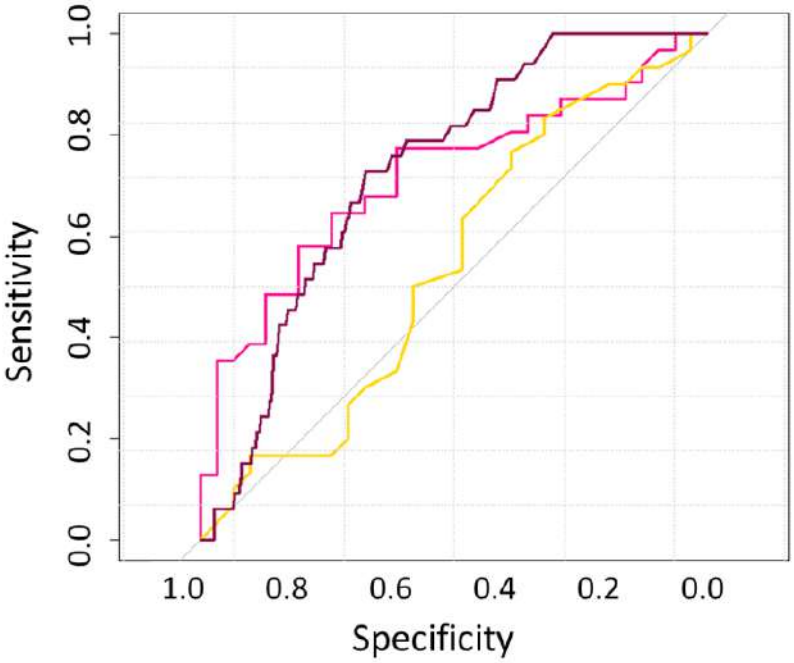


Figure 2.3 ROC curves for SHQ distance [pink line (laboratory cohort); dark pink line (laboratory–benchmark combined)] and nonverbal episodic memory [gold line (laboratory cohort)] predicting APOE genotype

Benchmark data validates an effect of APOE4 on wayfinding.

Having determined the diagnostic utility of SHQ for APOE genotype compared to standard memory test, we wanted to examine the utility of the population-level benchmark dataset as a normative control sample which could be used by clinicians in clinical settings. We took advantage of the fact that the benchmark SHQ dataset-as a representative of the population-predominantly includes ε3/ε3 carriers (75%) and performed a ROC curve with the *ε3ε4* and the benchmark data as a representative of non-risk controls. Area under the curve values indicated a very similar significant level of diagnostic accuracy as was demonstrated with the lab only cohort (AUC .701 SE .031 95% CI .639 - .759; see Figure 2.3 [dark pink curve]). Finally, to further representation the diagnostic utility of the benchmark population, we plotted each *ε3ε4* carrier's score over their age sex, education matched sub-population from the normal distribution of the UK population (see Figure 2.4). Controls can be found in Appendices: Supplementary Figure 2.4.

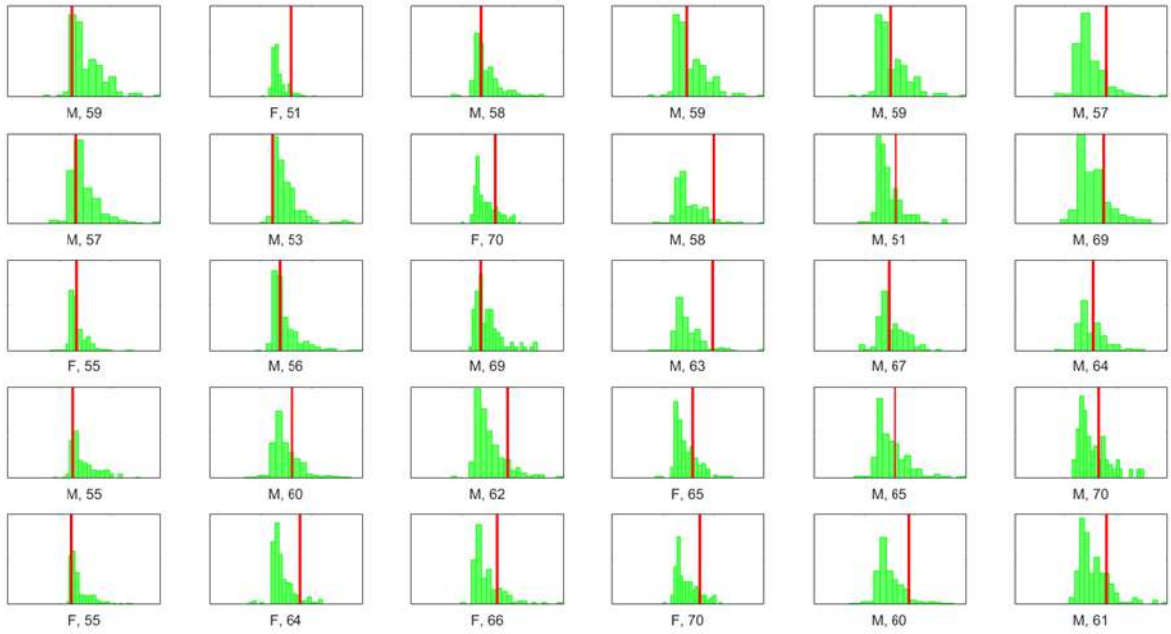


Figure 2.4. Each ε3ε4 carrier score (red line) on SHQ distance plotted against the normal distribution of scores from an age/sex/education-matched subpopulation of the benchmark dataset (green histogram). Wayfinding distance scores are on the x axis and frequency of the benchmark population on the y axis. Sex is represented as male (M) and female (F). Age is illustrated under each distribution right of sex.

Discussion

Our results show that i) we can replicate previous wayfinding changes in APOE gene carriers; ii) sex differences significantly impact on wayfinding behaviour but the effect of sex is negligible compared to APOE genetic risk; iii) healthy 'at-genetic-risk' of AD with no memory deficits can be distinguished on wayfinding measures on an individual level.

In more detail, using navigation benchmark Big Data and smaller APOE genotyped cohorts, we show that adults 'at-genetic-risk' of AD with no clinically detectable cognitive deficits, not only navigate further during wayfinding, but show a bias in navigating towards the border of the virtual SHQ environment in large open areas. This supports the hypothesis that suboptimal navigation performance is present in preclinical AD and that this is detectable on levels of the SHQ game, even when a closely matched demographic sample is provided by the global SHQ data set. We also show that while sex accounts for variation in navigation performance, sex does not reduce the sensitivity of SHQ to discriminate healthy ageing from genetically at-risk individuals of Alzheimer's disease.

Although adults at-genetic risk of AD deviate from the shortest route (often the Euclidean between the checkpoints) towards the environmental border of the SHQ environment, they successfully completed the wayfinding levels albeit sub-optimally. Thus, we propose that the navigational deficits detected here reflect an error corrective strategy (Hardcastle et al., 2015) for which environmental boundaries hold valuable navigational cues that aid the navigators' ability to self-localise and find their way through the environment when navigational uncertainty ensues. The neural substrates that give rise to the navigational uncertainty in the genetically at-risk group is most likely induced by errors in the grid cell system within the entorhinal cortex. The entorhinal cortex is not only one of the first cites of AD pathology in the brain (Braak and Del Tredici, 2015) but is also crucial for facilitating shortcut wayfinding behaviours and optimal navigation behaviour (Banino et al., 2018). Given that grid cells compute large-scale information and encode representations of self-location by measuring distance travelled by the navigator (Hafting et al., 2005; Moser et al., 2008), it is not surprising grid cell dysfunction results in navigational discrepancies in at-risk individuals of AD.

Given that phenotypic heterogeneity currently reduces the diagnostic and prognostic power of neurocognitive evaluations for early AD, we also sought to investigate if demographic and neuropsychology diversity impact navigation. The effect of the genotype that was most prominent when the environmental space was large and open (level 6 and 8). In terms of sex, we did find strong evidence of better performance in males on baseline navigation ability but no evidence to suggest that males at-genetic-risk were less vulnerable (in the preclinical stage at least) to the effect of the APOE ε4 genotype than women at-genetic-risk. This is a critical finding as it suggests that sex difference may not act on the phenotypic presentation of navigation deficits in the early asymptomatic stage of the disease. A recent meta-analysis (Neu et al., 2017) reports that women are particularly vulnerable to early underlying pathology between the ages of 55 and 70. Thus, whether sex and genotype interact to predict navigational ability on SHQ in later preclinical or prodromal stages of AD remains to be investigated. In the interest of diagnostic sensitivity, the time at which an increased female susceptibility to underlying pathology manifests behaviourally is a high priority. Although we found a sex-independent navigational deficit in adults at genetic risk of AD, evidence for strong spatial disparities on navigation performance across the sexes globally suggest that it is indeed appropriate to consider the need to stratify risk assessment by sex (Coutrot et al., 2018). For example, when genotype status is unknown, considering sex difference may hold

prognostic value as many high profile previous studies already suggest (Ferretti et al., 2018; Mielke et al., 2014; Snyder et al., 2016).

Based on data presented here on a population level and elsewhere, we now know that demographic diversity based on age, sex and nationality act on navigation proficiency, and men perform better at digital and real-life spatial navigation tasks (Coutrot et al., 2018). This finding, coupled with a plethora of pre-existing evidence for natural age-related decline in spatial navigation (Lester et al., 2017; Moffat, 2009), means that we must establish personalised normative measures to accurately assess spatial disturbances that have not been well-established as a underlying feature in preclinical AD pathology. From a clinical standpoint, clinicians and researchers should be advised to consider not only age, but also the sex of their putative patient before inferring pathological related spatial impairment. From a research perspective, researchers should work towards providing demographically corrected benchmarked scores for standardised neuropsychological test. To date, obtaining normative data of this nature has been challenged by heterogeneity in methodological approaches used to measure spatial navigation and uncertainty about population level differences in cognitive performance. Consistency across our non-risk control group and the benchmark scores is compelling evidence that SHQ may provide unique benchmarking data, on a global scale, by controlling for the demographical factors such as sex, advanced age and cultural background; factors which will alter how individuals perform on SHQ. Although level of education was included to refine the population data, education did not have a compelling effect on navigation performance in the global SHQ database. Further research is required to determine what demographic factors beyond age, sex and nationality will increase the sensitivity and specificity of navigation test for underlying preclinical AD.

Despite illustrating for the first time the clinical utility of new epidemiological data gathered on a global scale using the SHQ game, our study has several limitations. Firstly, we focus on preclinical rather than symptomatic AD, seeking to evaluate the prognostic value of SHQ rather than validate SHQ data as a potential diagnostic tool. However, given that many excellent cognitive diagnostics measures exist for symptomatic AD, we question whether navigation measures have true utility in this aspect. Instead, identification of subtle cognitive preclinical changes will be of greater future importance to complement other biomarkers as diagnostic and treatment outcome measures. Secondly, only 47% of all ε3ε4 carriers develop symptomatic AD by the age of 76 years on average. This is consistent with about 50% of the

ε3ε4 individuals in this study being impaired relative to the demographically corrected benchmark. Longitudinal studies are needed to truly determine how predictive spatial navigation combined with genotypic information is in the preclinical stages of the disease however. Further replication of our findings with preclinical cohorts defined by multiple cognitive, genetic and neurological markers is desirable, although it is promising that we replicate previous boundary findings (Kunz 2015). Moreover, although education was considered in the individualised approach to diagnosis of 'at-risk' AD, approx. 40% of the genotyped cohort has 15 years+ of education and 50% of the cohort are working in "professional" fields vs. skilled or low-skilled / manual, potentially leading to an over-representation at the educated individuals in this genotyped sample. Lastly, although best efforts were made to control for gaming proficiency, we cannot completely rule out a potential influence of previous gaming experience contributing to the observed male advantage in the data. Still, considering that we are investigating a 50-75 year old cohort, gaming proficiency should not play such a large role. More importantly, the difference of male and females in the SHQ data across ages does not change, suggesting that gaming proficiency plays only overall a minor role in assessing spatial navigation via an online App.

In conclusion, our work supports the hypothesis that navigational discrepancies are present in preclinical AD and can be captured by Sea Hero Quest available on iOS and Android platforms. We show for the first time promising evidence that normative data generated from the 4.5 million people who played SHQ worldwide, may in the future help us to create a prognostic test based on navigational proficiency – to help us to understand how the very earliest symptoms of AD is isolated from potentially confounding demographic factors, such as sex, advancing age, educational attainment or cultural background. This should reduce the problematic nature of phenotype variation obscuring the assessment of spatial disorientation as a first symptom of AD and offer the promise of individually tailored solutions in healthcare settings. Thus, spatial navigation emerges as a promising cognitive fingerprint, which will complement existing biomarker for future AD diagnostics and disease intervention outcome measures.

Chapter 3: Reduced connectivity between entorhinal and posterior cingulate cortices correlates with navigational deficits characteristic of at-risk Alzheimer's disease

Published Paper

Introduction

Current evidence shows that gold standard episodic memory tests fail to capture the first symptomatic manifestation of Alzheimer's disease (AD) (Coughlan et al., 2018b; Jessen et al., 2014; Zimmermann et al., 2019; Zimmermann and Butler, 2018). Thus, alternative diagnostic tools that do not require long training time and expensive equipment are urgently required. We have already seen that spatial navigation is a promising preclinical marker in Chapter 2. However, whether similar navigation deficits can be detected on another navigation test, the Virtual Supermarket Test (VST) (Tu et al., 2015), remains to be tested.

In Chapter 1, we discussed widespread navigation deficits in AD patients. More recent development of 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 or self-motion, 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).

Evidence now also suggests that on a cellular level, reduced grid cell representations in the entorhinal cortex correlate with 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 in Chapter 2, which discriminated ε4 carriers from non-carriers with a classification accuracy of 72%. However, no MRI data was included to pinpoint the neural changes that gave rise to ε4-related path integration deficits. In addition, while the field is largely focused on EC-mediate navigation impairments for the early detection of preclinical AD, functional connectivity (FC) changes also occur in 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 changes are understood to be functional responses to early AD pathology within the 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 cohorts. To address this gap, this investigation examined navigation performance and resting-state FC in APOE genotyped ε4 carriers and non-carriers, by testing four major navigation process using a short path integration paradigm called the VST. This study also examined the relationship between navigation performance on both the VST and Sea Hero Quest and FC between the EC, the hippocampus, the PCC and the precuneus. We propose that ε4-related navigation impairment would correlate with reduced edge of EC and one of more regions in the proposed functional neural network. As an additional measure, we investigated if subjective cognitive change (SCC), often considered a first symptomatic manifestation of disease (Jessen et al., 2014), accompanies navigation impairment and/or altered FC strength in the AD vulnerable neural network proposed here.

Methods

Participants

We recruited 150 participants between 50 and 75 years of age (M=61.92, SD=6.72) to participate in a research study at the University of East Anglia. Please refer to Chapter 2 for recruitment, screening criteria and APOE genotyping. See supplementary table 3.1 for group background characteristics. The participant sample size was 64 (including 32 ε3ε3 carriers and 32 ε3ε4 carriers) all of whom underwent VST and CCI cognitive testing. SHQ data was available for 60 participants. Twenty ε3ε3 carriers and 20 ε3ε4 carriers also underwent

structural and functional MRI. Homozygous APOE ε4 carriers and APOE ε2 carriers (15% of the UK population) were excluded. One ε3ε3 participant did not complete the scan due to distress and their data were excluded from the analysis. Two additional participants (one ε3ε3, one ε3ε4 carrier) 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%) original participants was used for neural analysis, reaching an acceptable fMRI sample size (Pajula and Tohka, 2016).

Measures and Materials

Paradigm overview

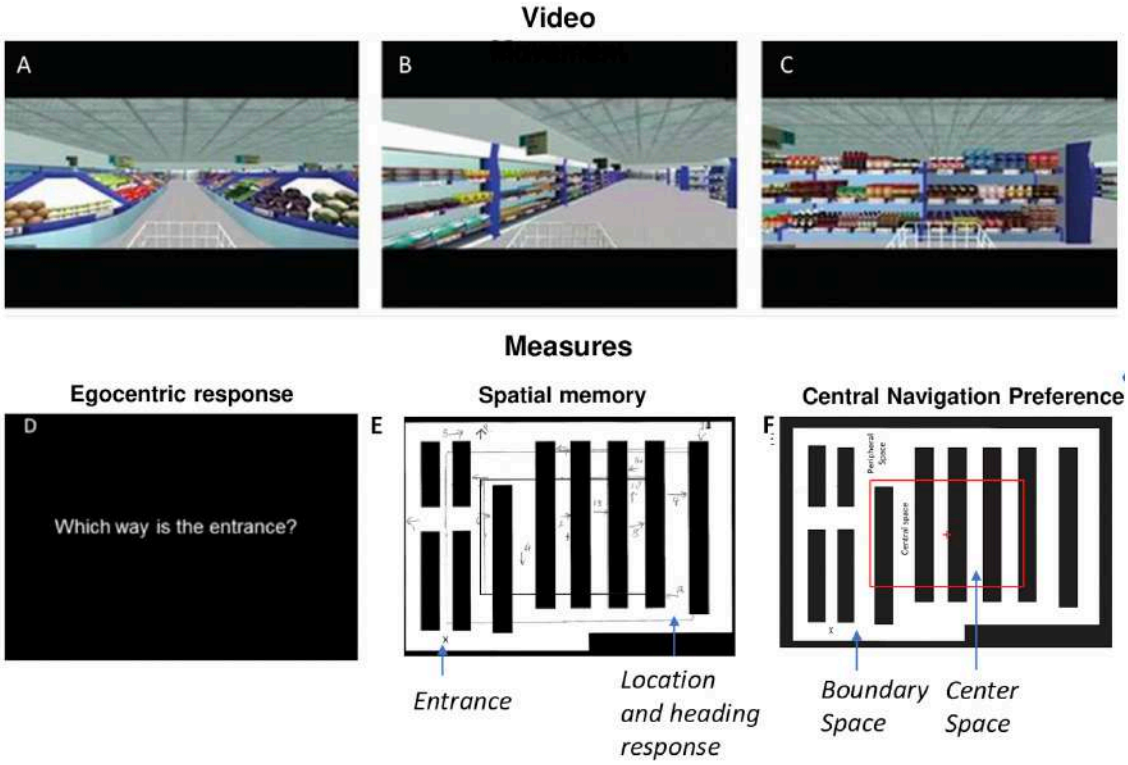


Figure 3.1 Spatial orientation was assessed using an ecological virtual supermarket environment. The layout of the virtual environment did not include any notable landmarks. An iPad 9.7 (Apple Inc., etc) was used to show participants 7-14-second video clips of a moving shopping trolley. All trials began at the same location in the supermarket but followed different routes to reach a different end point in each trial (**A**). Videos were presented from a first-person perspective and participants were taken to a set location while making a series of 90 degree turns (**B**). Once the video clip stopped (**C**), participants indicate the real-life direction of their starting point (**D**). Immediately following, participants indicate their finishing location (short-term spatial memory) and heading direction on a VST map (**E**). Number of location responses made in the central space and boundary spaces were recorded (**F**) Number of responses in the central vs peripheral space

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) place memory. 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 3.1 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 3.1 D). In a second step, participants indicate their finishing location (short-term spatial memory; Figure 3.1 E) and heading direction on a VST map. We extended our VST paradigm to a fourth spatial measure based on evidence of an entorhinal-mediated bias during path integration in at-risk AD. Based on a behavioural analysis by Kunz and colleagues (Kunz et al., 2015), we partitioned the environmental map into central versus boundary space. The central area was drawn by: i) determining the centre point by measuring half the width (e.g., 9.8cm) and half the length (e.g., 6.8cm) ii) creating the central and boundary areas by measuring half the width from the centre point (e.g., 4.9cm) and half the length from the centre point (e.g., 3.4cm) which allowed a centre/ boundary areas to be created. Participant-specific values of central navigational preference were then calculated as ratio scores:

Central navigation preference=
(N(centre))/(N(boundary))

where N(centre) is the number of responses made by a single participant in the centre of the arena and N(boundary) is the number of responses in the boundary of the supermarket map (Figure 3.1 E/F). Central navigation preference is a proxy for boundary-based place memory. The SHQ game described in Chapter 2 Methods section) was also included in the fMRI analysis to elucidate any neural correlation of SHQ performance.

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 (ROCF; with 3-min delayed recall) and the Four Mountains Test were used as secondary screening measures to assess any non-verbal episodic memory and spatial memory differences between both genetic groups (Chan et al., 2016; Shin et al., 2006).

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 seemly predictive of faster rates of memory decline (Samieri et al., 2014; Weston et al., 2018). The presence of SCC is also related to abnormal changes in Aβ and tau biomarkers in APOE ε4 carriers (Risacher et al., 2015b) and is thus considered important for early detection. Here, we measure SCC 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.

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 scanner artefacts. Please see appendices (Supplementary Information Chapter 3) pre-processing structural and functional MR images.

Anatomical ROI mask selection

Masks for the four AD vulnerable regions of interest (ROI) were created using Juelich historical atlas (i.e. right and left EC) and Harvard-Oxford cortical and subcortical structural atlases (i.e. right and left hippocampus, PCC, precuneus cortex). Masks were thresholded using 'fslmaths' to reduce potential overlap with neighbouring regions. ROIs were visually inspected by overlaying them on the MNI skull-stripped anatomical T1w data to ensure proper definition. A power spectrum for each ROI was generated for confirm that the functional MRI cleaning processes (detailed in the pro-processing section) adequately identified resting state signal between 0.01 and 0.08 Hz (see Appendices: Supplementary Figure 3.3)

Statistical approach

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. Chi square was used to assess 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 a main predictor of interest.

Based on the findings from chapter 2, age and sex were included 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). Nationality was not included as all participants were UK nationals. Education was not included as a

covariate. Education was tested in Chapter 2 and did not heavily influence navigation performance. Associations between VST, SHQ and CCI were tested using partial Pearson correlation in SPSS and 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 ROIs was 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. 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. 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 both 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).

Results

Neuropsychological assessment

As expected, no differences between the two genetic groups evident for on the neuropsychological assessment (Table 3.1) or on secondary characteristics (see Table 3.2), which confirmed that the impact of APOE genotype prior to clinically detectable MCI/AD symptomology could be measured.

Table 3.1 Primary demographic and neuropsychological profile the ε3ε3 carrier group and the ε3ε4 carrier group

	ε3ε3 (n=32)	**ε3ε4 (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.

Virtual Supermarket Test assessment

Heading direction (F = .799, P = .38) 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 (see Appendices: Supplementary Figure 3.1 for data visualisation). Egocentric orientation was significantly different between genetic groups, with ε3ε4 participants making fewer correct responses, compared with ε3ε3 participants (F = 4.18; P = 0.04). Place memory 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 3.2 A-C; Table 3.3 for mean values).

Table 3.2. Secondary characteristics of the ε3ε3 carrier group and the ε3ε4 carrier group

Measure		ε3ε3	ε3ε4	χ2 (df)	p value
Marital Status					
	Single	7	5	.413 (2)	*ns*
	Partner/married	25	27		
Education					
	>=8years	5	5	.169 (4)	*ns*
	=11years	6	5		
	=14years	7	11		
	=15years	14	11		
Blood pressure					
	Not medicated	24	26	.366 (1)	*ns*
	Medicated	8	6		
Cholesterol					
	Not medicated	26	28	.474 (1)	*ns*
	Medicated	6	4		
Family History of AD (missing n=6)	None	22	15	.856 (3)	*ns*
	One parent	7	7		
	Both parents	3	4		
Occupation (missing n=1)				.116 (3)	*ns*
	Manual/Unskilled	5	8		
	Skilled	8	10		
	Professional	18	14		

Secondary characteristics between genetic groups. No difference on any of the above listed characteristics were detected with Persons Chi square confirmed analysis.

Table 3.3 Effect of APOE genotype on Virtual Supermarket Test performance

	Mean (SD)	F	P value
Egocentric orientation			
ε3ε3	12.01 (2.3)	4.18	.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 place memory			
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 Place memory.

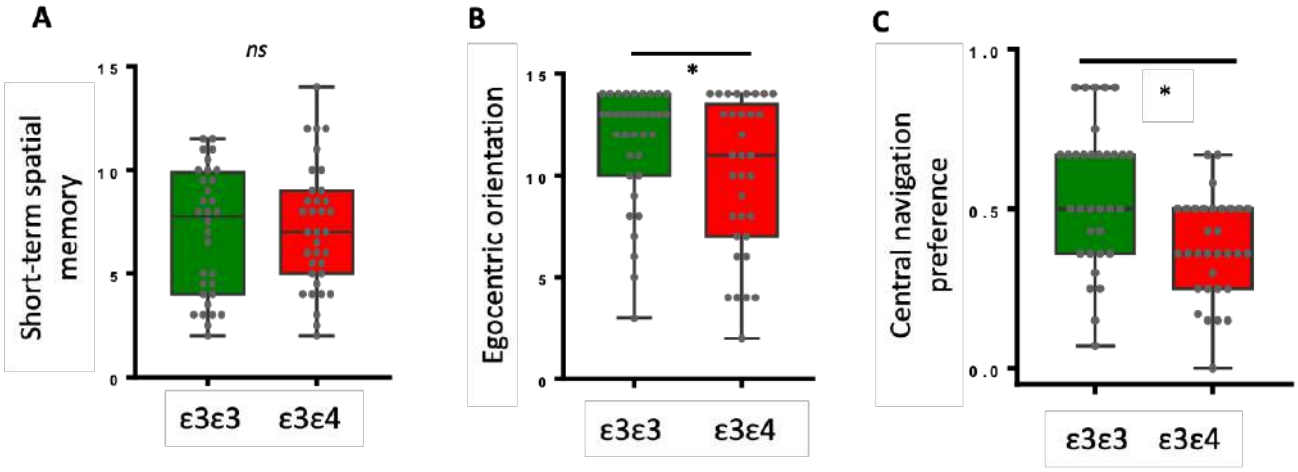


Figure 3.2 The effect of genotype on short-term spatial memory (non-significant), **B** egocentric orientation (significant), and **C** Place memory (significant).

Age and sex on spatial navigation

There was a significant effect of age on short-term spatial memory (F = 6.26; *P* = .002) and heading direction (F = 15.67; P<0.005). Mean performance values indicate that men outperformed women on all three aspects of the task: egocentric (M = 12.15, SD = 2.38,; M = 10.61, SD = 3.16; F = 3.39; *P* = .07); allocentric (M = 8.54, SD = 2.79; M = 7.44, SD = 3.21; F = 3.68, *P* = .06); and heading direction (M = 12.37, SD = 2.25; M = 11.72, SD = 2.24; F =

4.13; P = .04), although the sex effect on egocentric performance did not reach statistical significant.

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; Appendices: Supplementary Figure 3.1). 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 positively associated with self-reported executive function concern (r=-.347, p=.008).

Voxel based morphometry and 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 ε4-related navigation impairment on the VST or on SHQ 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 3.3 A). No difference was found between the genetic groups (see Supplementary Table 3.2 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).

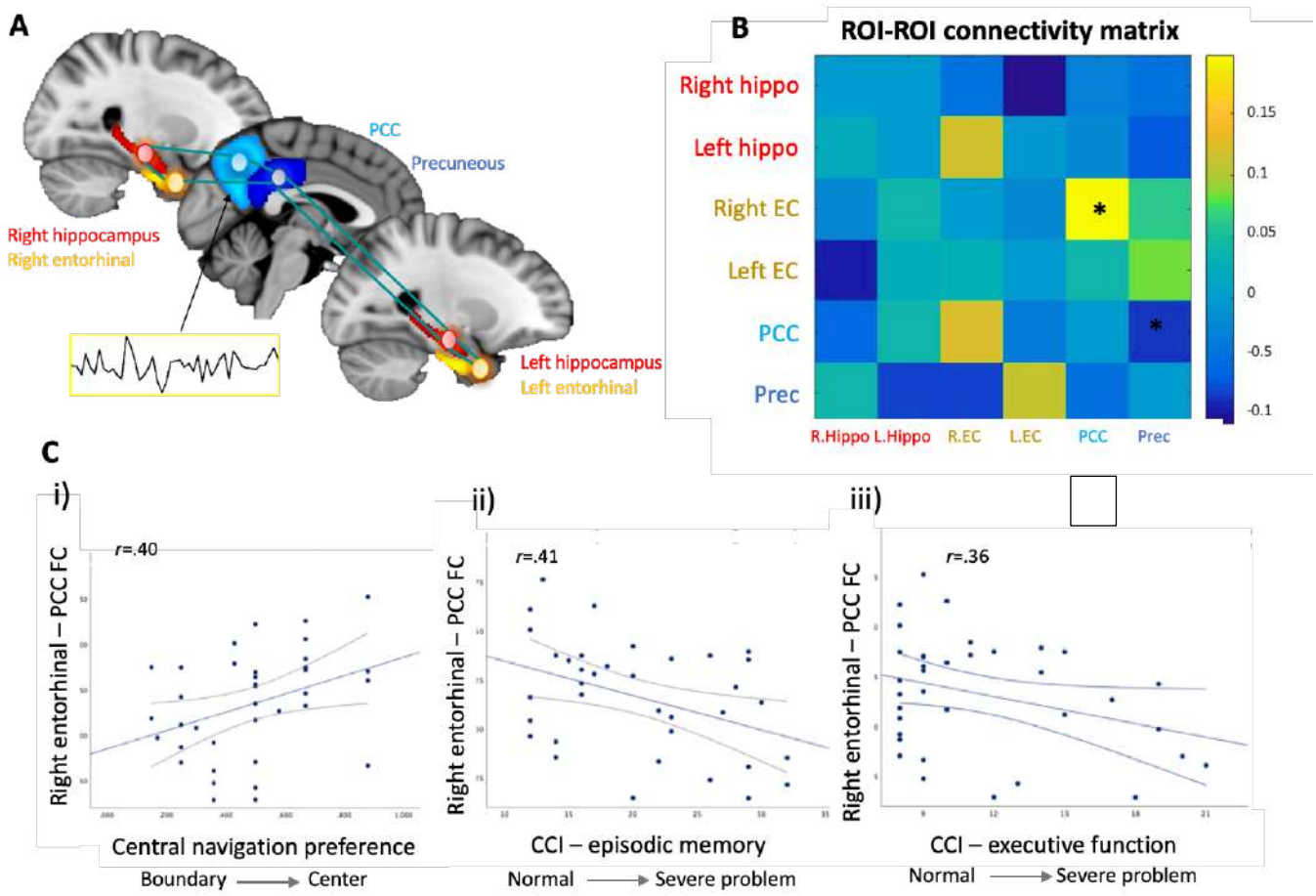


Figure 3.3 **A** APOE-dependent correlations in functional connectivity between selected ROIs: *right/left hippocampus, right/left EC, PCC, Precuneus*. **B** The 6node × 6node network matrix of correlation coefficients represents connectivity strength between nodal pairs in a dual regression to test 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.

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]; r squared=.228; P=035) (see Figure 3.2 B 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. T=This revealed that 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 appendices

for the independent ε3ε3 and ε3ε4 connectivity matrices (Appendices: Supplementary Figure 3.2).

Functional connectivity and ε4 sensitive Virtual Supermarket Test navigation processes.

Having determined altered FC changes in the EC, PCC and precuneus in the at-risk group, FC strength between each ROI pair was correlated with 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 3.3 B). No correlate in the pre-defined neural network for egocentric orientation was present. Left entorhinal-PCC connectivity (not significantly different between genetic groups) correlated with heading orientation performance (r=0.43, P_{FWE} =0.01). No ROI-ROI connectivity values correlated with egocentric orientation or short-term spatial memory.

ε4-related functional connectivity and Sea Hero Quest

We tested if the neural abnormalities uncovered correlate with SHQ performance focusing on distance travelled and duration to complete level 6, 8 and 11. Functional connectivity between the right EC-PCC and SHQ duration taken to complete levels approached significance (t=1.981, uncorrected *P*=0.05). No other associations in the correlation matrix approached significance. In the absence of a functional neural correlate, volumetric measures for each ROI was also correlated with performance. No functional correlate was discovered for SHQ distance travelled, although left EC volume (t=-1.948, uncorrected *P* =.06) and left PCC volume (t=2.054, uncorrected *P*=.05) showed a weak but non-significant association with distance travelled, adjusting for age, sex and total intracranial volume.

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 3.3 C).

Classifying genetic groups based on the Virtual Supermarket Test and functional connectivity

Although no neuro-functional correlate was identified for SHQ performance, VST egocentric orientation or subjective executive function, the neuro-functional correlate (EC-PCC) of the two strongest ε4 behavioural VST 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 Place memory 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 3.4 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 5 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 3.4 B).

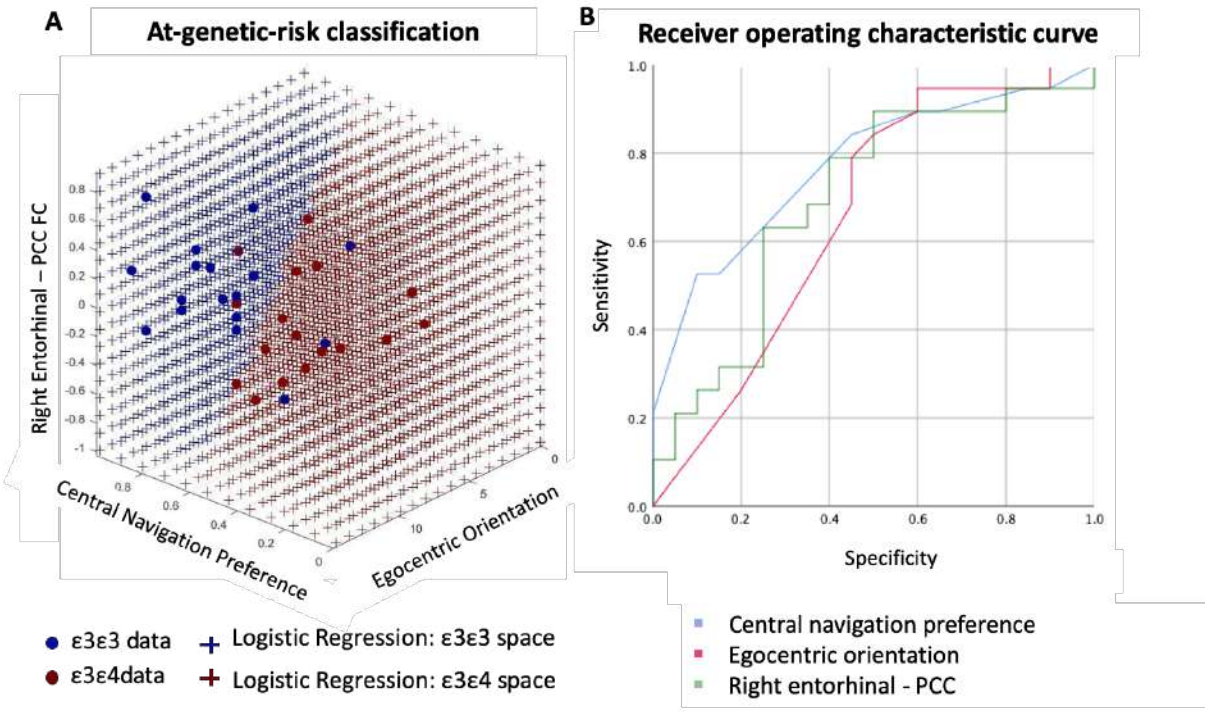


Figure 3.4 Logistic regression and ROC curves for right EC– PCC functional connectivity strength (green line) and VST cognitive measures central preference (blue) and egocentric orientation (red) predicting variants of the APOE genotype. **A** Logistic regression indicated that the regression model based 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.

Discussion

APOE ε4 is the strongest genetic risk factor for late-onset AD. Whether preclinical stage cognitive changes are detectable on a short clinically feasible task is yet 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, this shows that 1) navigation discrepancies following path integration co-exist with SCC in adults at-genetic risk of AD and 2) reduced network connectivity between the right EC and the PCC correlate with navigation discrepancies and subjective episodic memory concerns, which characterise the behavioural phenotype of at-genetic-risk.

Significant differences between the APOE genetic groups were found in two out of four of the VST spatial sub-measurements: Egocentric orientation and boundary-based place

memory. 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 boundary-based place memory as 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 place memory biases towards the boundary, compared to non-carriers.

Behaviourally, the ε4-related boundary-based place memory 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 SCC. (see Contreras et al., 2017 for more information on the CCI).

Based on the assumption that the border bias is driven largely by reduced grid-cell representations in the EC, it is surprising that SHQ distance travelled was not related to EC connectivity or volume. There are a number of potential reasons for this. Firstly, we used total distance travelled and duration as the metric of interest. If the neural correlates of each level differ, the this may explain the null effect. Moreover, the VST employs spatial memory during recall unlike SHQ which collects responses in real time. Thus, path integration in SHQ likely employs different neural computations compared to path integration in VST. Evidence for this is shown by the fact that measures on both tasks do not correlate.

Despite not discovering a neural correlate for SHQ, we found that 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 by 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 with 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 chapter.

The role of reduced EC – PCC functional connectivity in preclinical AD may not be surprising, as typically AD pathology, particularly 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). This is 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 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 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 show moderate levels of Aβ in the brain enhance FC due to compensatory brain mechanisms. This may explain why in ε4 cohorts, increased connectivity strength can be observed between the PCC and precuneus (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 investigating the neural correlates of ε4-related navigation impairment which prevents generalization. We also cannot rule out the possibility that boundary-based place memory is caused be another neural mechanism and/or the fact that boundary landmarks, although intentionally hidden in the VST map, may exert an influence toward the border in the ε4 group. Of course, longitudinally tracking these participants to confirm whether the 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. As we cannot say if navigation changes precede SCC 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 in AD have failed to identify disease-modifying agents with clinically significant benefit (Sevigny et al., 2016), the addition of multimodal diagnostic should facilitate earlier intervention and more sensitive treatment outcome measures. 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.

Chapter 4: Test-re-test reliability of Sea Hero Quest, the Virtual Supermarket Test and the Cognitive Change Index in a nonclinical sample with at-risk Alzheimer's disease

Submitted Unpublished Paper

Introduction

Upcoming clinical trials with pharmaceutical compounds (such as aducanumab) may acutely or sub-acutely alter navigation and memory function, which may serve as a predictor of long-term response to treatment (Atri et al., 2011; Laczó et al., 2016). Assessing treatment response crucially relies on the identification of cognitive markers that can detect a signal of treatment effect or efficacy (Husain, 2017). Although, current cognitive markers for MCI or clinical AD demonstrate good retest reliability, the reliability of novel preclinical tests used in cognitive neuroscience research, particularly spatial navigation tasks, is unknown. If diagnostic measures sensitive to preclinical cognitive changes will be translated into treatment outcomes measures in upcoming clinical trials, it will be crucial to establish test-re-test reliability of novel tests.

Virtual reality navigation tests demonstrate high ecological validity and are sensitive to abnormal changes along the functional gradient of entorhinal-hippocampal cortex in preclinical AD (Coughlan and Puthusseryppady, 2019; Kunz et al., 2015b; Zimmermann et al., 2019). Until now, spatial navigation or path integration studies have focused on cross-sectional group comparisons of spatial disorientation with prodromal or genetically-at-risk individuals and have overlooked the need to establish the test-retest reliability of navigation tasks in preclinical AD populations. If these tasks demonstrate test-retest reliability in the moderate to high range, then they may be a good means of assessing treatment response, particularly because reliabilities of commonly used memory tests such as the Rey Osterrieth

Complex Figure test (ROCF) and the Selective Reminding Task are poor (Bird et al., 2003; Mitrushina and Satz 1991).

Test–re-test reliability refers to the degree to which an assessment produces consistent results from one test session to another, in absence of any other change or intervention within the test population (O'neil-Pirozzi et al., 2012). Reliability is often negatively impacted by practice effects from repeated exposure to the same test trials (Crawford et al., 1989; Rawlings and Crewe, 1992). Thus, individuals may learn to apply strategies during re-testing, improving their navigation accuracy and efficiency, but decreasing test–re-test reliability (Lowe and Rabbitt, 1998; Wilson et al., 1998). Although the use of alternate forms of the same test measure is recommended to lower the problematic nature of retest effects, this solution may still be vulnerable to problem-solving strategies developed at baseline, resulting in improved performance across testing, even with alternate forms of the same test. To the best of our knowledge, none of the experimental cognitive tasks for preclinical AD or genetic vulnerability to AD have undergone reliability testing, which calls into question their future usefulness as treatment outcome measures.

Novel tests used in cognitive neuroscience research to assess spatial disorientation in preclinical AD groups include the Virtual Supermarket task (VST), developed to distinguish AD from other dementias, and Sea Hero Quest (SHQ), designed to measure navigation ability on a global scale. These tests were utilised in Chapter 2 and 3 as a marker of genetic vulnerability to AD (Coughlan et al 2019). The VST is a brief measure of path integration, including four tests measures and two alternative forms. Test administration consists of one learning trial and 14 tests trials that tap into egocentric orientation, central navigation preference (a proxy for boundary-based place memory), allocentric spatial memory and head direction performance. While at baseline (T1), a paper version of the supermarket map was used, an alternative form of the VST was employed at re-test (T2), to facilitate electronic and automatic recording of participant responses on a 9.7inch iPad. In addition to the VST, SHQ measures path integration through various wayfinding challenges that become more difficult over the course of the game (Coutrot et al., 2018). Multiple test scores may be obtained from each task and each task demonstrates feasibility in clinical populations. Therefore, we aimed to establish the reliability of these two measures to detect

AD related disorientation over two timepoints separated by an eighteen-month period. The Four Mountains test, a well-established measure of spatial memory in MCI, was included as a standard measure to compare the reliability of the novel spatial navigation tasks (Bird et al., 2010; Chan et al., 2016).

Subjective cognitive decline is often considered the first symptomatic manifestation of disease and it is present in the genetic at-risk group as reported in Chapter 3 (Jessen et al., 2014). Therefore, the Cognitive Change Index (CCI), a measure of subjective cognitive decline in episodic memory and executive function, was included in the test battery at baseline test (T1) and re-test (T2) (Contreras et al., 2017). Our hypothesis was undefined as we could not predict the performance of the tasks because test re-test reliability of navigation or path integration tests have not previously been investigated, to the best of our knowledge. We predicted that some scoring parameters would be more reliable than others because the neural correlates of VST and SHQ differ (as reported in Chapter 3), meaning that some measures may be less vulnerable to practice or novelty effects. We also predicted that demographic and genetic factors (i.e. APOE status) may influence test-re-test reliability, similar to many gold standard diagnostic tests (Ferretti et al., 2018; Husain, 2017).

Methods

Participants

In this cohort study, controls (ε3ε3) and genetically at-risk participants (ε3ε4) were assessed at enrolment and 18 months later. At baseline (May-December 2017) , and follow-up (September 2018-January 2019), participants underwent a neuropsychological examination and a novel spatial cognition test battery including novel and well-established (e.g., Four mountains) spatial cognition tests (Bird et al., 2010; Coughlan et al., 2019; Coutrot et al., 2018). At the follow-up (or re-test) analysis, we included participants who participated in both assessments (n=61; ε3ε3=33, ε3ε4=28), which equals 4 dropouts. One participant developed lupus (a systemic autoimmune disease) over the study period and was excluded. Mean age of participants at baseline was 61.92 ± 6.72 years and at follow-up was 63.71 ±

5.90 years. The average follow-up duration was 18 months ± 0.4 months. The recruitment and genotyping methods at baseline can be found in chapter 2.

Measures and materials

Neuropsychological assessment. The neuropsychology assessment consisted of the Addenbrooke's cognitive examination-III (ACE) version B at baseline and version C at follow-up. Different versions were administered at both timepoints in an attempt to limit retest effects and were primarily to ensure the cognition remained intact across timepoints (Matias-Guiu et al., 2017). Similarly, the ROCF was administered at baseline and the Taylor complex figure task was administered at follow up (see Table 4.1) (Hubley, 2010; Shin et al., 2006).

Spatial navigation performance assessment. Sea Hero Quest (SHQ) and the Virtual Supermarket Test (VST) were the key navigation tasks of interest given their sensitivity to variation in the APOE genotype at baseline. See supplementary video in appendix for the alternative electronic version of the VST. VST trials (1-14) in both versions were identical. Please refer to chapter two for a description of the SHQ measure. Please refer to chapter three for a description of the original VST test, and the Cognitive Change Index (CCI). The Four Mountains test (4MT) was included as a standard to measure against the reliability of the novel spatial navigation tasks: VST and SHQ. See table 4.2 for a list of nine scoring parameters in each cognitive task.

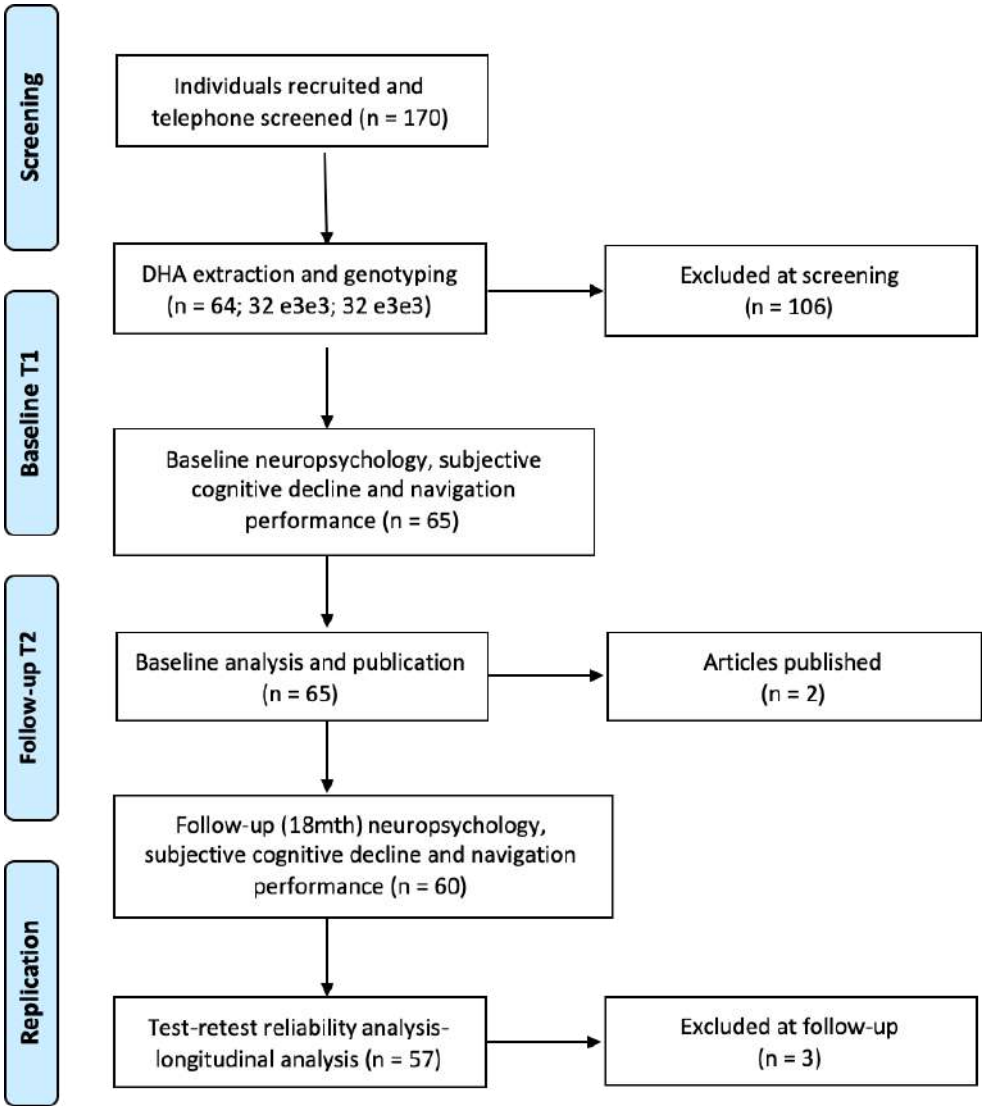


Figure 4.1 Longitudinal design at baseline (T1) and re-test (T2)

Statistical Analyses

We computed linear mixed effect models with random intercept and time slope per participant to test change on neuropsychological test performance over 18 months (delta Δ = [follow-up T2 – baseline T1]). We tested APOE groups separately given ε4 carriers' greater risk of cognitive decline compared to non-carriers (Corder et al., 1993). Fixed effects included the APOE genotype and sex. In accordance with recommendations, multiple comparisons were not corrected for because separate models were fitted for each performance outcome (Rothman, 1990). We report 2-sided P values with a significance of .05.

Test-retest reliability of each of continuous variable in the test battery from baseline (T1) to re-test (T2) was assessed using 2 complementary approaches: 1. Intraclass correlation coefficients (ICCs) and 95% confidence intervals were calculated according to McGraw and Wong as a measure of consistency between timepoints (McGraw, 1996). 2. Because high correlations may persist even in the presence of a change, or indeed demographic factors might influence change, repeated measures ANOVAS were used to determine whether effects of APOE or sex contributed to test-retest variability. Thus, interactions terms were included in a repeated-measures ANCOVA: APOE × timepoint and sex × timepoint. Including interactions tests for any variance due to an APOE/sex × time interaction that unless removed is pooled into the participant × time interaction error variance and inappropriately augments estimated unreliability and biases the ICC downward. All scoring parameter listed in table 4.2 were the dependent variables. A Bonferroni correction was made to determine the statistical significance of these multiple comparisons in the repeated measures.

RESULTS

Neuropsychological performance

Neuropsychological test performance at baseline and follow-up are presented in Table 4.1. There was no significant difference in change between the genetic groups from baseline to follow-up, expect on the ACE memory scale (t=2.410, p=0.02), with ε4 carriers' performance improving significantly more over the 18-month study period, compared to ε3 carriers. See Table 4.1 for mean neuropsychological scores across time points and mean change across time points in ε3ε3 and ε3ε4 carriers.

Table 4.1. Neuropsychological performance at baseline (T1) and re-test (T2)

Measure	Variable	Mean T2	Mean T1	Δ	P value
ACE	Total ε3ε3	93.70 ± 4.88	94.67 ± 3.67	-.97 ± 5.334	.06 (t=1.87)
	Total ε3ε4	94.37 ± 2.31	92.96 ± 3.82	1.41 ± 3.354	
	Memory ε3ε3	24.97 ± 1.43	24.70 ± 1.92	.27 ± 2.13	.02 (t=2.41)
	Memory ε3ε4	25.00 ± 1.07	23.70 ± 1.66	1.26 ± 1.75	

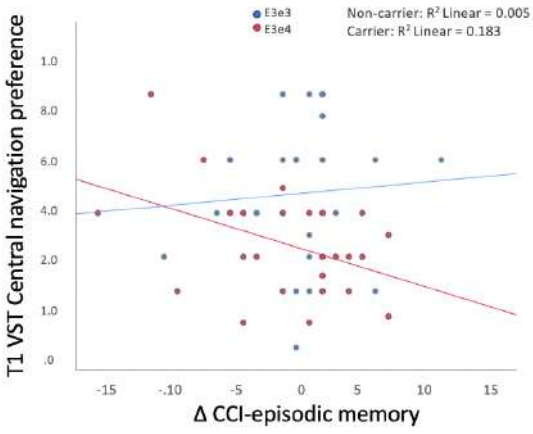
	Visuospatial ε3ε3	14.20 ± 1.32	14.93 ± 1.05	-.73 ± 1.34	*ns*
	Visuospatial ε3ε4	14.15 ± .94	14.85 ± 1.21	-.70 ± .99	
ROCF	Copy 3ε3	32.75 ± 2.84	33.23 ± 2.77	-.383 ± 2.976	*ns*
	Copy ε3ε4	32.15 ± 2.568	32.28 ± 2.62	-.185 ± 2.879	
	Recall ε3ε3	21.83 ± 5.337	20.51 ± 6.325	1.06 ± 4.733	*ns*
	Recall ε3ε4	21.50 ± 5.017	18.15 ± 6.111	2.60 ± 7.682	

T2 = follow-up; T1=Baseline; Δ Delta = T2 value - T1 value; p value= significant change between genetic groups; Rey = Rey Complex Figure Test

Based on the most sensitive APOE ε3ε4 parameters at baseline, we tested if baseline central navigation preference measured on the VST and SHQ predicted change on neuropsychology or subject cognitive concerns. We also included an APOE × baseline performance interaction term in the mixed effects model. There was significant interaction between central navigation preference on the VST and APOE genotype for change in episodic memory concerns (F=5.07, p=0.02), but not on executive function concerns. Independent models for each genetic group were then specified, revealing that less VST central navigation preference at baseline predicted worsening episodic memory concern over the study period in the ε3ε4 carriers (F=5.01, p=0.03), but not in ε3ε3 carriers (F=0.15, p=0.69; Figure 4.2)

Figure 4.2 Decreased central navigation preference (i.e. more boundary-based place memory) significantly predicts increased memory concern over 18 months in ε3ε4 carriers.

Test-retest reliability



Once confirmation that overall cognitive ability of the whole sample was intact at T2, test-retest reliability was measured. For all test measures in the test battery, intra-class correlation coefficients (mixed model) are presented in Table 4.2.

Table 4.2. Intra-class correlations coefficients (ICC) for test–re-test reliability of the Virtual Supermarket Test, Sea Hero Quest, the Cognitive Change Index and the Four Mountains Test

VST	ICC	95% CI	**SHQ**	ICC	95% CI	**CCI**	ICC	95% CI	**4MT**	ICC	95% CI
Egocentric	**.72**	.530-.838	Distance	**.50**	.058-.719	Memory	**.85**	.747-.913	Total	**.50**	.153-.703
Map drop	.06	-.82-.385	Duration	**.48**	.052-.718	EF	**.85**	.749-.914			
Heading	**.50**	.148-710									
CNP	.27	-.26-.576									

Bold=acceptable test-re-test reliability; ICC low test–re-test reliability (less than .50); ICC moderate test–re-test reliability (between .50–.80); ICC high test–re-test reliability (between .80–1.0) according to Koo and Li (2016)(Koo and Li, 2016). Each scoring parameter taps into performance on independent spatial process or self-report cognitive domain that are also interdependent.

Correlation coefficients ranged from 0.06 (extremely low reliability) to 0.85 (high reliability). Of the nine correlation coefficients, seven were statistically significantly greater than 0. Two of the nine test–re-test reliability correlation coefficients reflected high test–re-test reliability (greater than 0.80): CCI-episodic memory and CCI-executive function. VST egocentric orientation also approached high reliability. Three correlation coefficients reflected moderate test–re-test reliability (between 0.50–0.80): VST heading direction, SHQ distance travelled (level 6,8,11), and the 4MT total score. The remaining three: VST map drop, VST central navigation preference and SHQ duration reflected low test–re-test reliability (less than 0.50).

Post-hoc test–re-test reliability based on APOE and sex interactions

Repeated measures ANCOVAs specified APOE × time interactions and sex × time interactions to test if interactions were biasing the ICC results (Table 4.3).

Table 4.3. Mean scores and practice effects on the Virtual Supermarket Test, Sea Hero Quest, Cognitive Change Index and the Four Mountains Test

Test measure	Mean T1	Mean T2	Time × APOE (p)	Time × Sex (p)
VST				
Egocentric	11.02 ± 3.27	10.16 ± 3.29	.399	.245
Map drop error	07.53 ± 2.86	234.71 ± 97.21	.435	.543
Heading	11.68 ± 2.53	11.76 ± 2.50	.699	.882
CNP	00.48 ± 0.20	00.43 ± 0.11	**.050**	.835
SHQ				
Distance	4.081 ± .902	3.855 ± .612	.111	.852
Duration	4.964 ± 2.06	4.392 ± 1.31	.713	.599
CCI				
Memory	20.38 ± 6.71	20.11 ± 6.54	.227	.782
EF	11.57 ± 4.27	11.30 ± 4.03	**.028**	.905
4MT				
Total	09.76 ± 2.27	10.41 ± 2.18	.203	.446

Bold=p<0.05; SHQ, Sea Hero Quest; VST, Virtual Supermarket Test; CNP, Central Navigation Preference; CCI, Cognitive Change Index; EF, Executive function; 4MT, Four Mountains Test

Two measures showed an effect of time which suggests score instability across timepoints: VST central navigation preference and map drop error. This is attributable to re-retest effect (i.e. inconsistency across T1 and T2 test measures). Two measure also showed significant time × APOE interactions: VST central navigation preference and CCI self-report executive function. In the central navigation preference measure, the ε3ε3 group showed significantly worse performance over time (*T2* M=.465 ±.11; *T1* M=.562 ±.21; p=.02), while the ε3ε4 group showed no significant change over time (p=0.67). The ε3ε4 group showed significantly less concern on the executive function scale over time (*T1* M=12.78 ± 4.44; *T2* M=11.56 ± 3.74; p=.02), while the ε3ε3 group showed no significant change (p=.29).

Post-hoc reliability testing

As a final measure, we tested if APOE sensitive baseline measures were also sensitive at follow-up, adopting linear mixed effects models to account for inter-subject variability. Using a mixed model approach, with APOE and sex as a fixed effects and subject as the random effect, APOE predicted central navigation preference (t=-2.012, p=.03) with the ε3ε4 group (M=.465, SD=.11) displaying higher boundary-based place memory, than the ε3ε3 group (M=.402, SD=.09), consistent with the baseline effect (Appendices:

Supplementary figure 4.1). There was no main effect of APOE on egocentric navigation (t=-.852, p=.03) however. Despite reliability based on the ICC, sex did affect the egocentric navigation measure (t=-2.930, p= .01), with men (M=11.35 ± 3.38) outperforming women (M=9.06 ± 2.86) similar to the baseline performance. In SHQ, although the ε3 participants travelled a lesser distance, this was not significantly different from ε4 carriers T2 (see figure 4 for level 7 and level 8 visualisation; supplementary figure 4.2). Table 4.4 presents the mean scores on all VST and SHQ measures at T2. Please see Chapter 2 for baseline APOE effects on SHQ and Chapter 3 for baseline APOE effects on VST.

Table 4.4. Differences between the ε3ε3 carrier group and the ε3ε4 carrier group on Sea Hero Quest and Virtual Supermarket Test at re-test (T2)

Test measure	T2 Measure	ε3ε3 Mean (± SD)	ε3ε4 Mean (± SD)	*P* (t value)
SHQ	Distance level 6	.58 ± .16	.60 ± .11	.61 (t=0.26)
	Distance level 8	1.30 ± .26	1.42 ± .34	.17 (t=1.95)
	Distance level 11	1.89 ± 1.94	1.97 ± 2.03	.57 (t=0.31)
	*Distance level 7	.95 ± .31	1.01 ± .32	.45 (t=0.52)
	*Distance level 21	2.59 ± 1.1	2.72 ± .86	.69 (t=0.23)
VST (electronic version)	Egocentric	10.77 ± 3.23	9.50 ± 3.29	.14 (t=2.08)
	Map drop error	219.64 ± 92.33	246.41 ± 99.79	.09 (t=2.97)
	Heading direction	12.09 ± 2.55	11.28 ± 2.47	.23 (t=1.46)
	CNP	.465 ± .11	.402 ± .09	.03 (t=-2.01)

T2, Re-test; CNP, Central navigation preference; *SHQ levels newly introduced to the test battery at re-test.

Discussion

This study demonstrates the feasibility of implementing novel spatial navigation tests in upcoming RCTs as reliable and sensitive preclinical AD markers. Test-retest reliability was assessed in participants from Chapters 2 and 3, who underwent a re-test 18 months following baseline testing. Spatial navigation tests that were sensitive for preclinical AD, exhibited moderate test–re-test reliability in a nonclinical sample, with some scoring parameters being more reliable than others. Specifically, the CCI test–re-test reliability

correlation coefficients showed the highest test–re-test reliability while four of the navigation test measures showed moderate to high test–re-test reliability (VST egocentric orientation; VST heading direction; SHQ distance travelled and the 4MT total score). The remaining three metrics showed low test–re-test reliability (VST map drop error, VST boundary-based place memory and SHQ duration). Absolute performance was stable on six of the nine scoring parameters. In the other three measures (VST map drop error, VST boundary-based place memory and CCI executive function scale) individuals' mean scores significantly changed from the first to the second session or there was an interaction between timepoint and APOE.

For the VST map drop error parameter (a test of allocentric spatial memory), the individuals' mean scores changed significantly from the first to the second session. This was expected, as responses were recorded and scored differently at T1 and T2, explaining the poor stability across timepoints. The original allocentric measure used in T1 described by Tu and colleagues is sensitive but not specific for AD type dementia (Tu et al., 2017). Therefore, the scoring method was altered to capture more AD-sensitive drop placement error for allocentric memory of location responses. Although the mean drop error was larger in the ε4 carrier group compared to the non ε4-carrier group at T2 (which suggests more dispersed allocentric responses), this did not reach statistical significance.

The reliability of the VST boundary-based place memory and CCI executive function measures was dependent on the participant's genotype. On the place memory performance, while ε4 carriers remained stable across timepoints, ε3ε3 carriers performed worse at T2 compared to T1. This may indicate that participants actually use a different processing sequence at T2 and T1, due to changes made in the administration of the task measure from paper to computerized recording of the map location responses. Thus, the neural correlates of the test measure at T2 should be investigated to look for consistency with neural correlates at T1. Downstream analysis further showed that although the place memory measure appeared to be less sensitive to the APOE genotype at T2 compared to T1, ε4 carriers still displayed significantly lower place memory scores relative to non-carriers at T2. In terms of CCI executive function scale, there was instability across sessions with ε4 carriers reporting worse executive function concern at T2 compared to T1. Statistically

however, there was a high degree of association between participants test and re-test performance in the whole sample on both CCI parameters, suggesting this self-report scale may have utility in preclinical test batteries.

Despite different forms of VST administered at both timepoints, the egocentric orientation parameter demonstrated moderate-to-high test-re-test reliability, suggesting that this VST parameter translated well from the original form (at T1) to the fully electronic response form (at T2). The SHQ distance travelled measure also demonstrated moderate test-re-test reliability. However, post-hoc analysis showed that the effect of APOE on re-test performance was not replicated, suggesting score stability may not be entirely consistent across timepoints for VST egocentric orientation and SHQ distance travelled, despite high consistency across timepoints. This might be due to regression to the mean, which occurs when participants in the lowest quartile of cognitive performance at baseline improve more at re-test, compared to participants in the moderate to high quartile of cognitive performance. The APOE e4 effect on both these measures at baseline may then be partially driven by novelty effects such that, as a result of initial experience taking the test measure, the newness or novelty of that test disappears the second time, resulting in a small effect of APOE at re-test.

In similar cognitive studies, Goldberg and colleagues highlighted how practice/novelty effects reduce effect sizes at re-test and compromise the utility of preclinical AD test batteries to detect a signal of treatment effect or efficacy in randomized controlled trials (Goldberg et al., 2015). The smaller effect on central navigation preference measures (VST central preference and SHQ distance travelled) at re-test may also have a neural mechanistic explanation. Boundary correction that drives the effect as discussed in Chapter 2 is relevant in unfamiliar novel environments primarily (Hardcastle et al., 2015). Thus, at re-test, the novelty of the environment is lost, and thus grid cell organisations no longer require border cells input if there is repeated exposure to the same environment. This may explain why over both timepoints, the risk groups' grid code dependency on border cell input appears to lessen but not entirely dissipate.

Despite our best efforts to manage regression to the mean at T2 by careful selection of statistical methods and alternative forms of VST testing materials between timepoints, there are other statistical approaches to the problem of practice effects. For example, the reliable change index yields information on the number of participants in the sample who demonstrate improvement above and beyond practice. A confidence interval identifies the extent to which an individual participant would have to improve to demonstrate progress beyond a practice effect and beyond all reasonable doubt (Schatz and Ferris, 2013). Thus, this approach estimates the magnitude of change that exceeds the practice effect and could be explored in future studies.

Over the 18-month study period, we found very limited evidence of deteriorating cognition in the ε4 carrier group. This was expected as it takes up to a 12 years of amyloid accumulation for symptoms of prodromal AD or MCI to onset (Braak and Del Tredici, 2015; McKhann et al., 2011; Sperling et al., 2011). If AD pathology is indeed present in a proportion of midlife ε4 carriers who displayed disorientation at baseline, pathology would have not spread a significant amount throughout the 18-months. Our preliminary evidence does suggest that more boundary-based place memory on the VST predicts increasing SCC over the 18-month period in adult ε4 carriers only. This suggests that boundary-based place memory (described in Chapter 3) in genetically vulnerable individuals is predictive of worsening SCC. This is a significant finding as in cognitively normal individuals with elevated amyloid (aged 70 years) subjective cognitive complaints significantly predicts global cognitive decline over 4 years period (Amariglio et al., 2018). Future studies should examine whether APOE ε4, in combination with entorhinal-medicated disorientation, predicts dementia risk or prodromal onset in mid to late life adults.

In conclusion, the primary aim of this study was to establish the test-re-test reliability of a novel test battery as a sensitive diagnostic and treatment outcome measure for use in preclinical AD studies and randomised control trials. The secondary objective was to examine if a combination of biological (APOE) and novel cognitive (spatial disorientation) AD markers predict cognitive change over 18 months. While the self-reported cognitive decline test measure demonstrated the highest test re-test reliability, the novel VST egocentric orientation and SHQ distance travelled test measures also demonstrated

sufficient reliability, confirming their utility as a preclinical AD test. Boundary-based place memory may be indicative of worsening subjective memory decline in adults genetic at risk of AD, but its utility will need to be further investigated before a recommendation for use in clinical and research trials can be made.

Chapter 5: Low blood DHA concentration predicts better neurocognitive outcomes in at-risk Alzheimer's disease

Unpublished Paper

Introduction

Lifestyle changes may play a key role in the management of Alzheimer's disease (AD). For instance, lifestyle interventions include a number of preventative factors that an individual can engage in to lower their dementia and AD risk. These modifiable factors include cognitive training (to build cognitive reserve), neuroinflammatory reduction and adherence to a Mediterranean diet rich in docosahexaenoic acid (DHA) (Livingston et al., 2017) (See figure 5.1). The FINGER trial showed that a multidomain lifestyle intervention, similar to that shown in Figure 5.1, improved different cognitive domains, including processing speed, executive function and memory in elderly people (N=1,260). Importantly, the beneficial effect of diet, exercise, and vascular risk management was most notable in the APOE ε4 carrier group, suggesting that targeting these factors is an important preventative strategy for at-risk individuals (Rosenberg et al., 2018).

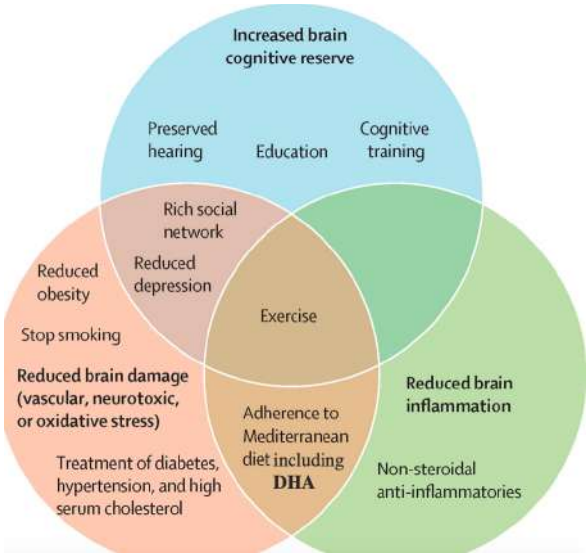


Figure 5.1 Potential lifestyle interventions for preventative strategies in Alzheimer's disease. Adopted from Livingston et al 2017.

Dietary intake is crucial to the maintenance of human health, in particular brain health. Higher long chain n–3 (ω-3) polyunsaturated fatty acids (LC ω-3 PUFA) helps to preserve memory in older age and lower the risk of developing AD (Ammann et al., 2017; Lim et al., 2006). The main dietary source of LC ω-3 PUFA is marine fish oils. The neuroprotective role of LC ω-3PUFA in old age was originally proposed based on rodent models showing that ω-3 PUFAs can reduce neuroinflammation, maintain synaptic plasticity and preserve the function of neuronal membranes that are fundamental to the conservation of healthy cognitive function (He et al., 2009; Yassine, 2017). Of special interest is DHA, a 22-carboxylic fatty acid that accounts for 30% to 40% of fatty acids in cortical grey matter. DHA is supplied to the brain from the systemic blood circulation. It is present in various lipid pools, including blood serum and erythrocytes (Figure 5.1) (Lacombe et al., 2018). While DHA is typically obtained from fish sources, it can also be synthesized in the liver from its shorter chain precursor alpha-linoleic acid (Domenichiello et al., 2015). The uptake of DHA in the brain has a range of structural benefits, as animal models showed (Calderon and Kim, 2004; Hu et al., 2010) beneficial effects of DHA on spatial navigation of the Morris water maze (He et al., 2009) in animals with and without amyloid deposition.

Rodent models show that DHA supplementation results in DHA accumulation in areas of the brain responsible for spatial navigation and memory, such as the hippocampus (Cutuli et al., 2016; Létondor et al., 2014; Xiao et al., 2005), where DHA is involved in the maintenance and restoration of neural membranes and facilitates functional interaction between neurons by modulating neurotransmission (Chung et al., 2008; Horrocks and Farooqui, 2004). In preclinical AD studies, long-term DHA supplementation in animals can reverse amyloid accumulation, protect against neuronal loss associated with AD pathology and improve overall navigation and spatial memory performance (He et al., 2009). Conversely, reduced ω-3PUFA levels lead to memory deficits and impaired hippocampal plasticity (Cutuli et al., 2016). Together, these findings suggest that DHA is beneficial in both normal and pathological ageing processes, particularly if DHA supplementation is

provided over a long period of time and in sufficiently high quality (Hooijmans et al., 2012; Y. P. Zhang et al., 2018).

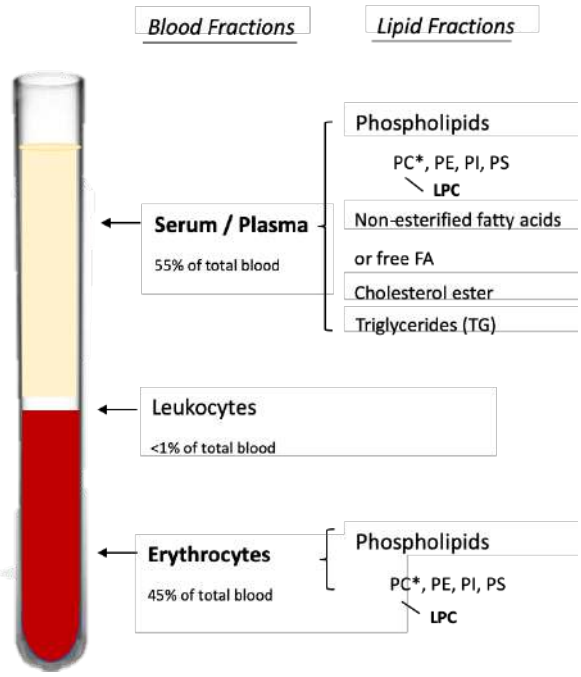


Figure 5.2 Bold: Blood fractions and lipid forms of interest. DHA measures include in this study are 1) Total DHA in serum 2) Total DHA in erythrocytes and 3) Total DHA in lysophosphatidylcholine form (LPC) form present in both serum and erythrocytes. As expected, a significant correlation between total DHA in erythrocytes and total LPC DHA was found ($r=.38$, $p<.001$). Total DHA in serum and total LPC DHA were not correlated ($r=.38$, $p<.001$), due to the presences of many other DHA forms in blood serum ($r=.05$, $p=.48$). LPC DHA is found in the blood serum and more predominately in the erythrocytes. DHA in the LPC form is considered the most important for the successful transport of DHA across the blood brain barrier (BBB) where it serve a neuroprotective mechanism in normal and pathology aging. LPC transport is not adversely affected by APOE ε4 presence, while the transport of DHA in other forms is negative effected by APOE ε4 presence
Abbreviations: Phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphoinositides (PI).

In human studies, the beneficial effects of DHA on brain health and cognition are less consistent. While high DHA concentration in human serum/plasma has been linked to a 47% reduction in the risk of developing all-cause dementia in the Framingham Heart Study (Schaefer et al., 2006), in a similar dementia-free Dutch cohort, dietary DHA intake was not associated with relative risk for AD (Devore et al., 2009). Danthiir et al., (2014) also found no associations between erythrocyte DHA concentration and cognitive performance. Self-

reported higher fish consumption actually predicted worse information processing speed in cognitively normal individuals (Danthiir et al., 2014). Moreover, in two RCTs involving 1043 healthy participants, no beneficial effect of DHA supplementation on cognitive function was observed (Sydenham E et al., 2012). Thus, many epidemiological investigations and randomized controlled trials (RCTs) have failed to replicate a beneficial effect of DHA on cognition or dementia risk (Danthiir et al., 2014; Devore et al., 2009; Kröger et al., 2009; Rogers et al., 2008) in both normal (Rogers et al., 2008; Witte et al., 2014) and pathologically ageing groups (Chiu et al., 2008; Quinn et al., 2010), despite strong the evidence for neurocognitive benefits proposed in animal models. The major studies investigating DHA and neurocognitive outcomes are summarized in Table 5.1.

There are several potential explanations for a lack of consistent evidence in human studies. Inconsistencies may be traced back to i) age effects, other methodological confounds whereby DHA is measured from ii) different blood fractions (e.g., plasma/serum or erythrocytes) and in iii) different AD populations (Otaegui-Arrazola et al., 2014). In the case of RCTs, supplementation is for relatively short periods of time of up to 6 months. Given that the half-life of brain DHA is over two years, it is likely that there was not enough time for DHA levels in the brain to increase sufficiently in order to affect the physiological processes in these shorter studies (Umhau et al., 2009). Another explanation links back to DHA transport system. DHA supplementation is typically administered in the form of triglycerides or cholesterol esters shown in Figure 5.2, which struggle to pass through the blood brain barrier (BBB) in humans. Long-chain phospholipids on the other hand are more efficient are passing the BBB and can obtained via dietary intake of fish (Lacombe et al., 2018) and not via supplementation. Thus, the form of ineffective brain DHA supplementation may explain failed RCT interventions to date.

Table 5.1 Major studies investigating DHA concentration in serum and erythrocytes and neurocognitive outcomes

Source	*Age*	*Cohort*	*DHA measure*	*Outcome measure*	*Power*	*Main findings*	*APOE effects*
Observational							

Barberger-Gateau et al., (2007)	65 years and over	CN	Weekly consumption of fish	Incident cases of dementia over 4 years	8,085	Fish consumption associated with a reduced risk of AD in non-carriers	Yes
Whalley et al (2008)	64–68 years	CN	Erythrocytes DHA	Cognition	120	DHA associated with cognitive benefits in non-carriers	Yes
Ammann et al., (2017)	65–80 years	CN	Erythrocytes DHA	Incident cases of dementia over 10 years	6,706 women	DHA associated with a significantly lower risk of dementia in APOE4 carriers and non-carriers	Yes
Tan et al., (2012)	67 ± 9 years	CN	Erythrocytes DHA	Cognition & brain volume	1,575	DHA associated with cognition and brain volume in *lowest* DHA quartile	No
Devore et al., (2009)	55 years and over	CN	Weekly consumption of fish	Incident cases of dementia over 9.5 years	5,395	Fish consumption and omega-3 PUFAs not associated with long-term dementia risk	No
Yassine eet al (2016)	67–88 years	CN	Serum DHA	Cognition, Hippocampal and entorhinal volume, Amyloid deposition	61	DHA associated with greater entorhinal and hippocampal volumes. DHA association with cognition modulated by APOE	Yes
RCT	*Age*	*Cohort, dosage*	*DHA measure*	*Outcome measure*	*Power*	*Main findings*	*APOE effects*
Quinn et al (2010)	76 (8.7)	MCI (18-mths f 2 g/d DHA)	Serum DHA	Cognition & total brain volume of	402	No overall effect on rate of cognitive and functional decline in patients. Faster rate of decline in APOE4	Yes
Stonehouse., et al (2013)	18–45 years	CN (6-mths/ 1.16 g DHA)	Erythrocytes DHA	Cognition	176	Episodic and working memory improved with DHA supplementation	No

Zhang et al., (2017)	65 years and over	MCI (12-mths/ 2g DHA	Serum DHA	Cognition & hippocampal volume	240	DHA associated with cognitive benefits and hippocampal volume	No
Zhang et al., (2018)	65 years and over	MCI (24-mths/ 2g DHA	Serum DHA	Cognition and amyloid beta	240	Daily DHA may improve cognition and change Aβ-mediated autophagy	No
Van de Rest., et al (2018)	65 years or older	CN (26 weeks/ 1,800 mg/d,	Serum DHA	Cognition	302	No significant changes on cognition irrespective of APOE	Yes
Andrieu et al., (2017)	70 years or older	CN (3 years/800 mg)	ERYTH ROCYT ES DHA	Cognition	1680	No significant effects on cognition over 3 years irrespective of APOE	Yes
Rogers et al., (2018)	18-70 years old	CN (26 weeks/1·5 g DHA)	LC DHA	Cognition	218	Negligible benefit LCPUFA on cognition or mood	No

Abbreviations: CN, cognitively normal; DHA, docosahexaenoic acid; APOE, apolipoprotein; PUFA, polyunsaturated fatty acids; LC, long chain; mths, months; RCT, Randomized clinical trial

The long-chain phospholipid form of DHA can be easily taken up in the brain and is contained in dietary fish. This DHA form may be a more promising method of DHA supplementation in future human trials. DHA in phospholipid form has a high conversion rate to lysophosphatidylcholine (LPC) DHA (see Figure 5.2). Patrick et al., (2019) suggests that LPC DHA is not only efficient at crossing the blood brain barrier (BBB), compared to other DHA forms, but that LPC DHA may also be immune to the negative effects of the apolipoprotein E (APOE) ε4 genotype on DHA BBB transport system (see Chouinard-Watkins and Plourde, 2014; Stonehouse et al., 2013) (Halliday et al., 2013; Nishitsuji et al., 2011; Patrick, 2019; Pontifex et al., 2018). The mechanism behind this APOE modulated response to phospholipid DHA are unknown.

The APOE gene is the main apolipoprotein modulating the transport of DHA through the BBB and within the wider central nervous system (Corder et al., 1993). Thus, the efficiency of DHA uptake to the brain depends on APOE status. The modulative role of APOE on DHA transport and uptake may also explain why fewer cognitive benefits are seen in APOE ε4 carriers compared to non-carriers. When DHA is supplemented in forms other than the easy-to-uptake phospholipid form, APOE4 carriers typically show fewer benefits than non-carriers despite both groups having similar levels of total DHA concentration in blood (Barberger-Gateau et al., 2007; Quinn et al., 2010; Whalley et al., 2008). Interestingly, some RCTs also suggest that APOEε4 carriers actually show more neurocognitive benefits as a result of supplementation of the easy-to-uptake phospholipid DHA than non-carriers (Patrick, 2019; Yassine et al., 2017). This finding lends support to the hypothesis that phospholipid DHA may be immune to the negative effects of the e4 genotype on DHA BBB transport and a preventative dietary strategy for individuals at high risk of AD.

Using a cross-sectional design, we include three different DHA measures: 1) total serum DHA (including DHA from phospholipids, triglycerides, cholesterol esters and free DHA 2) total erythrocyte DHA (including DHA primarily from phospholipids) and 3) total LPC DHA from both serum and erythrocytes (see fractions in figure 5.2). We measured the association of these DHA measures with neurocognitive outcome measures: i) hippocampal/entorhinal volumes and ii) egocentric navigation in three cohorts. Two were cognitively normal groups and one was a prodromal AD/mild cognitive impairment group (see Figure 5.3 Flowchart). We also examine a navigation performance as an outcome measure in one cognitively normal cohort. We propose that total serum DHA may be weakly associated with neurocognitive outcomes. In the case of total erythrocytes DHA and total LPC DHA, we hypothesis that APOE status would modulate a stronger association DHA levels and neurocognitive outcomes.

Methods

Participants

Three cohorts were investigated: 1) a small (n=46) and 2) a large (n=114) cognitively normal (CN) sample, as well as 3) a sample with prodromal AD (herein referred to as mild cognitive impairment group) (n=84); see Figure 5.3 flowchart). For a description of the recruitment and genotyping of the small CN cohort, please refer for to Chapter 2/3 (Mean Age=62; SD=0.6). 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. In the larger CN and Mild Cognitive Impairment (MCI) cohorts, participants were drawn from the ‘‘Cognitive Ageing, Nutrition and Neurogenesis [CANN]’’ study and recruitment took place across two research sites; the University of East Anglia (Norwich, UK) and the Swinburne University of Technology (Melbourne, Australia) (Irvine et al., 2018). CANN participants, aged 55-82 years (*CN cohort*: Mean Age=66; SD=0.6; *MCI cohort:* Mean Age=64; SD=0.6) underwent telephone screening, questionnaires, and an on-site screening visit. CANN participants then attended a clinical visit to undergo cognitive assessment and provide biological samples, including blood. The study obtained ethical approval from Bellberry Human Research Ethics Committee (Study ID 2015-03-227) and Swinburne University Human Research Ethics Committee (SHR Project 2015-208) for the Swinburne University of Technology site and the National Research Ethics Service Committee (Study ID 14/EE/0189) for the University of East Anglia site. All participants provided informed signed consent before participating.

Measures and materials

DHA analysis

In the small CN cohort, blood samples were collected immediately following the cognitive evaluation. DHA status was measured using a single drop of whole blood obtained via a finger prick collection kit (Faculty of Natural Sciences Institute of Aquaculture, University of Stirling). Blood samples were immobilised on a card and sent to the University of Stirling (Stirling, UK) for analysis. Please see Carboni et al., (2019) for a full description of

the Blood Spot PUFA analysis used to derive fatty acid concentrations (Carboni et al., 2019). In the larger CN and MCI CANN datasets, DHA status was measured from an overnight fasted blood sample as participants were part of a larger RCT. At the UEA site, 1 mL of blood was taken from ethylenediaminetetraacetic acid (EDTA) tubes and sent to the University of Stirling for analysis. At the Melbourne site 6 mL of blood was collected into a lithium heparin tube and sent to a commercial pathology laboratory for analysis of FA status (Australian Clinical Laboratories, Australia). Separation and quantification of fatty acids was done by gas-liquid chromatography (GLC; ThermoFisher Trace, Hemel Hempstead, UK) for participant samples across both databases. Details of the procedure FA measurement analysis has been previously published (Irvine et al., 2018).

APOE genotyping

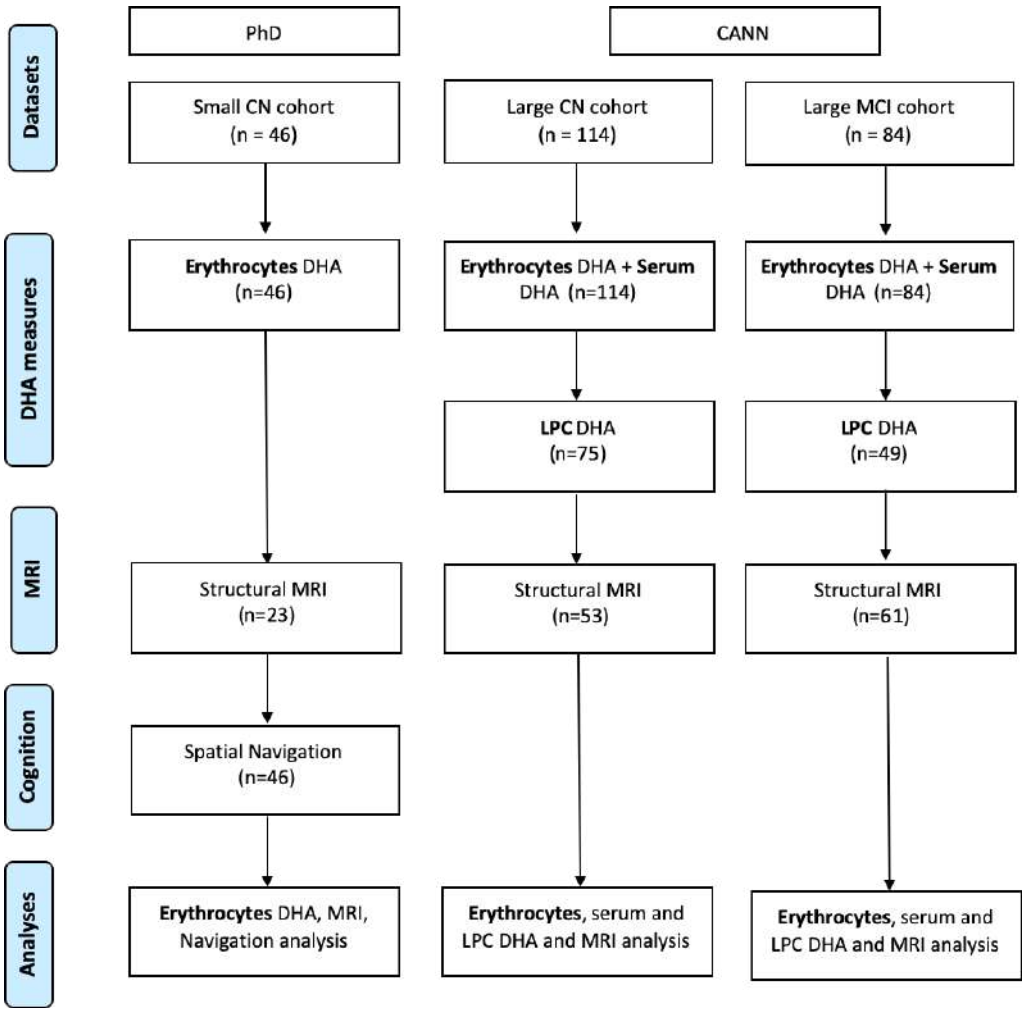
For *APOE* genotyping in cohort one, DNA was collected using a Darcon tip buccal swab (LE11 5RG; Fisher Scientific). Buccal swabs were refrigerated at 2–4 °C until DNA was extracted using the QIAGEN QIAamp DNA Mini Kit (M15 6SH; QIAGEN). In cohort two and three from CANN, the buffy layer (containing the white cell layer) was collected from the EDTA tube (BD Biosciences, San Diego, CA, USA) and genomic DNA was extracted using a DNA extraction kit (Qiagen, Hildenberg, Germany), following the manufacturer's instructions. In both cohorts, DNA was quantified by analyzing 2-µL aliquots of each extraction on a QUBIT 3.0 fluorometer (LE11 5RG; Fisher Scientific). High concentrations of DHA were diluted using MilliQ water (Millipore, Billerica, MA, USA) to achieve a concentration in the range of 1–10 ng. *APOE* genotype was determined by two real-time reverse transcription polymerase chain reaction (RT-PCR) single nucleotide polymorphism (SNP) genotyping assays, to determine the 112 T/C (rs429358) *APOE4* polymorphism and 158 C/T (rs7412) *APOE2* polymorphism per the Applied Biosystems (Foster City, CA, USA) TaqMan SNP Genotyping Assays protocol (2010). SNP identification were done on a 7500 Fast Real-Time PCR system (Applied Biosystems).

Food frequency questionnaire - retrospective recall

In all cohorts, self-report dietary intake was recorded using the EPIC Food Frequency Questionnaire (FFQ), a widely used and well-validated instrument to assess habitual diet over the previous year (Kroke et al., 1999). The questionnaire is based on 9-point scales

ranging from *never or less than once per month* to *6+ per day*. Oily fish intake was compared across genetic groups.

Figure 5.3. Flowchart of participant groups and outcome measures available



Abbreviations: CN, cognitively normal; CANN, cognitive ageing, nutrition and neurogenesis; DHA, docosahexaenoic acid; MRI, magnetic resonance imaging; LPC, lysophosphatidylcholine.

Structural magnetic resonance imaging

The raw MRI data were first converted to the NIFTI format (https://github.com/rordenlab/dcm2niix). Structural MRI acquisition is detailed in Chapter 3 and was acquired for all individuals in the small CN cohort (PhD). In CANN, approximately half of the participants at the two centres participated in structural MRI (N=115). At the Norwich site, the T1-weighted image is obtained using a three-dimensional

fast spoiled gradient echo brain volume imaging (FSPGR-BRAVO) sequence in the sagittal orientation, repetition time (TR)/ echo time (TE)/inversion time (TI) = 7,040/2.612/900 ms, 0.9 mm isotropic resolution, field of view (FOV) = 230 × 230 mm, number of excitations (NEX) = 0.5. The T2-weighted structural image is obtained using a three-dimensional CUBE fluid-attenuated inversion recovery (FLAIR) sequence in the sagittal orientation, TR/TE/TI = 6,000/125.8/1,863 ms, 0.9 × 0.9 in-plane resolution, with a slice thickness of 1 mm and 178 slices, FOV = 230 × 230 mm.

At the Melbourne site, the T1-weighted structural image is acquired using a three-dimensional magnetization prepared rapid gradient echo (MPRAGE) sequence, TR/TE/TI = 1,900/2.32/900 ms, 0.9 mm isotropic resolution, FOV = 230 × 230 mm, generalized autocalibrating partial parallel acquisition (GRAPPA), acceleration factor of 2. The T2-weighted structural image is obtained using a three-dimensional sampling perfection with application optimized contrasts using different flip angle evolution (SPACE) FLAIR sequences in the sagittal orientation, TR/TE/TI = 6,000/388/2,200 ms, 1.0 mm isotropic resolution, FOV = 256 × 256 mm, GRAPPA acceleration factor of 2. The SUT site also collected a high-resolution in-plane thick-slab T2-weighted structural scan in a partial volume centered on the temporal lobes. This T2-weighted structural image is acquired using an interleaved turbo spin echo sequence in the coronal orientation perpendicular to the long axis of the hippocampus, TR/TE = 4,230/109 ms, 0.5 × 0.5 in-plane resolution, 30 slices, slice thickness = 2.5 mm, FOV = 224 × 224 mm, bandwidth = 159 Hz/pixel, echo spacing = 13.7 ms, turbo factor = 19. These images are normalized using a pre-scan, as implemented by the scanner manufacturer.

Cognitive measures

Spatial navigation was measured using the Virtual Supermarket test (Tu et al., 2015) in the small CN cohort (PhD; see chapter 3 for test description). Spatial navigation data was not available from the CANN cohorts and thus cognition was not examined in the large CN cohort or the large MCI cohort.

Statistical Approach

Across all three cohorts, cortical surface reconstruction and hippocampal volumetric segmentation was performed with FreeSurfer image analysis suite (version 5.1) (http://freesurfer.net/) to acquire hippocampal subfield (see figure 2) and entorhinal cortex volumes. The technical details are described here (Iglesias et al., 2016). The processing stream includes motion correction, removal of non-brain tissue, automated Talairach transformation, intensity correction, volumetric segmentation, cortical surface reconstruction, and parcellation. The data were analyzed using RStudio (version 1.0.153). Simple linear regression models were fitted to examine the role of 1) DHA blood concentrations (total serum, total erythrocytes DHA and total LPC DHA) on hippocampal/entorhinal brain volume. Statistical models were specified and run using RStudio (version 1.0.153). Age, sex, total fat intake and socioeconomic status were controlled for in all cohort's analysis. Education was used as an index of socioeconomic status (Yassine et al., 2016). Given the larger samples, we had more degrees of freedom for greater number of comparisons. In this case the linear regression model was specified with DHA and APOE (including an interaction term) and the model was adjusted for age, sex, education and test centre (AUS/UK). All linear models examining MRI volumetric outcomes were also adjusted for total intracranial volume. Standardized residuals were extracted and plotted against fitted values to examine underlining assumption of normal distribution and heteroscedasticity before interpretation of the results. In the case of significant APOE*DHA interactions, post-hoc linear models were specified with APOE ε4 carriers (ε3ε4) and non-carriers (ε3ε3) separately. All statistical tests are two-tailed: $P < 0.05$. Example of a simple linear model testing DHA on brain volume:

$$Y_i = \beta_1 X_{i,1} + \ldots + \beta_n X_{i,n} + \varepsilon_i$$

where Y is a hippocampal subfield or entorhinal cortex volume. X (regression coefficients) are predictors including DHA, age, sex, socioeconomic status and total intracranial volume. ε_i is the normally distributed residual error. Partial eta squared (n_p^2) was used as a measure of effect size and was derived from lmSupport package in R (https://cran.r-project.org/web/packages/lmSupport). n_p^2 is the ratio of variance associated with an effect plus that effect and its associated error variance ($n_p^2 = SS_{effect} / SS_{effect} + SS_{error}$).

Results

Intact cognition in the small CN cohort was pre-confirmed at screening via telephone and test score on the Addenbrookes cognitive evaluation and the Rey-Osterrieth complex figure test (Matias-Guiu et al., 2017; Shin et al., 2006) (see Table 5.2 for Participant characteristics). In the CANN cohorts, CN and MCI were pre-classified with a modified telephone interview for cognitive status and Montreal Cognitive Assessment tool (Nasreddine, 2005). Details of the classification criteria have been previously detailed by Irvine et al 2018 (Irvine et al., 2018). The participant characteristics of both groups in cohort two are summarized in Table 5.3.

Association of DHA with MRI measures

Contra to predictions, in the small CN cohort, total erythrocyte DHA was not linearly related to any of the brain MRI measure including entorhinal cortex volume and hippocampal subfield volumes (see Supplementary Figure 5.1 for visual illustration of subfields), controlling for age, sex, total intracranial volume, socioeconomic status (data not shown). No interaction between total erythrocyte DHA and APOE was found (data not shown).

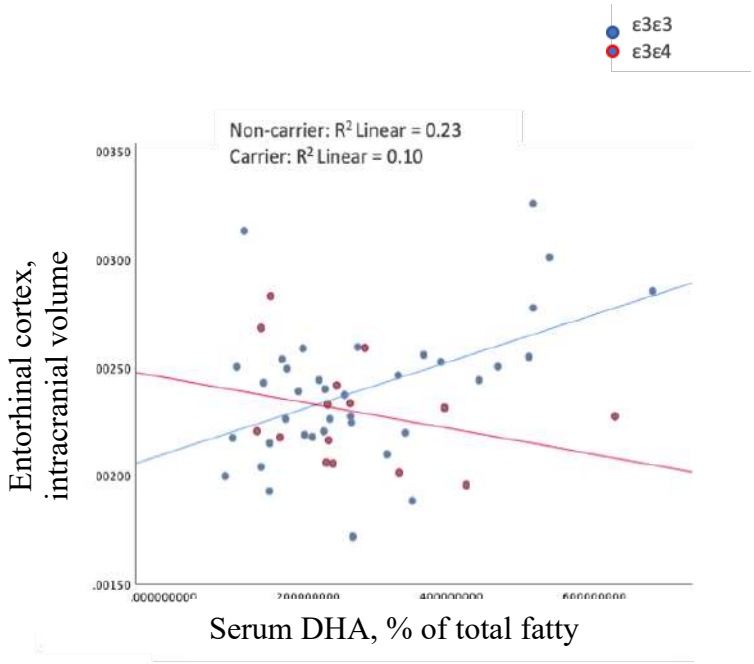


Figure 5.4 Effects of serum DHA on entorhinal cortex volume in cognitively normal individuals (n=53).

Table 5.2 Participant characteristics for the small cognitively normal (CN) cohort

	Mean (SD)			
		APOE genotype		
Characteristic	Total (n=47)	ε4 carriers (n=23)	non carriers (n=24)	*P* value
Age, y	61.30 (5.6)	60.82 (5.7)	61.75 (5.7)	.58
Sex (male/female)	15/31	4/18	11/13	
Blood pressure (missing=4)				
Not medicated	36	18	18	.61
Medicated	7	3	4	.10
ACE total	94 (3.7)	93 (5.4)	94 (2.1)	.55
Rey				
Copy	32 (2.8)	32 (2.8)	32 (2.9)	.55
Recall	19 (5.8)	17 (5.2)	20 (6.1)	.08
Cholesterol (missing=4)				
Not medicated	39	19	20	.55
Medicated	4	2	2	.81
Erythrocytes DHA (% of total FA)	2.64 (.71)	2.76 (.73)	2.52 (.62)	.25
Education, y	14.4 (5.4)	14.5 (2.9)	14.4 (3.6)	.72
Hippocampal volume (ratio of total intracranial volume, n=28)	.0046 (.00044)	.0046 (.00047)	.0047 (.00040)	.56
Entorhinal volume (ratio of total intracranial volume, n=28)	.0025 (.00031)	.0025 (.00038)	.0025 (.00028)	.84

Abbreviations: ACE, Addenbrookes cognitive examination; Rey, Rey–Osterrieth Complex Figure; FAs, fatty acids; Data are presented as mean (SD) for normally distributed data or median (IQR) for nonnormal distributions. The 2 groups were compared by an independent sample t test. 25/47 participants had hippocampal and entorhinal cortex measures.

In the second CN cohort, greater serum DHA (t= 2.15, p=0.03, n_p^2=0.10) but not total erythrocyte DHA (t=0.190, p=0.85) predicted greater right entorhinal volume. There was a significant interaction between total serum DHA and APOE genotype on left entorhinal volume (t=-2.20, p=0.03, n_p^2=0.10) and a weaker, non-significant interaction between serum DHA and APOE on right entorhinal volume (t=-2.00, p=0.05, n_p^2= 0.09), wherein the positive association between serum DHA concentration and entorhinal volume was significant in non-carriers (*left* t=2.101, p=0.04; *right* t=2.283, p=0.02) and not significant in carriers (Figure 5.4).

Table 5.3 Participant characteristics for the large CN and MCI cohort

	Mean (SD)			
		APOE genotype		
Characteristic				
Subjective memory impairment	Total (n=114)	ε4 carriers (n=38)	non carriers (n=76)	*P* value
Age, y	65.35	64.56 (5.9)	64.63(6.9)	.82
Sex (male/female)	52/62	18/20	34/42	
Blood pressure, mm Hg	68.44	68.72 (9.1)	68.32 (8.6)	
Systolic	133 (17)	121 (23)	126 (14)	.61
Diastolic	77 (8.8)	72 (7.3)	75 (7.8)	.10
BMI (missing n=3)	26.99 (4.29)	27.38 (4.61)	26.78 (3.84)	.85
MoCA total	26 (1.7)	26.5 (1.7)	27.6 (1.8)	.19
Fasting blood glucose level, mmol/l	5.19 (.55)	5.16 (.54)	5.21 (.57)	.57
Cholesterol mmol/l				
Total	5.19 (1.0)	5.12 (.92)	5.22 (1.1)	.55
HDL	1.41 (4.5)	1.39 (5.2)	1.44 (3.9)	.81
TG level mmol/l	1.21 (0.5)	1.13 (47)	1.17 (.37)	.78
BDNF	18958 (4676)	19359 (4702)	18144 (4589)	.13
Oily fish portion intake	0.675 (.657)	0.592 (0.556)	0.789 (0.788)	.06
Erythroytes DHA (% of total FA)	3.81 (.82)	3.79 (.95)	3.79 (.72)	.65
Serum DHA	.305 (.24)	.308 (.15)	.299 (.16)	.72
LPC DHA (% of total FA)	.072 (.7)	.068 (.08)	.079 (.06)	.91
Education, y	14 (3.2)	14 (2.5)	14 (3.6)	.72
Hippocampal volume (ratio of total intracranial volume, n=112)	.00449 (.000385)	.00443 (.000468)	.00454 (.000356)	.36
Entorhinal volume (ratio of total intracranial volume, n=112)	.00235 (.00036)	.00229 (.00006)	.00239 (.00005)	.56
Mild cognitive impairment	Total (n=84)	ε4 carriers (n=27)	non carriers (n=57)	*P* value
Age, y	66.33	66.67 (6.9)	66.00 (6.4)	.82
Sex (male/female)	33/51	11/16	22/35	
Blood pressure, mm Hg		67.71 (9.3)	71.39 (8.9)	
Systolic	125 (12.1)	127.82 (12.4)	124.81 (11.5)	.61
Diastolic	75.99 (7.6)	76.06 (8.1)	75.45 (6.8)	.10
BMI (missing n=3)	26.99 (4.29)	26.21 (4.48)	27.31 (4.43)	.85

MoCA total	26 (2.12)	25.56 (2.1)	26.21 (2.2)	.09
Fasting blood glucose level, mmol/l	5.44 (1.11)	5.32 (.55)	5.48 (1.28)	.57
Cholesterol mmol/l				
Total	5.20 (1.1)	5.5 (1.02)	5.07 (1.12)	.55
HDL	1.44 (49)	1.49 (3.5)	1.45 (4.3)	.81
TG level mmol/l	1.21 (0.5)	1.35 (.79)	1.24 (.72)	.78
BDNF	18962 (4756)	19072 (4848)	18697 (4642)	
Oily fish portion intake	0.626 (0.599)	0.500 (0.416)	0.745 (0.726)	.07
Erythroytes DHA (% of total FA)	3.81 (.82)	3.92 (.66)	3.79 (.89)	.65
Serum DHA	.331 (.15)	.338 (.14)	.328 (.17)	.72
LPC DHA (% of total FA)	.072 (.7)	.078 (.08)	.061 (.08)	.91
Education, y	14 (3.4)	14 (4.1)	14 (3.6)	.72
Hippocampal volume (ratio of total intracranial volume, n=112)	.00444 (.00047)	.00434 (.00063)	00441 (.00052)	.56
Entorhinal volume (ratio of total intracranial volume, n=112)	.00216 (.00038)	.00209 (.00038)	.00229 (.00038)	.07

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); MoCA, Montreal Cognitive Assessment; Mm/pL micromoles per liter; HDL, high-density lipoprotein; TG, triglyceride; BDNF, Brain-derived neurotrophic factor; LPC, Lysophosphatidylcholine; FAs, fatty acids; Data are presented as mean (SD) for normally distributed data or median (IQR) for nonnormal distributions. The 2 groups were compared by an independent sample t test. 112/198 participants had hippocampal and entorhinal cortex measure

We then tested the association of total LPC DHA with brain MRI measures. *Note LPC DHA data was only available in the UEA site (and not the SUI site), reducing the sample size (N=45). LPC DHA predicted right hippocampal volume (t=2.51, p=0.02, n_p^2=0.23), with a significant LPC DHA ×APOE interaction (t=-2.51, p=0.02 n_p^2= 0.23). Post-hoc analysis further investigating the LPC DHA ×APOE interaction showed DHA and right hippocampal volume were not linearly related in non-carriers and were inversely related in carriers (Figure 5.5A). Strongest LPC DHA associations were found in CA1 (t=3.44, p=0.002, n_p^2=3.84) and the molecular layer subfields (t=2.619, p=0.012, n_p^2=2.65) of the hippocampus. There was no effect of LPC DHA on the entorhinal cortex volume.

Validation of DHA with MRI associations in a mild cognitive impairment group

We then examined volumetric outcomes and LPC DHA associations in MCI cohort. There was a significant interaction effect between LPC DHA and APOE on right hippocampal

volume (t=-3.793, p<0.001, n_p^2=0.26) and on the left hippocampal volume (t=-2.783, p=0.007, n_p^2=0.24), in a direction consistent with the large CN group.

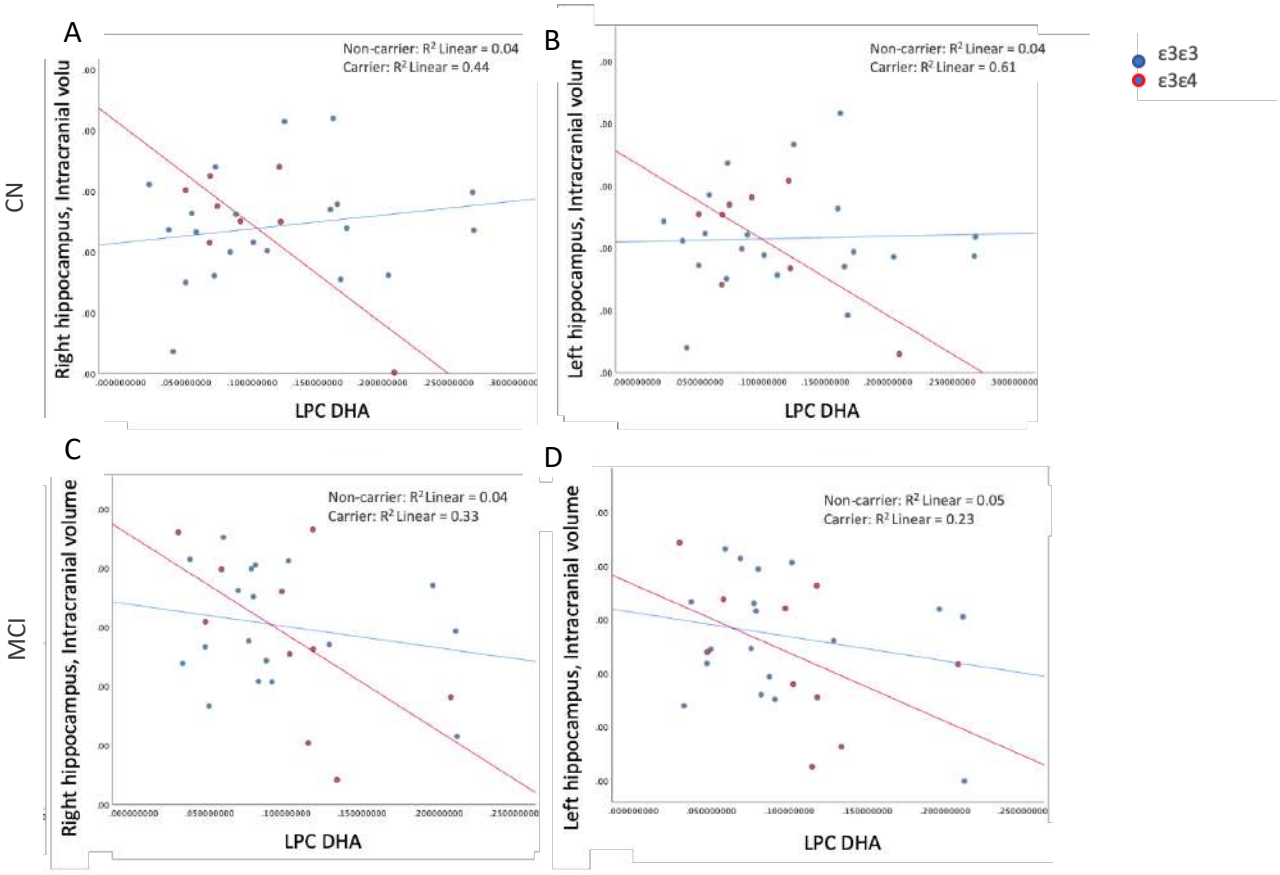


Figure 5.5 DHA in lysophosphatidylcholine form (LPC DHA) was significantly associated with **A |** right hippocampal volume not **B |** left hippocampal volume in cognitively intact ε3ε4 carriers. There was an association between LPC DHA and **C |** right hippocampal and **D |** left hippocampal volume in the ε3ε4 MCI carriers. There were no significant associations between LPC DHA and brain volume in the cognitively intact or the MCI ε3ε3 carriers.

When independent models were specified to examine the effect of LPC DHA in each group (APOE ε3ε3 and APOE ε3ε4) as a fixed effect, a significant inverse relationship between LPC DHA and hippocampal volumes was found in ε4 carriers (*right hippocampus t*= -2.665, p= 0.02; *left hippocampus* t=-2.361, p=0.03; Figure 5.5 C and D) but not in non-carriers, consistent with the pattern of effect in the MCI free CN group. No relationships between LPC DHA and entorhinal volume were found in the MCI or CN group.

Association of DHA with spatial navigation

Given the strong association between DHA in blood fractions containing long chain phospholipids and hippocampal volume, we were motivated to examine the relationship between total erythrocyte DHA and navigation performance. Navigation data was only available in the smaller CN cohort (n=46), and thus any findings represent proof of concept. There was no main effect of erythrocyte DHA on navigation performance, but there was a significant interaction between erythrocyte DHA and APOE on egocentric navigation (t=-2.01, p=0.05). Again, separate models were then specified for both genotype groups, revealing that DHA was inversely associated with egocentric performance (*b*=-.834, t=-3.445, p=0.003) in the ε4 carriers, but not in non-carriers (*b*=.31, t=1.487, p=.153), adjusting for age, sex and oily fish intake (see figure 5.6). That is, higher erythrocyte DHA levels predicted worse performance on the egocentric navigation in the ε4 carrier group. As a final step, we examined the association between egocentric performance and hippocampal subfields and entorhinal volumes to elucidate the neural correlates of the task measure. Using Pearson's partial correlation, we found a significant association between performance and subfield volumes: cornu ammonis (CA1) 1 (r=.47, p=.017) and the subiculum (r=.53, p=.006), covarying for total intracranial volume. We also found a significant weaker association between egocentric performance and entorhinal volume (r=.41, p=.03).

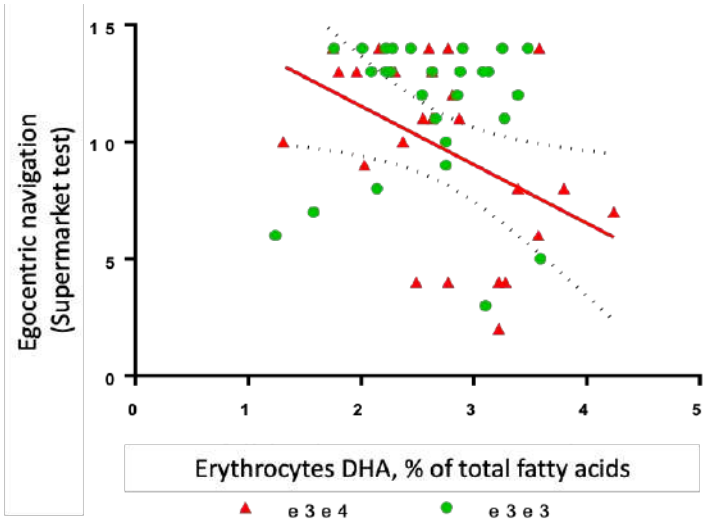


Figure 5.6 DHA in erythrocytes was inversely related to egocentric performance on the Virtual Supermarket Test

Discussion

In this study, we tested the effect of three DHA measures on neurocognitive health: 1) total serum DHA (including DHA from phospholipids, triglycerides, cholesterol esters, free DHA 2) total erythrocytes DHA (including DHA primarily from phospholipids) and 3) total LPC DHA from both serum and erythrocytes. Our results suggest that APOE modulates the relationship between phospholipid DHA and hippocampal brain volume in normal aging and prodromal AD. There was also preliminary evidence that APOE modulates the association between phospholipid DHA and hippocampal-dependant egocentric navigation performance. The modulative effect showed that decreased phospholipid DHA predicts higher hippocampal volume and better egocentric performance in APOE ε4 carriers only. Reduced levels of blood phospholipid DHA may be a sign of higher uptake of DHA in the brain, which in turn could help compensate for APOE ε4 presence by protecting hippocampal volume and navigation ability. We also report a positive association between total serum DHA (which includes many non-phospholipid forms of DHA) and entorhinal volume consistent with a major study by Yassine and colleagues (2016). This effect was strongest in the APOE ε3 carriers and adds support to the theory that APOE ε4 carriers cannot gain from the neuro-beneficial effects of non-phospholipid DHA form, most of which are found in the blood serum.

Serum DHA that includes all forms of DHA such as non-phospholipid forms with poor BBB penetration was positively associated with entorhinal volume and was modulated by APOE status. This positive association is consistent with reports of a similar pattern of association with higher serum DHA levels predicting greater entorhinal volumes (Yassine et al., 2016) Samieri et al., (2011) also found a positive relationship between serum DHA and subsequent slower cognitive decline over 7 years (Samieri et al., 2011). A 3-year retrospective study further showed that hippocampal volumes were also maintained in 800 older adults on DHA supplementation who had either normal cognition, MCI or AD. Consistent with the association between serum DHA and left entorhinal volume found here, Daiello and colleagues also found that in APOE ε4 noncarriers only, fish oil supplementation (rich in all forms of DHA like that found in serum DHA) was associated with preservation of brain volume including the hippocampus (Daiello et al., 2015).

Based on this pattern of association, it would seem contradictory that lower levels of the easy-to-uptake phospholipid enriched DHA would predict greater hippocampal volumes (particularly in the CA1 subfield) and better hippocampal-mediated navigation performance. However, unlike the positive association between plasma DHA and brain volume, the negative association between DHA and hippocampal volume outcomes was only found in the APOE ε4 carriers. Preservation of AD vulnerable brain volumes specifically in APOE ε4 carriers who have low levels of DHA in blood, is most likely due to an APOE ε4 dependant compensatory response. According to Yassine et al (2017), DHA's incorporation from blood into the entorhinal and hippocampal brain regions is up to 34% stronger in ε4 carriers compared to ε3ε3 carriers (Yassine et al., 2017). This likely results in a lower concentration of DHA circulating in the blood fractions of ε4 carriers with preserved brain volume, as shown in this study.

This inverse relationship between blood DHA and hippocampal volume was strongest when DHA was measured in the phospholipid form. According to Patrick et al (2019), the transport of LPC DHA, across the BBB in APOE ε4 carriers is intact, while the transport of DHA from triglycerides and cholesterol esters usually found in serum is impaired. This may then explain why only LPC DHA is linked to increased hippocampal brain volume and why there is no association between serum DHA levels and hippocampal brain volume in APOE ε4 carriers. Moreover, the LPC DHA effect on brain volume in CN APOE ε4 carriers was strong than that seen in the MCI APOE ε4 carriers. This may reflect less DHA update in the ε3ε4 MCI group due to significant hippocampal atrophy. If APOE ε4 uptake is indeed higher due to an increased requirement of DHA to maintain brain volume, than this may help explain why the inverse association between LPC DHA and hippocampal brain volume is stronger in CN ε4 carriers who have brain tissue to maintain unlike the MCI ε4 carriers who have already lost substantial amounts of hippocampal brain tissue.

Higher phospholipid DHA was associated with i) less hippocampal brain volume and ii) poor hippocampal-mediated navigation performance. Both these outcomes are characteristics of incipient AD (Coughlan et al., 2018b; Laczó et al., 2009). This suggests higher circulating phospholipid enriched DHA may represent an AD biomarker in normal cognition and mild cognitive impairment APOE ε4 carriers. In contrast, lower plasma DHA appears to be a biomarker for entorhinal volume loss in cognitively normal non ε4 carriers

only. Thus, APOE appears to modulate which class of lipid DHA will be neuroprotective in normal and prodromal aging.

This was the first study to link phospholipid DHA from erythrocytes to navigation performance in humans, although the sample size is a limitation. This association was dependent on APOE genotype, whereby lower concentrations of phospholipid enriched DHA from erythrocytes signified better egocentric navigation performance in carriers, again suggesting that greater circulating DHA marks depleted brain DHA in adult ε4 carriers. The neural substrates underlying egocentric performance included the CA1 and subiculum subfield volumes and LPC DHA was also linked to CA1 volumes. Mouse models show that increased brain DHA significantly enhances CA1 neurogenesis and neuritogenesis, accompanied with improved navigation performance on the Morris Water Maze. If our theory that depleted LPC DHA marks increased brain DHA in APOE ε4 carriers particularly in the hippocampus is accurate, then our results support the hypothesis in the hippocampus may provide a basis for the beneficial effect of DHA on spatial performance (He et al., 2009)

The findings produced in our study have some limitations. The relationships between LPC DHA (a specific form of phospholipid DHA) and navigation performance could not be directly measured. LPC DHA and brain MRI analysis included 38 non-carriers and 15 carriers in the cognitively intact adults, and 31 non-carriers and 18 carriers in the MCI adults and thus require additional validation. The MCI group had hippocampal and entorhinal atrophy, as well as episodic memory impairments compared to the dementia free group (see Appendices: supplementary results). Nevertheless, we cannot say for certain that MCI is of the AD type, given that amyloid or tau pathology could not be measured. The key strength of the study lies in the different blood and lipid fractions investigated which will inform nutrition precision in upcoming dietary intervention trials. It will also help establish measurable DHA levels as AD biomarkers.

Future studies should examine if DHA, when supplemented in the phospholipid form over a sufficient period and in sufficient quantities, can indeed have neuroprotective effects on brain structure and cognition. Further, the compensatory mechanism proposed here, which suggest a greater metabolic demand for DHA in the brain from blood among APOE ε4

carriers, might be less effective in older populations (Yassine et al., 2016). This would predispose older APOE ε4 carriers (e.g., 70+ years and above) to reduced uptake of brain DHA and increased risk of cognitive decline and dementia, as seen in other studies. This is consistent with the average age of cognitive decline due to AD in APOE ε4 carriers. For a decade before symptom onset, the brain is engaging in compensatory activities to preserve cognition and brain volume despite the presence of Aβ. Further, future investigations should examine if LPC DHA could be used as a marker for amyloid levels in cognitive normal and prodromal ε4 cohorts.

In conclusion, the present results show that higher serum DHA concentration predicts greater entorhinal volume, one of the first regions affected by AD pathology in the preclinical stage of disease (Braak and Del Tredici, 2015; Howett et al., 2019). It also provides novel evidence that among adults genetically at risk of AD, higher phospholipid DHA levels predicts poorer neurocognitive outcomes that are characteristic of incipient AD.

Chapter 6:

General Discussion

Summary

The primary goal of this thesis was to establish if navigation deficits precede the onset of verbal and non-verbal episodic memory deficits. The secondary goal was to examine if the neural correlates of preclinical navigation deficits are consistent with the spread of Alzheimer's disease (AD) pathology. Finally, we move beyond investigations of neurocognitive diagnostic tools, and towards prevention; examining if marine fish oils help preserve AD vulnerable brain regions in the preclinical stage of disease. Chapter one synthesizes pre-existing evidence that spatial disorientation is a sensitive and specific test of prodromal and clinical AD. Chapter two showed that individuals with preclinical AD without memory deficits show boundary-based navigation patterns on the SHQ game and

that these individuals also differ from population level benchmark data, generated via the public SHQ game. In chapter three, boundary-based place memory impairments (which coincided with subjective cognitive concerns) in preclinical adults were found using the virtual supermarket task. Functional connectivity between the EC and the PCC underpinned the cognitive phenotype comprised of boundary-based navigation and memory concern in the preclinical group. Chapter four comprised of an 18-month test-re-test reliability study and confirmed moderate reliability of the navigation parameters to detect at-genetic-risk AD at re-test. The final experimental chapter, Chapter five, suggests that low DHA blood concentration may indicate preserved hippocampal volume and egocentric function in those with an APOE ε4 genotype. Figure 6.1 provides a conceptual overview of the key findings.

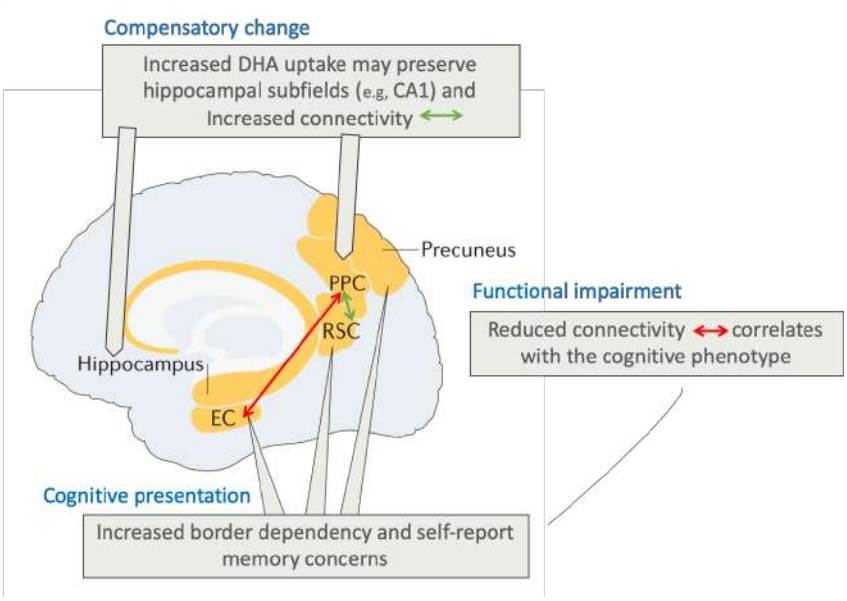


Figure 6.1. The neurocognitive profile of preclinical disease encapsulates several neural and compensatory changes including boundary-based navigation and boundary-based place memory, as well as increased neural activity and DHA uptake from blood to brain. These mechanisms mean symptomology remains dormant for up to a decade after pathological insults appear in the brain. Reduced functional connectivity between the entorhinal cortex and the PCC is a neural correlate of the neurocognitive changes in preclinical AD. Figure adapted from Coughlan et al., 2018.

Towards preclinical diagnosis

The neurocognitive profile of preclinical Alzheimer's disease

The overarching goal of this thesis was to establish if navigation deficits precede the onset of episodic memory deficits using tests typically used in a clinical setting. The findings from Chapters 2 and 3 show that spatial disorientation and co-morbid subjective memory concerns are present in the absence of verbal and non-verbal episodic memory deficits and that these abnormalities should be considered diagnostically relevant to preclinical AD. Disorientation and subjective memory concerns correlated with reduced connectivity between the EC and the PCC. This pattern of functional abnormality resembles the spread of disease as described by Braak and Del Tredici (2015) and Heidi et al., (2018), and lends further credibility to spatial navigation as a reliable first marker of AD. Further attention should be paid to whether subjective memory concerns appear before or after spatial disorientation during the preclinical stage of disease (Braak and Del Tredici, 2015; Jacobs et al., 2018).

Subtle spatial disorientation was captured using novel virtual reality cognitive tests and presented as i) boundary driven navigation strategies during path integration and boundary-driven place memory following path integration and ii) egocentric orientation inaccuracies immediately following path integration. These findings dovetail those reported by Kunz and colleagues (2015), who report a similar profile of behavioural and functional disturbances in APOE ε4 carriers, which they linked to the entorhinal cortex (Kunz et al., 2015a). On the other hand, egocentric orientation inaccuracies have not been previously documented in the preclinical literature, although egocentric cells can also be found in the mEC. Although Tu et al., (2015) noted that egocentric performance partially relies on the volumetric integrity of the RSC in AD patients who have significant hippocampal and entorhinal atrophy, the neural substrate for egocentric orientation in preclinical AD may indeed rely on the functional gradient of the hippocampal-entorhinal axis, as the structural integrity of these two regions is still preserved in early disease (Tu et al., 2015).

Additional virtual reality navigation tasks tap into AD vulnerable entorhinal-hippocampal function. These include the human Morris Water Maze (Laczó et al., 2014), the virtual path

integration task (Howett et al., 2019) and the spatial memory task (Doeller et al., 2008), which are sensitive to prodromal AD or preclinical AD. The virtual path integration task predicts CSF amyloid-b and total tau in MCI individuals and the human Morris Water Maze is currently being used to assess navigation abnormalities in preclinical individuals with pathological AD biomarkers. The spatial memory task is perhaps the most similar to the thesis test battery and identifies boundary-based navigation in genetically vulnerable individuals, although the task length (64 trials) reduces its clinical capacity. The spatial navigation tasks mentioned above and included in this thesis, may be a useful tool for the early detection of AD. Their role in future diagnostic criteria will be discussed in more detail in the upcoming sections.

A neuro-mechanistic model underlying the spatial phenotype of preclinical Alzheimer's disease

On a cellular level, the pattern of spatial disorientation in the preclinical group can be well explained using a neural attractor-network model presented by Hardcastle and colleagues (2015), which provides electrophysiological evidence that abnormal functional activity in the medial-entorhinal cortex leads to border corrective navigation behaviour. The boundary correction model suggests that grid cells (responsible for coding self-motion) accumulate error over time and distance travelled and that these errors must be re-set by encounters with environmental boundaries. Border cells in the hippocampus fire close to environmental boundaries and provide externally generated input that can correct grid-cell coding errors, stabilise place fields and facilitate self-localisation and successful path integration (Hardcastle et al., 2015). In turn, the border cell input results in a boundary-based navigation or place memory preference as discovered in Chapter 2 and 3 respectively. A recent study supports such neuro-behavioural inferences and shows that removal of border cell input via absent boundary cues results in impaired path integration in a human preclinical AD (Bierbrauer et al., 2019). Boundary correction crucially relies on the intactness of the hippocampus, as hippocampal lesion patients are unable to utilities boundary-based place memory as a compensatory mechanism during path integration (Vikbladh et al., 2019). Therefore, while boundary-based navigation is found in preclinical individuals, patient groups with hippocampal atrophy (e.g., amnestic MCI) likely will not show this behavioural compensatory mechanism. Of course, the proposed neuro-

mechanistic model for preclinical disorientation pertaining to defected EC function and compensatory hippocampal function is not complete. We still do not know the role of the PCC, if any, in the boundary corrective model. Perimeter (or annulus cells) located in the cingulate cortex apparently fire near all environmental boundaries like border cells. Grieve and Jeffery suggest that these cells also stimulate another cell group that fires only in the centre environmental, perhaps forming a precursor to boundary cells (Grieves and Jeffery, 2017). Further Bierbrauer et al (2019), very recently showed that the RSC is involved in the recruitment of boundary corrective navigation. Understanding the role of cells in cingulate cortex to entorhinal grid cell dysfunction is an important next step in completing the neuro-mechanistic picture of spatial disorientation in preclinical AD (Figure 6.2).

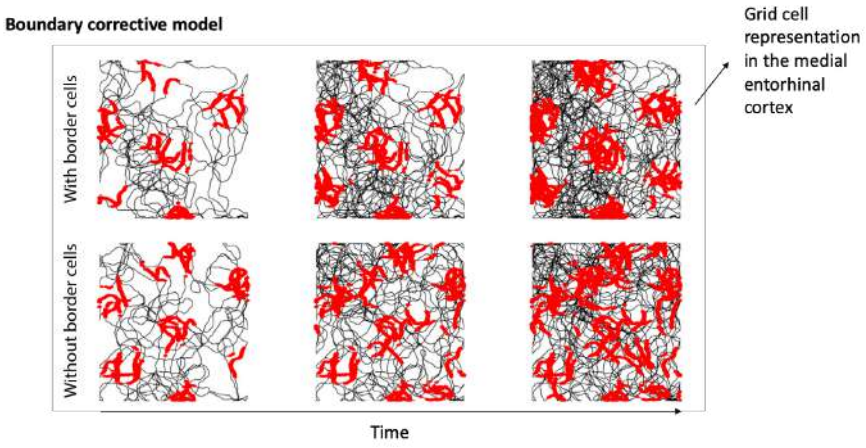


Figure 6.2. Rodent electrophysiological representation of grid cells with and without border cell input reflecting the spatial phenotype of preclinical AD. Figure adapted from Hardcastle et al., 2015.

Supporting the neuro-mechanistic model underlying the spatial phenotype of preclinical disease presented in this thesis, the border effect attenuated when novelty of the environment was lost, as shown in chapter four. Hardcastle states that the boundary correction model is particularly relevant in *unfamiliar* novel environments and thus, the familiarity of the environmental tasks at re-test should result in less boundary navigation preference at re-test, consistent with the results in chapter four (Hardcastle et al., 2015). This effect somewhat, but not entirely, dilutes the sensitivity of the novel VR navigation tasks. In terms of the future clinical use of the novel tasks, attention should be paid to novelty effects and whether they can be overcome in future testing. This would lend further

creditability to the measures as treatment outcome tools; going beyond ‘one time only’ diagnostic tests.

Sex effects on the spatial phenotype and prevalence of preclinical Alzheimer’s disease

Navigation differences driven by demographic factors, such as sex, influence variability in spatial ability irrespective of age or nationality (Coutrot et al., 2018). In Chapters 2 and 3, we found that different aspects of human spatial navigation are sensitive to preclinical AD and to sex. Allocentric memory and heading direction performance were influenced by a participant’s sex, corroborating current notions that men outperform women on cognitive map formation. On the other hand, boundary driven navigation and place memory were exclusively influenced by genetic risk, supporting Kunz et al (2015)’s findings, who first theorized that non-risk controls outperform high-risk APOE4 carriers on processes that relates to the boundary correction model present above (see figure 6.3). Thus, sex may not play an important role in diagnostics if clinicians can correctly dissociate between female sex-related shortcomings from preclinical AD-related shortcomings on navigation (Figure 6.3). In terms of egocentric navigation, genetic risk also influenced performance and there was a trend towards a significant sex effect found in chapter three. Chapter two and the global navigation study by Coutrot et al., (2018) suggest that egocentric navigation in SHQ is influenced by sex, so whether sex needs to be accounted for when interpreting AD-related egocentric impairment needs future investigation.

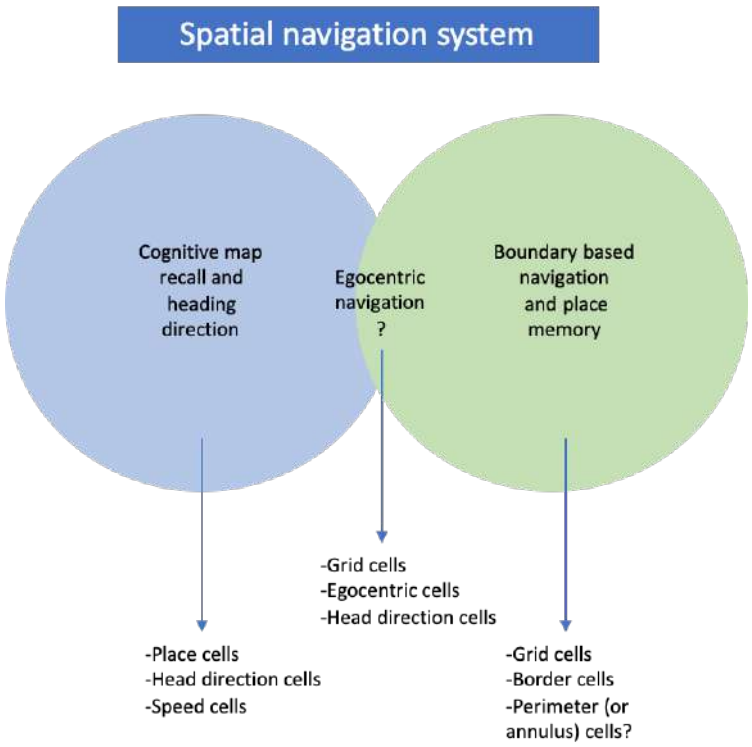


Figure 6.3. Variance in the spatial navigation system accounted for by sex (*blue*) and variance accounted for by preclinical disease (*green*). Sex accounts for variance dependent on more age-related processes and pathological insults account for variance that remains relatively unaffected by age-related processes.

The relevance of sex effects on navigation performance may also be applied to AD prevalence. Neuroimaging studies show that cerebral blood flow and functional connectivity is higher for men in the visual and motor cortices. This may explain a male advantage on spatial navigation tasks that employ visual-motor function as it appears to be exercised more in men than in women throughout the lifespan (Gur et al., 1995). Moreover, men are believed to favour hippocampal-entorhinal mediated allocentric navigation processes during navigation over egocentric parietal-mediated process favoured by women (Moffat, 2009). These preferences may provide a neuroprotective service for hippocampal-entorhinal brain areas in men, which subsequently allow men to retain cognitive function better than women, despite similar pathological insults in the brain of both sexes (Barnes et al., 2005). In this thesis, sex did not modulate the effect of genetic vulnerability on navigation performance, although in other larger studies, the APOE ε4 allele is associated with more hippocampal pathology, functional connectivity changes, cortical thinning, and

memory impairments in women compared with men along the AD continuum (Farrer, 1997; Ferretti et al., 2018). Thus, sex differences in brain structure and function may facilitate a higher threshold of AD tolerance in men, and a superior navigation ability, irrespective of other demographic factors, such as age, nationality or genetic components. This then holds important questions regarding the effectiveness of targeted spatial cognitive training in younger at-risk adults. If lifelong navigation strategies do lead to more AD resilience in men, than this begs the questions: can increasing entorhinal-hippocampal navigation proficiency via training serve a neuroprotective mechanism in preclinical AD? Cognitive training is a specific interventional approach designed to address difficulties associated with cognitive decline in dementia, but it is not widely used in clinical practice despite the urgent need to retain functioning in early to late AD (BaharFuchs et al., 2013).

Overdiagnosis of preclinical Alzheimer's disease: Identification modalities and pitfalls

Most navigation studies in cohorts of patients with genetic risk factors for AD have been conducted with APOE genotyped individuals, due to the association between the APOE ε4 allele and AD risk. However, there are other means of identifying a preclinical cohort, which include cerebrospinal fluid evaluation and positron emission tomography scans as mentioned in chapter one, as well as additional genetic biomarkers such as the polygenic risk score. Each modality picks up on preclinical disease, either by direct measurement of AD pathology in the case of the two former examples, or indirect inference of AD pathology via established correlations with brain pathology as in the case for the latter example. For instance, polygenic risk scores comprise of 31 single nucleotide polymorphisms. Using polygenic approach, it's possible to accurately (82%) predict which individuals with MCI will convert to clinical AD (Chaudhury et al., 2019). Polygenic risk scores can also predict amyloid accumulation and entorhinal cortex volume loss in MCI and preclinical AD, irrespective of APOE status (Tan et al., 2019). Thus, means of identifying at-risk individuals are varied. Future research must explore which combination of spatial phenotypic markers, alongside these genotypic and biological markers, will lead to an accurate prediction of conversion from preclinical to AD dementia. This is crucial as in 2017, 36 million adults 50 years or older from the US (more than 30% of the US population) had elevated levels of brain amyloid based on a PET scan but had no cognitive

impairment. Although each individual with elevated amyloid levels meets the criteria for preclinical AD, only 31% of women and 23% of men with elevated amyloid will go on to develop dementia based on estimation of lifetime risks using biomarkers for preclinical disease (Brookmeyer and Abdalla, 2018). Treatment for even half of the 36 million adults would lead to health care expenditures of more than $100 billion per annum and would result in administering treatment to those who do not require it (Langa and Burke, 2019). Incorporating spatial navigation and genetic screening tools to separate preclinical individuals with a higher probability of dementia from those with a lower probability of dementia may alleviate this financial burden. For example, epidemiological studies have shown that on average 47% of APOEε4 carriers convert to clinical AD by the age of 76 (Liu et al., 2013). Thus, if we thus assume that a navigational deficit is a result of underlying preclinical AD pathology and not the just the presence of the APOE ε4 gene, we would assume that about half of the ε4 carriers from this thesis would show the AD related spatial phenotype. This is consistent with the number of preclinical individuals that exhibit spatial disorientation on the Sea Hero Quest game at detailed in chapter two and thus lends further support to the utility of the novel cognitive tasks for usage in clinical or medical settings.

Beyond diagnosis

Docosahexaenoic acid as a neuroprotective agent in preclinical Alzheimer's disease

Non-pharmaceutical compounds have received considerable attention as ways to slow neurodegeneration since current pharmaceutical treatments fail to slow AD progression and instead work to alleviate symptoms. This thesis presents a proof of concept study, pertaining to the effectiveness of marine fish-based fatty acids, namely DHA, to preserve entorhinal and hippocampal brain volume in genetically vulnerable individuals. Chapter six offers novel evidence to suggest that genetically vulnerable adults with preserved hippocampal brain volume and intact navigation may absorb more DHA from the blood to the brain where it promotes neurogenesis in the hippocampus (particularly in the CA1 subfield), compared to non-genetically at-risk adults with similar hippocampal preservation.

Previous studies suggest that low blood DHA concentration is indicative of preclinical pathology, since low blood DHA levels are indicative of low intakes, low DHA availability and thus low uptake to the brain (Tan et al., 2012). Based on the findings in this thesis, an alternative theory is plausible: Low blood concentration is a result of increased brain uptake that is stimulated by preclinical pathology. Thus, low blood concentration may reflect a beneficial compensatory mechanism, as opposed to a causal mechanism that drives pathological process in the brain. Additional explanations also exist. It could be that lower blood DHA in APOE ε4 carriers is due to greater DHA oxidation (which has been shown) or there are simply greater DHA uptake in adipose tissue for storage in APOE ε4 carriers. All three mechanisms are plausible and warrant further investigation.

Behaviourally, the findings support the pre-existing hypothesis that the hippocampus provides a basis for the beneficial effect of DHA on spatial performance, which was first proposed in a rodent model offered by (He et al., 2009). This beneficial effect may also work on the cellular efficiency of the hippocampal-entorhinal system including the boundary correction model discussed earlier. DHA was linked to egocentric performance, suggesting the DHA may also influence AD vulnerable egocentric cells in the medial entorhinal cortex.

In terms of the therapeutic utility of DHA in preclinical AD, the form of DHA supplementation in future trials is important, as phospholipid DHA appears to share a closer relationship with brain volume in genetically vulnerable individuals. Further research should examine the influence of demographic factors, such as sex and nationality. Although sex is already considered in many research studies (Fisk et al., 2018; Minihane et al., 2000; Schaefer et al., 2006; Vauzour et al., 2017), nationality has not been investigated due to the increased data collection burdens. Nationality largely determines dietary patterns and may be partially explaining differences in worldwide dementia rates. Thus, lifelong interactions with total dietary intake and DHA (in addition to other variables discussed above, including APOE and sex) may help to explain inconsistency in nutritional literature.

Bench to bedside translation

This thesis offers a rationale for the further investigation into how blood DHA, brain DHA and preclinical disease relate. The most prudent next step, however, is to include a

navigation test in future diagnostic and treatment (nutritional and pharmaceutical) studies. The Virtual Supermarket task is now used in the by the European Prevention for Alzheimer disease and by the PREVENT study to develop conversion markers in preclinical individuals (Ritchie et al., 2016, 2010). Further, both Sea Hero Quest and the Virtual Supermarket Task will be used in the deep and frequent phenotyping study, to examine the relationship between task performance and CSF biomarkers and PET imaging biomarkers (Koychev et al., 2019). This will produce a more comprehensive assessment of preclinical AD, extending beyond APOE genetic risk. In terms of immediate bench to bedside translation, the demenTia Research And Care Clinic (TRACC https://www.uea.ac.uk/health-sciences/research/projects/tracc) will make immediate use of the novel spatial tasks, where they are currently being used to distinguish early AD from other dementia types. Moreover, the task may be incorporated in centres such as the Brain Health Centre (https://oxfordhealthbrc.nihr.ac.uk/help-us-develop-oxfords-new-brain-health-centre), which aims to offer patients access to the most up to date diagnostic tools, after initial referral from a GP based on memory concerns. Although the contribution of spatial navigation may identify incipient AD earlier, like any novel addition to diagnostics, caution not to abandon standard protocol is important. Gold standard episodic memory tests may be used in conjunction with novel navigation tasks and self-report complaints until evidence accumulates to suggest navigations tools detect disease earlier than episodic memory tests. Further, it is important to investigate if a combination of cognitive assessment methods will together offer higher sensitivity for future conversion to clinical AD states. Clearly, a body of research evidence is required before amendments to preclinical diagnostic criteria can happen. Finally, in the interest of global utility and across culture validity, large-scale studies across nations should be carried out to determine if navigation tests are indeed independent of language and background, which would then facilitate the accurate comparison of results across clinical trials in the UK (e.g. PREVENT; Ritchie et al., 2010) and Finland (e.g. FINGER; Ngandu et al., 2015), for example.

Methodological considerations and future research recommendations

While limitations of each experimental design were discussed in the preceding chapters, some overarching limitations should be addressed. This thesis offers evidence in preclinical

AD defined by the APOE genotype, cognitive symptoms are present in otherwise healthy adults, thus holding the promise of early detection (Coughlan et al., 2019; Grilli et al., 2018; Kunz et al., 2015b; Schoemaker et al., 2017). However, the biggest limitation of this experimental approach is that it remains unclear if this neurocognitive model is simply a product of the effect of APOE on neural development and cognition in midlife adults, or if this model truly reflects the biological presence of 'preclinical' neuropathology (Belloy et al., 2019; Jack et al., 2018). Future work should build on the neuro-mechanistic model presented here and test the accuracy of the proposed preclinical model to determine which individuals meet the National Institute on Aging-Alzheimer's Association staging criteria for preclinical AD. This will require the usage of cerebrospinal fluid and PET imaging biomarkers, as well as upcoming newly developed blood evaluations for tau pathology. Such projects should also account for disease modulators, such as cardiovascular risk factors and risk genes beyond APOE (e.g., the polygenic risk score), which may provide additional insight into the specificity of spatial disorientation and subjective cognitive complaints for the early detection of disease. Future research should see SHQ cut-offs established for clinicians who adopted the upcoming medical version of SHQ, which will be based on the demographic population data. The cut off values for the VST rely on future data collection and standardisation on the electronic format. Together this will provide a personalised risk profile such as that proposed by Scheltens and colleagues (Scheltens et al., 2016).

Implications for preclinical, prodromal and clinical Alzheimer's disease

While the main promise of spatial navigation tasks lies in early detection, navigation tools may implicate research over the entire disease life course. For example, despite failures to identify an accepted cognitive marker for preclinical neuropathology, recent rodent models showed that spatial memory deficits occur before mature tau tangles spread beyond the entorhinal cortex (Fu et al., 2017). This is a key finding in the search for a link between cognitive markers and early AD pathology, and future investigations will continue to examine the association between spatial deficits and pathological spread from the medial temporal to the highly connected parietal lobes. Future RCTs will also examine a number of questions pertaining to: 1) the degree to which navigation impairments predict amyloid and or tau pathology? 2) which factors (such as personal sensation of cognitive decline)

alongside navigation impairment, determine when a patient will develop dementia? 3) do all amyloid-positive patients suffer the same pattern of navigation decline? 4) can protective factors, such as dietary intervention, slow down navigation and memory decline in preclinical/prodromal AD? (Scheltens et al., 2016). Among already demented individuals, spatial navigation tools may crucially be used to inform dementia-related missing incidents, which are highly prevalent but still poorly understood. Future studies will examine if (and what) spatial layouts and navigational cues may prevent a missing incident (Puthusseryppady et al., 2019). This will hold important implications for the safeguarding of AD patients and for the well-being of their families.

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Appendices

Supplementary Information Chapter 2

The effect of homozygote genotype status on navigation in SHQ

We tested the effect of genotype on Wayfinding Distance and duration with the inclusion of the e4e4 carriers, which resulted in strong effect of genotype on both Wayfinding Distance (b=0.36,p<.0001) and wayfinding duration (b=0.56, p<.0001). We then tested whether the inclusion of the e4e4 group would diminish the effect of sex on duration however the effect sex still held although the coefficient dropped by .49 to .27. Although we found no interactive effects between genotype and sex in the base cohort on Wayfinding Distance or duration, an interactive effect between age and genotype did approach significance for Wayfinding Distance (b=-0.021, p=0.08) and duration (b=-0.05, p=0.06) with the inclusion of the e4/e4 group.

Supplementary Table 2.2. Mixed effects of APOE genotype and demographic factors on SHQ performance including a homozygote group

Demographic characteristics of the genetic groups from the lab cohort. No difference on any of the above listed characteristics were detected with Persons Chi square confirmed analysis (family history *p*=9.18, education .695, occupation .438).

SHQ Variable	Fixed Effect	b coefficient	Std. Error	F value	*p* value
Wayfinding Distance					
Model 2 (including ε4ε4 carriers)	APOE**	0.355	0.044	69.51	<.0001
	Sex	0.010	0.097	0.06	0.802
	Age	0.004	0.008	0.24	0.62
Wayfinding Duration					
Model 2 (including ε4ε4 carriers)	APOE**	0.562	0.099	36.90	<.0001

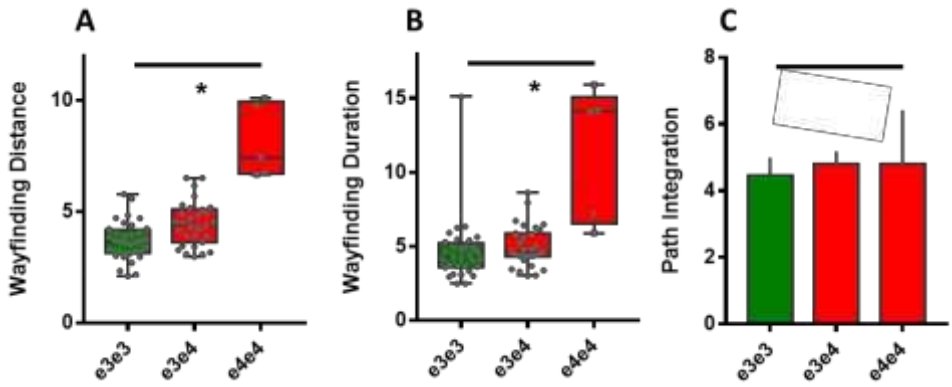
	Sex*	0.271	0.220	3.45	0.061
	Age	0.026	0.018	0.03	0.863
Path Integration					
Model 1 (excluding ε4ε4 carriers)	APOE	-0.094	0.122	0.005	0.943
	Sex*	-0.418	0.223	7.558	0.00

Supplementary Table 2.3 Mixed effect model for sex and age on SHQ performance in population-based sample.

Linear Mixed Effects	n	Fixed Effect	b coefficient	Std. Error	t value	p value
Wayfinding Distance	27308	Sex	0.076	0.001	40.993	**<.0005**
	27308	Age	0.005	0.001	30.775	**<.0005**
Wayfinding Duration	27308	Sex	0.051	0.001	21.70	**<.0005**
	27308	Age	0.004	0.002	21.03	**<.0005**
Path integration	27308	Sex	-0.059	0.002	-29.883	**<.0005**
	27308	Age	-0.003	0.002	-15.361	**<.0005**

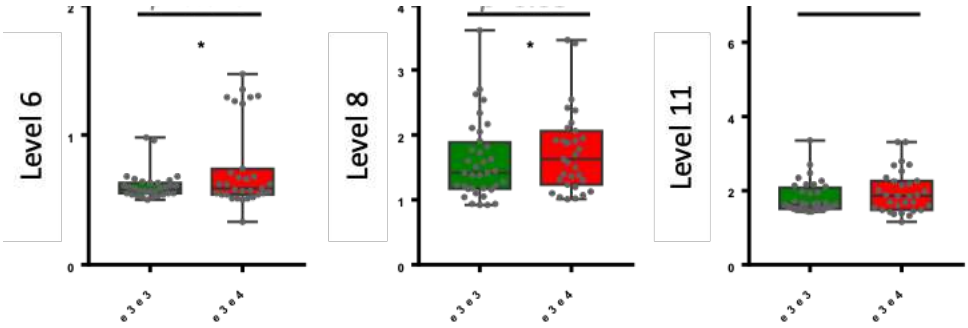
Linear mixed models applied to the population-based data illustrates a greater effect of sex relative to age on wayfinding levels and Path Integration

Supplementary Figure 2.1



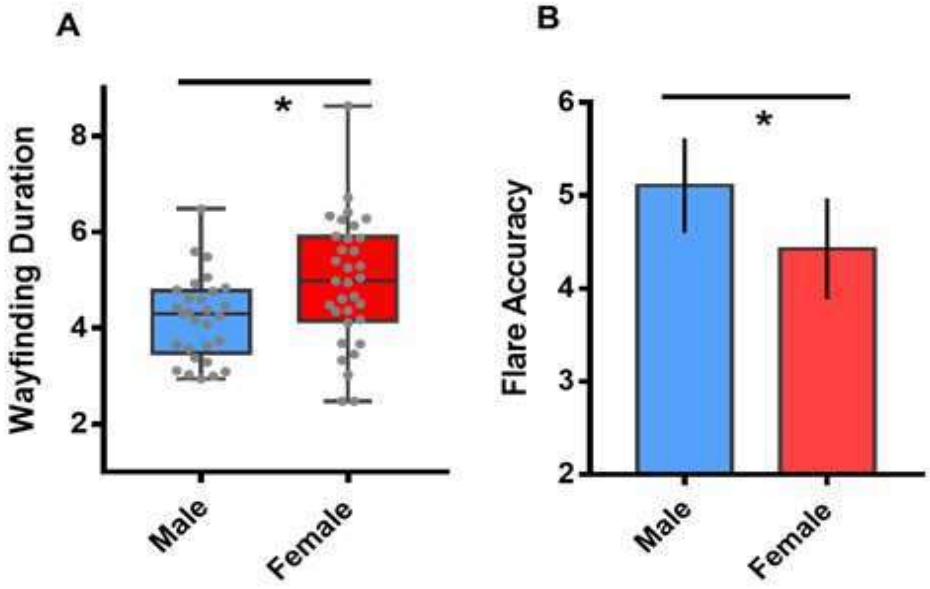
Using mixed effects models, a significant effect of homozygote APOE carriers' status on **A** wayfinding distance and **B** wayfinding duration was detected. No significant effect on **C** path integration was found.

Supplementary Figure 2.2



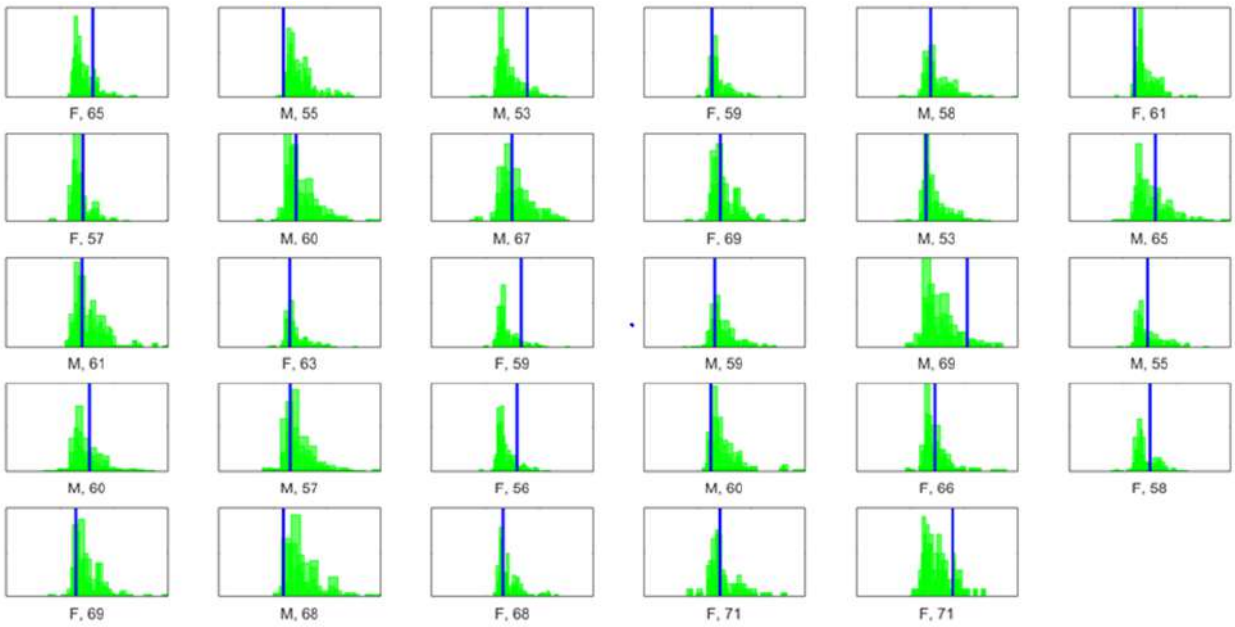
Main effect of APOE genotype on wayfinding levels 6 and 8. No significant effect of genotype on wayfinding level 11.

Supplementary Figure 2.3



A Main effect of sex on duration to complete wayfinding levels suggested that male participants completed wayfinding levels in less time via the boat acceleration function than female participants. **B** Male participants performed significantly better than female participants when required to integrate newly acquired allocentric information with egocentric viewpoint-based cues on flare accuracy levels.

Supplementary Figure 2.4



Each e3e3 carrier score (blue line) on SHQ distance plotted against the normal distribution of scores from an age-sex-education matched sub-population of the benchmark dataset (green histogram). Wayfinding distance scores are on the x axis and frequency of the benchmark population on the y axis. Sex is represented by M = male, F = female sex. Age is illustrated under each distribution right of sex.

Supplementary Information Chapter 3

Pre-processing structural and functional MR images.

MRI data were pre-processed using a pipeline written in bash, which incorporated several tools from the FMRIB Software Library (FSL, Oxford; Smith *et al*., 2004). In brief, we reoriented the structural image to Montreal Neurological Institute (MNI) space, applied the automatic 'robustfov' algorithm to the T_2w FLAIR and T_1w data to remove neck voxels, and then we used FLIRT (Jenkinson et al., 2002) to register the T_2w FLAIR to the T_1w, moved the FLAIR mask to the T_1w space, and used 'fslmaths' to apply the FLAIR mask to the T_1w. The 'BET' algorithm (Smith, 2002) was applied to remove any non-brain structures in the T_1w data and the whole brain was segmented into white matter, grey matter, and cerebrospinal fluid (CSF) using FAST v4.0 (Zhang et al., 2001). Using 'fslstats' we performed cranial volume estimation for grey and white matter across the whole image, in mm^3, to be used as a covariate in downstream fMRI analysis.

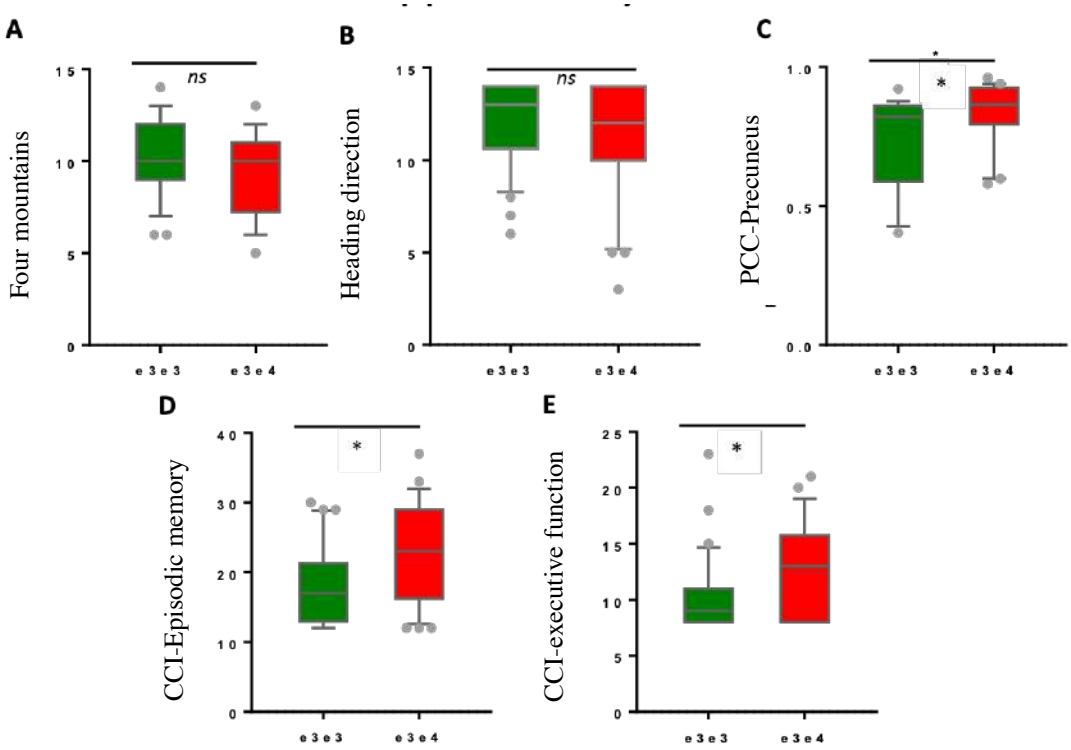
Functional image pro-processing was conducted with 'MELODIC' (Multivariate Exploratory Linear Optimized Decomposition into Independent Component, Beckmann, 2012) to remove most types of artefacts such as motion, physiology and scanner. Briefly, this included removal of the first 5 volumes (to account for signal steady-state transition and T_1w equilibration), slice timing correction, and head motion correction. To spatially normalise the functional EPI image, the T_1w images were used to register the functional data to their corresponding anatomical images, and the resulting aligned T_1w dataset was transformed into MNI space. Functional images were resampled to $2 \times 2 \times 2$ mm^3 voxels and spatially smoothed using a 3-mm full-width half-maximum (FWHM) Gaussian kernel. To correct for low frequency drifts, we applied temporal high-pass filtering (100 s or 0.01 Hz). Two trained members of the research team cleaned the data manually and, in accordance with standard guidelines (Bieterbosch et al., 2017), regressed out noise components using 'fsl_regfilt'. Two labellers were chosen in the interest of inter-labeller consistency. Given the modest sample size, automated approaches such as FIX and AROMA were not appropriate.

Supplementary Table 3.1. The effect of APOE on regional brain volume

	Mean (SD)	*P value*
Right entorhinal		
ε3ε3	1872.8 (355.5)	*ns*
ε3ε4	1674.4 (274.9)	
Left entorhinal		
ε3ε3	1935.0 (326.0)	*ns*
ε3ε4	1975.2 (398.5)	
Left PCC		
ε3ε3	2889.8 (328.3)	*ns*
ε3ε4	3087.1 (508.7)	
Right PCC		
ε3ε3	3000.1 (447.1)	*ns*
ε3ε4	3016.8 (407.2)	
Right precuneus		
ε3ε3	9645.8 (909.9)	*ns*
ε3ε4	9875.0 (1307.6)	
Left precuneus		
ε3ε3	9334.2 (818.8)	*ns*
ε3ε4	9317.8 (1067.2)	
Right hippo		
ε3ε3	3502.9 (325.5)	*ns*
ε3ε4	3483.4 (422.5)	
Left hippo		
ε3ε3	3416.7 (358.0)	*ns*
ε3ε4	3367.8 (390.1)	

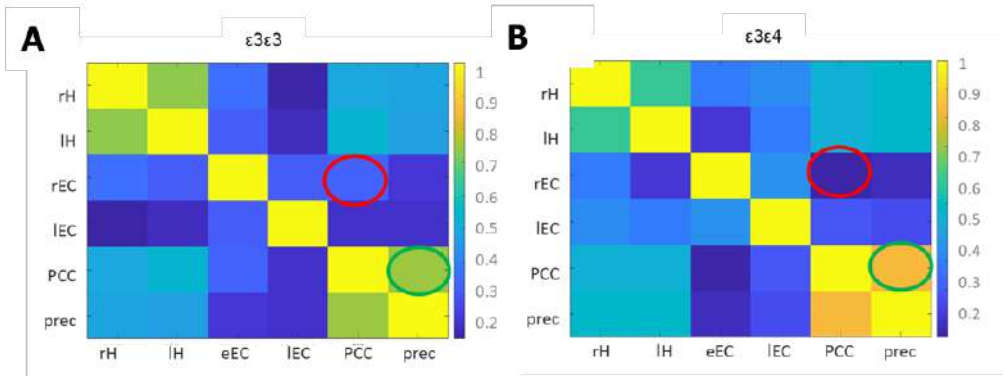
ANCOVA with age and sex and intracranial volume as covariates testing the significance of any difference in regional brain volume. Regional brain volumes are derived following FreeSurfer reconstruction (cortical parcellation and sub-cortical segmentation). Code available on request.

Supplementary Figure 3.1



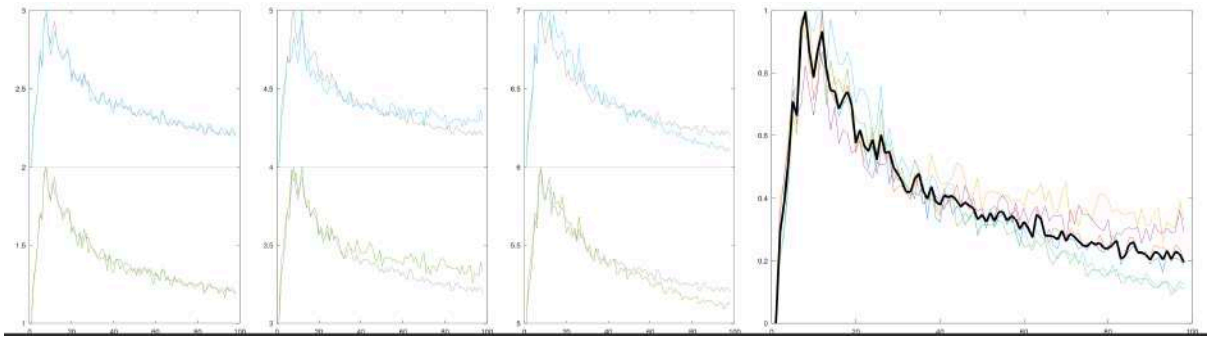
Genotypic effect on **A** Four mountains tests, **B** VST heading direction, **C** PCC-precuneus connectivity, **D** CCI episodic memory and **E** CCI executive function.

Supplementary Figure 3.2



Independent ε3ε3 and ε3ε4 connectivity matrices *red circle* = ε4 related reduced connectivity, *green circle* = ε4-related increased connectivity.

Supplementary Figure 3.3



Power spectrum generated after signal to noise cleaning in FSL Melodic for each of the network ROIs.

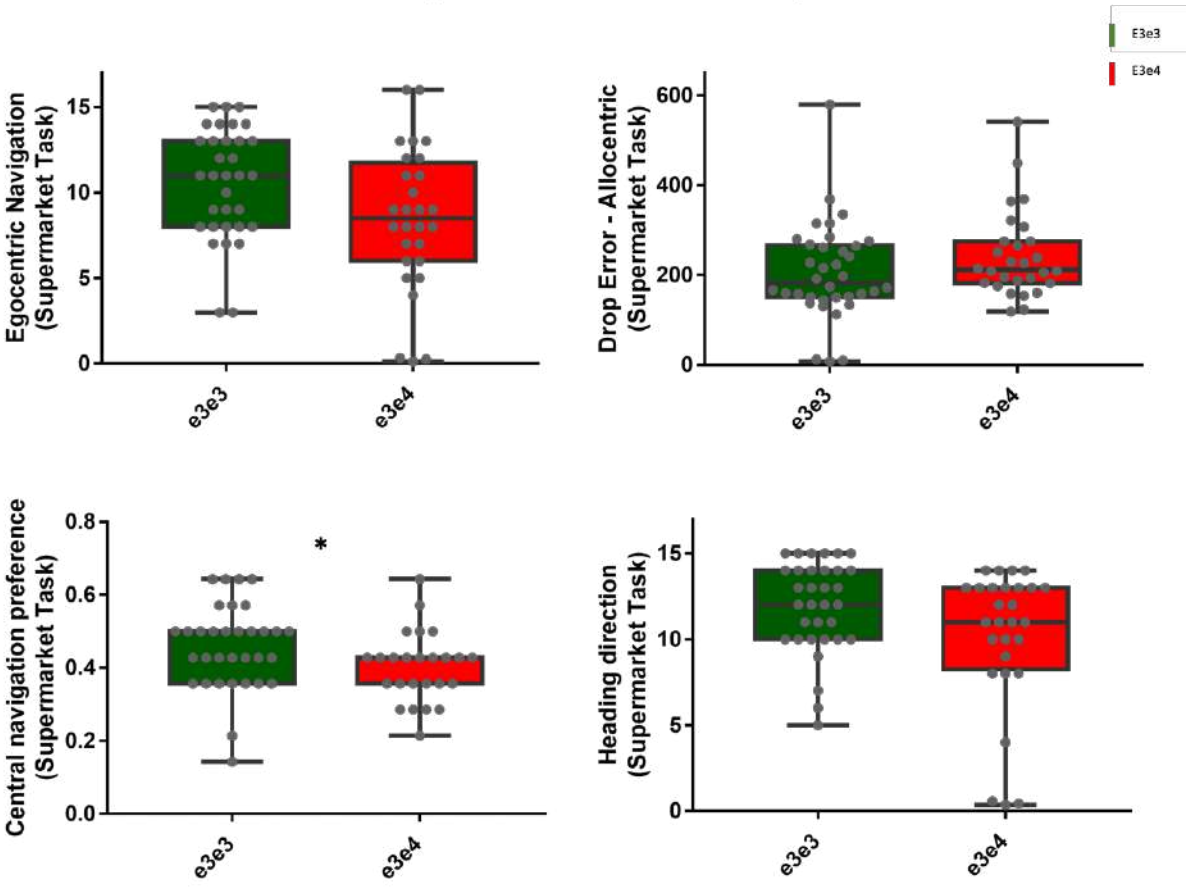
Supplementary Information Chapter 4

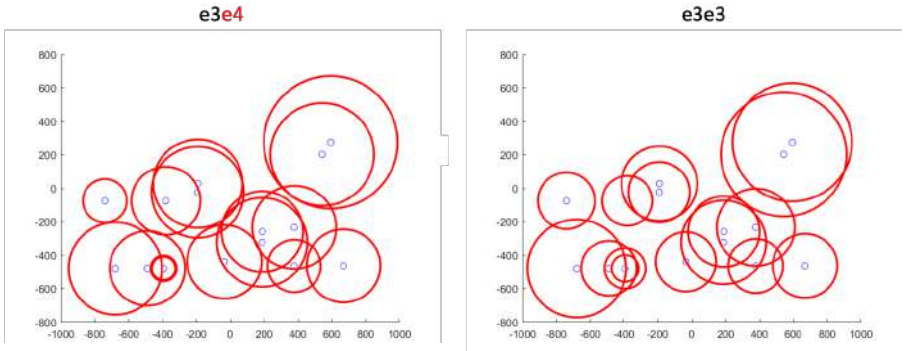
Supplementary Table 4.1. Navigation performance and subjective concern from T1 to T2

Measure	Variable	Mean ± T2	Mean ± T1	Δ	P value
SHQ ± missing n=8	Distance level 6 e3e3	0.58 ± .09	0.59 ± .09	-0.009 ± .08	*ns*
	Distance level 6 e3e4	0.60 ± .11	0.68 ± .29	-.075 ± .32	
	Distance level 8 e3e3	1.30 ± .26	1.42 ± .46	-.12 ± .51	*ns*
	Distance level 8 e3e4	1.42 ± .34	1.78 ± .72	-.36 ± .74	
VST ± missing n=2	Egocentric e3e3	10.77 ± 3.23	11.84 ± 2.43	-1.06 ± 2.93	ns
	Egocentric e3e4	9.50 ± 3.29	9.96 ± 3.98	-.60 ± 3.24	
	CNP e3e3	.465 ± .11	.562 ± .21	-.102 ± .231	.041
	CNP e3e4	.402 ± .09	.387 ± .14	.014 ± .159	
CCI	Episodic memory e3e3	19.04 ± 6.17	18.64 ± 6.42	.39 ± 4.12	ns
	Episodic memory e3e4	21.48 ± 6.76	22.44 ± 6.58	-.96 ± 5.41	
	Executive function e3e3	11.18 ± 4.37	10.54 ± 3.88	.64 ± 3.17	0.02
	Executive function e3e4	11.56 ± 3.75	12.78 ± 4.45	-1.22 ± 2.53	

SHQ; Sea Hero Quest; VST; Virtual Supermarket Test; CCI Cognitive Change Index; T2 = follow-up; T1=Baseline; Δ ± Delta = T2-T1; CNP; Central Navigation Measure; Distance is a proxy for less central navigation preference; Mixed effects models detected significant degree of change between groups on the VST central navigation measure. p value= significant change between genetic groups. *Note:* VST at T2 was recorded electronically

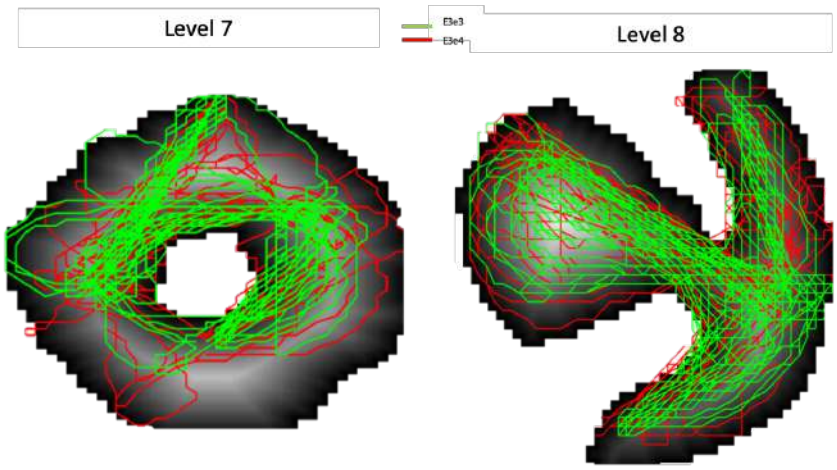
Supplementary figure 4.1: Virtual Supermarket Test performance at re-test





Correct allocentric response
Mean allocentric drop error on trial

Supplementary figure 4.2: SHQ level 7 and 8 performance at re-test



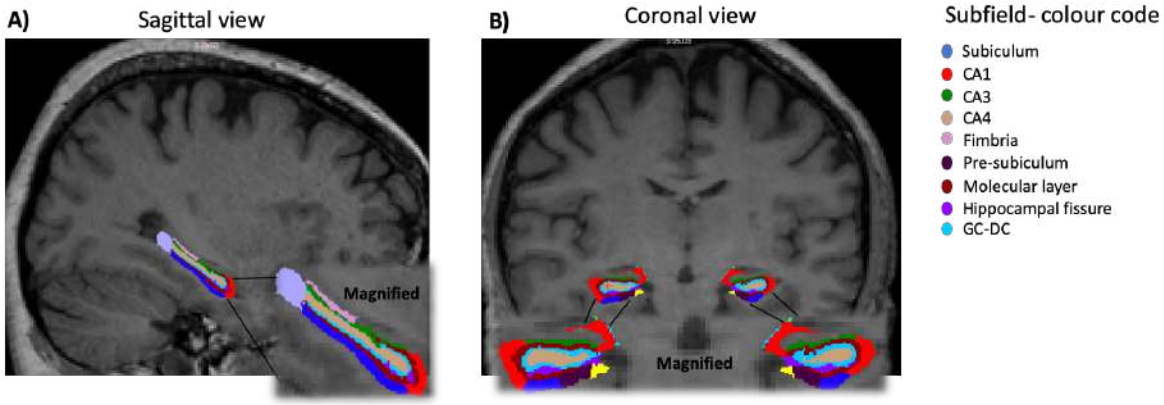
Supplementary Information Chapter 5

Supplementary results

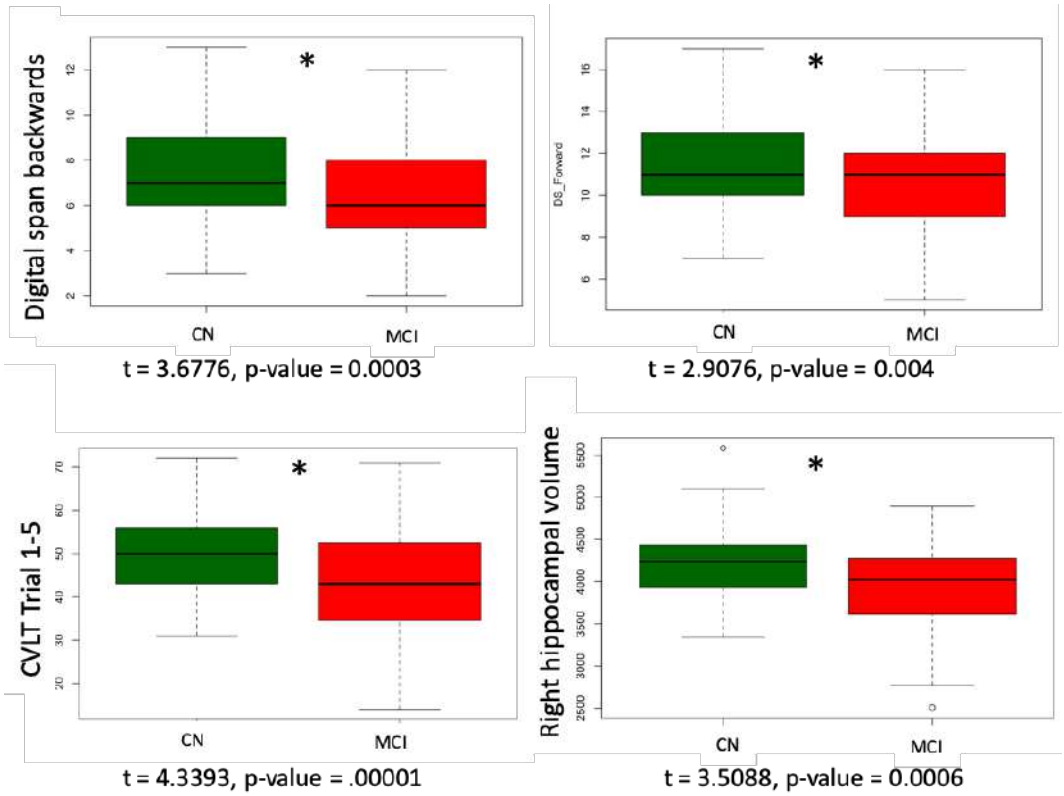
Confirming atrophy in the MCI group relative to the cognitively intact group (''CANN'').
There was an effect of the cognitive status (CN/MCI) on the left (t=-2.55 p>0.01, n_p^2=0.04) and right (t=-2.08 p>0.04, n_p^2=0.04) hippocampal volume, which was driven by the following subfields: subiculum (t=-2.080, p=0.04, n_p^2=0.04), molecular layer (t=-2.053 p=0.04, n_p^2=0.04) and the fimbria (t=-2.270 p=0.02, n_p^2=0.05). There was also an effect of cognitive status on the right entorhinal volume (t=-2.429, p=0.01, n_p^2=0.06) but not on the left entorhinal cortex volume (t=-1.796, p=0.075, n_p^2=0.03). In all cases, the CN group showed higher brain volume compared to MCI group.

Confirming memory impairment in the MCI group relative to the cognitively intact group (''CANN'').
There was a main effect of cognitive status on free and cued recall after long delay as well as digital span (see *Supplementary Figure 5.2*) In all cases, the CN group outperformed the MCI group.

Supplementary Figure 5.1. Hippocampal subfields



Supplementary Figure 5.2



California Verbal Learning Test (CVLT) and digital span confirms cognitive impairment and hippocampal atrophy in the MCI group compared for the CN group