Measurement of allocentric processing in mild cognitive impairment and early Alzheimer's disease using a virtual reality object location paradigm

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Thesis declaration form

I confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Overview

This thesis, a joint project with Elizabeth Harding, investigates the neural correlates of allocentric, hippocampal-based spatial processing in healthy older adults and those with Mild Cognitive Impairment (MCI) and early Alzheimer's Disease (AD). Part 1 systematically reviews studies looking at the relationships between performance on allocentric spatial tasks and structural or functional imaging data, of the hippocampus in particular, in healthy older adults. The review confirms the oft-reported deficit in allocentric processing in older adults and the majority of the reviewed studies support the hypothesis that such impairments are accompanied by atrophy, metabolic or microstructural alterations and underactivity of the hippocampal areas.

Part 2 reports a quantitative study of a novel allocentric object location memory task, using immersive virtual reality (iVR) technology, that was tested on older adults with amnestic MCI (characterised by memory loss) and age-matched healthy controls. Hippocampal and entorhinal subfield volumes were measured using structural MRI. MCI patients displayed a clear deficit in recalling object locations compared to controls that was significantly explained by hippocampal volume. The results highlight the potential of using innovative iVR spatial tasks to estimate hippocampal functioning and improve the diagnostic process for people with MCI and AD.

Part 3 discusses some of the issues encountered during the course of the research, including recruiting and conducting experiments with cognitively impaired elderly participants, the challenges and benefits of working with multiple collaborators across institutions, and the difficulties and learning process of using iVR technology, analysing and interpreting structural imaging data as a beginner.

Impact statement

This thesis, a joint project with Elizabeth Harding, investigates the neural correlates of allocentric, hippocampal-based spatial processing in healthy older adults and those with amnestic Mild Cognitive Impairment (aMCI, characterised by memory loss) and early Alzheimer's Disease (AD). The main experiment consists of a novel allocentric object location memory task, using immersive virtual reality (iVR) technology, that was tested on older adults with aMCI and age-matched healthy controls. MCI patients displayed a clear deficit in recalling object locations compared to controls that was significantly explained by hippocampal volume.

Due to its high controllability and ecological validity, iVR is likely to play an increasingly important role in basic neuropsychological research and clinical interventions. It is especially well-suited to investigating spatial processing due to the important influence of proprioception, head direction and motor feedback in spatial memory and navigation, and the strong hippocampal and subiculum contribution to performance in aMCI patients highlights its value as a sensitive measure of allocentric processing and clinical memory impairment, showing good validity and effectively discriminating between aMCI patients and controls.

Hippocampal volume was strongly correlated to and significantly predicted a simple outcome of the iVR task, mean displacement error. This is of great importance diagnostically, as the current best-practice process of determining MCI and subjective memory impairment is complex and multimodal, involving clinical interviews with the patient and informants, cognitive assessment, neuroimaging and CSF biomarker testing. Structural MRI and CSF testing are considered particularly important in the earlier stages to determine disease progression and identify underlying AD (Albert et al., 2011; de Toledo-Morrell et al., 2004;

Dubois et al., 2014; Frisoni et al., 2010; Shaw et al., 2009), however remain rather costly and invasive for widespread use in community settings. In recent decades, spatial memory and navigational tasks have emerged as strong contenders for improving the sensitivity of diagnosis, as they target the brain regions initially affected by AD neuropathology and are much more affordable and acceptable to patients. This study demonstrates the utility and viability of spatial tasks using immersive VR in the target populations of older adults with and without memory impairment, as well as the strong correlation between performance and HC integrity, suggesting that such tasks can be valuable additions to the diagnostic process.

Furthermore, there are numerous published studies and ongoing research on cognitive training and remediation interventions for MCI and AD, with reported effects in improving various aspects of cognitive functioning (Reijnders, van Heugten, & van Boxtel, 2013). Previous studies have already shown an improvement in allocentric memory and navigation when participants were allowed to actively engage or explore the environment (Carassa et al., 2002; Plancher et al., 2012) – the overwhelming verisimilitude of immersive VR in simulating the spatial experience of reality and the ability to track participants over larger areas are critical advantages for designing clinical interventions. Specific training in allocentric memory and navigation can greatly benefit both healthy older adults and preclinical or MCI patients (Lövdén et al., 2012; Serino et al., 2017).

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Part 1: Literature Review

Spatial memory and navigation in ageing:

A systematic review of MRI and fMRI studies in healthy participants

Abstract

Aim

Spatial navigational and memory deficits are widely observed in normal ageing and early Alzheimer's disease. This review aimed to systematically examine current neuroimaging evidence for structural and functional differences, particularly in the hippocampus (HC), associated with changes in allocentric spatial abilities due to normal ageing.

Methods

PsychInfo, Medline and Embase searches were conducted for peer-reviewed studies on allocentric spatial navigation and memory in normal ageing that reported structural magnetic resonance imaging (MRI) or functional magnetic resonance imaging (fMRI) data. 15 eligible studies were identified after applying exclusion criteria and assessing for quality.

Results

There was a marked deficit in allocentric spatial processing and trend towards egocentric strategies in older adults when compared to young controls or across the lifespan, which was associated in the majority of studies with changes in HC volume, metabolic or microstructural indicators, and underactivity. A few studies reported no significant correlations.

Conclusion

Findings confirm the consensus in the literature of an age-related deficit in allocentric spatial processing and a shift towards egocentric strategies. A larger proportion of studies supported the contribution of HC atrophy, microstructural/metabolic alterations or functional changes to the allocentric spatial impairment. There is a need for more sensitive imaging techniques to detect subtle changes in the HC as well as piloting of more ecologically valid spatial tasks.

Introduction

Increasingly, countries across the world are dealing with ageing populations, with a quarter of Europe over 60 and the rest of the world except Africa expected to reach that proportion by 2050 – an estimated 2.1 billion people (United Nations Department of Economic and Social Affairs, 2017). This is already resulting in shifting demands in social and medical care to reflect the needs of an older populace, and in the behavioural and neurosciences, there is growing research into the normal and pathological changes in cognition that accompany ageing, especially given the burden placed by conditions such as Alzheimer's disease (AD) on individuals, families and societies.

Memory decline, central to diagnosing dementia (McKhann et al., 1984), has also long been associated with normative ageing (Craik, 1994). The medial temporal lobe (MTL), particularly the hippocampus (HC), underlie both episodic memory and the spatial memory and navigational system in humans and other animals (Burgess, Maguire, & O'Keefe, 2002) and is one of the regions most sensitive to the effects of ageing (Lister & Barnes, 2009; Raz et al., 2005). Episodic memory, or the recollection of specific, autobiographical events as opposed to semantic or procedural memory, experiences decline with ageing (Levine, Svoboda, Hay, Winocur, & Moscovitch, 2002; Nyberg, Backman, Erngrund, Olofsson, & Nilsson, 1996) that is linked to reduced MTL and HC functioning (Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2005). Although there is debate in the literature about whether the marked deterioration of episodic memory seen in AD is underpinned by the same or distinct neurodegenerative processes seen in healthy ageing (Bishop, Lu, & Yankner, 2010; Brayne & Calloway, 1988; West, Coleman, Flood, & Troncoso, 1994), there is consensus about the core role of the

hippocampus. Since the HC is also essential for spatial memory and navigation, it is not surprising that this capacity deteriorates alongside episodic memory in both normative ageing and prodromal AD, and can in fact serve as an early warning sign and easily quantifiable way of assessing HC functioning and transition into AD, compared to assessments of episodic memory (Gazova et al., 2012; Monacelli, Cushman, Kavcic, & Duffy, 2003).

The concept of Mild Cognitive impairment (MCI) was introduced to provide a diagnostic staging post between healthy ageing and dementia (Petersen, 2004) and has proven to be a valuable concept for research into AD. Amnestic MCI, with a 10 to 15-fold risk of developing AD (Petersen, 2009), is characterised by memory loss and cognitive decline greater than expected in normal ageing, with sub-clinical impact on daily functioning. As may be expected, the spatial deficits observed in MCI patients are intermediate between healthy older adults and those with early AD (Serino, Cipresso, Morganti, & Riva, 2014).

Correspondingly, there is a self-reported and observed deterioration in navigational abilities in non-demented older adults (P. C. Burns, 1999; Moffat, 2009) which has been garnering more interest in a field formerly dominated by psychometric testing and measures such as visuospatial memory and mental rotation, which are not directly translatable to real-life behaviours such as spatial memory and wayfinding (Hegarty, Montello, Richardson, Ishikawa, & Lovelace, 2005). Although there have been early studies using real-world paradigms (Evans, Brennan, Skorpanich, & Held, 1984; Wilkniss, Jones, Korol, Gold, & Manning, 1997), the advent of technology and the ability to economically create virtual environments to assess spatial memory and navigation has led to many more studies investigating this ability in younger participants, AD patients, and older adults.

Declines in spatial memory and navigation, an early hallmark of Alzheimer's disease, also occur during the course of normal ageing and can serve as an easily quantifiable estimate of hippocampal functioning.

1.1 Spatial reference frames: egocentric, allocentric, cognitive maps

Spatial orientation and navigation can depend on two different reference frames – egocentric and allocentric. Egocentric processing is self-to-object and habitual, based on encoding locations and objects in relation to the individual, and retains the same perspective as the initial representation. For example, memorising a certain route by the sequence of left-right turns and where landmarks appear relative to oneself. This form of representation is independent of any higher-level model of the environment. Meanwhile, allocentric processing is object-to-object and based on encoding relationships of objects and environmental characteristics to each other. The related concept of a "cognitive map" (Tolman, 1948) refers to the underlying allocentric representation of the environment which is perspective-independent, allowing more flexible navigation in novel environments. Through exploration, an animal builds a cognitive map of the spatial relationships between different proximal, distal landmarks and any boundaries in a local environment, while also tracking their own location in relation through sensory feedback. The map allows them to navigate efficiently to a certain goal from anywhere in the space through their global knowledge of the interrelationships between different features of the environment. These two frames are sometimes referred to as response or place learning, route or survey, non-spatial or spatial; however for consistency this review has kept to the terms egocentric and allocentric. There is much evidence indicating that a deficit in utilising an allocentric reference frame underlies the

spatial impairment found in older adults and AD patients (Colombo et al., 2017; Gazova et al., 2012; Serino et al., 2014).

In many spatial memory and navigational tasks it is possible to use either an allocentric or egocentric strategy, and individual differences exist in preference and ability (Bohbot, Gupta, Banner, & Dahmani, 2011). However tasks in which good performance depends on one representation or the other can shed light on the neural dependencies of spatial memory and navigation in participants and tease out preference for or impairments in either type of processing that occur with ageing. A classic task of spatial memory and hippocampal function, the Morris Water Maze (R. Morris, 1984), was first devised for rodents. Animals had to learn the location of a hidden platform in a circular pool surrounded by distal cues. Allocentric processing is suggested by successful recall of the platform location from novel starting locations, as rodents would have to remember the platform position in relation to the distal cues and work out another path from their new location in the pool.

Spatial navigation can utilise an egocentric or allocentric reference frame. Egocentric processing encodes directions and movements only in relation to the self. Allocentric navigation depends on a 'cognitive map' – a perspective-independent neural representation of proximal, distal landmarks and boundaries of the environment in relation to each other.

1.2 Neural correlates of spatial memory and navigation

Numerous animal lesion and cell recording studies and experimental studies in normal and clinical human populations have implicated different brain regions and networks in egocentric and allocentric processing: the former is linked to striatal structures and activation in the

caudate nucleus, precuneus, frontal and posterior parietal cortices (Boccia, Nemmi, & Guariglia, 2014; Burgess, 2008; Hartley, Maguire, Spiers, & Burgess, 2003), while allocentric processing and navigation is primarily subserved by the medial temporal lobe and hippocampal system (Packard & McGaugh, 1996), which creates and updates the cognitive map (O'Keefe & Nadel, 1978).

A large array of neuronal cell types support this complex ability. "Place cells" in the HC (Ekstrom et al., 2003; O'Keefe, 1976), forming the basis of the cognitive map, fire selectively at specific locations (place fields) in an environment independent of head orientation, and can remain stable for weeks or "remap" if the environment changes (Fyhn, Hafting, Treves, Moser, & Moser, 2007). Grid cells, mainly found in the medial entorhinal cortex (MEC), pre- and parasubiculum (Hafting, Fyhn, Molden, Moser, & Moser, 2005; Jacobs et al., 2013), fire repeatedly across an environment to form a grid-like pattern of equilateral triangles. Border cells are sensitive to the distance and direction of boundaries and are found in in the EC, subiculum, pre- and parasubiculum (Lever, Burton, Jeewajee, O'Keefe, & Burgess, 2009; Solstad, Boccara, Kropff, Moser, & Moser, 2008). Other cells underlying the supporting function of *path integration* (estimating one's location while navigating based on self-motion information) include head direction cells in the dorsal presubiculum (Jeffrey S. Taube, 2007) and speed cells in the HC and MEC (Kropff, Carmichael, Moser, & Moser, 2015), which depend on environmental cues to correct calculation errors that accumulate over time. Furthermore, the retrosplenial cortex (RSC) is hypothesized to mediate between the allocentric and egocentric frames of reference (Byrne, Becker, & Burgess, 2007).

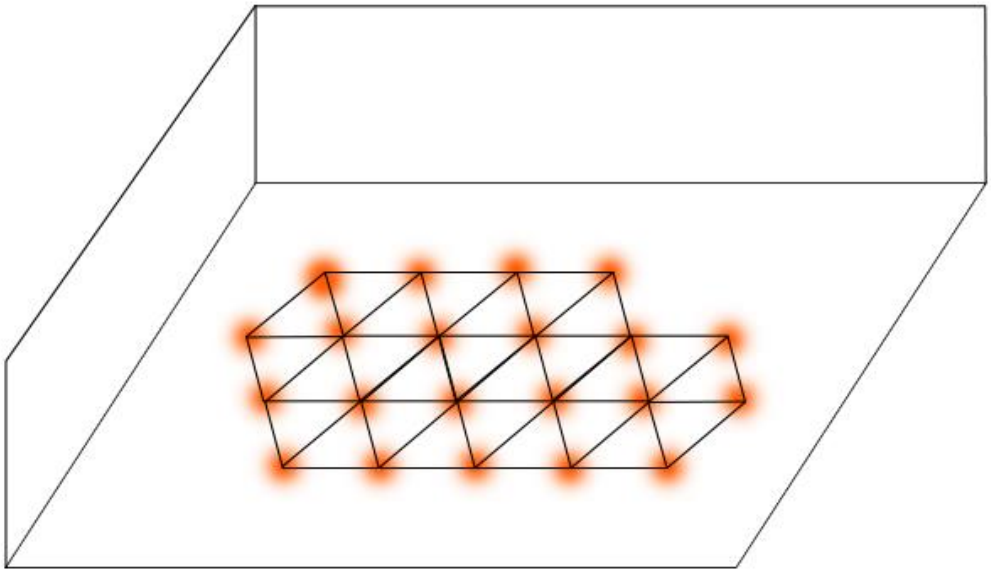

Figure 1. *Grid cell firing patterns from single neuron recordings. Grid cells support the animal in constructing a representation of its local space.*

Allocentric processing is supported by specialised spatial cells in the hippocampal formation and entorhinal cortex, such as 'place cells', 'grid cells', 'boundary vector cells'. These work together to construct a cognitive map of the environment.

1.3 Pattern of changes in memory with ageing

Ageing impacts different types of memory disparately, with working, associative, contextual and spatial memory impaired in older adults (Craik, 1994). This pattern of deficits reflect both general trends such as cognitive slowing and neurobiological changes in the regions of the brain that support respective types of memory, like the MTL and frontal-striatal systems (Buckner, 2004). The deterioration in spatial memory and navigational abilities in older adults has been well-characterised in many studies, the main observations being a decline in allocentric processing, a preference and increased reliance on egocentric strategies (Colombo et al., 2017) and impairments in switching between frames of reference (Harris &

Wolbers, 2014) within the context of wider difficulties in set switching associated with the ageing PFC (Meiran, Gotler, & Perlman, 2001). Subtle impairments in egocentric processing (route learning) may be due to age-related deterioration in the caudate nucleus (Betts, Acosta-Cabronero, Cardenas-Blanco, Nestor, & Düzel, 2016). However, in comparison to allocentric strategies, egocentric processing appears to be relatively intact in older adults.

While more basic visual functions such as distance and object perception are maintained (Lester, Moffat, Wiener, Barnes, & Wolbers, 2017), older adults show impairments in vestibular processing, perception of self-motion and pronounced deficits in path integration (Harris & Wolbers, 2012; Mahmood, Adamo, Briceno, & Moffat, 2009). Along with declines in allocentric spatial working memory linked to the medial PFC (Lester et al., 2017), these disproportionately affect the components of allocentric processing and contribute to poorer spatial navigation performance. In addition, older adults experience greater difficulty in encoding and retrieval of spatial information in long term memory in large-scale environments requiring exploration to fully comprehend (Head & Isom, 2010; Lövdén et al., 2012), prefer proximal to distal cues or boundaries (Moffat & Resnick, 2002; Schuck, Doeller, Polk, Lindenberger, & Li, 2015), and are slower to learn and encode a cognitive map (Daugherty et al., 2015; Moffat & Resnick, 2002). The underlying cellular mechanisms are well elucidated in numerous animal studies (Lester et al., 2017).

Aside from global memory and cognitive decline, ageing specifically affects allocentric processing and the ability to switch between reference frames. Older adults show impairment in several components of allocentric processing such as path integration and long term memory of spatial information.

1.4 Structural and functional changes in the hippocampus with ageing

Although the relationship between hippocampal volume and poorer memory in ageing was unclear in a large meta-analysis (Van Petten, 2004), more recent studies have reported shrinkage of the HC, EC and prefrontal cortex (PFC) with ageing, particularly from middle age onwards (A.-T. Du et al., 2006; Raz & Rodrigue, 2006) and associations to memory performance were found longitudinally (Rodrigue & Raz, 2004). Furthermore, the studies in the meta-analysis mostly utilised verbal memory tasks when declines in episodic and spatial memory are more marked with ageing (Gazova et al., 2012; Hedden & Gabrieli, 2004). Changes to prefrontal functions such as working memory, functional connectivity, long-term potentiation (LTP – the process believed to underlie learning in hippocampal pyramidal cells) in the HC and place cell firing stability may all contribute to the decline in performance of older individuals on hippocampal tasks (Lester et al., 2017). A decrease in pattern discrimination and speed of cognitive mapping (i.e. spatial learning) in place cells have been reported in aged rats (Hok, Chah, Reilly, & O'Mara, 2012; Schimanski, Lipa, & Barnes, 2013). Risk and protective factors such as hypertension (Korf, White, Scheltens, & Launer, 2004), cognitive reserve (Buckner, 2004; Tucker & Stern, 2011) and genetic variation (Beaudet et al., 2015) can greatly affect hippocampal integrity and functioning and subsequently, episodic, spatial memory and navigation with ageing.

The MTL experiences shrinkage with ageing which has been linked to memory decline. Cell atrophy, changes in functional connectivity, decreased efficiency of LTP, poorer pattern discrimination and speed of cognitive mapping contribute to lower hippocampal functioning as do risk and protective factors.

1.5 Review rationale and research questions

What we know about the healthy navigational system and the impact of ageing has been reviewed extensively (Klencklen, Després, & Dufour, 2012; Moffat, 2009), most recently in a broad overview including animal studies and discussing cellular mechanisms (Lester et al., 2017) and a systematic review of allocentric and egocentric spatial studies with healthy young and older subjects (Colombo et al., 2017). However, there has not been a systematic review of papers focusing on spatial memory and navigation in healthy older participants with imaging data yet, except a review of fMRI findings in young adults (Boccia et al., 2014). Furthermore, relatively less is known about how the morphological and functional changes in the HC and medial temporal lobes that occur with ageing map onto the widely observed age-related behavioural differences in spatial memory and navigation, such as the impairment in allocentric processing, reliance on egocentric strategies, and difficulty in switching between reference frames, either in cross-sectional samples comparing young and older subjects, or longitudinally, tracking and correlating the neural and behavioural changes in spatial memory and navigation as individuals age.

This review aims to fill the gap in the existing literature, provide an overview of the MRI and fMRI studies investigating spatial memory and navigation in older adults, with or without younger control groups, and longitudinal studies comparing individuals across time, and summarise the current evidence on neural correlates of age-associated spatial and allocentric deficits. Lastly, it discusses the findings in context of our latest understanding of the ageing navigational system, strengths and limitations of the review and included studies, and suggests directions for future research.

This review's main objectives are:

1. To examine the characteristics of allocentric and egocentric navigation in healthy older adults and age-related differences relative to younger adults;
2. To review the structural imaging findings on neural changes with ageing, focusing on hippocampal and surrounding areas, and the structural and functioning imaging evidence on neural correlates of allocentric processing in healthy older and younger adults.

Methods

A systematic review was conducted on existing peer-reviewed literature to investigate our current understanding of spatial memory and navigational changes in normative ageing and their structural and functional neural correlates. The PRISMA guidelines for systematic reviews (Moher, Liberati, Tetzlaff, & Altman, 2009) were followed for this paper.

2.1 Inclusion criteria

The inclusion criteria for the review were as follows: (1) the main experimental paradigm assessed spatial navigation and/or memory with regard to allocentric and egocentric referencing, (2) the sample included healthy elderly participants with or without younger control groups, (3) the study reported structural magnetic resonance imaging (MRI) or functional magnetic resonance imaging (fMRI) data for the elderly participants, (4) the study was a full-length article published in a peer-reviewed journal, (5) in English with full text available. As this review aimed to examine the neural correlates of changes in spatial memory and navigation accompanying normal ageing, studies comparing healthy elderly to pathological samples or focusing on participants with AD and mild cognitive impairment (MCI) were excluded. Studies involving interventions targeted at spatial navigation and/or memory were only included if they reported baseline measurements fulfilling the inclusion criteria.

Studies that met the inclusion criteria then underwent formal quality assessment using an appropriate critical appraisal tool. Studies with a rating above 55% were included in the systematic review.

2.2 Search methodology

To identify qualifying studies, a database search was conducted in November 2017. PsycInfo, Ovid MEDLINE and Embase databases were searched for entries containing the following terms in all fields: (“allocentric” or “spatial memory” or “third person perspective” or “egocentric” or “spatial orientation (perception)” or “spatial navigation”) AND (“aging” or “geriatrics” or “gerontology” or “geropsychology” or “human development” or “older adult*” or “elder*” or “age differences”). The detailed search strategy is presented in **Table 1**.

Search terms were purposefully inclusive so that studies employing less common terminology would be found as well. Terms referring to neuroimaging, MRI or fMRI were not included in the database search and studies were assessed for criterion 3 during the screening stage instead, as key papers fulfilling inclusion criteria identified whilst preparing for the systematic review used variable terms to refer to neuroimaging and/or made little mention of imaging data in the title and keywords.

	Terms	Results
1	allocentric.mp.	3524
2	"spatial memory".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, nm, kf, px, rx, ui, sy, tc, id, tm]	33455
3	"third person perspective*".mp.	637
4	egocentric.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, nm, kf, px, rx, ui, sy, tc, id, tm]	7776
5	"spatial orientation (perception)"/	7081
6	"spatial navigation".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, nm, kf, px, rx, ui, sy, tc, id, tm]	4556
7	1 or 2 or 3 or 4 or 5 or 6	51677
8	aging/ or geriatrics/ or gerontology/ or geropsychology/ or human development/	596290
9	"older adult*".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, nm, kf, px, rx, ui, sy, tc, id, tm]	167415
10	elder*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, nm, kf, px, rx, ui, sy, tc, id, tm]	746865
11	age differences/	73122
12	8 or 9 or 10 or 11	1382167
13	7 and 12	4069
14	limit 13 to english language	3977
15	limit 14 to human	2087
17	remove duplicates from 15	1532

Table 1. *Search terms*

Results

3.1 Search results

The database search produced 4069 articles which was reduced to 2087 after limiting results to English articles and human studies (**Figure 2**). After removing duplicates 1532 articles remained. A further 17 articles meeting search term criteria were identified in the references section and citation searches (using Google Scholar) of recent reviews and key papers (Beaudet et al., 2015; Colombo et al., 2017; Driscoll et al., 2003; Lester et al., 2017). The titles and abstracts of these 1549 articles were screened and 20 articles were considered to have met the inclusion criteria. The full-text versions were then assessed to ascertain their eligibility, with five articles being excluded after this step (reasons given in **Figure 2**). The excluded articles are listed in **Appendix 1**. The remaining 15 articles were subject to the formal quality assessment.

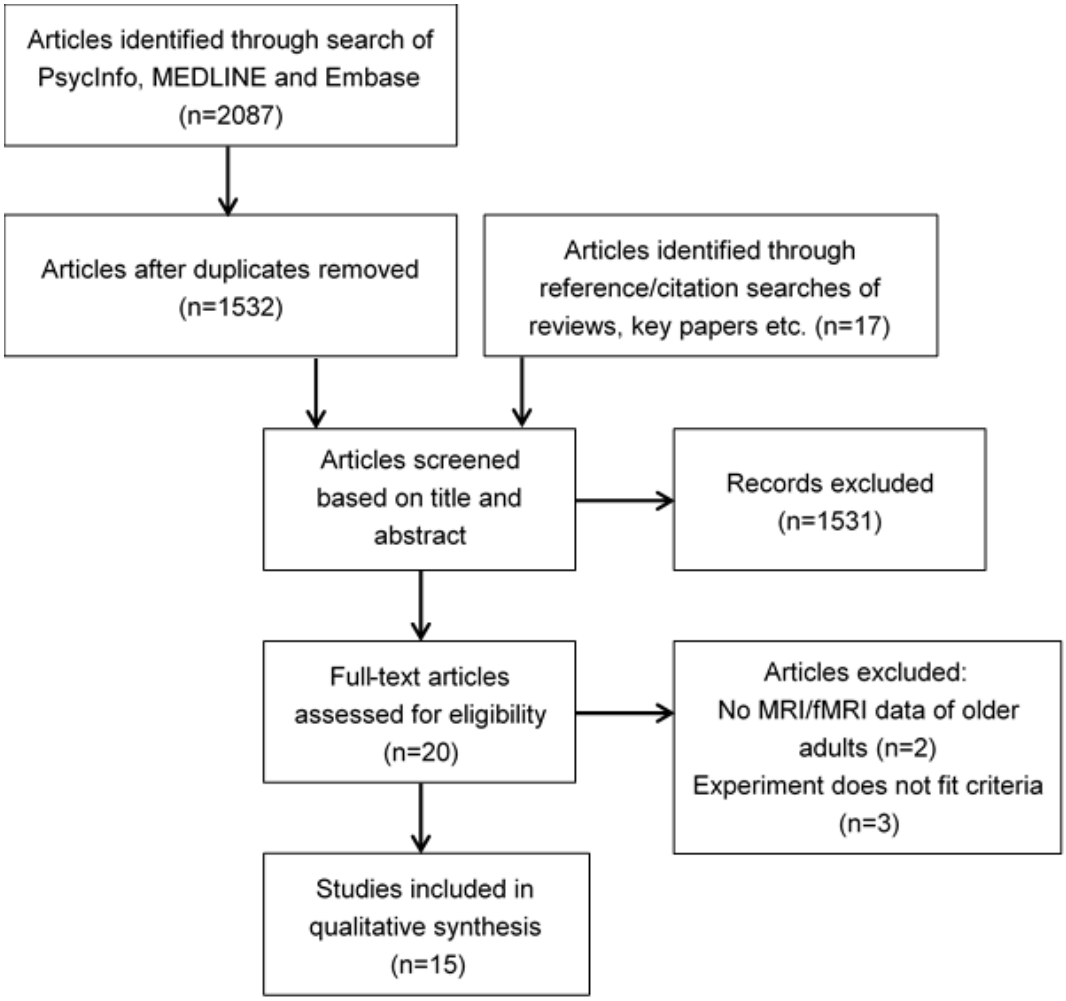

Figure 2. *Flow chart of selecting papers for review following PRISMA guidelines*

3.2 Quality assessment

There are a variety of tools available to researchers for assessing randomised controlled trials or intervention studies (Downs & Black, 1998; Higgins & Green, 2008), however fewer are designed to evaluate experimental or quantitative studies with non-randomised groups or correlational designs, such as those examined in this review. The “Qualsyst” tool (Kmet, Lee, & Cook, 2004) was developed for health science researchers to critically appraise the quality, based on the construct of internal study validity, of quantitative and qualitative studies using a standardised checklist and produces an overall quality score out of 100% for each study (see **Appendix 2** for the checklist and scoring instructions). It was selected for this systematic

review because it is a general appraisal tool suitable for assessing a wide range of different study designs, as identified papers included cross-sectional as well as longitudinal designs. The authors suggest cut-off points ranging from 55% to 75% depending on the constraints of the systematic review. As the quality scores of the studies ranged from 73-86%, and considering the low number meeting inclusion criteria, all studies were included in the systematic review (**Table 2**). Weaknesses in studies with 'Medium' quality (scores >75%) and caveats were mentioned in the Discussion, however given the narrow range, quality ratings could not add significantly to the critical analysis. Detailed scores for the assessed studies are presented in **Appendix 3**.

Study Author(s)	Year	Type	Overall Score	Quality
Driscoll et al.	2003	MRI	0.73	Medium
Meulenbroek et al.	2004	fMRI	0.77	High
Moffat et al.	2006	fMRI	0.82	High
Moffat et al.	2007	MRI	0.86	High
Antonova et al.	2009	MRI/fMRI	0.73	Medium
Head and Isom	2010	MRI	0.77	High
Lövdén et al.	2012	MRI	0.81	High
Konishi et al.	2013	fMRI	0.77	High
Konishi and Bohbot	2013	MRI	0.73	Medium
Daugherty et al.	2015	MRI	0.77	High
Schuck et al.	2015	fMRI	0.77	High
Daugherty et al.	2016	MRI	0.77	High
Korthauer et al.	2016	MRI	0.82	High
Daugherty and Raz	2017	MRI	0.73	Medium
Konishi et al.	2017	MRI	0.82	High

Table 2. *Quality ratings*

The 15 studies included 10 studies reporting structural MRI and neurophysiological data (iron accumulation, N-acetylaspartate/creatine [NAA/Cre] levels) obtained with separate imaging, four studies reporting fMRI data obtained from scanning during the experiment, and one study with both MRI and fMRI data (Antonova et al., 2009). Although 'egocentric' was included in the search strategy, studies predominantly focused on investigating allocentric processing in ageing with spatial memory or navigational paradigms.

3.3 Summary tables of reviewed studies

Many of the reviewed papers originated from several key research groups, with the radial maze studies led by Bohbot at McGill University, consisting of three studies (Konishi et al., 2013; Konishi, Mckenzie, Etchamendy, Roy, & Bohbot, 2017; Konishi & Bohbot, 2013), and the Morris Water Task studies driven by Moffat and Resnick or Raz (for path complexity) at Wayne State University, consisting of two (Moffat, Elkins, & Resnick, 2006; Moffat, Kennedy, Rodrigue, & Raz, 2007) and three studies (Daugherty et al., 2015; Daugherty, Bender, Yuan, & Raz, 2016; Daugherty & Raz, 2017) respectively. Details of the studies and the main findings are presented, separately for MRI and fMRI, in **Tables 3a** and **3b**. The study with both MRI and fMRI data (Antonova et al., 2009) has been included in both tables.

Study Author(s)	Year	Sample (N)*	Study Design	Sample characteristics	Mean age (SD or range)	Female (%)	Imaging modality
Driscoll et al.	2003	32	Cross-sectional with comparison group	16 younger adults (YA), 16 older adults (OA), non-APOE ε4 allele carriers	YA: 26.1 (20-39) OA: 77.6 (60-85)	YA: 50 OA: 50	MRI MRSI (HC NAA/Cre)
Moffat et al.	2007	68	Cross-sectional with comparison group	32 younger adults, 36 older adults	YA: 24.5 (0.91) OA: 68.5 (0.92)	YA: 75 OA: 67	MRI
Antonova et al.	2009	20	Cross-sectional with comparison group	10 younger adults, 10 older adults	YA: 23.6 (1.78) OA: 72.14 (5.33)	N/A	MRI (VBM) fMRI
Head and Isom	2010	47	Cross-sectional with comparison group	29 younger adults, 63 older adults with MRI data for 47 OA	69.9 (8.55)	72.3	MRI (VBM)
Lövdén et al.	2012	91	Randomised intervention with comparison group	44 younger adults, 47 older adults	YA: 26.0 (2.8) OA: 65.0 (2.8)	All male	MRI DTI
Konishi and Bohbot	2013	45	Cross-sectional, single group	Older adults only	64.38 (4.0)	51.1	MRI (VBM)
Daugherty et al.	2015	139	Cross-sectional, single group	Age range from 18-77	48.52 (15.85)	66.2	MRI
Daugherty et al.	2016	65	Cross-sectional, single group	Age range from 19-75	44.99 (16.31)	67.7	MRI
Korthauer et al.	2016	22	Longitudinal & cross-sectional with comparison group	51 recruited at 8-year FU, 22 with MRI: 9 middle-aged, <60 (MA), 13 older adults, >60 (OA)	Baseline: 59.7 (30-83) MA: 50.8 (40-59) OA: 67.9 (60-78)	MA: 66.7 OA: 53.8	MRI DTI MRSI (NAA/Cre)
Daugherty and Raz	2017	213 (131)	Longitudinal, single group	2-year FU sample from Daugherty et al. (2015), aged 18-77 at baseline. n=40 had hypertension	Baseline: 51.27 (15.52) FU: 55.74 (14.28)	74.0	MRI R2* relaxometry
Konishi et al.	2017	49	Cross-sectional, single group	107 older adults (55-80), subset (n=49) with MRI	66.04 (4.41)	55.1	MRI

YA: younger adult; OA: older adult; FU: follow up; MRI: magnetic resonance imaging; MRSI: magnetic resonance spectroscopy imaging; VBM: voxel based morphometry; DTI: diffusion tensor imaging

* Sample size with both behavioural and imaging data

Table 3a. *Summary of structural MRI studies*

Primary spatial paradigm	Screening and Cognitive tests	Main findings
Virtual Morris Water Task (vMWT) (Hamilton and Sutherland,1999), Transverse Patterning Discrimination Task (TPDT)	Extensive battery of cognitive measures in ageing study, MMSE	Age-related deficits in both hippocampal tasks. Age-related reduction in HC volume & NAA/Cre levels. Correlation between HC volume and performance on vMWT was non-significant after controlling for age and HC activity. Significant contribution of HC volume to TPDT performance after controlling for age and HC activity.
Virtual Morris Water Task (vMWT) (Moffat and Resnick, 2002)	7 tests grouped into Processing Speed, Working Memory, Spatial Memory and Executive Control indices	Age-related reduction in left PFC, HC, CN, CB, PFW volume. Age-related deficits in vMWT first trial, learning and search accuracy. Non-significant correlation between HC volume and vMWT performance. vMWT performance correlated with CN, PFW volume, executive function, working and spatial memory.
Virtual task (MWT analogue) requiring encoding and recall of location of pole in a circular arena using distal environmental cues	MMSE screen for OA WASI, OA significantly higher IQ	Age-related deficit in accuracy of location recall. No age-related reduction in HC volume, but several other regions larger in YA. Attenuation of HC and perirhinal activation in OA during encoding and retrieval accompanied by poor task performance.
Virtual maze with landmarks and wallpaper recall measures (Hartley et al., 2003): wayfinding condition allowed free exploration, route learning condition prescribed path taken	Short Blessed Test for gross cognitive impairment	Age-related deficits in wayfinding and route learning. HC volume significantly associated with wayfinding performance. CN volume significantly associated with route learning performance. However no significant difference between above two associations and other ROI.
Virtual zoo linked to treadmill, participants were required to search for cued animals	Large neurocognitive battery including measures of allocentric ability	Age-related decline in navigation significantly improved by 4-month training and somewhat maintained 4m post-test. Improvement did not transfer to allocentric paper task, which did not correlate with baseline VR performance. No age differences in HC volume, higher diffusivity for OA. HC size/MD not associated with VR performance.
Concurrent Spatial Discrimination Learning Task (CSDLT), a 12-arm radial maze with objects in half the arms. Differentiates allocentric or egocentric strategies.	MMSE, MoCA screen Rey Auditory Verbal Learning Test (RAVLT), Rey-Osterrieth Complex Figure Task (RO)	CSDLT performance (allocentric strategy use) positively correlated with right HC volume, but RAVLT and RO performance did not. Caudate volume not negatively correlated with CSDLT. Increased HC volume covaried with increased right OFC, AMG, PHG volumes.
Virtual Morris Water Task (vMWT) (Moffat and Resnick, 2002)	MMSE and Center for Epidemiological Study Depression Questionnaire (CES-D)	Age and sex-related increases in time and path complexity. Age-related deficit in first trial but no effect on learning across trials. Smaller HC volume associated with higher path complexity and greater travel time. HC and PHG volume associated with change in path complexity i.e. learning.
Virtual Morris Water Task (vMWT) (Moffat and Resnick, 2002)	MMSE and Center for Epidemiological Study Depression Questionnaire (CES-D)	Age-related reduction in HC subfields and EC. Age-related deficit in learning speed and overall performance. Subiculum and EC was associated with faster decrease in path complexity, while CA1-2 associated with faster path shortening. HC subfield volumes did not contribute to overall age-related poor performance.
Virtual Morris Water Task (vMWT)	Extensive neurocognitive battery: Memory, Attention, Executive Functioning, Language, Visuospatial tasks	No age-related decline at FU of any vMWT measures. vMWT latency correlated with right HC, medial OFC and thalamus after controlling for demographics. vMWT latency correlated with right HC and thalamus only in MA, and with right mOFC in OA. Bilateral UF FA significantly correlated with latency after controlling for age, speed.
Virtual Morris Water Task (vMWT) (Moffat and Resnick, 2002)	MMSE and Center for Epidemiological Study Depression Questionnaire (CES-D)	Learning rate and absolute improvement (path length, PL) declined, but reduction in path complexity (FD) improved, all worse in OA at FU. CN, HC, PHG, CB significantly reduced at FU. Greater CN iron, smaller CB and CN volume predicted longer PL, great HC iron and smaller PHG associated with more variable FD. Iron accumulation, not HC volume, predictive of longitudinal decline.
Virtual wayfinding task: required to navigate from one landmark to another in virtual town (n=107, MRI n=49), CSDLT (n=93, MRI n=47)	MMSE screen MoCA	Age-related reduction in right HC and CN volume. Age negatively correlated with wayfinding but not CSDLT performance. MoCA correlated with HC volume, wayfinding performance independent of age, and CSDLT performance. Generally, wayfinding and CSDLT performance correlated with HC volume.

HC: hippocampus; PHG: parahippocampal gyrus; EC: entorhinal cortex CN: caudate nucleus; PFC: prefrontal cortex; CB: cerebellum; PFW: prefrontal white matter; OFC: orbitofrontal cortex; AMG: amygdala; UF: uncinate fasciculus; ROI: region of interest; FA: fractional anisotropy; MD: mean diffusivity; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; WASI: Wechsler Abbreviated Scale of Intelligence

Study Author(s)	Year	Sample (N)*	Study Design	Sample characteristics	Mean age (SD or range)	Female (%)	Imaging modality
Meulenbroek et al.	2004	40	Cross-sectional with comparison group	20 younger adults, 20 older adults 	YA: 23 (2.8) OA: 63 (7.2)	YA: 50 OA: 50 	functional MRI
Moffat et al.	2006	51	Cross-sectional with comparison group	30 younger adults, 21 older adults 	YA: 27.07 (5.46) OA: 68.43 (5.56)	YA: 50 OA: 52.4 	functional MRI
Antonova et al.	2009	20	Cross-sectional with comparison group	10 younger adults, 10 older adults 	YA: 23.6 (1.78) OA: 72.14 (5.33)	N/A 	MRI (VBM) functional MRI
Konishi et al.	2013	52	Cross-sectional with comparison group	23 younger adults, 29 older adults 	YA: 23.8 (3.8) OA: 64.2 (4.7)	YA: 60.9 OA: 48.3 	functional MRI
Schuck et al.	2015	48	Cross-sectional with comparison group	26 younger adults, 22 older adults 	YA: 28.1 (3.9) OA: 67.2 (3.9)	All male 	functional MRI

YA: younger adult; OA: older adult; MRI: magnetic resonance imaging; VBM: voxel based morphometry

* Sample size with both behavioural and imaging data

Table 3b. *Summary of functional MRI studies*

Primary spatial paradigm	Screening and Cognitive tests	Main findings
Video sequences of fixed routes through virtual homes and with arrow cues at intersections during encoding; participants asked to recall left, right or straight during recognition. Control condition had repeated travel down empty corridor with arrows.	Nil	Small but significant age-related deficit in route recognition. Stronger activation in YA in posterior fusiform/PHC areas, and weaker recognition-related activity in anterior PHC possibly linked to route encoding advantage in YA. OA showed higher activity in left perisylvan region and ACC, possibly related to attentional deficits.
Virtual building with 6 objects dispersed, participants asked to navigate shortest route to recalled location of objects	Nil	Age-related deficit in object location recall. OA showed reduced activation in posterior HC, PHC gyrus, RSC, parietal regions and greater frontal lobe (ACC, medial frontal cortex) activation. Increased navigational accuracy is associated with greater activation in posterior PHC gyrus, RSC and precuneus overall. Strategy use was not assessed.
Virtual task (MWT analogue) requiring encoding and recall of location of pole in a circular arena using distal environmental cues	MMSE for OA WASI, OA significantly higher IQ	Age-related deficit in accuracy of location recall. No age-related reduction in HC volume, but several other regions larger in YA. Attenuation of HC and perirhinal activation in OA during encoding and retrieval accompanied by poor task performance.
Concurrent Spatial Discrimination Learning Task (CSDLT), a 12-arm radial maze located in a larger environment, with objects in half the arms. Distinguishes between use of allocentric or egocentric strategies.	MMSE screen MoCA	OA slower acquisition to criterion but matched YA performance. YA had HC activation in beginning of learning, OA had CN activation at end of learning. OA using spatial strategy had HC activation in learning while OA using response strategy had CN activation, suggesting a shift to response strategies accounts for age-related deficits.
Object location task - 5 objects in a virtual circular arena, boundary and landmark conditions manipulated to investigate influence on recalled object locations	Nil	Age-related deficit in object location recall and learning rate. YA performance predicted by a boundary-processing model and OA showed no significant influence of boundary manipulations. Greater HC/PHC activity associated with boundary model predictions. Greater CN activity in OA associated with landmark processing (using lenient threshold). Additional HC activity for landmark learning in high-performing OA.

HC: hippocampus; PHC: parahippocampal cortex; ACC: anterior cingulate cortex; RSC: retrosplenial cortex; CN: caudate nucleus; MWT: Morris Water Task; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; WASI: Wechsler Abbreviated Scale of Intelligence

Glossary

OA: Older adults	**YA:** Younger adults
HC: Hippocampus	**PHG/PHC:** Parahippocampal gyrus
EC: Entorhinal cortex	**CN:** Caudate nucleus
CB: Cerebellum	**LPFC:** Lateral prefrontal cortex
RSC: Retrosplenial cortex	**ACC:** Anterior cingulate cortex
OFC: Orbitofrontal cortex	**AMG:** Amygdala

3.4 Experimental designs of structural MRI studies

Eleven studies included structural MRI (sMRI) data on participants, and four studies included other measurements such as magnetic resonance spectroscopy imaging (MRSI), diffusor tensor imaging (DTI) and R2* relaxometry. Eight of the studies had cross-sectional samples while three had intervention or longitudinal data, with one of the longitudinal studies being a follow up (FU) of a large sample from a cross-sectional study two years later (Daugherty et al., 2015, 2016). Six studies recruited younger controls for comparison to an older adult group and five of those also had imaging data for the younger participants, while three studies had samples with a wide age range and estimated the influence of age in later analyses. Two studies only looked at neural correlates of spatial performance in older adults.

Seven studies (Antonova et al., 2009; Daugherty et al., 2015, 2016; Daugherty & Raz, 2017; Driscoll et al., 2003; Korthauer et al., 2016; Moffat et al., 2007) used virtual reality analogues, presented on computer screens and controlled by keyboard or joystick, of the Morris Water Task (vMWT), a test of spatial memory. They mainly investigated allocentric processing through randomised start points, although individual studies focused on examining different outcome measures. The task starts with a training phase where participants explored and learnt the position of a hidden platform within a circular pool. Distal cues that could be used to

encode the platform position allocentrically were located in the larger room enclosing the pool, and once participants located the platform, they were notified of success through a tone and/or the platform appearing. Participants' starting positions and orientations were randomised to discourage egocentric strategies, and there was a time limit imposed on trials. The protocols included probe trials where the platform was removed and participants' search paths were recorded, and some studies had control conditions where the platform was visible.

The four remaining sMRI studies employed a dual-solution navigation task – a 12-armed radial maze (Concurrent Spatial Discrimination Learning Task, CSDLT), whose training phase can either be egocentrically or allocentrically encoded, however distinguishes between the two strategies using probe trials (Konishi & Bohbot, 2013; Konishi et al., 2017), or spatial navigation tasks in complex virtual environments with multiple local and distal landmarks such as zoos (Lövdén et al., 2012), towns (Konishi et al., 2017) or interconnected rooms (Head & Isom, 2010). In the wayfinding (allocentric) conditions, participants were allowed to freely explore the spaces, guided by the experimenters to ensure exposure to all landmarks (Head & Isom, 2010; Konishi et al., 2017), or were required to navigate through the entire environment to search for cued stimuli (Lövdén et al., 2012). The main outcome measures were flexible shortest-route navigation from any landmark to another, and accuracy in navigating to cued landmarks, which depend on allocentric processing, i.e. the effective formation and maintenance of a cognitive map. Head and Isom (2010) employed route learning as a control condition while the other two studies did not have specific controls.

3.5 Overview of structural MRI findings

3.5.1 Age-related behavioural indicators of differences in allocentric processing

All nine studies comparing spatial task performance between younger adults, usually in their 20s-30s, and older adults (Antonova et al., 2009; Driscoll et al., 2003; Head & Isom, 2010; Korthauer et al., 2016; Lövdén et al., 2012; Moffat et al., 2007) or comprising of samples with a large age range (Daugherty et al., 2015, 2016; Daugherty & Raz, 2017) found pronounced age-related deficits in performance, an outcome that confirms the consensus in wider literature (Colombo et al., 2017). Generally speaking, older adults (OA) tend to travel more circuitous routes, spend more time searching, and be less accurate in recalling the trained location in vMWT, and when required to plan a novel route between two landmarks, deviate more from the optimal distance, as well as successfully locating the cued landmarks less. Three studies from the same researchers (Daugherty et al., 2015, 2016; Daugherty & Raz, 2017) highlighted the additional explanatory power of path complexity, a novel vMWT measure independent from path length and search time, that is quantified by fractal dimensionality (FD), already used in studies of ecological animal behaviour (Gautestad, 2011).

Studies that recruited only OA or those of middle age and above had mixed findings of the effect of age within this group, with most of the studies reporting null results – two studies that examined the relationship between age and CSDLT performance in OA samples, largely in their 60s, did not find a significant effect of age on performance (Konishi & Bohbot, 2013; Konishi et al., 2017), and neither did a study looking at a subset of participants stratified into middle-aged (40-59 years old) and OA (60-78 years old) find any age-related differences in

performance on the vMWT (Korthauer et al., 2016), suggesting that the commonly found significant differences between younger adults (YA) and OA may already be somewhat established in middle age. In contrast, in a sample of OA aged 55-80 years old in Konishi et al. (2017) there was a significant effect of age on performance on a wayfinding task set in a virtual town, particularly in the distance travelled in excess of optimal routes between two landmarks, and to a lesser degree the accuracy of locating cued landmarks. It is likely that the wayfinding task is more cognitively challenging in comparison to the vMWT and CSDLT, where only one location or choice in a smaller space has to be encoded at one time and there is less demand on abilities such as working memory and executive function, important for encoding and recall of multiple objects scattered in a large-scale space as well as route planning involving knowledge of the possible paths in between. However, it is also important to avoid over-extrapolating from the findings of only three studies and the positive result of one particular study.

The three studies containing longitudinal data reported largely negative results of decline in allocentric processing across time. The interventional study that tested participants at baseline, after 4 months of training on an allocentric “virtual zoo” task, and 4 months post-training (Lövdén et al., 2012) reported consistent marginal improvement in both the YA and OA control walking-only groups that suggested a test-retest effect. However, it is difficult to say whether the short study window (8 months) limited any meaningful findings about deterioration with age. The two longitudinal studies with longer FU periods of two years (Daugherty & Raz, 2017) and 8 years (Korthauer et al., 2016), both employing the vMWT, presented no clear evidence that ageing negatively affects performance on spatial tasks. The first study reported inconsistent intra-individual reliability of vMWT across time and had mixed

longitudinal findings: vMWT learning rate and absolute magnitude of improvement declined while reduction in path complexity improved at FU. Lastly, Korthauer et al. (2016) did not find any difference in vMWT performance at FU after 8 years. It should be noted that both studies had samples with a wide age range (baseline age ranges 18-77 and 30-83) which may have occluded any decline in the older participants in overall analyses of the main effect of age, as well as experiencing high dropout rates of about 40%, which the first study handled through statistical estimation of the missing data and the second through controlling for significant difference factors in the regression analyses. There is a clear need for more longitudinal studies of allocentric processing tracking individuals as they grow older, and minimising participant dropout, in order to elucidate the consistently significant age differences in spatial navigation that has been observed in cross-sectional samples.

Consistent with the literature, there is a pronounced deficit in allocentric processing when comparing young and older adults or across the lifespan. Studies with middle-aged to older adults had mixed findings, with some reporting an influence of age in that population. The few longitudinal studies did not find a deterioration in performance across time.

3.5.2 Age-related differences in hippocampal neural structure and biochemistry

When looking at cross-sectional age-related differences in regional volumes and biochemistry of the HC and surrounding areas, the findings present a mixed picture, with a larger proportion of studies supporting cross-sectional and longitudinal age differences in hippocampal and surrounding regional volumes. Significant age differences were found in bilateral, left, right and posterior HC volumes, N-acetylaspartate and creatine ratios

(NAA/Cre) in the HC and frontal white matter regions (Driscoll et al., 2003), lateral prefrontal cortex grey matter (LPFC), prefrontal white matter (PFW), caudate nucleus (CN), and cerebellum (CB) (Moffat et al., 2007), and hippocampal mean diffusivity (Lövdén et al., 2012) while age correlated with right HC volume (Konishi et al., 2017) and with smaller HC subfield and EC volumes (Daugherty et al., 2016). Four studies did not report simple comparisons or correlations of regional volumes or biochemistry with age (Daugherty et al., 2015; Head & Isom, 2010; Konishi et al., 2013; Korthauer et al., 2016) while two studies did not find differences in HC volumes or NAA/Cre between YA or middle aged and OA (Antonova et al., 2009; Lövdén et al., 2012). This appears consistent with the literature as there is high individual variability in the volume of HC and surrounding regions (HC/PHG) in OA (Van Petten, 2004), as well as many influencing factors on HC/PHC volume such as genetics, health conditions and fitness (Persson et al., 2014; Raz et al., 2005; Raz & Rodrigue, 2006) that may lead to mixed results due to different inclusion criteria and sample heterogeneity.

The two studies with longitudinal structural MRI data both found significant differences in HC volume across time. Lövdén et al. (2012) observed declines in left and right HC volumes in the control group consistent with previous longitudinal studies while the intervention group displayed stable hippocampal volumes post-training and 4 months after. Daugherty & Raz (2017) reported significant shrinkage in the HC, PHG as well as CN and CB, but not LPFC, after two years consistent with longitudinal studies, although again there was significant missing MRI data (72/213 and 49/131 at baseline and FU) that was estimated statistically.

About half of the studies reported significant age differences in HC volume, metabolic or microstructural indicators, while the rest did not directly compare regional volumes or found no differences. The two longitudinal studies reported shrinkage in line with previous literature.

3.5.3 Neural correlates of allocentric spatial memory and navigation in ageing

All studies examined brain-behaviour correlations in an attempt to characterise the neural (volumetric/metabolic) correlates of allocentric spatial memory and navigation, with the majority of studies finding significant associations between task performance and hippocampal and parahippocampal areas.

Eight studies reported mixed or positive findings on hippocampal associations with allocentric processing. Driscoll et al. (2003) reported that HC NAA/Cre, but not volume, accounted for a significant amount of variance in vMWT performance, while HC volume, NAA/Cre and age all accounted for significant variance in performance on a non-spatial HC-dependent task. Head and Isom (2010) found a significant age-related impairment in wayfinding (allocentric condition) on a virtual maze task, with HC volume associated with better wayfinding performance and CN volume with route learning (egocentric condition) in the subset of OA scanned; however between-association differences were non-significant. Building on a previous YA study that found correlations between allocentric strategies with HC volume and egocentric strategies with CN volume in CSDLT (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007), Konishi and Bohbot (2013) investigated neural correlates of spontaneous strategy use in an OA sample and found a correlation between right HC volume and allocentric strategy use, but not between CN volume and egocentric strategy use. In a large OA sample, Konishi et al. (2017) found a negative correlation between age, wayfinding ability

and HC volume, but again no associations between CN volume and egocentric strategies. Higher right HC volume was correlated with younger age. Wayfinding task performance (allocentric processing) was positively correlated with right, left and total HC volume. CSDLT probe trial performance correlated with left and total HC volume. Neither task correlated with total cerebral or CN volume.

Eight out of 11 sMRI studies reported mixed or positive findings. Three studies reported significant associations between HC volume and allocentric spatial ability (vMWT performance, wayfinding, radial maze) and one study found an association only with HC metabolism. Little evidence supported the relationship between egocentric processing and CN volume.

Daugherty et al. (2015) demonstrated the additional explanatory power of vMWT path complexity (FD) as FD change significantly correlated with hippocampal areas, while path length did not. HC volume was positively associated with smaller vMWT trial 1 FD, shorter search time, and larger FD change across trials, while PHG volume also positively correlated with FD change. Greater search time was associated with smaller CB volumes. Daugherty et al. (2016) examined the relationship between age, HC subfields CA1-2, EC, CA3-dentate gyrus volumes, and vMWT search path length and FD, and found that regional volumes were not associated with average path length, FD, first trial performance or absolute improvement. Independent of age, larger subiculum and EC volumes correlated and trended respectively with greater reduction in FD, while CA1-2 volumes correlated with greater reduction in path length but not FD. In the two year FU of Daugherty et al. (2015), Daugherty and Raz (2017) found that vMWT learning rate and performance declined across time but path complexity improved quicker. Advanced age, higher pulse pressure, smaller CB and CN volumes and

greater CN iron were associated with less efficient search paths while path complexity improvement was predicted by lower HC baseline iron and larger PHG volumes. Korthauer et al. (2016) found that total vMWT latency was negatively associated with grey matter volume in right HC, left and right thalamus, right medial OFC, while distance was negatively associated with right HC volume. In middle aged subjects, total latency was negatively correlated with right HC while in OA it correlated with right medial OFC. Total latency negatively correlated with fractional anisotropy in the left and right uncinate fasciculus after controlling for age and speed.

Three studies investigated vMWT path complexity (FD) and variously reported that FD reduction was associated with HC, PHG, EC and subiculum volumes, while only HC iron and PHG volume predicted FD change longitudinally. One study found associations between HC grey matter or total volume and vMWT latency and path length, although HC's role is unclear in OA.

Three studies reported no significant associations between hippocampal regions and spatial task performance. Moffat et al. (2007) found a non-significant main effect of HC volume on vMWT performance, where the influence of HC volume was only significant in YA on the first trial, while prefrontal grey, white matter, and CN volumes explained significant variation in vMWT performance independent of age with a trend for CB. As there was no difference found between YA and OA on HC volumes, Antonova et al. (2009) did not perform correlations with vMWT performance. Lövdén et al. (2012) did not find any significant associations between HC volumes or mean diffusivity with virtual zoo performance in the YA and OA intervention groups, either together or separately.

Three out of 11 studies found no significant correlations between HC volume or microstructure and allocentric spatial ability. One study reported a correlation with CN volumes.

3.6 Experimental designs of functional MRI studies

Five papers reported fMRI data on studies of spatial memory and navigation, all cross-sectional designs comparing healthy OA samples, ranging from their late 50s to 70s, with younger control groups in their 20s to early 30s (Antonova et al., 2009; Konishi et al., 2013; Meulenbroek, Petersson, Voermans, Weber, & Fernández, 2004; Moffat et al., 2006; Schuck et al., 2015). Diverse experimental paradigms targeting allocentric processing were employed, including a MWT analogue task “Arena” (Antonova et al., 2009), CSDLT (Konishi et al., 2013), a route learning task (Meulenbroek et al., 2004), a “virtual maze” task requiring flexible landmark-to-landmark navigation (Moffat et al., 2006), and an object location memory task manipulating landmark location and boundaries (Schuck et al., 2015). All participants were trained on familiarisation tasks, e.g. for key presses or joysticks, closely modelled on the experimental paradigms before scanning.

Meulenbroek et al. (2004) showed subjects 14 video sequences of fixed routes through virtual homes of similar size and topography, training them to remember and press the corresponding key of the direction (left, right, straight) signalled by yellow arrows at five decision points. In the recognition condition, the sequences were shown again and subjects indicated by keypress the direction taken. Interspersed with rest periods, the control condition was passive viewing of a straight corridor with arrows. In Moffat et al. (2006), six objects were scattered in a virtual environment (VE) consisting of several rooms and hallways and

participants were instructed to fully explore and encode all object locations, aware they would be tested on their “map knowledge” to encourage allocentric encoding. They were required to navigate to specified objects by the shortest of several routes. The control condition was following a designated path in a visually similar VE using floor markers. Antonova et al. (2009) used “Arena”, a MWT analogue where participants had to navigate to and remember the location of a pole within a circular arena with abstract coloured patterned walls, equivalent to MWT distal cues. After six training trials participants were placed randomly in the arena without the pole and had to navigate to the recalled location. This was interspersed with rest epochs and a visual control period of passively watching static abstract coloured patterns.

Konishi et al. (2013) administered the CSDLT with fMRI to 52 younger and older adults. YA data was published separately (Etchamendy, Konishi, Pike, Marighetto, & Bohbot, 2012). Participants were trained to use the keyboard on a practice VE beforehand and OA were given a mock fMRI scan with a non-transferable CSDLT analogy task for familiarisation. A visuomotor control condition (navigate down one of two arms with an object, no distal environment) was interspersed with CSDLT trials, and participants simultaneously performed a working memory counting task to disrupt learning during the control condition. To investigate landmark (striatal) and boundary (hippocampal) information processing in ageing, Schuck et al. (2015) applied a computational model of boundary processing derived from Burgess & O’Keefe (1996) and a model of landmark processing (Doeller & Burgess, 2008) to generate predictions and compare them to behavioural data on a VR object location task. Male participants navigated in a circular outdoor arena surrounded by walls and distal cues e.g. mountains, clouds, with a landmark (traffic cone) and five randomised objects. During encoding trials, participants navigated from the centre to collect objects (only one appeared at

a time) and learn their locations. On feedback trials, six for each object, they navigated to the recalled location of a cued object, with the object then appearing in the real location and collected again. In the three types of transfer trials, the boundary was increased or decreased by 20%, or the landmark was shifted, and participants navigated to recalled locations of cued objects without feedback.

3.7 Overview of fMRI findings

3.7.1 Age-related differences in allocentric processing

Similar to the structural MRI studies described above, all studies except one found significant age differences in performance on spatial memory and navigation tasks in keeping with findings from numerous other studies without imaging. Meulenbroek et al. (2004) reported both groups performed well above chance in route recognition with a slight but significant age-related deficit. Moffat et al. (2006) recorded significantly reduced speed overall and greater mean number of errors during object location recall for OA. Antonova et al. (2009) found that OA were significantly worse at recalling the pole location compared to YA. Schuck et al. (2015) calculated vectors for displacement between recalled and correct object locations during the final feedback trial and three types of transfer trials and compared them to vectors predicted by the landmark and boundary models. He reported that YA performed significantly better, with an age x trial interaction in the feedback phase, i.e. more absolute improvement in YA. YA showed behaviour consistent with the boundary model and a smaller effect of landmark processing, while OA showed the opposite pattern. Visual inspection of vectors showed that YA consistently shifted their remembered locations in response to boundary change while OA did not. The remaining study (Konishi et al., 2013) reported

similar final performance for both groups on the CSDLT but slower learning for OA, requiring significantly more training trials to reach criterion.

Four out of five fMRI studies found significant age differences in performance on spatial memory and navigation tasks, including route learning and object location memory in simple and complex environments. One study reported slower acquisition for OA in a radial maze but no difference in final performance.

3.7.2 Age-related differences in functional activation during allocentric processing

Generalising from the fMRI studies, there are fairly consistent findings regarding reduced activity in OA of the HC and parahippocampal areas during spatial tasks, with one study reporting a positive association between HC/PHG activation and task performance (Moffat et al., 2006). Two studies observed increased activation in frontal areas such as the ACC in OA (Meulenbroek et al., 2004; Moffat et al., 2006).

Meulenbroek et al. (2004) reported activation of a neural network involved in spatial memory and navigation in both groups (dorsal and ventral visual streams) during the task, and diminished activation in dorsal and ventral visual processing streams, posterior fusiform, parahippocampal and parietal areas in OA during route encoding, which they inferred as the neural basis of the small age-related deficit in route encoding, as these areas are known to support memory formation of complex visual stimuli with a spatial component (Ekstrom et al., 2003; Weis, Klaver, Reul, Elger, & Fernández, 2004). OA also had undiminished anterior parahippocampal activity compared to YA, thought to indicate an abolished familiarity signal, diminished perisylvian deactivation during encoding and stronger activation of the ACC

during route recall, hypothesised to be related to failure to inhibit distractions and irrelevant information.

Moffat et al. (2006) also found increased activation in frontal and striatal areas, including the ACC and frontal medial cortex, which may be due to a more general compensatory shift from medial temporal regions supporting navigation to reliance on frontal areas (Gutchess et al., 2005), and reduced activation in the HC, PHG, retrosplenial cortex and parietal areas in OA, again consistent with existing fMRI literature on memory in OA (Daselaar et al., 2005). Furthermore, superior spatial navigation task performance was associated with increased activation in the posterior PHG, RSC and precuneus, supporting the hypothesis that the observed age-related impairment in spatial tasks is underpinned by reduced functionality in these areas.

Antonova et al. (2009) found that for encoding versus rest, YA had greater activation in the bilateral HC, left PHG, right anterior frontal pole and dorsolateral PFC (DLPFC), while OA had greater activation of the corpus striatum. During retrieval, YA activated the medial temporal lobe structures and right DLPFC while OA activated the anterior medial cingulate gyrus. Despite no difference in hippocampal volumes, there was age-related attenuation in HC and perirhinal activity for encoding and retrieval, although the difference did not survive between-group analysis of variance. The authors noted the concordance of their findings on age-related attenuation of HC/PHG activity with previous studies and suggested that the performance deficits seen in OA are due to subtler changes in neurogenesis and functional connectivity in the HC rather than atrophy.

Two studies focused on the dissociation between hippocampal and striatal systems, believed to support allocentric and egocentric navigation respectively (Iaria, Petrides, Dagher, Pike, & Bohbot, 2003), and found generally attenuated HC and increased striatal activity in OA (Konishi et al., 2013; Schuck et al., 2015). Konishi et al. (2013) reported a time effect with HC being recruited early on in the encoding phase by YA while CN activated towards the end, more so in OA. The authors hypothesised that ageing creates preference and reliance on the caudate/striatal system of stimulus-response learning (egocentric) due to reduced cognitive resources (Iaria et al., 2003; Nadel & Hardt, 2004), and their results fit with the characterisation of the hippocampal and striatal systems being fast, resource-intensive and slower acting (i.e. developed through experiencing reward contingencies) respectively (van der Meer, Johnson, Schmitzer-Torbert, & Redish, 2010). Schuck et al. (2015) reported largely similar results and manipulated boundaries and landmarks associated with each system to demonstrate OA reliance on proximal cues and insensitivity to distal elements such as boundaries. However, they also found that OA landmark processing was related to HC activity in addition to striatal areas (putamen) and the thalamus, suggesting HC function may be altered by ageing. Interestingly, high-performing OA in both studies also activated hippocampal in addition to striatal areas, suggesting that HC involvement in OA may either indicate allocentric strategies or supplement landmark-based/egocentric learning.

The five fMRI studies mostly reported reduced HC and PHG activity in OA during allocentric tasks. OA had more activation in frontal and striatal areas in all studies, while one study observed a positive association between HC activation and performance.

Discussion

4.1 Allocentric processing in old age

With reference to the two main objectives of the review, it is possible to conclude for the first that in agreement with the overall findings in wider research, healthy older adults show a consistent performance gap compared to young adults in spatial tasks in almost all measures collected by the studies examined, including Morris Water Maze analogues and wayfinding in virtual mazes. The only exception is the study by Konishi et al. (2013) which found similar final performance between OA and YA but still a slower learning rate in OA. Older adults have longer, more complex paths, spend more time searching and are less accurate in learning the platform location in the MWT; they take less efficient paths and are less accurate in recalling object locations in other virtual navigation tasks. These differences appear to be driven by general factors including slower processing and movement speed causing OA to take more time, poorer executive functioning for demands including route planning and poorer working memory, as well as spatial and navigational characteristics including primarily egocentric and local landmark-based strategies (Bohbot et al., 2012) and difficulty in selecting or switching to an allocentric reference frame (Harris, Wiener, & Wolbers, 2012; Harris & Wolbers, 2014; Wiener, Condappa, Harris, & Wolbers, 2013).

Results of studies involving middle aged (MA) to older adults only and longitudinal studies in this review are very ambiguous taken together and not clarified the trajectory of how observed cross-sectional age differences develop. There were only three studies that looked at OA only or examined MA and OA, with two null results and one reporting significant age effect, and it may be that the studies are underpowered due to small sample sizes or the

tasks used may not be sensitive enough to identify subtle changes. Alternatively, the significant age deficits may have begun prior to or during middle age, however that is at odds with reported trends in HC atrophy which accelerate in older age (Raz et al., 2005). The three longitudinal studies had very short time windows and/or significant participant attrition that appeared non-random, possibly affecting findings if lower-performing subjects or those with greatest age-related decline in spatial ability dropped out. Given the difficulties of conducting rigorous longitudinal studies, there is a clear need for more resources to be devoted to this area, perhaps including allocentric processing as a standard measure in large cohort studies of ageing, especially considering the importance of spatial memory and navigation deficits as an early indicator of dementia (Gazova et al., 2012).

4.2 Neural correlates of spatial processing: trends from MRI, fMRI and other findings

The 11 structural studies presented a varied picture, with the majority of studies supporting age-related regional volume differences and longitudinal shrinkage in the HC and surrounding areas, as well as offering evidence for age differences in caudate nucleus, cerebellar and prefrontal regional volumes. The positive evidence is distributed across cross-sectional and longitudinal studies, with one of the two studies reporting no age difference having a very small sample size (Antonova et al., 2009). Three out of four studies that included measures such as NAA/Cre and DTI reflecting metabolic or microstructural alterations have also supported age-related differences (one did not report direct comparisons), suggesting that more subtle changes in hippocampal synaptic plasticity, neurotransmission, and LTP that are known to affect functions such as cognitive mapping in animals (Lester et al., 2017) are likely contributing to age-related changes in humans as well.

When correlational or regression analyses are conducted with task performance, several main findings can be surmised: (1) Total (and right) HC volume, as well as to a lesser extent PHG areas, on balance correlate with performance on allocentric spatial navigational tasks, while mixed results were reported on the subgroup of studies that investigated performance correlation with CN volume and HC biochemical/microstructural estimates, (2) Attenuated activation of the HC and PHG, and increased activation in striatal areas in OA during spatial tasks, and to a lesser extent increased frontal activation, accompany age-related deficits, (3) High-performing OA appear to activate the HC in addition to striatal areas when compared to low-performing OA. Taken together with other findings from the literature (Colombo et al., 2017), there appears to be reasonable support for an age-related reduction in HC/PHG volume and activity, mediated through a shift away from allocentric processing to reliance on egocentric strategies, ultimately contributing to the poorer performance of OA in spatial memory and navigation tasks. The right HC, investigated and significantly associated with task performance in three studies, supports memory for specific locations in environments (Burgess et al., 2002; Maguire, Frackowiak, & Frith, 1997). There is considerable difficulty in translating differences in biochemical (NAA/Cre, iron content) or DTI measurements (Alexander, Lee, Lazar, & Field, 2007; Daugherty & Raz, 2015) into meaningful inferences about neural microstructure and metabolic damage, which along with the mixed results reported in this review, restricts the interpretation of findings.

Similarly, it is hard to assess with much confidence findings on extrahippocampal neural correlates of spatial task performance as they were not investigated in all reviewed studies. However, there is moderate evidence across MRI and fMRI studies supporting an association between reduced integrity/volume and increased activity in frontal regions and OA spatial

task performance that may be mediated by factors such as poorer spatial working memory, attentional deficits, executive functioning and goal planning. The route planning that is required in large virtual mazes recruits the RSC to monitor routes during navigation, mediate between egocentric and allocentric processing, and PFC for unexpected detours in addition to the HC (Spiers & Maguire, 2006). The caudate nucleus and striatum is another region that attracted significant attention in the reviewed studies given the central role it holds in egocentric or response learning. Volumetric correlations with spatial task performance were mostly null in OA only studies (Head & Isom, 2010; Konishi & Bohbot, 2013; Konishi et al., 2017), with one positive association (Moffat et al., 2007) while fMRI findings suggested increased activation in OA corresponding to their bias toward egocentric strategies.

4.3 Strengths and limitations of review and included studies

The current review systematically and comprehensively examined all studies investigating the impact of age on allocentric processing in spatial memory and navigation and the relationship to hippocampal and other regional volumes and activity. One strength is that the reviewed articles were similar in experimental design and paradigms used, with the majority employing the MWT and others using radial mazes or larger and more complex virtual environments with multiple landmarks. This allows results to be generalised more easily across studies. Furthermore, the quality assessment tool produced a narrow range of scores that indicated the overall quality of the reviewed studies is fairly homogenous and of an acceptable standard, although a third of studies fell just below the ‘High’ threshold, mostly due to small sample sizes, lack of information about participant selection/control and incomplete reporting of statistical variance. Therefore, scores from the assessment tool was

of limited use in eliminating studies of poor quality or in determining the emphasis of the review given such a narrow range.

There were three studies including or scanning OA only that would have been strengthened by inclusion of young comparison groups, while the two long-term studies suffered significant and non-random dropout limiting the applicability of findings. Repeated administration of the MWT, a conceptually simple task, in the longitudinal studies may have produced test-retest learning, although the effect should be limited. The performance measures and factors entered into regression analyses also varied considerably across studies, with some focusing on conventional outcomes including distance and length of time while others investigated change rates, path complexity, and consistency with model predictions. Aside from the HC, there was heterogeneity in the ROI selected, making it difficult to compare findings as different regions were included in analyses. The studies were often underpowered with sample sizes too small for the number of predictors entered. Thus, it is necessary to be cautious when interpreting and ascribing meaning to increasingly complex interrelationships between multiple regional/functional measures and task performance. The varied imaging methodology due to technological advances and different protocols also introduces inevitable variance to the results.

The virtual reality tasks in the reviewed studies have the obvious advantage of greater ecological validity compared to pen and paper tests for assessing real-life spatial memory and navigation, allowing experimenters to collect much more detailed and extensive data regarding participant behaviour. Studies incorporating both real and computer versions of egocentric/allocentric spatial tasks report strong correlations in performance and similar

predictive and differentiating power (Burgess et al., 2002; Serino et al., 2014), and the same neural networks and regions are likely recruited. However, key differences remain between VR and real-life spatial tasks, including gaps in proprioceptive and self-motion feedback using joysticks or keyboards versus exploring by walking, and the lack of sensory immersiveness of computer screens compared to a real world environment. Furthermore, the advantage of being able to investigate functional activation during spatial navigation using VR tasks is counterbalanced by the reduced validity due to immobilisation in a brain scanner during testing.

4.4 Strengths and limitations of spatial tasks and study methodology

All the reviewed studies aimed to investigate the neural correlates of allocentric processing in ageing and older adults, however it is based on an assumption that better performance on the tasks corresponds to allocentric processing. Despite Meulenbroek et al. (2004) presenting their task as a measure of allocentric processing, the aspects of the task thought to rely on that may actually have involved other processes such as encoding semantically in working memory or procedurally as a sequence of egocentric movements, and the lack of participant agency in terms of navigation decisions might also have affected encoding (Plancher, Tirard, Gyselinck, Nicolas, & Piolino, 2012) and differentiated the task from the rest of the studies. In fact, another study (Head & Isom, 2010) employed a similar procedure as an egocentric control for the allocentric condition of wayfinding.

Although commonly regarded as a measure of allocentric processing or cognitive mapping, it is actually somewhat difficult to pinpoint the specific processes involved in solving the allocentric MWT due to the small scale of the space that conceivably allows successful recall

of the platform location using sensory matching, encoding self-to-environmental cue relations at goal location in earlier learning trials, or multiple distal-cue-to-platform encoding without self-localisation within an allocentric reference frame (Wolbers & Wiener, 2014). This problem of defining what constitutes allocentric navigation also occurs in other “vista” scale spaces (Montello, 1993) that can be perceived from a single location, although dual-solution tasks such as the CSDLT purport to distinguish between the use of reference frames. The computations performed in an allocentric vista space task merely constitute a subset of the processes involved in navigating “environmental” scale spaces, employed by a third of all reviewed studies. In environmental scale spatial navigation, self-localisation in addition to goal localisation is necessary and higher demands are placed on working memory (knowledge of the junctures between two landmarks) and executive functioning (novel route planning, route monitoring and re-planning). Both the hypothesised overall decline in computational resources (Craik, 1986) and reduction in processing speed (Salthouse, 1996) that occur with ageing disproportionately impact frontal functions such as executive planning, working memory and also allocentric navigation, which requires more attentional resources (K. D. Wilson, Woldorff, & Mangun, 2005) and is considered less automatic and elementary than egocentric processing (Pouliot & Gagnon, 2005). Again, these changes would be more apparent in environmental scale versus vista scale spatial tasks due to the additional cognitive demands of the former. However, a disadvantage of environmental scale tasks is the lack of standardisation, as most paradigms are formulated by individual research groups, rarely directly replicated, and vary considerably in size of the environment, protocol, and number and type of stimuli. In comparison, the experimental properties and outcome measures of MWT have been well validated through numerous studies. Despite this limitation,

the consistency in findings implicating hippocampal volume and attenuated activity across studies using the MWT, radial mazes and larger scale spaces provides strong evidence for the hippocampus's central role in processing contextual/environmental information to support allocentric navigation.

Lastly, very few reviewed studies interviewed participants about actual strategy use and considered this factor in their analyses, or included comparison conditions aimed at tapping into egocentric processing systematically, except Head & Isom (2010). The control condition in the MWT does not require much spatial learning including egocentric, since the goal (platform) is always visible to the participant. Thus, the caveats of the differences in task parameters and what specific processes they are measuring have to be held in mind when understanding the findings of this review.

4.5 Conclusion and future directions for research

Spatial navigation in complex environments is an ancient and essential ability for survival that has been garnering a lot of attention in the last few decades in the context of the neurobiological changes that occur with ageing. Human behavioural research inspired by animal behavioural neuroscience, and more recently structural and functional imaging of the brain, have revealed its crucial connection with the hippocampus. This systematic review examined all studies that investigated the role of age in allocentric spatial navigation and its neural correlates. The results show a pronounced deterioration of spatial memory and navigation with normal ageing on tasks that depend on encoding distal environmental cues and require formation of a cognitive map, as well as reasonable evidence linking the deficit to age-related alterations in hippocampal morphology and attenuated activation.

Despite the barriers and resource demands, more rigorous longitudinal studies of spatial navigation and neuroimaging should be conducted to investigate how these age differences evolve and are interrelated over time. Information should be collected about spontaneous allocentric or egocentric strategy use and individual tendencies, through self-report or interviews, and care taken to control for this variable in tasks where either or a mixture can be used. The development of more ecologically valid paradigms set in large scale spaces is also important as there are a larger set of demands that may not be fully reflected in standard paradigms such as the Morris Water Maze. The advent of commercially available immersive virtual reality (iVR) with its ability to track movement is another significant advance beyond computer-based VR tasks used to date in spatial navigation research, as experimental paradigms can be hugely improved in ecological validity both in terms of highly convincing three dimensional visual and audio input, responsiveness to head movements, and by incorporating self-motion and proprioceptive feedback.

In light of the rapidly growing research into the functions supported by different hippocampal subfields and surrounding regions of the medial temporal lobe such as the entorhinal (Deshmukh & Knierim, 2011) and parahippocampal cortex (Bohbot et al., 1998; Vann, Brown, Erichsen, & Aggleton, 2000), there is a clear need for more studies focusing on examining associations between multiple spatial task outcomes with HC/PHG subfield volumes and activity in older adults, to validate the many promising results from animal studies.

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Part 2: Empirical Paper

Measurement of allocentric processing in mild cognitive impairment and early Alzheimer's disease using a virtual reality object location paradigm

Abstract

Aim

Mild cognitive impairment (MCI) and Alzheimer's Disease (AD) are major contributors to disability in old age and defined in the early stages by spatial memory deficits associated with hippocampal (HC) and entorhinal (EC) atrophy. Currently diagnosis occurs late in the process which limits efficacy of interventions. This study investigated the neural correlates of a novel object location task (OLT) in immersive virtual reality (iVR).

Methods

Twenty amnestic MCI (aMCI) patients and twenty two healthy controls were tested on the iVR OLT, underwent neuropsychological testing and structural MRI scanning. OLT performance and HC, EC subfield volumetric data were compared between groups, and correlational analyses of HC/EC volumes and performance were conducted.

Results

Participants with aMCI were significantly impaired in object location recall and object recognition compared to controls. They had significantly smaller total HC, subiculum, CA1, EC and perirhinal volumes. There was a significant interaction of group in analysis of neural correlates: OLT performance was strongly predicted by total HC and subiculum volumes in patients only. EC subfields were not significant predictors of performance.

Conclusion

Performance on the novel OLT in immersive VR is a good indicator of HC integrity in older adults with amnestic MCI and can improve the diagnostic process for people with MCI and AD in the future.

Introduction

1.1 Alzheimer's Disease

Alzheimer's disease (AD) is the commonest cause of dementia, affecting 35.6 million people worldwide in 2010 – a number that is set to increase to an estimated 115.4 million by 2050 (Prince et al., 2013). Given the expected growth trajectory and high economic impact of health and social care needed to support people with dementia (Wimo et al., 2011), improving early identification and intervention for AD is an important priority, as modifiable lifestyle risk factors such as diet and exercise can ameliorate cognitive decline (Ngandu et al., 2015), potentially delaying illness onset and significantly reducing the burden of care (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007).

Like other dementias, AD is a chronic neurodegenerative disease that initially manifests as memory loss, mild language impairment, and executive difficulties (A. Burns & Iliffe, 2009). A decline in short-term and episodic memory, the recall of autobiographical events set in a particular time and place, is often the first noted symptom. The symptoms of AD are attributed to neuronal loss and atrophy in areas of the cerebral cortex including the medial temporal lobe (MTL), thought to result from the accumulation of beta amyloid-Aβ plaques and tau protein neurofibrillary tangles observed in vivo and post-mortem studies (Tiraboschi, Hansen, Thal, & Corey-Bloom, 2004; Verhoeff et al., 2004). It is currently diagnosed through progressive memory impairment, supported by cerebrospinal fluid (CSF) biomarker testing, MRI or amyloid PET imaging (Dubois et al., 2014). However, by the time memory symptoms manifest, pathophysiological disease processes have usually caused significant and irreversible damage to the brain.

Converging evidence suggests that the neurobiological changes underpinning AD start many years before memory symptoms appear (Jack et al., 2010) and that treatment may be most effective when given pre-clinically (Sperling et al., 2011). In light of recent advances in medications that tackle AD neuropathology like amyloid plaques (Sevigny et al., 2016), the need to develop quicker, less invasive and more cost-effective tests to detect AD before the onset of clinical symptoms becomes ever more pressing.

1.2 Mild Cognitive Impairment

Mild cognitive impairment (MCI), a category describing the intermediate stage between normal ageing and dementia, is characterised by memory loss and cognitive decline greater than expected for ageing yet not meeting criteria for clinically probable AD, preserved general cognition and no impact on functioning (Petersen, 2004). The subgroup defined by memory loss is known as amnestic MCI (aMCI), in contrast to non-amnestic MCI (naMCI) where domains other than memory are primarily affected, and is linked to higher likelihood of AD (Csukly et al., 2016; Jungwirth, Zehetmayer, Hinterberger, Tragl, & Fischer, 2012). People with amnestic MCI are 10-15 times more likely to develop AD than the general population (Petersen et al., 2009), making it a natural target for research into early detection and intervention. At the aMCI stage, the extent of MTL atrophy in structural MRI can serve as a diagnostic marker for AD (Frisoni, Fox, Jack, Scheltens, & Thompson, 2010). Currently, diagnosing MCI can be a lengthy, resource-intensive and invasive process, involving clinical interviews at multiple time points, detailed cognitive testing, MRI scans and possible lumbar punctures for CSF biomarker testing. There is great potential for additional assessment methods to improve diagnostic accuracy and affordability as the global population ages.

1.3 Alzheimer's Disease, spatial navigation and normal ageing

One of the earliest clinical symptoms of AD is topographical disorientation – a decline in spatial memory and ability to navigate (Serino et al., 2014). Spatial disorientation manifests very early in the onset of Alzheimer's, often appearing before any noticeable decline in episodic memory or other cognitive functions, and can occur as a result of landmark agnosia (inability to orient using salient features in the environment), egocentric disorientation (inability to represent object locations with respect to self), heading disorientation (inability to represent direction of orientation with respect to external environment) or anterograde disorientation (inability to create new representations of the environment).

The early manifestation of spatial difficulties is unsurprising given that the first neuropathological changes in AD are seen in the trans- and entorhinal cortex (EC) of the MTL, spreading to the hippocampus (HC) (Braak, Braak, & Bohl, 1993). These areas are crucial to both spatial and episodic memory, and because of the comparative ease of quantifying spatial deficits experimentally, as well as the rich literature on spatial memory and navigation in animals, it has emerged as one of the most promising areas of research into predictors of developing AD.

General theories of cognitive ageing hypothesise declines in computational resources (Craik, 1986) and reduction in processing speed (Salthouse, 1996). More recently, the Scaffolding Theory of Aging and Cognition (STAC-r, Reuter-Lorenz & Park, 2014) explains imaging and behavioural findings in ageing cognition through the dual processes of neurobiological challenges, e.g. general cortical atrophy, and functional deterioration, e.g. decreased specificity of brain activity, with compensatory scaffolding processes operating differently in

each individual. The revised theory incorporates constructs of neural enrichment (cognitive reserve, cardiovascular health) and depletion (genetic risk factors for AD plaques and tangles, stress/cortisol's impact on the hippocampus), all of which are useful ideas for more focal areas of research such as MTL and spatial processing degeneration in older adults.

1.4 Spatial reference frames: allocentric and egocentric

Spatial orientation and navigation can utilise two different reference frames: egocentric and allocentric. Egocentric processing is self-to-object, based on encoding locations and objects in relation to the self, and retains the same perspective as the initial representation. An example is memorising a certain route by the sequence of left-right turns and where landmarks appear relative to oneself. It is linked to greater activation in the precuneus, frontal and posterior parietal areas (Boccia et al., 2014; Burgess, 2008). Meanwhile, allocentric processing is object-to-object, based on encoding relationships of objects and environmental characteristics relative to each other, and perspective-independent, allowing for flexible navigation in novel environments. Allocentric processing and navigation is subserved by the hippocampal system, where "place cells" (PC) that fire at specific locations in a local environment independently of individual orientation were first observed in mice (O'Keefe, 1976) and later humans (Ekstrom et al., 2003). These findings bolster the concept of a "cognitive map", an allocentric, perspective-independent representation of the environment in the hippocampus (O'Keefe & Nadel, 1978; Tolman, 1948).

1.5 Allocentric processing deficit as a predictor of Alzheimer's disease

Allocentric processing is of particular importance for research into early detection due to two lines of evidence: firstly, histological and neuroimaging studies point to neuropathological,

structural and functional changes associated with AD beginning in the anterior MTL, specifically in the lateral and medial EC and spreading to the HC (Braak et al., 1993; A. T. Du et al., 2001; Khan et al., 2014), with atrophy and hypometabolism also observed in areas of the parietal lobe and cingulate gyrus such as the retrosplenial cortex (RSC) (Vlček & Laczó, 2014). Being the site of initial pathology, EC volume has consistently been found to correlate with memory performance in MCI patients and to predict conversion to AD (de Toledo-Morrell et al., 2004; Risacher et al., 2010; Schmidt-Wilcke, Poljansky, Hierlmeier, Hausner, & Ibach, 2009). The subiculum and CA1 subfields neighbouring the EC are the next affected – focal atrophy of the CA1, subiculum and presubiculum are reported in volumetric studies of patients with MCI and early AD (de Flores, La Joie, & Chételat, 2015), and the subiculum has been linked in recent studies to non-spatial memory impairments in participants with MCI and pre-MCI subjective cognitive decline (Carlesimo et al., 2015; Hirjak et al., 2017; Lindberg et al., 2017). Cellular and imaging studies have linked the subiculum and EC to specific components of allocentric processing, discussed in the next section.

Secondly, consistent with the findings regarding EC and HC function, allocentric and to a lesser extent, egocentric, memory performance differentiated patients with early AD from other dementias and controls (Bird et al., 2010; Burgess, Trinkler, King, Kennedy, & Cipolotti, 2006), distinguished between aMCI patients with or without underlying AD (Moodley et al., 2015), and furthermore predicted transition into MCI (Verghese, Lipton, & Ayers, 2017) strongly highlighting its potential as an early predictor of incipient AD, likely due to the critical importance of the EC, subiculum and HC in supporting allocentric processing.

A systematic review by Serino et al. (2014) identified 16 studies of allocentric and egocentric processing in MCI/AD patients. The majority of studies focused on comparing controls, AD and MCI patients, with high-risk groups such as early onset AD, genetic susceptibility (ApoE4+) or aMCI being of special interest (Albert et al., 2011). The review concluded that a prevalence of allocentric impairment is apparent in the earliest stages of AD and in MCI patients, with some studies suggesting a specific deficit in switching between reference frames, mediated by the RSC (Byrne et al., 2007; Ruggiero, Iavarone, & Iachini, 2018). The authors noted the importance of research on even earlier stages of AD such as "preclinical AD", where elevated biomarkers are the only indicator of risk, as well as designing more ecological tasks that have bearing on real-world symptoms such as topographical disorientation. Recent studies on preclinical AD confirm that MTL functions such as allocentric spatial navigation are the first affected, even before any other observable impairments (Allison, Fagan, Morris, & Head, 2016).

1.6 The role of hippocampus and entorhinal subfields

A wide variety of functional connections and specialised neurons in the HC complex and adjacent cortices underlie the ability to represent spatial components allocentrically. The HC is critical for spatial learning, as shown by decades of research in animal lesion and human studies (Burgess et al., 2002). It has major reciprocal connections with the cortex through two distinct pathways: the medial (MEC) and lateral EC (LEC), with studies suggesting a functional dissociation of the two (Deshmukh & Knierim, 2011). Characterisation of EC subfields in humans is in its early stages – recent imaging and functional connectivity studies indicate that the human anterolateral EC (alEC) and posteromedial EC (pmEC) are

analogous to the rodent LEC and MEC, and connect the HC to the perirhinal (PRC) and parahippocampal cortices (PHC) respectively (Maass, Berron, Libby, Ranganath, & Düzel, 2015; Navarro Schröder, Haak, Zaragoza Jimenez, Beckmann, & Doeller, 2015).

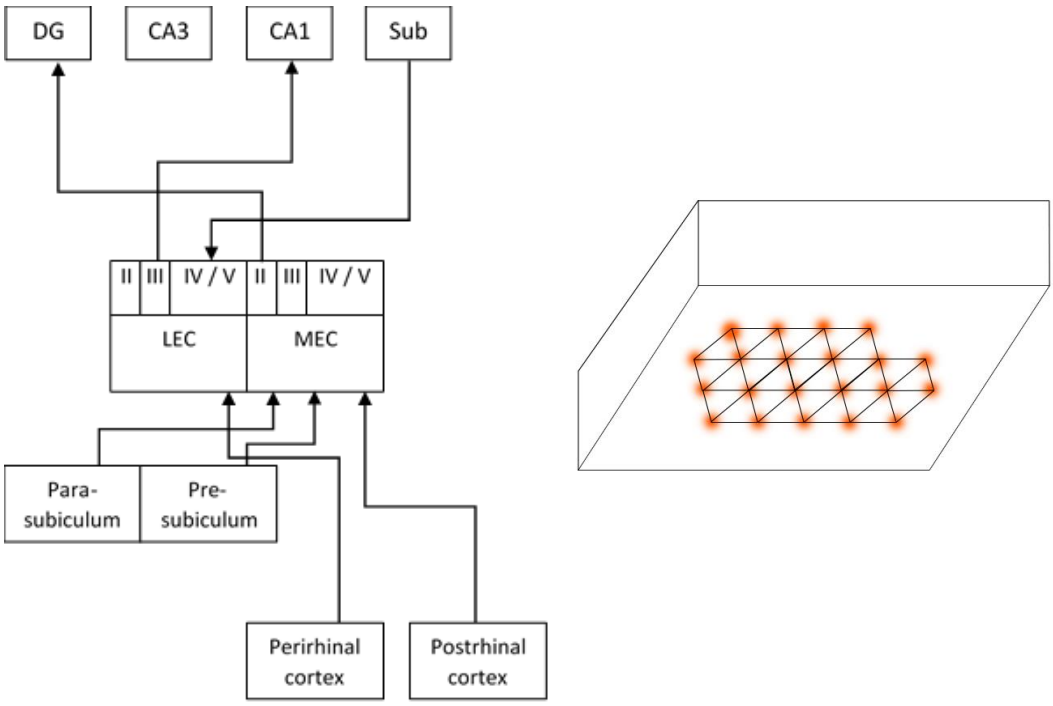

Figure 1. *(1) Anatomy of the HC formation, (2) grid cell firing patterns.*

The specialised functions and cell types found in the EC and HC are summarised below.

- **Lateral/anterolateral entorhinal cortex (LEC/alEC):** This area has been linked to discriminating and recalling configural or spatial properties of objects and their locations as the apex of the ventral visual stream. LEC neurons are found to support object location recall, and animal lesion studies suggest that LEC damage affects the ability to represent and recall objects and their spatial and temporal contexts (Tsao, Moser, & Moser, 2013; Van Cauter et al., 2013).

- **Medial/posteromedial entorhinal cortex (MEC/pmEC):** This area is thought to complement allocentric/HC functions such as cognitive mapping (Knierim, Neunuebel, & Deshmukh, 2014). MEC damage lowers stability and precision of HC PC and impairs global spatial learning with a navigational or path integration component (Hales et al., 2014).

- **Place cells (PC):** Neurons found in the HC, usually CA1 and CA3, that fire whenever an animal enters a specific location in an environment. PC firing is affected by external information such as boundaries and distal cues as well as receiving input from specialised cells in the EC below.

- **Grid cells (GC):** Found in the pmEC/MEC, HC pre- and parasubiculum (Boccara et al., 2010; Hafting et al., 2005; Jacobs et al., 2013), GC are sensitive to external cues, firing periodically across an environment to form a grid-like pattern of equilateral triangles. Reduced GC activity in the pmEC was reported in high-risk populations and associated with poor spatial memory performance, decades before AD onset (Kunz et al., 2015).

- **Border and boundary vector cells (BVC):** Found in the pmEC/MEC, HC subiculum, pre- and parasubiculum (Lever et al., 2009; Solstad et al., 2008), BVC are sensitive to the distance and direction of boundaries.

- **Head direction cells (HDC):** Discovered in the rodent postsubiculum (J S Taube, Muller, & Ranck, 1990) and primate presubiculum (Robertson, Rolls, Georges-François, & Panzeri, 1999) HDC are orientation-specific, location-invariant and may underlie our “sense of direction” (J S Taube, 1998).

Unlike PC, which remap with environmental changes, GC and HDC display relatively stable firing activity between environments or in absence of external sensory input and are likely involved in maintaining an estimate of location based on self-motion information while navigating, i.e. *path integration* – the ability to return to a starting point in the absence of environmental visual information, relying on optic flow, vestibular, motor and proprioceptive cues. The pmEC/MEC is hypothesised to be the site of integrating directional (HDC) and positional (GC, PC) information for representing location and self-localisation (Witter & Moser, 2006). Path integration is impaired in older adults and disproportionately in people with MCI/AD (Adamo, Briceño, Sindone, Alexander, & Moffat, 2012; in prep, Castegnaro; Mokrisova et al., 2016).

1.7 Self-motion and immersive Virtual Reality (iVR)

Ever since realistic 3-dimensional environments can be simulated on computers, virtual reality has been used to investigate the neural correlates of spatial navigation, sometimes combined with real time functional imaging (Burgess, Maguire, Spiers, & O'Keefe, 2001; Maguire et al., 1998). Serino et al. (2014) emphasised the potential of virtual reality technology in assessing spatial deficits, as the many advantages it had over traditional cognitive or real-world testing included greater ecological validity, multisensory stimulation, control over environments and stimuli and opportunity for active exploration. Virtual reality is already commonly utilised in spatial memory studies due to greater ecological validity over paper tests, rich behavioural information, and cost effectiveness over biomarker testing and imaging. The majority of studies incorporating both real and computer versions of allocentric/egocentric spatial tasks report strong correlations in performance and similar

predictive and differentiating power (Cushman et al., 2008; Serino et al., 2014), suggesting that the same neural networks and areas are activated.

The arrival of affordable immersive virtual reality (iVR) systems in the last few years creates exciting possibilities for fine-tuning spatial tasks to explore the behavioural correlates of different EC and subiculum subfields, and investigating allocentric processing influenced by self-motion, proprioceptive and motor feedback as computer VR tasks cannot adequately simulate these aspects of real-world experiences. Although published studies using iVR to investigate spatial processing are scarce due the novelty of the technology, a study by Plancher et al. (2012) recreated the experience of driving, requiring participants to operate a driving apparatus to explore a virtual town. It was hypothesised that the improved recall of allocentric context in all groups (AD, MCI, control) as well as better temporal memory performance in MCI patients in the active exploration (driver versus passenger) condition is due to additional self-motion and procedural information. Other studies (Carassa, Geminiani, Morganti, & Varotto, 2002; Winter, Mehlman, Clark, & Taube, 2015) also suggest that normal GC firing is dependent on self-motion cues and allocentric memory is enhanced by active exploration.

1.8 The current study

Considering what we know about AD pathology beginning in the EC and subiculum, containing specialised cells that support allocentric processing through path integration and object-related contextual memory, and the reported associations of these areas with non-spatial (verbal, autobiographical) memory impairments in preclinical, MCI and AD patients, it can be hypothesised that these regions are also implicated in the observed

allocentric deficit. Furthermore, new advances in our understanding of LEC and MEC analogues in humans and finer HC/EC segmentation protocols have enabled in-depth investigation of the differential contributions of individual EC and HC subfields to allocentric spatial memory. Knierim et al. (2014) hypothesised that the MEC, in a network alongside subiculum regions and RSC, provides information about actions and self-localisation, while the LEC provides information about external (e.g. object) cues and where they are in relation to the self (Deshmukh & Knierim, 2011; Yeung et al., 2017).

Therefore, the current study employed a novel iVR object location task (OLT) to investigate object location memory in healthy controls and aMCI patients, aiming to tap into functions understood to be HC-dependent such as cognitive mapping, path integration and object-context recall. Associations between performance and individual HC and EC subfields, obtained using high resolution imaging, were explored. The study demonstrates iVR's utility for and sensitivity in detecting established allocentric processing deficits in MCI patients, as well as identifying which subregions are the strongest predictors of components of these deficits, which would be hugely significant steps towards improved early identification of people at risk of developing AD. The findings will have important clinical implications and can potentially provide another source of information during the process of diagnosing MCI and AD as well as enhancing the experience for patients.

1.8.1 Regions of interest (ROIs)

The few previous studies exploring neural correlates of allocentric deficits in people with MCI/AD have largely only looked at hippocampal volumes, and some reported significant associations of spatial performance with total or right HC volumes or activity (Migo et al.,

2016; Moodley et al., 2015; Nedelska et al., 2012; Weniger, Ruhleder, Lange, Wolf, & Irle, 2011). In light of the cumulative evidence outlined in the introduction, the current study specifically examined pmEC, alEC, PRC (area 35), subiculum and presubiculum volumes in addition to EC and total HC volumes. The parasubiculum was not included due to lower reliability in automated segmentation protocols for small subregions (Whelan et al., 2016).

1.8.2 Research questions and hypotheses

Jointly with project partner E.H.:

1. *Does the performance of aMCI participants on the OLT differ significantly from those of matched healthy controls?*

   Since the design of the OLT was meant to assess allocentric spatial memory, it was expected that aMCI patients would perform significantly worse than healthy controls on the task, as measured by mean displacement error between objects' actual and recalled locations. As this research question was shared between projects it was only briefly presented and discussed.

Individually:

2. *What are the group differences in intracranial and regional volumes, especially hippocampal (HC) and entorhinal (EC) subfields?*

   Previous research has indicated support for significant differences in HC and EC volumes between MCI patients and controls, although the evidence is considerably stronger for distinguishing mild AD from MCI patients and controls. Other measures may be better at classifying group membership than grey matter volume (Zhang et al., 2013). According to

the literature, it was expected that aMCI patients would have significantly smaller HC/EC subfield and total volumes than healthy controls, especially in areas affected earliest by AD pathology e.g. EC and subiculum.

3. *What is the relationship between EC and HC subfield volumes and OLT performance?*

   According to existing research, a significant association between HC and EC atrophy shown in neuroimaging (i.e. AD progression) and poorer OLT performance was expected in the aMCI sample. The relationship between HC/EC volumes and performance might not be prominent in healthy controls (Van Petten, 2004), as there is mixed evidence in the literature of neural correlates of HC-dependent tasks in healthy older adults, suggesting that other measures such as fMRI, metabolic or microstructural indicators may reveal subtler deficits in HC functioning (Daugherty et al., 2016; Driscoll et al., 2003; Head & Isom, 2010; Korthauer et al., 2016; Moffat et al., 2007). The individual regional volumes that were expected to correlate with performance in aMCI patients include alEC, pmEC, EC, area 35, subiculum and presubiculum.

Methods

2.1 Design

The study employed a cross-sectional design to compare performance of aMCI patients and age and education-matched healthy controls on a behavioural measure of allocentric spatial memory, the Object Location Task (OLT). Total and subfield volumes of the ROIs: presubiculum, subiculum, total HC, alEC, pmEC, total EC and area 35 were obtained from participants through structural magnetic resonance imaging (MRI) and their relationship with OLT performance was explored.

2.2 Participants

2.2.1 Recruitment

Amnestic MCI patients were recruited through a university-affiliated NHS memory clinic in Cambridge and gave informed consent to be contacted for research studies and for additional structural data to be collected for research during their clinical MRI scan. They were then invited by research staff to participate in the current study. Healthy controls were recruited from Join Dementia Research or were spouses of the patients. Informed consent was obtained for this specific study from all participants prior to testing, and capacity to consent was ascertained by the researchers in accordance with the Mental Capacity Act (2005). Patients and controls were scanned at brain imaging facilities located at the university or the same hospital. Participants were offered reimbursement for their travel expenses. Some aMCI patients and healthy controls had previously participated in a different research study utilising immersive VR technology.

2.2.2 Diagnostic assessment of Mild Cognitive Impairment

Amnestic MCI was diagnosed by a neurologist according to the Petersen criteria (Petersen, 2004), diagnosis of which requires: i) subjective cognitive complaint, ii) objective evidence of cognitive impairment, iii) preserved activities of daily living, iv) functional independence and v) absence of dementia. Objective cognitive decline was evaluated using the Addenbrooke's Cognitive Examination – Revised (ACE-R, Mioshi, Dawson, Mitchell, Arnold, & Hodges, 2006), scoring .5 on the Clinical Dementia Rating scale (CDR) (J. C. Morris, 1997). In most cases patients underwent an MRI scan to provide supplementary information. The cases were discussed in a multidisciplinary setting to determine a diagnosis, and patients were informed by their assessing clinician.

Eight aMCI patients underwent CSF amyloid/tau biomarker studies (β-amyloid$_{1–42}$, total tau, phosphorylated tau) as part of their clinical diagnostic workup. CSF biomarker studies were undertaken using ELISA assay kits (Innotest, Innogenetics, Ghent, Belgium) as outlined elsewhere (Shaw et al., 2009). Thresholds for negativity or positivity were set as CSF amyloid > 550 pg/ml, CSF tau < 375 pg/ml with a CSF tau: amyloid ratio of < .8 (Mulder et al., 2010). Patients were stratified into biomarker-positive (MCI+, n=4) and biomarker-negative (MCI-, n=4) groups. The remaining 12 aMCI patients did not undergo CSF studies as part of their clinical workup.

2.2.3 Inclusion criteria

All participants met the following criteria:

- Fluent in English.
- Aged 50 years or above.

- No uncontrolled hypertension or diabetes.
- No significant psychiatric, substance misuse, neurological problems or learning disabilities that would interfere with neuropsychological or VR testing.
- No sensory or motor difficulties that would interfere with testing.
- Capacity to consent to take part in the study.

Although all aMCI patients scored 75 or above on the Addenbrooke's Cognitive Examination – Revised (ACE-R) (Larner, 2007) and above 26 on the Mini-Mental State Examination (MMSE) at when initially diagnosed, three patients scored below 75 on the ACE-R and five scored below 26, the cut-off for mild dementia, on the MMSE in their closest assessment to testing. This reflects the variable lengths of time patients had been diagnosed and the degenerative nature of AD, and was not an exclusion criteria in recruitment. All cognitive screening tests were performed within six months of participation in the current study.

2.2.4 Ethics

The study was undertaken in line with the regulations outlined in the Declaration of Helsinki and was approved by the NHS Cambridge South Research Ethics Committee (REC reference: 16/EE/0215). Researchers A.L. and E.H. obtained Visiting Researcher status on 18 August 2017 from CUH Cambridge University Hospitals NHS Foundation Trust (see **Appendices 4** and **5** for letters granting ethical approval and Visiting Researcher status).

2.2.5 Sample size

A preliminary power analysis in the proposal for this research project suggested a sample size of around 20 per group to detect medium (.5-.6) correlations between HC/EC regional

volumes and performance, as typically reported in previous studies on allocentric spatial memory in MCI/AD populations (Moodley et al., 2015; Nedelska et al., 2012). Power analysis for a multiple regression with three predictors was conducted in GPower to determine a sufficient sample size using an alpha of .05, a power of .80, and a medium effect size (f^2 = .15) (Faul, Erdfelder, Lang, & Buchner, 2007). Based on the stated assumptions, the desired sample size is 77. The study initially aimed to recruit 25 participants per group due to time and resource constraints. The final recruited samples were 20 aMCI patients and 22 healthy controls for the OLT, and MRI data was available for 16 patients and 22 controls. Of the four missing patients, two declined or cancelled their MRI scans, one could not be scanned due to claustrophobia, and one patient's scans were excluded from data analysis due to significant missing cortical matter from a previous operation.

2.3 Procedure

Participants were tested by A.L., E.H., and D.H., E.B., Z.A. from Cambridge in one session lasting from one to two and a half hours depending on their speed in completing the various tasks. Initially, the experimental procedure was explained and their consent obtained by the researcher, after which demographic information was collected. Participants were then administered the OLT and a battery of neurocognitive tests, with the order of administration counterbalanced across participants. Participants were able to take breaks in between tasks as needed. MRI scans were obtained on a separate occasion, as part of patients' diagnostic workup, or arranged with healthy controls when they volunteered as research participants. All participants' MRI scans were conducted within six months of participation in the current study. Large population studies suggest annual EC and HC atrophy rates of .5-.8% for healthy older

controls and 1.6-2.4% for MCI patients (McDonald et al., 2009), which is not likely to have a significant confounding effect on this study's findings.

2.4 Behavioural measures

2.4.1 Neuropsychological assessment battery

All participants were administered a neurocognitive battery (described elsewhere in the thesis of E.H.) intended as comparators to the OLT. The following tests were included in chronological order:

- The National Adult Reading Test (NART), an estimate of premorbid IQ
- The Rey Complex Figure Test (RCFT), a test of visual working memory
- The Trail Making Test B (TMT-F), a test of executive functioning
- The Digit Symbol (DS) from the Wechsler Adult Intelligence Scale
- The Four Mountains Test, an HC-dependent spatial memory task (Hartley et al., 2007).

2.4.2 Object Location Task

2.4.2.1 Materials

The HTC Vive VR system was used in the study, as it allowed participant movement and tracking in a controlled space. The system included a headset with a resolution of 1080x1200 pixels per eye and a 90Hz refresh rate, two hand-operated controllers and two 'lighthouses' which define the tracked space. The headset contained in-built head direction and location sensors, while the lighthouses tracked hand movement via markers on the controllers and participant location via headset markers to create an immersive VR experience. The headset

was large enough to accommodate spectacles. In order to avoid trailing wires and associated safety hazards, the VR headset was plugged into a wire-free computer that was designed as a backpack, the MSI VR ONE Desktop, while the lighthouses and hand controllers were wirelessly connected. The running of the VR task on the backpack was remotely controlled by researchers through a 15-inch MacBook with a resolution of 1400x900 pixels. Participants wore the backpack along with the headset while performing the task. A 3.5 metre square area was configured in a large room with the two base stations in order to track participants' movement.

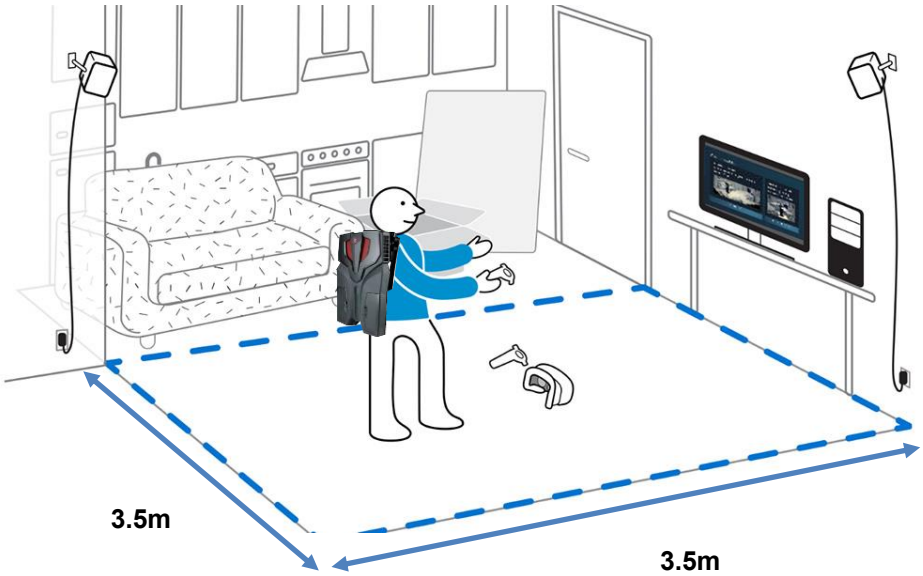

Figure 2. *Set up of the VR and tracking system.*

2.4.2.2 Pilot testing

The OLT was piloted by A.L., E.H. and A.C. on young volunteers in the Institute of Cognitive Neuroscience, University College London. Initial feedback from ten participants indicated the task was well-tolerated and there were no dizziness or vertigo issues sometimes reported with prolonged use of VR headsets. An identical VR setup was used for an earlier path

integration task in Cambridge and was similarly well-tolerated by older adults and aMCI patients.

2.4.2.3 Part 1: Object Location

After a verbal explanation of the task and reading illustrated instructions, participants were fitted with the headset and backpack and placed in a grey featureless virtual room with a welcome message. They were allowed time to acclimatise to the headset, after which the experiment was initiated by the researchers and they were placed in the practice virtual environment (VE). The practice and four test VEs consisted of a square enclosure surrounded by walls customised to 80% of a participant's height (constraining them to the area tracked by the VR system) and open landscapes beyond the walls with natural scenery e.g. mountains, trees, to serve as distal landmarks. After one minute in which they were free to look and walk around to familiarise themselves with the VE, participants completed three encoding trials and two recall trials in succession. In every environment VE three unique objects were randomly selected for each participant from a pool of 15.

Encoding trial: An object appeared on a pedestal within the enclosure. Participants were reminded they were allowed as much time as they needed in order to remember the object's location. They then walked into the object to make it disappear and a new object appeared in a different location. A third object appeared after the participant finished with the second one. Participants were instructed to return to the centre of the enclosure, indicated by a yellow cone, after each encoding trial. Order of appearance of the three objects were randomised for each encoding trial.

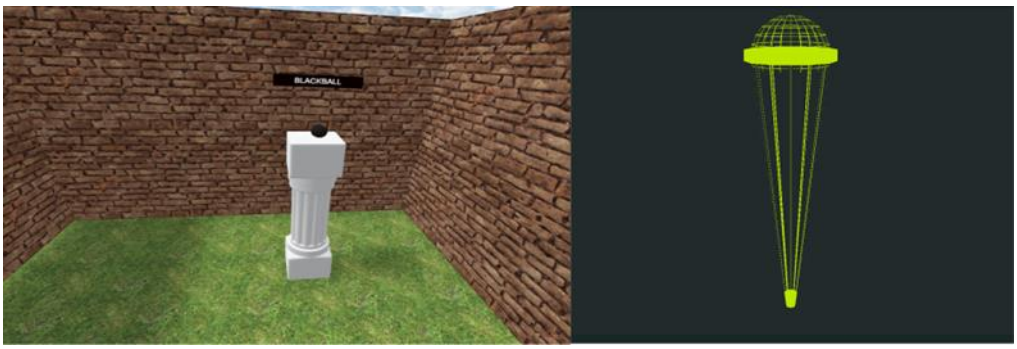

Figure 3. *On the left, a screenshot of the participant's view of an object presented on a pedestal. On the right, the yellow cone in the enclosure instructing participants to return to centre.*

Recall trial: Participants were taught how to use the hand controller, which projected a hologram of the pedestal when the button is pressed, allowing them to specify a location within the enclosure by releasing the button. To minimise invalid responses, they were carefully instructed in the practice environment and reminded through the task of how to use the controller. They were prompted by a small picture of an object on the lower right corner of their visual field and indicated their best guess of its location, doing it for all three objects in a random order. They were allowed as much time as needed. Participants were instructed to return to the centre of the enclosure, indicated by a yellow cone, after each recall trial.

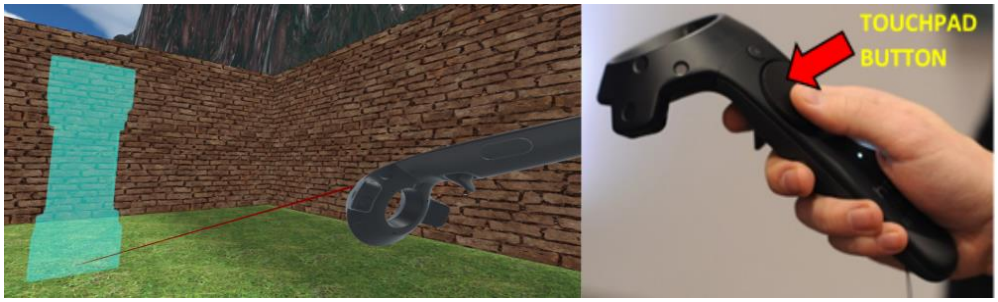

Figure 4. *On the left, a demonstration of how participants specified a location with the hand controller. On the right, detailed view of the hand controller.*

All participants performed three encoding and two recall trials each for the practice environment and four test environments. They were therefore exposed to 15 unique objects in total, which was the same for all participants although allocated to different environments for individuals. The number of trials was determined through pilot testing in young and older controls to ensure a good level of performance and reasonable task duration (one hour). Environments were presented in a fixed order for all participants, and varied in landscape features, time of day, and textures of the ground and enclosure walls. Participants were allowed to take breaks in between environments if they wished.

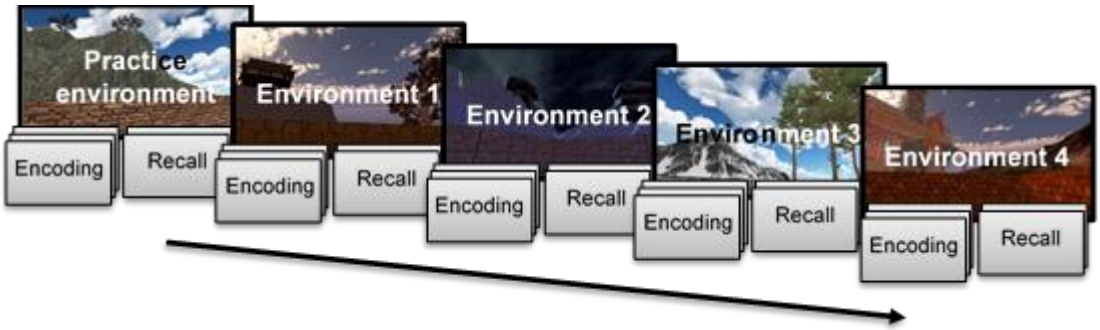

Figure 5. *All participants completed one practice environment and four test environments. Each environment had three encoding and two recall trials.*

2.4.2.4 Part 2: OLD/NEW object familiarity task

Participants were then given a computerised task, written in Matlab, on the same 15-inch MacBook testing their recall of objects seen in Part 1 of the experiment. They were shown an object and asked to indicate whether it was OLD (seen in Part 1) or NEW (never seen before) by pressing labelled keys. They were instructed to use a strict criterion i.e. objects had to be exactly identical in colour and style. If the object had appeared in Part 1, regardless of the participant's answer, they were shown the four environments and required to choose which one they saw it in by pressing 1-4. The environments' positions onscreen were randomised to

prevent a spatial response bias. There was a timeout of 60 seconds for responding and participants were instructed to make their best guess. Participants had a learning phase when they received feedback on the three objects from the practice environment, before completing the test phase containing the 12 objects from the four environments along with 12 foils.

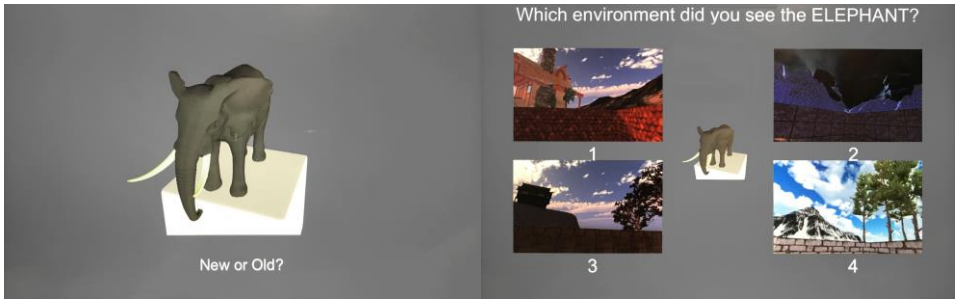

Figure 6. *Object familiarity task: old or new (left); which environment it appeared (right).*

2.5 Volumetric MRI

2.5.1 Imaging

Participants underwent MRI scanning on 32 channel Siemens 3T Prisma scanners in Cambridge. Scans were undertaken using the same acquisition parameters and included a 1x1x1mm T1-weighted MPRAGE (TA 5:12, TR 2300ms, TE 2.96ms) and high-resolution .4x.4x2 mm T2-weighted scans through the hippocampal formation, with scans aligned orthogonally to the long-axis of the hippocampus (TA 8.11, TR 8020ms, TE 50ms).

2.5.2 Hippocampus

High-resolution T2-weighted MRI scans of participants were analysed using Freesurfer Version 6, an open source software package for imaging analysis. Cortical reconstruction, including reconstruction of the whole hippocampus and its neighbouring subcortical regions, and volumetric segmentation were performed in Freesurfer; after HC reconstruction an

automated subregion parcellation protocol was used to segment specific subregions of the HC formation (Iglesias et al., 2015). The protocol predicts the location of 12 HC subregions using a refined probabilistic atlas based on a combination of manual delineations of the HC formation from ultra-high resolution *ex-vivo* MRI scans and manual annotations of surrounding subcortical structures from an independent dataset. The segmented HC subregions are the CA1, CA2/3, fimbria, subiculum, presubiculum, CA4/DG, hippocampal tail, hippocampal fissure, the parasubiculum, the molecular layer, granule cells in the molecular layer of the DG (GC-ML-DG) and the hippocampal-amygdala transitional area (HATA) (passage referencing Whelan et al., 2016).

Freesurfer's estimated total intracranial volume (eTIV) for each participant was taken as their intracranial volume (ICV). All regional volumes were summed bilaterally, corrected for head size by dividing by ICV and multiplied by a thousand to facilitate data analysis.

2.5.3 Entorhinal and perirhinal cortex

Manual segmentation of the EC was performed by A.L., D.H., and Z.A. on high-resolution T2-weighted MRI scans of the participants. Raters were blinded to the identities of participants, cognitive scores and OLT performance. Volumetric segmentation of the EC and PRC (area 35) was performed on high-resolution T2-weighted 3T MRI scans according to the protocol described in Berron et al. (2017). Volumetric analysis of the EC subregions was conducted based on a protocol developed based on connectivity data (Maass et al., 2015). The approach prioritised high-confidence segmentation of terminal slices over unreliable segmentation of the putative subparts. The alEC and pmEC were manually segmented on high resolution T2-weighted 3T MRI scans of aMCI patients and healthy controls. The alEC

was segmented on three initial slices of EC and the pmEC on the three terminal slices of EC (**Figure 7**). The alEC segmentation started two slices anterior to appearance of subiculum, posteriorly reaching the first slice with visible subiculum (**Figure 7**). The pmEC segmentation was based on the landmark of the uncal separation, extending for one slice anterior and one posterior to the slice with uncal apex i.e. two last slices of hippocampal head and the first slice of the hippocampal body (**Figure 7**). The anterior superior border was constituted by the lateral end of ambiens gyrus, and posteriorly, the superior border was determined by the inferior part of medial subiculum. Inferiorly, the EC was segmented until the base of the Collateral Sulcus (CS) on half the angle between the CS and the parahippocampal gyrus. Bright voxels representing cysts, CSF or the meninges were excluded.

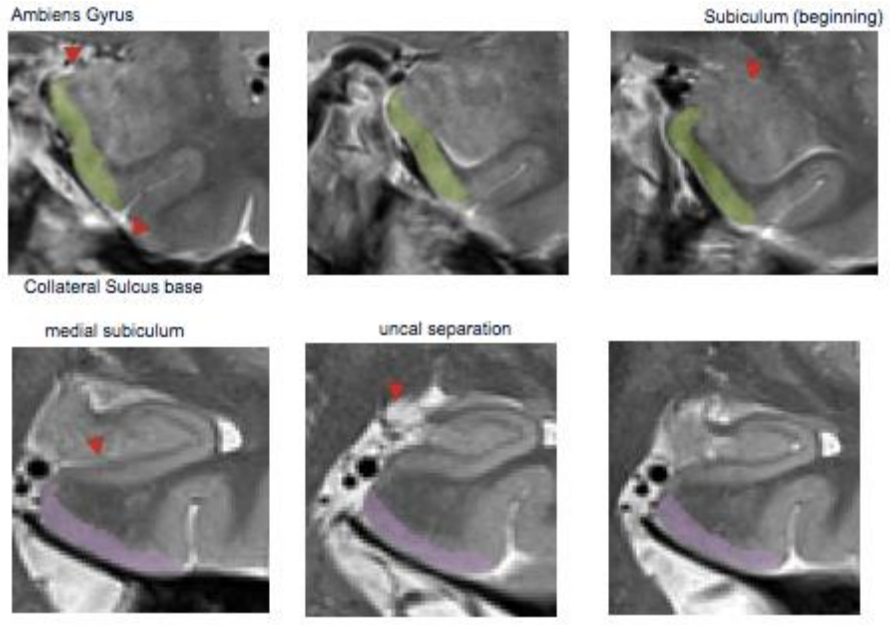

Figure 7. *Illustrated protocol for alEC and pmEC segmentation. Top: Segmentation of alEC, starting two slices anterior to appearance of subiculum, and posteriorly reaching the first slice with visible subiculum. Bottom: Segmentation of pmEC, based on the landmark of the uncal separation, extending for one slice anterior and one posterior to the slice with uncal apex. The anterior superior border is constituted by the lateral end of ambiens gyrus, and posteriorly, the*

superior border is determined by the inferior part of medial subiculum. Inferiorly, the EC is segmented until the base of the CS. Figure courtesy of David Howett (2017).

Manual volumetric segmentations were performed using ITK Snap 2.20 software package (http://www.itksnap.org) under standardised settings. All volumetric measurements were summed bilaterally and corrected for head size by dividing by ICV and multiplying by a thousand. Inter-rater reliability was estimated with the intra-class correlation coefficient ICC(2,k) (Shrout & Fleiss, 1979) and the Dice metric (Dice, 1945) using three scans segmented by all the raters. Values ranged from moderate to good reliability (Dice: .62 – .69; ICC: .69 – .78).

2.6 Data analysis

Data analysis was carried out with Statistical Package for the Social Sciences (SPSS) version 24.0, and graphical representations were generated using SPSS and Microsoft Office Excel 2016. Participant demographic, clinical, neurocognitive and OLT data were entered into a database alongside hippocampal and entorhinal total and subfield volumes. T-tests for independent samples were used to compare demographic, OLT and volumetric data between groups. Chi-square tests were used to compare categorical variables. Pearson's correlation analyses were run to explore associations between OLT performance, HC and EC volumes. Multiple regression analyses were used to investigate neural predictors of performance on the OLT while controlling for other factors such as age, gender and premorbid IQ.

The false discovery rate (FDR) method (Benjamini & Hochberg, 1995) was employed to correct for multiple comparisons within each family of tests, using a detection threshold of .1. The FDR is a less conservative method than the Bonferroni method and more appropriate for

planned comparisons where several significant results are anticipated as well as exploratory research. When there was a within-group significant correlation, the correlation coefficients of the two groups were compared for significant differences with *cocor*, an online calculation tool coded with R using the Fisher r-to-z transformation (Diedenhofen & Musch, 2015).

Results

3.1 Demographics and cognitive outcomes

A final sample of 20 aMCI patients and 22 controls were tested on the OLT. There were no significant differences in age, years of education or gender between groups, and controls scored significantly higher on the MMSE and ACE-R (**Table 1**).

	aMCI (SD)	**Control (SD)**	***p-value***
Age	68.30 (9.6)	65.68 (7.8)	.339
Gender (% female)	35	59	.118
Education (years)	15.15 (4.1)	15.59 (3.8)	.719
MMSE	27.60 (2.5)	29.90 (.3)	**.001****
ACE-R	86.65 (8.5)	97.90 (2.9)	**<.001****

***p* < .05, **p* < .01 (FDR-adjusted)*

Table 1. *Demographic and cognitive characteristics of participants*

There were four CSF+ and four CSF- patients and 12 with unknown status. Five aMCI patients had hypertension, all controlled by medication, two had historical depression and anxiety and one had treated diabetes. Their demographic and clinical details are summarised in **Table 2**. There were no significant differences in a one-way ANOVA. As the groups were too small all patients were considered together in subsequent analyses.

	CSF+ (SD)	**CSF- (SD)**	**Unknown (SD)**	***p-value***
N	4	4	12	
Age	70.50 (11.6)	67.00 (5.9)	68.00 (10.6)	.876
Gender (F/M)	2/2	0/4	5/12	>0.05†
Education (years)	15.76 (5.5)	14.00 (4.1)	15.33 (3.9)	.825
MMSE	26.00 (3.1)	29.50 (1.0)	27.45 (2.4)	.141
ACE-R	83.50 (14.3)	90.00 (4.7)	86.45 (7.8)	.292

***p* < .05, **p* < .01 (FDR-adjusted),* † Fisher's exact probability test

Table 2. *Demographic and cognitive characteristics of aMCI subgroups*

3.2 Research question 1: Group differences in OLT performance

3.2.1 Part 1: VR Object Location

The results of this research question are shared with project partner E.H. and are presented briefly by both of us in order to provide context for our individual investigations. Displacement error, the linear distance between actual and recalled locations of objects, was the main performance outcome in Part 1 of the OLT. The mean displacement error was derived from 24 responses in the eight recall trials across four test environments for all participants except from the first six patients. The first six patients completed a version of the task with four objects per environment (i.e. 32 recall trials in total) and their responses were averaged across those. Any responses that were invalid (e.g. outside of the enclosure, towards the horizon etc.) were discarded. The mean path length (metres) and mean time spent (seconds) were calculated based on participant movement and time elapsed between placing consecutive objects in the recall trials respectively.

aMCI patients performed significantly worse in terms of mean displacement (metres) and time spent (seconds). There was no significant difference in terms of path length (**Table 3**).

	aMCI (SD)	**Control (SD)**	***p-value***
Mean displacement	.89 (0.6)	.40 (0.2)	**.001****
Mean path length	1.66 (0.6)	1.53 (1.0)	.636
Mean time spent	16.34 (5.9)	10.44 (4.4)	**.001****

***p* < .05, **p < .01 (FDR-adjusted)*

Table 3. *Performance on OLT VR object location*

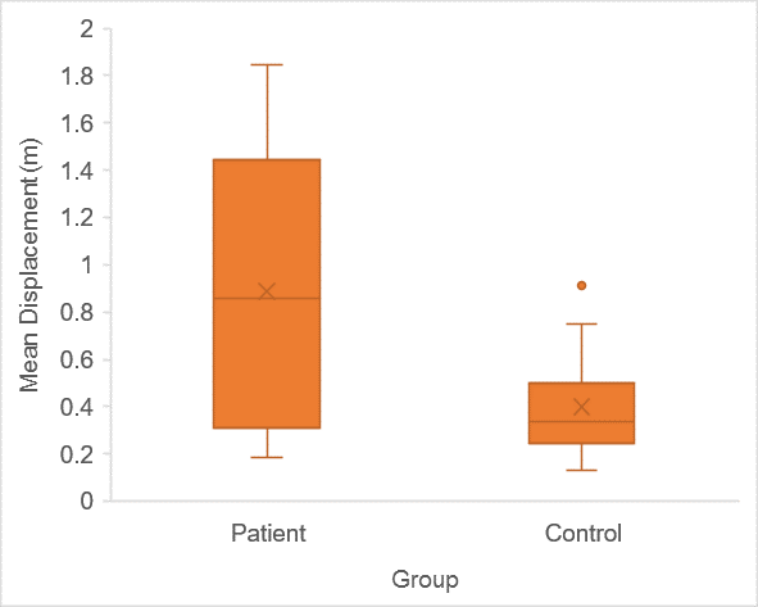

Figure 8. *Performance on VR object location. Patients had significantly poorer object location memory as measured by displacement error and greater variance compared to controls.*

3.2.2 Part 2: OLD/NEW object familiarity task

Due to an early technical error in the Matlab computer task code that was subsequently identified and corrected, data was unrecorded for seven patients and one control. The performance of the remaining 13 patients and 21 controls is presented in **Table 4**.

	aMCI (SD)	**Control (SD)**	***p-value***
N	13	21	
Old/New correct (%)	82.45 (13.3)	91.47 (6.8)	**.038***
Environment correct (%)	41.83 (18.4)	36.90 (17.6)	.442

***p* < .05, ****p* < .01 (FDR-adjusted)*

Table 4. *Performance on OLD/NEW object familiarity task*

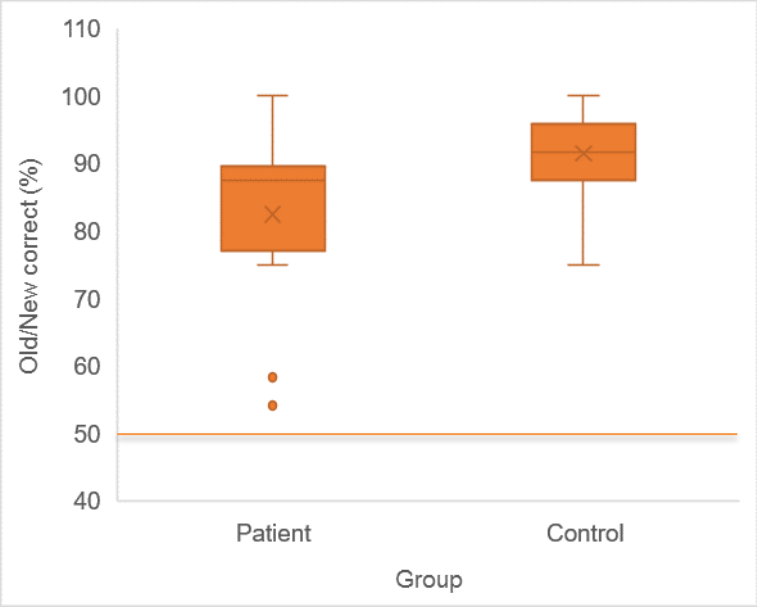

Figure 9. *Object recognition performance on OLD/NEW object familiarity task. Patients had significantly worse recognition of familiar objects. The line indicates chance (50%).*

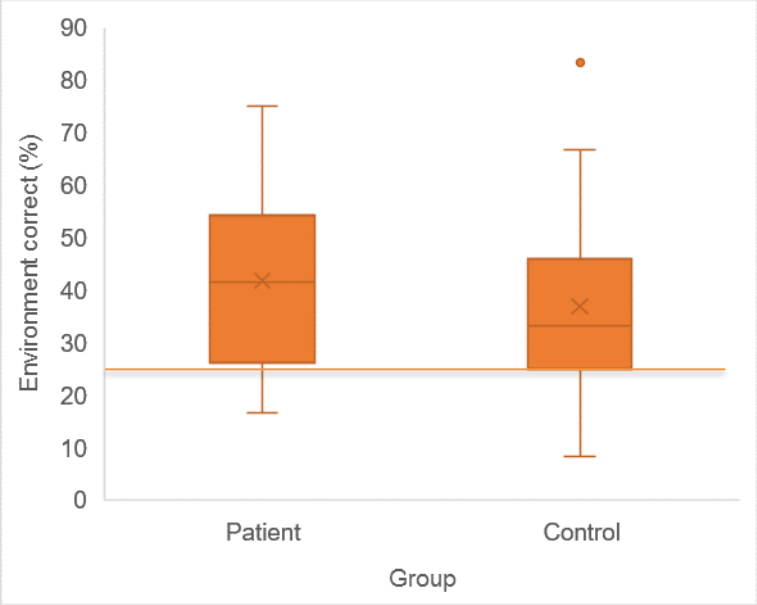

Figure 10. *Environment recognition performance on OLD/NEW object familiarity task. Patients and controls performed equally poorly on recognising the environmental context for specific objects. The line indicates chance (25%).*

3.3 Research question 2: Group differences in regional volumes

3.3.1 Hippocampus

Independent t-tests were used to compare total HC, presubiculum and subiculum volumes across groups. CA1 was also included in this analysis due to well-documented changes in people with aMCI (de Flores et al., 2015). Due to the exploratory nature of the study, both raw and ICV-corrected volumes are summarised below in **Table 5**. Raw total HC, CA1 and subiculum volumes were significantly smaller in aMCI patients but these differences became non-significant after correcting for ICV. Presubiculum volumes were not significantly different between groups. ICV was slightly but significantly smaller in patients ($t_{(36)}$ = -2.17, p = .037), which has been found in some but not all studies in AD (Lo & Jagust, 2013).

	Raw			Corrected		
	aMCI (SD)	**Control (SD)**	***p-value***	**aMCI (SD)**	**Control (SD)**	***p-value***
N	16	22		16	22	
Total HC	5944.04 (767)	6720.50 (720)	**.003****	4.04 (.52)	4.27 (.39)	.361
CA1	1183.20 (158)	1347.22 (159)	**.003****	.8 (.11)	.9 (.08)	.120
Presubiculum	493.56 (81)	535.58 (72)	.099	.336 (.06)	.341 (.04)	.133
Subiculum	762.87 (119)	856.32 (101)	**.013***	.519 (.08)	.544 (.05)	.202

***p** < .05, ****p** < .01 (FDR-adjusted)*

Table 5. *HC volumes of participants. Patients had significantly smaller total HC, CA1 and subiculum volumes before correction for head size.*

3.3.2 Entorhinal and perirhinal cortex

Entorhinal cortex and area 35 were significantly smaller in patients before correcting by head size and the inter-group alEC volumetric difference approached significance (**Table 6**). There

were no significant differences between groups in any subregional volumes after normalisation.

	Raw			Corrected		
	aMCI (SD)	**Control (SD)**	***p-value***	**aMCI (SD)**	**Control (SD)**	***p-value***
N	16	22		16	22	
EC	676.54 (242)	854.82 (216)	**.022***	.460 (.16)	.542 (.13)	.090
alEC	533.37 (121)	603.63 (99)	.057	.363 (.08)	.386 (.08)	.359
pmEC	311.60 (122)	322.67 (75)	.732	.213 (.09)	.206 (.05)	.753
Area 35	903.28 (195)	1104.04 (175)	**.002****	.614 (.13)	.681 (.15)	.168

***p** < .05, ****p** < .01 (FDR-adjusted)*

Table 6. *EC volumes of participants. Patients had significantly smaller EC and area 35 volumes before correction for head size.*

3.4 Research question 3: OLT performance and HC/EC volumes

3.4.1 Neural correlates of OLT VR object location performance

Pearson's correlation coefficients were used to investigate the relationships between the ROIs and OLT VR object location performance in aMCI patients and controls separately. ICV-corrected volumes were used for all correlations and bivariate outliers were identified using residuals and a Tukey 1.5 hinged spread analysis. As a result one patient was excluded from whole HC, presubiculum, subiculum and area 35, two from EC and alEC, and four from pmEC analyses (**Table 7**).

Mean displacement	**aMCI *r***	***p-value***	**Control *r***	***p-value***	***cocor sig.***
N	12-15		22		
Total HC	-.867	**<0.001****	.101	.654	**<0.001****
Presubiculum	-.753	**0.001****	.134	.553	**0.0025****
Subiculum	-.852	**<0.001****	.028	.902	**<0.001****
EC	-.659	**.010***	-.205	.359	0.114
alEC	-.417	.138	-.124	.582	

pmEC	-.301	.342	-.260	.242	
Area 35	-.735	**.002****	-.355	.105	0.123

***p* < .05, ****p* < .01 (FDR-adjusted)*

Table 7. *Correlations between VR object location performance and ROIs*

After correcting for multiple comparisons using FDR, total HC, presubiculum, subiculum, EC and area 35 volumes were significantly associated with OLT displacement in patients, but none of the correlations were significant in controls. Total HC, presubiculum and subiculum strongly correlated with OLT displacement error in patients. Comparing coefficients indicated that the group differences between total HC, presubiculum and subiculum were highly significant while EC and area 35 approached significance.

3.4.2 Neural correlates of OLT OLD/NEW object familiarity task performance

The available data for OLT OLD/NEW object familiarity task performance was also investigated using bivariate correlations, although due to the small sample sizes the findings can only be considered exploratory (**Table 8**).

	Old/New % correct		**Environment % correct**	
	aMCI *r* *(p-value)*	Control *r* *(p-value)*	aMCI *r* *(p-value)*	Control *r* *(p-value)*
N	12	21	12	21
Total HC	.138 (.67)	.227 (.322)	.467 (.126)	-.034 (.885)
Presubiculum	.469 (.124)	.415 (.061)	.392 (.207)	.180 (.436)
Subiculum	.307 (.332)	.359 (.110)	**.625 (.030*)**	.123 (.596)
EC	-.06 (.853)	-.122 (.597)	**.593 (.042*)**	-.091 (.695)
alEC	-.349 (.266)	.286 (.208)	-.014 (.965)	-.092 (.692)
pmEC	-.399 (.199)	-.100 (.666)	.284 (.371)	-.047 (.841)
Area 35	.016 (.960)	-.002 (.992)	.239 (.454)	.271 (.235)

***p* < .05, ****p* < .01 (FDR-adjusted)*

Table 8. *Correlations between OLD/NEW object familiarity task performance and ROIs*

There were no significant correlations with regional volumes for OLT OLD/NEW object familiarity task in controls. After FDR correction, the correlation between environment recall with EC and subiculum volumes approached significance but was not below threshold. Examination of the scatterplots of these two regions (**Figure 14**) revealed that there were no significant outliers and the significant associations in patients may indicate underlying relationships between larger subiculum and EC volumes and more accurate recall of environmental contexts. However, this certainly needs to be further investigated with a larger sample.

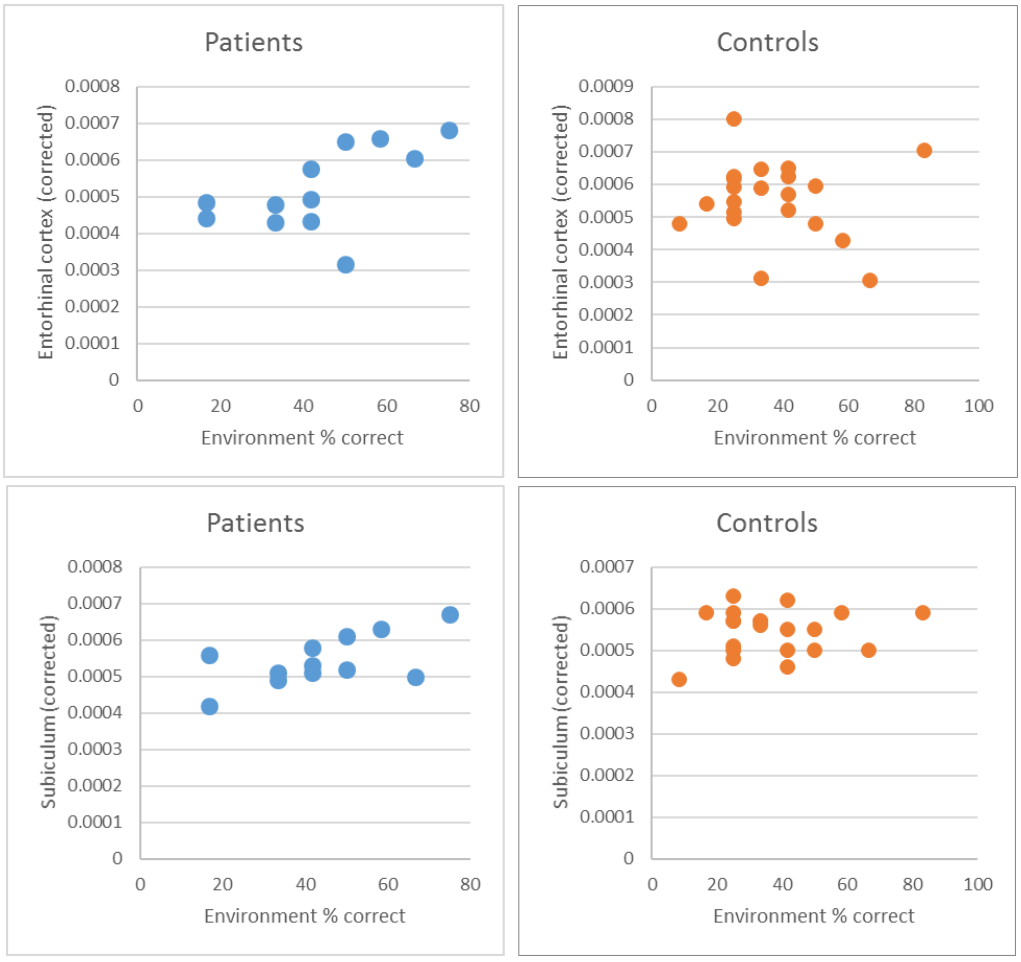

Figure 11. *Scatterplots of corrected EC and subiculum volumes against environment recognition performance for patients and controls. Patients showed trends of larger EC and subiculum correlating with better recall of environmental context.*

3.4.3 Multiple regression analyses

Multiple regression analyses were conducted to investigate whether HC and EC subfield and total volumes were significant predictors of variance in OLT VR object location performance after other factors were accounted for. Due to the small sample size and high collinearity between regional volumes, separate stepwise regressions were run with one ROI as a predictor each time to minimise the number of predictors. Linear regressions were run for the significant correlations found in 3.4.1., i.e. total HC, presubiculum, subiculum, EC and area 35 with outliers excluded.

As the correlation analyses suggested that there may be a group × regional volume interaction, interaction terms using corrected volumes were computed for the patient and control ROIs listed above. In order to avoid multicollinearity all the ROIs were centred on the mean before calculating interaction terms. The tolerance values for all regression analyses were satisfactory and all above .2.

For all the models run, the dependent variable was mean displacement in OLT. Age, sex, premorbid IQ and group were entered into the first block. The two interaction terms were then entered into the second block. The results are presented in **Table 9**.

	Mean displacement				
Predictors	*β*	*t*	*p*	Sr	
Age	.162	1.31	.201	.130	
Sex	-.089	-.827	.415	-.083	
Premorbid IQ	-.010	-.079	.937	-.008	R^2 = .701, R^2_{adj} = .641,
Group	-.210	-1.62	.116	-.162	$F_{(6,30)}$ = 11.72, p < **.001****
Int. Total HC (patient)	-.661	-5.64	**.000****	-.563	
Int. Total HC (control)	.072	.703	.487	.070	
Age	.289	2.166	**.038***	.243	
Sex	-.025	-.208	.837	-.023	
Premorbid IQ	-.051	-.349	.730	-.039	R^2 = .623, R^2_{adj} = .547,
Group	-.314	-2.227	**.034***	-.250	$F_{(6,30)}$ = 8.25, p < **.001****
Int. Presubiculum (patient)	-.527	-4.281	**.000****	-.480	
Int. Presubiculum (control)	.109	.935	.357	.105	
Age	.139	1.01	.320	.106	
Sex	-.037	-.328	.745	-.035	
Premorbid IQ	.023	.167	.868	.018	R^2 = .668, R^2_{adj} = .601,
Group	-.273	-2.046	.050	-.215	$F_{(6,30)}$ = 10.06, p < **.001****
Int. Subiculum (patient)	-.626	-5.018	**.000****	-.528	
Int. Subiculum (control)	.054	.479	.636	.050	
Age	.427	2.635	**.013***	.365	
Sex	.028	.187	.853	.026	
Premorbid IQ	.036	.199	.844	.028	R^2 = .443, R^2_{adj} = .327,
Group	-.289	-1.625	.115	-.225	$F_{(6,30)}$ = 3.84, p = **.006****
Int. EC (patient)	-.242	-1.604	.120	-.222	
Int. EC (control)	-.093	-.667	.510	-.092	
Age	.385	2.262	**.031***	.324	
Sex	.017	.107	.915	.015	
Premorbid IQ	.112	.614	.544	.088	R^2 = .383, R^2_{adj} = .259,
Group	-.410	-2.296	**.029***	-.329	$F_{(6,30)}$ = 3.1, p = **.018***
Int. Area 35 (patient)	-.075	-.502	.619	-.072	
Int. Area 35 (control)	-.022	-.138	.891	-.020	
Age	.129	1.129	.268	.104	
Sex	-.050	-.499	.621	-.046	
Premorbid IQ	.035	.289	.774	.027	R^2 = .744, R^2_{adj} = .692,
Group	-.226	-1.880	.070	-.174	$F_{(6,30)}$ = 14.49, p < **.001****
Int. Total HC (patient)	-.845	-6.349	**.000****	-.587	
Int. EC (patient)	.290	2.358	**.025***	.218	

***p** < 0.05, ****p** < 0.01*

Table 9. *Multiple regression analyses*

The regression equations with total HC (R^2_{adj} = .641, $F_{(6,30)}$ = 11.72, p < .001), presubiculum (R^2_{adj} = .547, $F_{(6,30)}$ = 8.25, p < .001) and subiculum (R^2_{adj} = .601, $F_{(6,30)}$ = 10.06, p < .001) interactions as predictors were highly significant. Total HC (β = -.661, p < .001), presubiculum

(β = -.527, p < .001) and subiculum (β = -.626, p < .001) volumes separately and significantly predicted OLT performance in patients but not in controls, explaining 31.7%, 23.0% and 27.9% of the variance in patients' OLT performance respectively when age, sex, group and premorbid IQ were controlled for. EC and area 35 volumes were not significant predictors of performance. However, EC volume significantly predicted additional variance in patient OLT performance (β = .290, p = .025) alongside total HC volume in a regression analysis.

Discussion

4.1 Summary of main findings

The current study investigated the relationship between hippocampal and entorhinal subfields and performance on an immersive virtual reality task of allocentric spatial memory in aMCI patients and healthy controls. The existing evidence in the literature led us to predict significant differences in task performance, HC/EC volumes, as well as significant correlations between the two in aMCI patients but not controls. Our results were to a large extent in line with predictions, although there are some unexpected findings that warrant further consideration and research.

Firstly, there was a statistically significant and meaningful difference between patients and controls in terms of memory for object locations in a VR environment. Patients were substantially less accurate in recalling the location of previously seen objects, even when allowed as much time as they needed. There was a large variance in patient performance, reflecting the heterogeneous nature of the aMCI sample, however that did not detract from the large performance gap observed between groups. In a post-task immediate recall assessment, patients were also less able to distinguish between previously seen objects and foils. Memory for environmental contexts in which familiar objects were seen were comparable between groups and exhibited a floor effect.

Secondly, there is a preponderance of studies showing significant differences in HC/EC subregional volumes in people with MCI/AD versus healthy older adults. In the current study, patients had significantly smaller raw volumes of total HC, CA1, subiculum, EC and PRC (area 35) compared to controls, while the difference in alEC approached significance. There were no

significant differences between patients and controls in any regional volumes after normalising by head volume.

Thirdly, there were strong, consistent associations between task performance and hippocampal and to a lesser extent entorhinal subregional volumes in aMCI patients. Pearson's correlation analyses showed significant associations between patient OLT performance (mean displacement error) with volumes of all HC ROIs, EC and area 35. Regression analyses indicated that after controlling for age, sex, premorbid IQ and group, total HC, presubiculum and subiculum volumes significantly explained variance in patient OLT performance, while EC and area 35 did not. EC significantly explained additional variance in OLT performance on top of total HC volume. Group and age were also significant predictors in several of the regression models. All HC models showed good fit and explained large amounts of total variance in OLT performance according to the R-squared statistics, suggesting a significant hippocampal contribution to OLT performance. The lack of explanatory power of entorhinal volumes was unexpected, however both EC and area 35 volumes were highly correlated with age in patients, which may explain the non-significance after controlling for demographic factors.

4.2 Interpretation of results and contextualising with previous research

The current study piloted a novel object location memory task employing immersive VR, which has only very recently begun to be widely used in psychological research due to increasing affordability and offers unparalleled experiential simulation of real world spatial memory and navigation. Although significant limitations exist for research on humans compared to the level of detail in animal single cell recording studies, immersive VR allows

experimenters to obtain much more controlled, detailed and ecological behavioural data compared to non-immersive VR paradigms, thus enabling fine-grained targeting and analysis of the differential functions and subregions of the MTL memory system, which is particularly valuable for elucidating the subtle preclinical memory deficits in Alzheimer's Disease.

We found a significant difference in performance on the OLT between aMCI patients and controls. This is consistent with the copious literature in the field of spatial memory and navigation in aMCI and early AD patients documenting allocentric deficits in the Hidden Goal Task/Morris Water Maze (Hort et al., 2007; Kalová, Vlček, Jarolímová, & Bureš, 2005; Laczó et al., 2012; Laczó et al., 2009, 2014), object location memory (Serino, Morganti, Di Stefano, & Riva, 2015), and navigation in more complex environments (Plancher et al., 2012; Weniger et al., 2011). The OLT required participants to encode and retrieve three sequentially-presented object locations with the absence of local cues ("intra-maze" cues in the terminology of rodent studies) and while minimising the effectiveness for egocentric or procedural memory strategies (e.g. memorising turn directions or body movements from a fixed start point) by randomising the order of presenting and recalling objects. Performance is thus more dependent on allocentric encoding of distal environmental cues ("extra-maze" cues), such as trees and mountains, in relation to the objects and constructing a cognitive map of the enclosure. Our findings confirm longstanding evidence of a specific allocentric processing deficit in people with MCI and the earliest stages of AD that is rooted in MTL and HC functional deterioration and atrophy.

For the second research question, our findings are contrary to the prediction of significantly smaller total HC and subfield volumes in aMCI patients which have been reported in most

studies. The lack of significant differences in HC/EC volumes after normalisation becomes more explicable in light of the fact that many studies do not control for brain size when reporting differences between MCI patients and healthy controls, only accounting for ICV in later analyses (Carlesimo et al., 2015; Hirjak et al., 2017; Lindberg et al., 2017; Schmidt-Wilcke et al., 2009). It is worth noting that despite being more heavily female, the control group had significantly larger brains, which has been hypothesised to be a proxy for cognitive reserve and appears to be a protective factor against developing AD (Lo & Jagust, 2013). Along with the normalisation method used (dividing by head size) tending to over-correct MTL volumes compared to other methods (Voevodskaya et al., 2014), this may mathematically reduce the differences seen and account for the discrepancy between what has been reported in previous studies and our current findings. If raw volumes are considered, total HC, CA1, subiculum, EC and perirhinal cortices are all significantly smaller in aMCI patients – corresponding to the areas of focal atrophy noted in numerous studies to date (de Flores et al., 2015). The appearance of total HC volume may suggest that our aMCI participants were not in the earliest stages of disease, as general hippocampal atrophy was sufficiently advanced to produce a significant difference between groups. This would seem to be supported by several patients scoring close to or below the threshold for mild AD in the MMSE and ACE-R. Another factor to consider is the small number of patients included in this study; studies of similar size correcting for ICV have reported null results, perhaps as a consequence of low statistical power (Kerchner et al., 2013; Wisse et al., 2014).

The OLT was based on the paradigm investigating neural correlates of boundary and landmark-based object location memory in Doeller et al. (Doeller, King, & Burgess, 2008) with the design influenced by rodent studies aimed at dissociating the functions of the LEC and

MEC, especially to explore the alEC/LEC's role in object spatial memory and object-context recall (Deshmukh & Knierim, 2011; Tsao et al., 2013) in aMCI patients and healthy older adults. Researchers found LEC neurons that fired when animals are near locations where objects were previously placed or if familiar objects were relocated or replaced by unfamiliar ones (Tsao et al., 2013; D. I. G. Wilson, Watanabe, Milner, & Ainge, 2013), pointing to their role in associative encoding of object-location-spatial context relationships and tracking changes. The OLT attempted to quantify this by assessing post-task environmental context recall for each object, however due to a programming error resulting in significant missing data, as well as a floor effect in both patients and controls, it is hard to draw firm conclusions from the existing findings. The floor effect may indicate that the environmental context was of limited use in solving the task, thus less attended to and recalled by all participants, and additionally the cognitive load might have been too high for any meaningful differences to emerge, as participants saw twelve unique objects. Based on informal feedback, some participants have mostly engaged with the enclosure and not depended heavily on environmental cues during the task. The enclosure with its tall walls was highly salient and might have facilitated spatial processing of the small square area as a local space and prompted participants to use boundary-dependent distances and direction vectors as a strategy to encode object locations using HC place cells in accordance to the boundary vector cell model of place cell firing (Barry et al., 2006). This is supported by the total HC volume being the strongest correlate and predictor of OLT performance. Furthermore, compared to cell recordings volumetric MRI is a very crude estimate of brain activity and function, and therefore may not be the best measure to assess LEC's role in object-context memory. There is also the unavoidable variance introduced by the manual segmentation of

the EC by several people, which is less consistent than autosegmentation in Freesurfer. Although there was no significant correlation between alEC and object-context memory, the trends towards significance with EC and subiculum volume in patients are intriguing and should be investigated in a larger sample.

Similarly, contrary to predictions there was a lack of any significant associations between alEC, pmEC and OLT VR object location performance. Part of the issue may be due to different manual segmentation protocols of the EC into anterolateral and posteromedial parts, a consequence of the novelty and rapidly-changing landscape of research into the human EC. The current protocol, based on connectivity data, was determined by coronal slices and produces three subregions of the EC – alEC, pmEC and the largest EC, while an alternative protocol divides the EC into alEC and pmEC only in gradating manner (Olsen et al., 2013; Palombo et al., 2013). This means that the areas designated EC in the current study correspond to parts of alEC and pmEC in other publications, several of which reported significant associations with cognition or object memory (Olsen et al., 2017; Yeung et al., 2017). The simpler narrative of an MEC-mediated spatial/where pathway concerned with idiothetic (i.e. internal or self-motion) cues and a LEC-mediated non-spatial/what pathway concerned with object-related cues is beginning to be altered by recent findings that suggest a more interdependent and complex picture (Save & Sargolini, 2017). The GC-containing MEC appear important for both idiothetic functions like path integration as well as allocentric referencing, i.e. responding to distal landmarks and boundaries (Van Cauter et al., 2013). GC firing is particularly affected by polarised local environments such as square enclosures (Krupic, Bauza, Burton, Barry, & O'Keefe, 2015). The PRC may work with the HC to bind object locations to a cognitive map (Connor & Knierim, 2017).

The EC correlated or trended with both with mean displacement error and object-context recall in the OLT, which is an encouraging sign there may be some contribution of alEC/pmEC to OLT performance. However, the significant correlation between OLT mean displacement and the PRC (area 35), associated with alEC and object contextual memory (Burwell, Saddoris, Bucci, & Wiig, 2004), was eliminated when age was controlled for and thus may not be due to a true group difference.

Total HC and subiculum volumes showed by far the most striking correlations with OLT mean displacement in patients, and were also highly significant predictors of variance in subsequent regression analyses. The OLT, although influenced by paradigms investigating LEC, can arguably be conceptualised as a primarily hippocampal, allocentric task requiring repeated cognitive mapping of several specific locations within a local environment mostly using distal cues and boundary-related information (Doeller et al., 2008), as participants encountered the three objects in a random order every trial, and were asked to return to the centre between trials, reducing the contribution of idiothetic information as conveyed by GC in the pmEC. The most dominant characteristic of the OLT virtual environment were the square walls, followed by distal landmarks, because there were no local landmarks within the enclosure to aid spatial encoding, nor were the objects presented simultaneously allowing participants to easily encode their relative positions in the enclosure. This may account for the weak contribution of the alEC and EC to object location memory. Save and Sargolini (2017) note in their review that the MEC and LEC are not absolutely crucial for processing spatial and non-spatial information, as suggested by mixed findings from rat lesion studies of the EC (Burwell et al., 2004; Steffenach, Witter, Moser, & Moser, 2005), and that their involvement is modulated by environmental factors such as higher cognitive load and environmental

complexity due to number of unique objects (Kuruvilla & Ainge, 2017). This is also supported by evidence that MEC lesions or GC incapacitation do not obliterate HC place cell activity (Bush, Barry, & Burgess, 2014).

Therefore, we can hypothesise that the highly salient boundaries (walls) excited BVC activity in the subiculum, presubiculum and pmEC/EC to support HC place cell encoding of object locations, with pmEC grid cell and alEC output playing complementary but not essential roles in PC accuracy and remapping in context of discriminating between OLT's geometrically similar environments, e.g. when switching environments (Bostock, Muller, & Kubie, 1991; Bush et al., 2014; Lu et al., 2013). The correlation with HC subfield volumes in patients can be attributed to pathological AD-associated neuropathology and atrophy in these regions which disrupted normal PC functioning and thus strengthened the predictive relationship between object location memory and regional volumes compared to healthy controls. Other studies on allocentric spatial memory in aMCI and early AD patients have mixed findings on significant associations between allocentric processing, HC volume and activity (Migo et al., 2016; Moodley et al., 2015; Nedelska et al., 2012; Weniger et al., 2011). Nedelska et al. (2012) reported an association between right HC volume and performance on Morris Water Maze only in aMCI patients but not controls, and Moodley et al. (2015) reported a correlation between performance on the Four Mountains Test, an HC-dependent task requiring participants to recognise images of several mountains from different perspectives and lighting, and bilateral HC volume that did not survive correction. Migo et al. (2016) found significantly reduced fMRI activity in aMCI patients in a widespread spatial processing network including bilateral HC despite no difference in performance on a VR analogue of the Radial Arm Maze, while Weniger et al. (2011) did not find a significant association between HC volume and

aMCI patients' performance on a virtual park navigation task. Given the relatively few studies investigating neural correlates of allocentric processing in people with aMCI, our positive findings are a valuable addition to the evidence linking early AD neuropathology and atrophy in the HC and EC to well-characterised spatial impairments.

The subiculum has been investigated in recent years as one of the areas of initial focal atrophy along with CA1 in large MCI samples, and significant correlations to semantic (Carlesimo et al., 2015; Lindberg et al., 2017; Zammit et al., 2017) and importantly autobiographical memory (Hirjak et al., 2017) have been reported. Accordingly, the subiculum was one of the HC subfields (along with CA1 and granule cells in the molecular layer) with the strongest correlations to OLT mean displacement in our participants with aMCI (r > .80). It contains many of the spatial cells described above, crucially boundary vector cells and head direction cells, which play key roles in modulating PC activity especially in a small enclosure like the OLT, and furthermore is the major output pathway for CA1, receiving input from the EC, PRC and prefrontal cortex (O'Mara, Sanchez-Vives, Brotons-Mas, & O'Hare, 2009). De Flores et al. (2015) discussed evidence indicating that subiculum and CA1 volumetric reduction preceded cognitive deficits and predicted conversion from MCI to AD. This suggests that the higher discriminating power of HC subfield analysis over the whole HC is mostly restricted to the predementia stage. Thus in light of our findings, further research is needed on the subiculum and other HC subfields and their relationship to CSF status, AD progression and allocentric processing in aMCI or even preclinical samples.

4.3 Scientific and clinical implications

The current study represents a successful proof of concept of the use of immersive VR technology in healthy older adults and amnestic MCI patients, and demonstrated a consistent group difference in a novel object location memory paradigm. Due to its high controllability and ecological validity, immersive VR is increasingly going to be used in basic neuropsychological research and clinical interventions (Rus-Calafell, Garety, Sason, Craig, & Valmaggia, 2018). It is particularly well-suited to investigating spatial processing due to the important influence of proprioception, head direction and motor feedback in spatial memory and navigation, and the strong hippocampal and subicular contribution to OLT performance in aMCI patients highlights its value as a sensitive measure of allocentric processing and clinical memory impairment, showing good validity and effectively discriminating between aMCI patients and controls.

Hippocampal volume was strongly correlated with and significantly predicted a simple outcome of the current OLT, mean displacement error. This is of great importance diagnostically, as the current best-practice process of determining MCI and subjective memory impairment is complex and multimodal, involving clinical interviews with the patient and informants, cognitive assessment, neuroimaging and possible CSF biomarker testing. Structural MRI and CSF testing are considered particularly important in the earlier stages to determine disease progression and identify underlying AD (Albert et al., 2011; de Toledo-Morrell et al., 2004; Dubois et al., 2014; Frisoni et al., 2010; Shaw et al., 2009), however remain rather costly and invasive for widespread use in community settings. In recent decades, spatial memory and navigational tasks have emerged as strong contenders

for improving the sensitivity of diagnosis, as they target the brain regions initially affected by AD neuropathology and are much more affordable and less invasive for patients. This study demonstrated the realistic possibility of using immersive VR spatial tasks in the target populations of older adults with and without memory impairment, as well as the strong correlation between performance and HC integrity, suggesting that such tasks can be valuable additions to the diagnostic process.

Furthermore, there are numerous published studies and ongoing research on cognitive training and remediation interventions for people with MCI and AD, with reported effects in improving various aspects of cognitive functioning (Reijnders, van Heugten, & van Boxtel, 2013). Previous studies have already shown an improvement in allocentric memory and navigation when participants were allowed to actively engage or explore the environment (Carassa et al., 2002; Plancher et al., 2012) – the overwhelming verisimilitude of immersive VR in simulating the spatial experience of reality and the ability to track participants over larger and larger areas can only be advantages for designing clinical interventions. Specific training in allocentric memory and navigation, if efficacious in delaying the onset of AD or ameliorating topographical disorientation in early AD, can potentially benefit both healthy older adults and preclinical or MCI patients (Lövdén et al., 2012; Serino et al., 2017).

4.4 Limitations

One of the main methodological issues with the current study is the small sample size, especially of MRI data from aMCI patients, which amounted to 12-15 after participant attrition and eliminating outliers. Underpowered studies risk finding significant effects that may not be true (Button et al., 2013) and conducting multiple statistical analyses also increases the

probability of Type 1 errors, although the study tried to control for multiple comparisons using the FDR. However, the technical error that caused significant missing data for the OLD/NEW object familiarity task meant that results could not be analysed with quantitative methods. Furthermore the number of predictors entered into the regression models is also high for the sample size. Therefore the next step must be to replicate our findings in a larger sample of aMCI patients and controls, preferably with larger numbers of CSF positive and negative patients to enable within-group analysis of the role of OLT performance and HC and EC subfields in differentiating AD pathology.

Moreover, there was a significant difference in NART scores between the final healthy control and patient groups as IQ was not a characteristic the study set out to match. Due to time and resource constraints a NART matched control group was not recruited. Although the study attempted to control for premorbid IQ in the regression analyses, this could have contributed to the significant difference in OLT performance as a non-spatial or MTL-specific factor, for example by facilitating performance through better working memory or processing speed.

The current study did not observe any correlations between regional volumes and OLT performance in healthy controls, a somewhat unexpected finding in light of the not insubstantial literature on allocentric deficits and its neural correlates in healthy ageing (Colombo et al., 2017; Lester et al., 2017). However, the restricted age range and demographic characteristics (i.e. high IQ) of the control group may have served to minimise the effect of individual differences in HC/EC volumes on task performance, as many other studies either compare young controls with older adults or include samples with a wide age

range. We did not look at other ROIs such as the caudate or prefrontal cortices which may have shed more light on OLT performance.

Lastly, although commonly accepted as a valuable tool for diagnosing MCI (Frisoni et al., 2010), structural MRI is a gross anatomical measure of neuronal atrophy and may not reveal functional irregularities or microstructural changes that precede volume reduction. Combining structural MRI with other techniques such as diffusion tensor imaging (DTI) and functional MRI can increase our ability to characterise the neurobiological changes that accompany cognitive decline in AD (Zhang et al., 2013). Different MRI segmentation protocols, both automatic and manual, inevitably introduce variability in delineation of subfields and the chosen method of correcting regional volumes by head size can also considerably influence resulting data (Voevodskaya et al., 2014). The study has followed conventions of the affiliated research group based in Cambridge to maximise comparability across experiments.

4.5 Future research

Our findings from the current study would be strengthened if they were replicated in a larger sample of aMCI patients and controls, preferably with sufficient numbers of both CSF-positive and negative participants, which would allow for comparing OLT performance and exploring potential differences in neural correlates. Greater numbers may also clarify any role of the EC subfields, PRC and PHC in task performance, as would the inclusion of other relevant ROIs such as the precuneus, RSC and prefrontal areas. Additionally other techniques such as DTI, fMRI and biomarker levels may also shed light on predictors of OLT performance, as atrophy is likely a signifier of a neurodegenerative process that began much earlier.

The task itself may be subject to many modifications, for example controlling for strategy use by including an egocentric condition for comparison, or altering the geometry and salience of the boundaries by lowering the walls or changing the enclosure shape. This can help elucidate the neuropsychological components of allocentric processing in aMCI patients and healthy controls and their relationships to regional volumes. To investigate the roles of the LEC and MEC in more detail tasks from animal studies can be replicated in the VR setup – in fact a path integration task had already been successfully piloted prior to the current study, with a difference established between aMCI patient and control groups. Similarly paradigms targeting the LEC can be refined based on the current OLT, for example having proximal cues, multiple objects concurrently, and changing object identity or location and tracking change in participant behaviour and path characteristics. Furthermore, tasks that have already been proven to be effective discriminators in 2-dimensional VR (King, Burgess, Hartley, Vargha-Khadem, & O'Keefe, 2002; Moodley et al., 2015) can be replicated in immersive VR.

An important area for future research is integrating and validating the utility of immersive VR spatial tasks in the normal diagnostic process of a memory clinic. The current study has shown that it is viable, safe and cost-effective to use such technology with older adults and MCI patients, only requiring the space of a regular room to set up, and possible to complete tasks within a reasonable timeframe. Importantly, task performance is significantly linked to a gold standard measure of diagnosing MCI – HC volume as estimated in structural MRI. The next step would be to quantify OLT's sensitivity and specificity in differentiating aMCI patients, compare it to traditional cognitive testing, and investigate its relationship to CSF biomarker status and structural MRI data in larger samples representative of a memory clinic's referrals.

4.6 Conclusion

The current study found a reliable group difference between amnestic MCI patients and healthy controls in performance on a task assessing allocentric object location memory, using innovative immersive virtual reality. We also report significant group differences in raw HC, subiculum, CA1, EC and perirhinal volumes, which confirms a pattern of focal atrophy originating from the EC and spreading to the subiculum and CA1. Crucially, total HC and subiculum volume significantly predicted substantial percentages of the variance in aMCI patient task performance, demonstrating the potential of the current task to reflect hippocampal atrophy in people with amnestic MCI and highlighting the need for further research on preclinical populations and the benefits of immersive VR in improving the diagnostic approach in this field.

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Part 3: Critical Appraisal

Introduction

This appraisal considers several key practical and theoretical issues that arose during the process of conducting this research. The first section covers the topic of piloting and conducting research in this particular population, namely older adults with memory impairments, and discusses the challenges in recruitment, using VR with the elderly and adaptations to the study protocol for this population.

The second section considers the benefits and difficulties of working with multiple collaborators from different research groups and institutions in the context of resource constraints and the doctorate's limited timeline, including the process of overcoming issues with communication and balancing the priorities of all parties involved.

The last section discusses the various challenges and advantages of conducting research using novel VR technology and imaging data, and reflects on my learning curve during the process of data collection, analysis and writing up.

Research with older adults and patients with Mild Cognitive Impairment

1. Recruitment

Common barriers in recruiting older adults for research came up during the course of this study, including poor health, logistical difficulties and high participant attrition (McMurdo et al., 2011; Mody et al., 2008). The initial planned sample size of 25 per group, which was already a lower goal when considering the numbers needed for sufficiently powered multiple regression analyses, could not be met within the study timeline partly because of cancellations and rescheduling of testing sessions due to health appointments, health-related

issues e.g. recovery from operations, arranged transport falling through and caring for ageing spouses. The aspect of recruiting participants with memory impairment also contributed to delay and attrition, as several participants forgot appointments or confused the times and locations of testing, requiring rescheduling. Although we attempted a telephone reminder the day before, sometimes participants could not be reached due to age-related reasons such as not having mobile phones or voicemail functionality on their landlines. Exclusion due to participants' inability to consent did not occur, perhaps because testing took place relatively quickly after their memory clinic appointment where their capacity for informed consent was assessed. In addition, the secondary attrition from missing MRI scans (four out of 20 MCI patients had missing or excluded data) meant that the final sample size for analysis was smaller than ideal.

Moving forward, the simplest remedy would be to extend the period of recruitment and testing and follow best practice guidelines (McMurdo et al., 2011) in aiming to screen three times the required sample size and accounting for dropout rates of at least 10-20%. This is ambitious for a DClinPsy project since the available period for research is limited compared to a PhD, however there is room for extension given our recruitment and testing took place over six months. Future studies including MRI data should consider the secondary attrition when planning their recruitment goals, as well as the time between diagnosis of MCI and testing, as the capacity to give informed consent may deteriorate with longer delays.

2. Use of immersive VR technology with older adults

Immersive virtual reality (iVR) is not a new technology by any means – earliest utilisation of VR tracking head and eye movements in research can be traced back decades (Bohil, Alicea,

& Biocca, 2011; Loomis, Blascovich, & Beall, 1999). However, it remained a niche area due to the high cost of equipment. In the last few years, developments in affordable iVR headsets such as Oculus Rift, spearheaded by the gaming industry, lowered the barriers to accessible VR technology for the general population and simultaneously facilitated scientific research in diverse areas. Many research groups are now piloting immersive VR in neuroscience research, cognitive training and clinical interventions (Atherton et al., 2016; Rus-Calafell et al., 2018). However, due to the dominance of entertainment and gaming in the arena, there has been relatively less focus on whether the technology is suitable for, and can be adapted to the needs of older people.

Use of immersive VR has been linked to “motion sickness” side effects of nausea and dizziness earlier on (Regan, 1995), although this has been ameliorated by technological advances such as higher frame rates and better spatial tracking (Arguinbaev, 2017; Unity FAQ, 2018). This was an initial concern in the planning and piloting stages of the study, given the declines in vestibular and motor stability that affect MCI and AD patients (Tangen, Engedal, Bergland, Moger, & Mengshoel, 2014) and older adults more generally. Piloting conducted in younger participants went smoothly with no significant side effects; a very visually and experientially similar path integration task was also successfully tested in aMCI patients and controls prior to our study, and well tolerated in both groups, which alleviated these concerns somewhat.

When introducing participants to the experiment, we were cognizant of immersive VR’s novelty and ensured they had as much time as they needed to become comfortable with the equipment and acclimatize to wearing the headset. We explained the exact stages of putting

on the VR and what they were expected to experience, and checked in with them after every step e.g. adjusting the position or tightness of the headset and backpack straps. We also verbally guided them through the practice and test environments as much as necessary. It was helpful that some of the participants had previously done the VR path integration task and were able to quickly adapt. We made sure that they were able to take breaks in between environments, an opportunity that many of the more elderly participants took as the backpack was around two kilograms and can feel heavy after a prolonged period. With these adjustments, all of the healthy controls and most MCI patients except one were able to complete the whole experiment.

A risk of exceeding the boundaries of the 3.5 metre square was flagged up in the pilot with young volunteers, as the wider VR environment beyond the enclosure may tempt participants into trying to walk through the walls. A warning message was added to the VR experiment when participants reached the virtual walls and we explained beforehand that we may lightly touch a participant's shoulder to indicate they are near the edges. However this did not prove to be a significant issue with the older participants, possibly due to their lower speed and greater salience of the enclosure walls as real barriers.

3. Adjusting the process for elderly participants and cognitive impairments

Because of the wide variability in memory impairment amongst our sample of MCI patients, it was challenging for some of them to complete the VR section and object familiarity task as they sometimes forgot the instructions. Instructions appeared onscreen at the commencement of each phase, however participants occasionally struggled to retain them. Thus we repeated them frequently to remind participants and verbally "walked through" tasks

with those forgot, in accordance with common cognitive strategies to improve memory (Belleville, 2008; Troyer, Murphy, Anderson, Moscovitch, & Craik, 2008). We also increased the time out for responding in the object familiarity task to one minute after the first few MCI patients struggled to respond quickly. The inclusion of practice blocks in both tasks were very helpful in allowing cognitively impaired participants to learn task demands and should be made more extensive in the future.

Use of the hand controller was harder than anticipated, as some MCI patients struggled to hold down and release the button correctly due to difficulties with fine motor control and remembering instructions. We attempted to address this by frequent verbal reminders and demonstrating physically (guiding their hands and fingers on the control) during the recall trials in the practice environment and continuing in the test environments if necessary. Furthermore, having two recall trials maximized the collection of usable data as invalid responses outside the enclosure had to be discarded.

In the future the motor complexity of task demands should be taken into consideration, and simplified if possible. The current instructions require participants to hold down the hand controller button to project the pedestal and to release it at the recalled object locations in order to record their response. This could be simplified to an automatic projection from the hand controller that requires no prolonged pressing and a button press for response. Any responses outside the enclosure should also be recognised as invalid by the task and participants allowed to respond again immediately, instead of discarding the data. Instructions can be adapted to always appear onscreen to assist participants with memory impairments.

Benefits and difficulties of working with multiple collaborators

1. Multidisciplinary and multi-institutional collaboration

Literature reviews on collaboration (Mattessich & Monsey, 1992) especially in scientific research (Bukvova, 2010; Cummings & Kiesler, 2005) highlight potential benefits such as access to expertise and resources, exchange of ideas, higher quality of results and acquiring new skills, as well as influencing factors such as frequency and quality of communication, coordination, decision-making, division of labour and credit. Multi-university collaborations introduce complicating factors including more difficulties in communication and coordination that may impact on outcomes. The current study involved multiple disciplines and institutions – the VR experiment was created by a PhD student in engineering and neuroscience at UCL, while the testing location, equipment and participants were provided by the collaborating research team in Cambridge, led by a neurology consultant and coordinated by a neuroscience PhD student. There are clear benefits to collaboration as it allowed each team to access VR technology/experiments and control and patient participants respectively, as well as pooling personnel and resources for testing; however, as a junior member in the organizational structure, I also had to manage challenges resulting from communication between teams and individuals, at different levels of command, and try to balance competing priorities of the parties involved in the collaboration.

2. Effective communication and balancing competing priorities

Due to the nature of how the collaboration was organised and my role in it, I was dependent on other parties in many aspects such as initial recruitment, logistical arrangements such as contacting and scheduling participants for VR testing, MRI scans, booking rooms, and access

to participants' demographic and clinical data e.g. CSF status, screening test scores and medical history. Any technical alterations that had to be made to the VR were dependent on the PhD student who coded the tasks.

This was challenging for several reasons: my experiment had to be changed from the initial proposed Town Square Task due to an unexpected hiatus of the UCL PhD student who created the VR tasks. As the UCL PhD student was not onsite during test sessions in Cambridge, the rare technical issues, such as glitches in the VR and object familiarity task causing unrecorded respondent data, were too complex for me to remedy on the spot and sometimes required hours of troubleshooting, highlighting the advantages as well as pitfalls of multidisciplinary collaboration.

To maintain a satisfactory level of oversight of the recruitment and testing timeline and ensure suitable participants are being tested, my project partner and I had to work closely with the Cambridge PhD student to clarify concerns and voice our needs. In order to centralize and streamline the process, one person on the Cambridge team was designated as a key contact for participants. On reflection it would have been helpful to discuss and decide on a clearer allocation of responsibility before embarking on the project, as ideally we would be more actively involved with participant recruitment and scheduling. Sometimes, ambiguity in the internal decision-making and communication process within the Cambridge team impacted us (e.g. a miscommunication about which CSF status to preferentially recruit), and when brought to our notice, we tried to resolve through quick, proactive liaison and clarification, underscoring the importance of effective communication in research collaborations (Bozeman, Gaughan, Youtie, Slade, & Rimes, 2016).

As DClinPsy students, our project was also on a much tighter timeline compared to the senior researchers and PhD students, which meant logistical problems impacted us disproportionately and accentuated some of the competing priorities of the different groups. At some point there was a difficulty with room availability for testing, meaning that the rate of recruitment slowed to several participants a month. This was much more problematic for us due to the limited time we have, and understandably caused anxiety about completing data collection on time. After discussion with my supervisor, the urgency of the problem was communicated through the senior level and another location was consequently sourced, allowing more participants to be scheduled.

Furthermore, balancing competing priorities of individuals and teams involved in the study was an important theme that arose. When initial data analysis revealed a floor effect in object environmental context recognition, a senior researcher suggested that the VR task should be modified to encourage participants to rely more on environmental cues in encoding object locations. From a research-oriented perspective, the advantages of modifying the task immediately are clear – it enables a better version that is sensitive to potential alEC functioning to be piloted sooner, and conserves the pool of naïve MCI patients, as an alEC-dependent spatial task is of more scientific significance than a hippocampal one. However, because my partner and I based our project around the initial version of the OLT, it would have had a negative impact on the consistency of our study to alter task parameters partway through. Eventually, after discussion at the senior level, we were able to recruit up to 20 patients to ensure that we had a decent sample size to complete our data analysis.

Striking an appropriate balance between competing priorities is always difficult in

collaboration, especially when the unforeseen conflicts arise during the process, and when one's needs clash with a highly prioritised goal. I feel that I handled this issue to the best of my ability while being mindful of my position in the organisation – I reflected my concerns to my supervisor who was able to communicate with other senior researchers, and they had the power to negotiate a mutually satisfactory resolution.

Nevertheless, working with collaborators from different disciplines and institutions enabled my project to be more ambitious in the scope of what it achieved, and considerably smoothened the ethics approval process and recruitment of MCI patients that can prove time-consuming, challenging and potentially unfeasible if I were attempting this study on my own. Similarly, access to VR technology and structural MRI data would not have been possible without collaborating with a research team with greater resources.

Conducting research with VR and MRI

1. Resolving technical issues during testing

Introduced to virtual reality technology as a complete newcomer, I naturally encountered challenges during the course of this study. The immersive VR system (HTC Vive) in this project was produced for mass market use, and thus was fairly intuitively designed and easy to learn how to set up and use. However, I did not have a good understanding of the functioning of the VR experiment and object familiarity task, as they were programmed by others and required understanding of C# and Matlab. Although I was familiar with some other programming languages, there was insufficient time to get myself up to speed with the technical aspects in this study before testing began. This was a problem when the programs sometimes malfunctioned during testing, as I was unable to troubleshoot on the spot and

there was data loss on one or two occasions due to this. It was quite a stressful situation for myself as an experimenter, and a disorienting experience for the participants who had to wait open-endedly as we tried to resolve the issue. Luckily, in the vast majority of cases simple solutions such as restarting were effective and technical issues merely lengthened the testing.

Another issue was the technical skill required for data extraction and analysis – we were reliant on scripts written by the PhD students to obtain the mean displacement error, percentage correct etc., which meant that a programming error (no information recorded about which environment a participant selected in the object familiarity task, as the four choices were spatially randomized) was not picked up until partway through testing. This resulted in lost data for several participants in the beginning, which restricted later data analysis. This could have been avoided by working out the data extraction at the beginning of testing with the PhD students, and ensuring that I had a good understanding of how to do so myself. Extracting and analyzing data on an ongoing basis starting from the first participant would also have revealed problems earlier.

2. MRI volumetric analysis

I faced a steep learning curve with the manual segmentation of the entorhinal cortex. From the beginning, I was very keen to be involved in segmentation as I felt that with my project being based on structural MRI, it was important that I had a thorough understanding of how the MRI data was analysed and participated in the process as much as I could.

I had no previous experience with volumetric MRI analysis and thus relied very much on the neuroscience PhD student when starting to learn how to use the segmentation software

ITKSNAP. He was very helpful and patiently explained the protocols to me and the other experimenters, as well as arranging sessions where we could segment the same scans and feedback on one another's work. It was essential to have a good grasp of the 3-dimensional anatomical structure of the hippocampus and entorhinal cortex in order to properly segment the MRI scans, and this was an aspect I struggled the most with, requiring a lot of refinement and guidance from the PhD student.

Conclusion

The issues discussed in this appraisal reveals the demands and benefits of engaging older adults with or without cognitive impairment in research, demonstrating that with appropriate adjustments and forward planning, it is both feasible and rewarding to introduce them to cutting edge technologies like immersive VR. The appraisal also highlights common advantages and difficulties of multidisciplinary and multi-institution collaborations, reflecting on how problems were managed and could have been mitigated by adhering to best practices indicated by research, as well as considers my personal experience of learning how to perform MRI analysis and my appreciation for the support of other collaborators.

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Appendices

Appendix 1: Studies excluded based on inclusion and exclusion criteria

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Appendix 2: Quality assessment tool checklist and scoring instructions

Table 1. Checklist for assessing the quality of quantitative studies

Criteria		YES (2)	PARTIAL (1)	NO (0)	N/A
1	Question / objective sufficiently described?				
2	Study design evident and appropriate?				
3	Method of subject/comparison group selection *or* source of information/input variables described and appropriate?				
4	Subject (and comparison group, if applicable) characteristics sufficiently described?				
5	If interventional and random allocation was possible, was it described?				
6	If interventional and blinding of investigators was possible, was it reported?				
7	If interventional and blinding of subjects was possible, was it reported?				
8	Outcome and (if applicable) exposure measure(s) well defined and robust to measurement / misclassification bias? Means of assessment reported?				
9	Sample size appropriate?				
10	Analytic methods described/justified and appropriate?				
11	Some estimate of variance is reported for the main results?				
12	Controlled for confounding?				
13	Results reported in sufficient detail?				
14	Conclusions supported by the results?				

Appendix A: Manual for Quality Scoring of Quantitative Studies

Definitions and Instructions for Quality Assessment Scoring

How to calculate the summary score

- **Total sum** = (number of "yes" * 2) + (number of "partials" * 1)
- **Total possible sum** = 28 – (number of "N/A" * 2)
- **Summary score:** total sum / total possible sum

Quality assessment

1. *Question or objective sufficiently described?*

   Yes: Is easily identified in the introductory section (or first paragraph of methods section). Specifies (where applicable, depending on study design) *all* of the following: purpose, subjects/target population, and the specific intervention(s) /association(s)/descriptive parameter(s) under investigation. A study purpose that only becomes apparent after studying other parts of the paper is *not* considered sufficiently described.

   Partial: Vaguely/incompletely reported (e.g. "describe the effect of" or "examine the role of" or "assess opinion on many issues" or "explore the general attitudes"...); *or* some information has to be gathered from parts of the paper other than the introduction/background/objective section.

   No: Question or objective is not reported, or is incomprehensible.

   N/A: Should not be checked for this question.

2. *Design evident and appropriate to answer study question?*

   (If the study question is not given, infer from the conclusions).

   Yes: Design is easily identified and is appropriate to address the study question / objective.

   Partial: Design and /or study question not clearly identified, but gross inappropriateness is not evident; *or* design is easily identified but only partially addresses the study question.

   No: Design used does not answer study question (e.g., a comparison group is required to answer the study question, but none was used); *or* design cannot be identified.

   N/A: Should not be checked for this question.

3. *Method of subject selection (and comparison group selection, if applicable) or source of information/input variables (e.g., for decision analysis) is described and appropriate.*

   Yes: Described and appropriate. Selection strategy *designed* (i.e., consider sampling frame and strategy) to obtain an unbiased sample of the relevant target population or the entire target population of interest (e.g., consecutive patients for clinical trials, population-based random sample for case-control studies or surveys). Where applicable, inclusion/exclusion criteria are described and defined (e.g., "cancer" -- ICD code or equivalent should be provided). *Studies of volunteers:* methods and setting of recruitment reported. *Surveys:* sampling frame/ strategy clearly described and appropriate.

   Partial: Selection methods (and inclusion/exclusion criteria, where applicable) are not completely described, but no obvious inappropriateness. Or selection strategy is not ideal (i.e., likely introduced bias) but did not likely seriously distort the results (e.g., telephone survey sampled from listed phone numbers only; hospital based case-control study identified all cases admitted during the study period, but recruited controls admitted during the day/evening only). Any study describing participants only as "volunteers" or "healthy volunteers". *Surveys:* target population mentioned but sampling strategy unclear.

   No: No information provided. Or obviously inappropriate selection procedures (e.g., inappropriate comparison group if intervention in women is compared to intervention in men). Or presence of selection bias which likely seriously distorted the results (e.g., obvious selection on "exposure" in a case-control study).

   N/A: Descriptive case series/reports.

4. *Subject (and comparison group, if applicable) characteristics or input variables/information (e.g., for decision analyses) sufficiently described?*

   Yes: Sufficient relevant baseline/demographic information clearly characterizing the participants is provided (or reference to previously published baseline data is provided). Where applicable, reproducible criteria used to describe/categorize the participants are clearly defined (e.g., ever-smokers, depression scores, systolic blood pressure > 140). If "healthy volunteers" are used, age and sex must be reported (at minimum). *Decision analyses:* baseline estimates for input variables are clearly specified.

   Partial: Poorly defined criteria (e.g. "hypertension", "healthy volunteers", "smoking"). Or incomplete relevant baseline / demographic information (e.g., information on likely confounders not reported). *Decision analyses:* incomplete reporting of baseline estimates for input variables.

   No: No baseline / demographic information provided.
   Decision analyses: baseline estimates of input variables not given.

   N/A: Should not be checked for this question.

5. *If random allocation to treatment group was possible, is it described?*

Yes: True randomization done - requires a description of the method used (e.g., use of random numbers).

Partial: Randomization mentioned, but method is not (i.e. it may have been possible that randomization was not true).

No: Random allocation not mentioned although it would have been feasible and appropriate (and was possibly done).

N/A: Observational analytic studies. Uncontrolled experimental studies. Surveys. Descriptive case series / reports. Decision analyses.

6. *If interventional and blinding of investigators to intervention was possible, is it reported?*

Yes: Blinding reported.

Partial: Blinding reported but it is not clear who was blinded.

No: Blinding would have been possible (and was possibly done) but is not reported.

N/A: Observational analytic studies. Uncontrolled experimental studies. Surveys. Descriptive case series / reports. Decision analyses.

7. *If interventional and blinding of subjects to intervention was possible, is it reported?*

Yes: Blinding reported.

Partial: Blinding reported but it is not clear who was blinded.

No: Blinding would have been possible (and was possibly done) but is not reported.

N/A: Observational studies. Uncontrolled experimental studies. Surveys. Descriptive case series / reports.

8. *Outcome and (if applicable) exposure measure(s) well defined and robust to measurement / misclassification bias? Means of assessment reported?*

Yes: Defined (or reference to complete definitions is provided) and measured according to reproducible, "objective" criteria (e.g., death, test completion – yes/no, clinical scores). Little or minimal potential for measurement / misclassification errors. *Surveys:* clear description (or reference to clear description) of questionnaire/interview content and response options. *Decision analyses:* sources of uncertainty are defined for all input variables.

Partial: Definition of measures leaves room for subjectivity, or not sure (i.e., not reported in detail, but probably acceptable). Or precise definition(s) are missing, but no evidence or problems in the paper that would lead one to assume major problems. Or instrument/mode of assessment(s) not reported. Or misclassification errors may have occurred, but they did not likely seriously distort the results (e.g., slight difficulty with recall of long-ago events; exposure is measured only at baseline in a long cohort study). *Surveys:* description of questionnaire/interview content incomplete; response options unclear. *Decision analyses:* sources of uncertainty are defined only for some input variables.

No: Measures not defined, or are inconsistent throughout the paper. Or measures employ only ill-defined, subjective assessments, e.g. "anxiety" or "pain." Or obvious misclassification errors/measurement bias likely seriously distorted the results (e.g., a prospective cohort relies on self-reported outcomes among the "unexposed" but requires clinical assessment of the "exposed"). *Surveys:* no description of questionnaire/interview content or response options. *Decision analyses:* sources of uncertainty are not defined for input variables.

N/A: Descriptive case series / reports.

9. *Sample size appropriate?*

Yes: Seems reasonable with respect to the outcome under study and the study design. When statistically significant results are achieved for major outcomes, appropriate sample size can usually be assumed, unless large standard errors (SE > ½ effect size) and/or problems with multiple testing are evident. *Decision analyses:* size of modeled cohort / number of iterations specified and justified.

Partial: Insufficient data to assess sample size (e.g., sample seems "small" and there is no mention of power/sample size/effect size of interest and/or variance estimates aren't provided). Or some statistically significant results with standard errors > ½ effect size (i.e., imprecise results). Or some statistically significant results in the absence of variance estimates. *Decision analyses:* incomplete description or justification of size of modeled cohort / number of iterations.

No: Obviously inadequate (e.g., statistically non-significant results and standard errors > ½ effect size; or standard deviations > _ of effect size; or statistically non-significant results with no variance estimates and obviously inadequate sample size). *Decision analyses:* size of modeled cohort / number of iterations not specified.

N/A: Most surveys (except surveys comparing responses between groups or change over time). Descriptive case series / reports.

10. *Analysis described and appropriate?*

Yes: Analytic methods are described (e.g. "chi square"/ "t-tests"/"Kaplan-Meier with log rank tests", etc.) and appropriate.

Partial: Analytic methods are not reported and have to be guessed at, but are probably appropriate. Or minor flaws or some tests appropriate, some not (e.g., parametric tests used, but unsure whether appropriate; control group exists but is not used for statistical analysis). Or multiple testing problems not addressed.

No: Analysis methods not described and cannot be determined. Or obviously inappropriate analysis methods (e.g., chi-square tests for continuous data, SE given where normality is highly unlikely, etc.). Or a study with a descriptive goal / objective is over-analyzed.

N/A: Descriptive case series / reports.

11. *Some estimate of variance (e.g., confidence intervals, standard errors) is reported for the main results/outcomes (i.e., those directly addressing the study question/objective upon which the conclusions are based)?*

Yes: Appropriate variances estimate(s) is/are provided (e.g., range, distribution, confidence intervals, etc.). *Decision analyses:* sensitivity analysis includes all variables in the model.

Partial: Undefined "+/-" expressions. *Or* no specific data given, but insufficient power acknowledged as a problem. *Or* variance estimates not provided for all main results/outcomes. *Or* inappropriate variance estimates (e.g., a study examining change over time provides a variance around the parameter of interest at "time 1" or "time 2", but does not provide an estimate of the variance around the difference). *Decision analyses:* sensitivity analysis is limited, including only some variables in the model.

No: No information regarding uncertainty of the estimates. *Decision analyses:* No sensitivity analysis.

N/A: Descriptive case series / reports. Descriptive surveys collecting information using open-ended questions.

12. *Controlled for confounding?*

Yes: Randomized study, with comparability of baseline characteristics reported (or non-comparability controlled for in the analysis). *Or* appropriate control at the design or analysis stage (e.g., matching, subgroup analysis, multivariate models, etc). *Decision analyses:* dependencies between variables fully accounted for (e.g., joint variables are considered).

Partial: Incomplete control of confounding. *Or* control of confounding reportedly done but not completely described. *Or* randomized study without report of comparability of baseline characteristics. *Or* confounding not considered, but not likely to have seriously distorted the results. *Decision analyses:* incomplete consideration of dependencies between variables.

No: Confounding not considered, and may have seriously distorted the results. *Decision analyses:* dependencies between variables not considered.

N/A: Cross-sectional surveys of a single group (i.e., surveys examining change over time or surveys comparing different groups should address the potential for confounding). Descriptive studies. Studies explicitly stating the analysis is strictly descriptive/exploratory in nature.

13. *Results reported in sufficient detail?*

Yes: Results include major outcomes and all mentioned secondary outcomes.

Partial: Quantitative results reported only for some outcomes. *Or* difficult to assess as study question/objective not fully described (and is not made clear in the methods section), but results seem appropriate.

No: Quantitative results are reported for a subsample only, or "n" changes continually across the denominator (e.g., reported proportions do not account for the entire study sample, but are reported only for those with complete data -- i.e., the category of "unknown" is not used where needed). *Or* results for some major or mentioned secondary outcomes are only qualitatively reported when quantitative reporting would have been possible (e.g., results include vague comments such as "more likely" without quantitative report of actual numbers).

N/A: Should not be checked for this question.

14. *Do the results support the conclusions?*

Yes: All the conclusions are supported by the data (even if analysis was inappropriate). Conclusions are based on all results relevant to the study question, negative as well as positive ones (e.g., they aren't based on the sole significant finding while ignoring the negative results). Part of the conclusions may expand beyond the results, if made *in addition to* rather than instead of those strictly supported by data, and if including indicators of their interpretative nature (e.g., "suggesting," "possibly").

Partial: Some of the major conclusions are supported by the data, some are not. *Or* speculative interpretations are not indicated as such. *Or* low (or unreported) response rates call into question the validity of generalizing the results to the target population of interest (i.e., the population defined by the sampling frame/strategy).

No: None or a very small minority of the major conclusions are supported by the data. *Or* negative findings clearly due to low power are reported as definitive evidence against the alternate hypothesis. *Or* conclusions are missing. *Or* extremely low response rates invalidate generalizing the results to the target population of interest (i.e., the population defined by the sampling frame/strategy).

N/A: Should not be checked for this question.

Appendix 3: Detailed scores for studies included in systematic review

Study	Criteria													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Driscoll et al.	2	2	1	2	Not applicable			2	1	2	0	1	2	2
Meulenbroek et al.	1	1	1	2				2	2	2	1	2	2	1
Moffat et al. (2006)	2	1	2	2				2	1	2	1	2	2	1
Moffat et al. (2007)	2	2	2	2				2	2	2	1	1	2	1
Antonova et al.	2	2	1	2				2	1	2	1	1	1	1
Head & Isom	2	1	1	2				2	1	1	1	2	2	2
Lövdén et al.	2	2	1	2	2	0	*na*	2	2	2	1	2	2	1
Konishi et al. (2013)	2	1	1	2				2	1	2	1	2	2	1
Konishi & Bohbot	2	1	0	2				2	2	2	2	1	1	1
Daugherty et al. (’15)	2	1	2	2				2	2	2	1	1	1	1
Schuck et al.	2	2	1	2				2	1	2	1	1	2	1
Daugherty et al. (’16)	2	1	1	2				2	2	2	1	1	2	1
Korthauer et al.	2	2	1	1				2	1	2	2	1	2	2
Daugherty & Raz	2	1	1	2				1	1	2	2	1	2	1
Konishi et al. (2017)	2	2	0	2				2	2	2	2	1	2	1

Appendix 4: Ethics amendment approval letter

NHS
Health Research Authority

East of England - Cambridge South Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

16 February 2018

Mr David Howell
Herchel Smith Building
Robinson Way
Cambridge
CB2 0SZ

Dear Mr Howell

Study title:	**VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function in early Alzheimer's disease (VIRTECH-AD)**
REC reference:	**16/EE/0215**
Amendment number:	**SA3**
Amendment date:	**18 January 2018**
IRAS project ID:	**193437**

The above amendment was reviewed at the meeting of the Sub-Committee held in correspondence on 09 February 2018

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	SA3	18 January 2018

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

16/EE/0215: Please quote this number on all correspondence

Yours sincerely

Dr Leslie Gelling
Chair

E-mail: nrescommittee.eastofengland-cambridgesouth@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Dr Rachel Kyd, Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge*
Mr David Howell

East of England - Cambridge South Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 09 February 2018

Committee Members:

Name	*Profession*	*Present*
Dr Leslie Gelling	(Chair) Reader in Research Ethics	Yes
Dr Kate Williams	Senior Research Associate	Yes

Also in attendance:

Name	*Position (or reason for attending)*
Mr George R. Martin	REC Manager (Minutes)

Appendix 5: Visiting Researcher status approval letter

Cambridge University Hospitals NHS
NHS Foundation Trust

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HR Manager: Nacha Samaila
01223 274660
nacha.samaila@addenbrookes.nhs.uk

29th August 2017

Dear Adrienne

Letter of access for research – A093863 - VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function in ageing and mild cognitive

This letter confirms your right of access to conduct research through Cambridge University Hospitals NHS Foundation Trust for the purpose and on the terms and conditions set out below. This right of access commences on **18th August 2017** and ends on **30th September 2018** unless terminated earlier in accordance with the clauses below.

You have a right of access to conduct such research as confirmed in writing in the letter of permission for research from this NHS organisation. Please note that you cannot start the research until the Principal Investigator for the research project has received a letter from us giving permission to conduct the project and you have provided the Trust's R&D department with written evidence that you have completed GCP training from an EU institution before you start your research.

The information supplied about your role in research at Cambridge University Hospitals NHS Foundation Trust has been reviewed and you do not require an honorary research contract with this NHS organisation. We are satisfied that such pre-engagement checks as we consider necessary have been carried out.

You are considered to be a legal visitor to Cambridge University Hospitals NHS Foundation Trust premises. You are not entitled to any form of payment or access to other benefits provided by this NHS organisation to employees and this letter does not give rise to any other relationship between you and this NHS organisation, in particular that of an employee.

While undertaking research through Cambridge University Hospitals NHS Foundation Trust, you will remain accountable to your place of work **University of Cambridge** but you are required to follow the reasonable instructions of **Dr Dennis Chan and Rodney Laing** in this NHS organisation or those given on their behalf in relation to the terms of this right of access.

Where any third party claim is made, whether or not legal proceedings are issued, arising out of or in connection with your right of access, you are required to co-operate fully with any investigation by this NHS organisation in connection with any

such claim and to give all such assistance as may reasonably be required regarding the conduct of any legal proceedings.

You must act in accordance with Cambridge University Hospitals NHS Foundation Trust policies and procedures, which are available to you upon request, and the Research Governance Framework.

You are required to co-operate with Cambridge University Hospitals NHS Foundation Trust in discharging its duties under the Health and Safety at Work etc Act 1974 and other health and safety legislation and to take reasonable care for the health and safety of yourself and others while on Cambridge University Hospitals NHS Foundation Trust premises. You must observe the same standards of care and propriety in dealing with patients, staff, visitors, equipment and premises as is expected of any other contract holder and you must act appropriately, responsibly and professionally at all times.

If you have a health condition or disability which may affect your research role and which might require reasonable special adjustments to your role, if you have not already done so, you must notify your employer and the Trust's R&D HR Office prior to commencing your research role at the Trust.

You are required to ensure that all information regarding patients or staff remains secure and *strictly confidential* at all times. Personal identifiable data must be carried securely at all times and mobile devices must be encrypted. You must ensure that you understand and comply with the requirements of the NHS Confidentiality Code of Practice (https://www.gov.uk/government/publications/confidentiality-nhs-code-of-practice) and the Data Protection Act 1998. Furthermore you should be aware that under the Act, unauthorised disclosure of information is an offence and such disclosures may lead to prosecution. Data controllers could also be fined for a breach of the Data Protection Act 1998. You must familiarise yourself with the Trust's Information Governance Code of Conduct.

You must keep confidential any information regarding the design, conduct or management or results of any research unless authorised in writing by the Trust to disclose it. You must acknowledge the Trust's contribution in any publication arising out of this Agreement.

Subject to any agreement with your employer to the contrary (e.g. as part of a multi-centre study), any Intellectual Property (IP) resulting from research carried out under this Agreement will be the property of the Trust and you will do all things necessary or desirable to give effect to the assignment of this IP.

You should ensure that, where you are issued with an identity or security card, a bleep number, email or library account, keys or protective clothing, these are returned upon termination of this arrangement. Please also ensure that while on the premises you wear your ID badge at all times, or are able to prove your identity if challenged. Please note that this NHS organisation accepts no responsibility for damage to or loss of personal property.

We may terminate your right to attend at any time either by giving seven days' written notice to you or immediately without any notice if you are in breach of any of the terms or conditions described in this letter or if you commit any act that we reasonably consider to amount to serious misconduct or to be disruptive and/or prejudicial to the interests and/or business of this NHS organisation or if you are convicted of any criminal offence. You must not undertake regulated activity if you

are barred from such work. If you are barred from working with adults or children this letter of access is immediately terminated. Your employer will immediately withdraw you from undertaking this or any other regulated activity and you MUST stop undertaking any regulated activity immediately.

Your substantive employer is responsible for your conduct during this research project and may in the circumstances described above instigate disciplinary action against you.

Cambridge University Hospitals NHS Foundation Trust will not indemnify you against any liability incurred as a result of any breach of confidentiality or breach of the Data Protection Act 1998. Any breach of the Data Protection Act 1998 may result in legal action against you and/or your substantive employer.

INDUCTION AND MANDATORY TRAINING
You are responsible for familiarising yourself with the Trust's policies and mandatory training courses such as Moving and Handling, Health and Safety, Fire Training etc and be aware of the responsibility to maintain a safe environment for patients, staff and visitors

Your host Manager will ensure that you receive a comprehensive Departmental Induction. She/he will also provide you with details of Corporate Induction, research specific induction and annual Mandatory Refresher Training.

If your letter of access is for more than 3 months, you must attend Corporate Induction. Where your letter of access is for more that 12 months, you must attend annual Mandatory Refresher Training.

If your current role or involvement in research changes, or any of the information provided in your Research Passport changes, you must inform your employer through their normal procedures. You must also inform your nominated manager in this NHS organisation.

Yours sincerely

Stephen Kelleher
R&D Manager, Cambridge University Hospitals NHS Foundation Trust

cc: Dr Dennis Chan, Dept of Clinical Neurosciences, dc598@medschl.cam.ac.uk
University HR, via e-mail

Enc: ID Badge Form

Appendix 6: Study Information Sheets

7th October 2016
Version 5

UNIVERSITY OF CAMBRIDGE

Herchel Smith Building for Brain and Mind Sciences
Forvie Site, Robinson Way
Cambridge CB2 0SZ
Tel: 0044 (0)1223 760697
Fax: 0044 (0)1223 336 581

Department of Clinical Neurosciences

Dr Dennis Chan PhD MD FRCP

PARTICIPANT INFORMATION SHEET
for control participants

Study title: VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function (VIRTECH)

Part 1 - Background:

We would like to invite you to take part in a research study which aims to identify changes in brain function associated with early Alzheimer's Disease (AD) using a new test of spatial navigation and memory. We would like to ask for your involvement as a "control" participant, so that we can obtain a set of normal scores against which the scores of people with early AD can be compared. Your participation in these tests is for research purposes only and will *not* be used for any clinical use or determining your risk of developing Alzheimer's Disease. The results will be anonymised, kept strictly confidential and only accessed by approved researchers involved in this study. Your GP will not be informed of your participation or have access to the data collected".

Before you decide to take part we would like you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. You can talk to others about the study if you wish or ask one of the researchers for further information.

What is the purpose of the study?

The study aims to determine whether a test of spatial navigation (the ability to make one's way within an environment) and spatial memory (the ability to

remember the location of objects within an environment) can help predict whether people may develop AD in the future. These tests have been chosen because spatial navigation and memory are abilities controlled by regions of the brain called the entorhinal cortex and the hippocampus, which are some of the first brain regions to be affected by AD. As such, when people are in the earliest stages of AD, it is proposed that these abilities will be impaired and so if we can identify this impairment we may be able to identify AD earlier than is the case at present.

The problem is that it is not practical to test people's spatial navigation and memory in real world settings such as parks or streets. Virtual reality (VR) technology provides a solution to this problem. VR headsets (Figure 1, left) give the wearer the experience of being immersed within a virtual environment (Figure 1, right), within which they can move around. This novel technology therefore provides a previously-unavailable opportunity to test spatial navigation and memory in a way that may be applied in clinical practice.

Figure 1. Left: the wearable VR headset. Right: an example of the immersive virtual environment visualised by the headset wearer.

However, to date VR has never been used for testing spatial navigation or memory in people at risk of AD. To do so, we need to undertake studies not only to establish the set of normal scores on this test but also to obtain any feedback on whether people experience any problems with this test, such as nausea or a sense of disorientation while moving around within the virtual environment. Any such feedback will be used to adjust the test in order to prevent these issues in the future.

Why have I been invited?

You have been invited to participate in this study because you are aged between 20 and 99 years and do not report any problems with memory or thinking.

Do I have to take part?

Participation in this study is entirely voluntary. We will describe this study to you and go through this information sheet, which will be given to you. Should you decide to take part, we will ask you to sign a consent form. You are free to withdraw from the study at any time, with no need to give a reason.

Part 2 – Further information

What does participating involve?

This study requires you to wear a VR headset and you will be asked to explore a virtual environment, for example of a grassy plain surrounded by mountains. Once you have familiarised yourself with the virtual environment, you will be shown a series of objects placed at specific locations within the environment and your memory for the location of these objects will be tested.

At the end of the testing period we will ask you a few questions about your experience during the task, and in particular whether you experienced any discomfort or disorientation during the task.

Expenses and Payments

Participants are responsible for any costs associated with travelling to the testing location. No extra expenses or payments will be incurred.

What are the possible disadvantages and risks of taking part?

There are no major disadvantages or risks associated with participation. However, some users do find VR makes them feel nauseous and/or disorientated. It is important that if you experience any of these problems that you make the researcher aware so they can stop the test.

What are the possible benefits of taking part?

There are no immediate benefits to you. However, if the study is successful in its aims, then it will help us diagnose AD in its very earliest stages which may allow for the earlier initiation of more effective AD treatments.

What if there is a problem?

If you have a concern about any aspect of the study or wish to make a complaint, you can speak to one of the researchers who will do their best to answer your questions or address your complaint. If this is not appropriate then please contact Dr Dennis Chan in writing, or by telephone

Confidentiality – who will have access to the data?

If you join this study your personal information and the data we collect will be stored on a secure network and only the research team will have access to it. It is possible that the anonymised data may be used by researchers working with the research group for other similar ethically approved research protocols, where the same standards of confidentiality will apply.

What will happen to the results of the research study?

The results of the study will be published in scientific journals. Participant data will be anonymised so that it is not possible for you to be identified in published articles.

Who is organising and funding the research?

This study is organised by Dr Dennis Chan. The study results will be analysed by Dr Chan's research group.

Contact details

If you have any queries, please contact Dr Dennis Chan in writing, or by telephone

Dr Dennis Chan MD PhD FRCP

University Lecturer and Honorary Consultant in Clinical Neurosciences

Chief Investigator

14 June 2016
Version 2

UNIVERSITY OF CAMBRIDGE

Cambridge University Hospitals NHS
NHS Foundation Trust

Department of Clinical Neurosciences

Dr Dennis Chan PhD MD FRCP

Herchel Smith Building for Brain and Mind Sciences
Forvie Site, Robinson Way
Cambridge CB2 0SZ
Tel: 0044 (0)1223 760669

PARTICIPANT INFORMATION SHEET

for patients with mild cognitive impairment

Study title: VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function in Alzheimer's Disease (VIRTECH-AD)

Part 1 - Background:

We would like to invite you to take part in a research study that uses virtual reality/augmented reality (VR/AR) technology to test spatial navigation (getting from A to B) and spatial memory (remembering the location of objects) in people with mild cognitive impairment.

You have been asked to participate in this research because your memory clinic specialists have diagnosed you with mild cognitive impairment.

Before you decide to take part we would like you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. You can talk to others about the study if you wish or ask one of the researchers for further information.

What is the purpose of the study?

Spatial navigation and memory are abilities controlled by regions of the brain called the entorhinal cortex and the hippocampus, which are the first brain regions to be affected by AD. As such, when people are in the earliest stages of AD, these abilities will be impaired and if we can identify this impairment will help detect AD in its very earliest stages.

The problem is that it is not practical to test people's spatial navigation and memory in real world settings such as parks or streets. However, current wearable VR/AR technology provides a solution to this problem. VR headsets (see Figure 1, left) give the wearer the experience of being immersed within a virtual environment (Figure 1, right), within which they can move around using hand-held controls. By comparison, AR headsets generate artificial objects and scenes (holographic images) superimposed on the real world (hence the term "augmented reality"). While this does not have the advantage of VR in creating a fully immersive simulated environment, the ability to perceive the real world as well as the simulated images may prove less disorientating.

Together, these novel technologies provide previously unavailable opportunities to test spatial navigation and memory in a way that may be applied in clinical practice.

Figure 1. Left: a wearable VR headset. Right: an example of the immersive virtual environment visualised by the headset wearer.

However, to date, VR/AR has not been used for testing spatial navigation or memory in people with early AD. To do so, we need to undertake studies to

investigate which VR/AR technology can be used to help diagnose early AD and also to obtain feedback on whether people experience any problems with these tests, such as nausea or a sense of disorientation while moving around within the simulated environment using the VR/AR headsets. Any such feedback will be used to adjust the test in order to prevent these issues in the future.

Do I have to take part?

Participation in this study is entirely voluntary. We will describe this study to you and go through this information sheet, which will be given to you. Should you decide to take part, we will ask you to sign a consent form. You are free to withdraw from the study at any time, with no need to give a reason.

What does participating involve?

This study requires you to wear a VR or AR headset. At the beginning of the study you will be invited to use a VR headset (the HTC Vive, currently available commercially). As the study proceeds, the study team will have access to an AR headset (the Microsoft Hololens, not yet available commercially) and you will be asked if you would like also to be tested using this headset.

It takes less than ten minutes to get used to the headsets. You will be asked to explore a simulated scene which a number of everyday objects (such as a vase or a ball) are placed at specific locations. Once you have familiarised yourself with this scene and with the VR or AR equipment, you will be tested on your ability to navigate within the simulated scene and your memory for the location of these everyday objects.

You will be tested once with the VR headset and once with the AR headset, if you agree to return at a later date for testing with the latter. Each test will take around 40 minutes.

At the end of the testing period we will ask you a few questions about your experience during the task, and in particular whether you experienced any discomfort or disorientation during the task.

Who will have access to my medical records?

Your medical records will be reviewed by the study team before you start the study to check your eligibility for the study.

In addition to the study team, your records may be reviewed as part of an auditing process carried out by the R & D department. These audits serve to uphold rules relating to good clinical practice.

Will I be told about the results from this study?

Yes. We will endeavour to inform you of the results of tests. Furthermore our aim is to present the study results at public-patient meetings and you will be invited to attend these meetings. These presentations will describe the overall study results rather than results on individuals and will not refer to you, or any other participant, personally or in any other way that would compromise your anonymity.

In the event that my condition deteriorates and my mental capacity is lost, what will happen to my personal data?

Assuming that you gave your consent at the beginning of the study, the research team would retain the study data collected and continue to use it confidentially in connection with the purposes for which consent is being sought.

Expenses and Payments

We will be able to contribute £20 to the cost of travel to our research site.

What are the possible disadvantages and risks of taking part?

There are no major disadvantages or risks associated with participation. However, some users may find VR headsets makes them feel sick and/or disorientated. It is important that if you experience any of these problems that you make the researcher aware so they can stop the test.

What are the possible benefits of taking part?

There are no immediate benefits to you. However, if the study is successful in its aims, then it will help us diagnose AD in its very earliest stages.

What if there is a problem?

If you have a concern about any aspect of the study or wish to make a complaint, you can speak to one of the researchers who will do their best to answer your questions or address your complaint, or by contacting Dr Dennis Chan in writing, or by telephone **01223 760696.** If you wish to express concerns or complaints to someone outside the research team then you are advised to contact the Patient Advisory and Liaison Service (PALS), Addenbrooke's Hospital, Cambridge.

Confidentiality – who will have access to the data?

If you join this study your personal information and the data we collect will be stored on a secure network and only the research team will have access to it. Since we work with other researchers worldwide in the study of AD, it is possible that these researchers may analyse *anonymised* data arising from this study, abiding by the terms of formal collaborations between the University of Cambridge and other academic institutions. In such instances your personal details will not be shared.

What will happen to the results of the research study?

The results of the study will be published in scientific journals. Participant data will be anonymised so that it is not possible for you to be identified in published articles.

Who is organising and funding the research?

This study is organised by Dr Dennis Chan and funded by the Medical Research Council and by Alzheimer's Research UK. The study results will be analysed by Dr Chan's research group.

Contact details

If you have any queries, please contact Dr Dennis Chan in writing, or by telephone 01223 760696.

Appendix 7: Study Consent Forms

7th October 2016
Version 2

UNIVERSITY OF CAMBRIDGE

Herchel Smith Building for Brain and Mind Sciences
Forvie Site, Robinson Way
Cambridge CB2 0SZ
Tel: 0044 (0)1223 760697
Fax: 0044 (0)1223 336 581

Department of Clinical Neurosciences

Dr Dennis Chan PhD MD FRCP

PARTICIPANT CONSENT FORM
Control Participants

Study title: VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function (VIRTECH).

Principal Investigator: Dr Dennis Chan, University Lecturer and Honorary Consultant in Clinical Neurosciences.

Please tick the boxes:

1. I confirm that I have read and understood the information sheet version 5 dated 7th October 2016 for the above study and have been given a copy to keep.	
2. I have had the opportunity to ask questions about the study and have received answers to my questions.	
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
4. I understand that my personal details and information about me that is gathered for this research study will be held on a secure, confidential computerised database that is only accessible to members of the research group. This is in accordance with the Data Protection Act 1998.	
5. I confirm that I have had sufficient time to consider whether or not I want to be included in the study.	
6. I give consent for additional virtual reality based tests of spatial navigation and memory to be performed, as outlined in the participant information leaflet.	
7. I understand that none of my results will be given to me and that I will not benefit financially from taking part	
8. I understand that the research data collected from the study will only be share within our research group and I will not be identified if the results are published.	
9. I agree to take part in the above study.	

7th October 2016
Version 2

CONSENT FORM (CONFIDENTIAL)

Title of project: **VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function (VIRTECH)**

Principal Investigator: Dr Dennis Chan, University Lecturer and Honorary Consultant in Clinical Neurosciences

Participant Identification Number:

Project ID Number:

Name of subject	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher (to be contacted (in the event of any problems)	Date	Signature

Comments or concerns during the study.

If you have any comments or concerns you may discuss these with the investigator.

If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, University of Cambridge University.

One form for Participant

One form to be kept as part of the study documentation

14 June 2016
Version 2

UNIVERSITY OF CAMBRIDGE

Cambridge University Hospitals NHS
NHS Foundation Trust

Department of Clinical Neurosciences
Dr Dennis Chan PhD MD FRCP

Herchel Smith Building for Brain and Mind Sciences
Forvie Site, Robinson Way
Cambridge CB2 0SZ
Tel: 0044 (0)1223 760697
Fax: 0044 (0)1223 336 581

PARTICIPANT CONSENT FORM

Study title: VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function in Alzheimer's Disease (VIRTECH-AD).

Principal Investigator : Dr Dennis Chan, University Lecturer and Honorary Consultant in Clinical Neurosciences

Please tick the boxes:

1. I confirm that I have read and understood the information sheet version 2 dated 14 June 2016 for the above study and have been given a copy to keep.	
2. I have had the opportunity to ask questions about the study and have received answers to my questions.	
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
4. I understand that my personal details and information about me that is gathered for this research study will be held on a secure, confidential computerised database that is only accessible to members of the research group. This is in accordance with the Data Protection Act 1998.	
5. I confirm that I have had sufficient time to consider whether or not I want to be included in the study.	
6. I give consent for the study tests of spatial navigation and memory to be performed, as outlined in the Participant Information Sheet.	
7. I understand that my medical records and the research data collected from the study may be looked at by the sponsor, by regulatory authorities or by the NHS Trust, as part of auditing policies.	
8. I understand that the research data collected from the study will be analysed on an anonymous basis.	

9. I understand that I will not receive any financial benefit from any intellectual property arising from this study.	
10. I agree to take part in the above study.	

CONSENT FORM (CONFIDENTIAL)

Title of project: **VIrtual Reality Testing of Entorhinal Cortex and Hippocampal function in Alzheimer's Disease (VIRTECH-AD).**

Principal Investigator: Dr Dennis Chan, University Lecturer and Honorary Consultant in Clinical Neurosciences

Patient Identification Number:

Project R&D Number:

Name of participant	Date	Signature
Name of Person taking consent	Date	Signature

One form for Participant

One form to be kept as part of the study documentation

Appendix 8: Joint project contribution

A.L. and E.H. jointly tested all the aMCI patients, including neuropsychological testing and running the VR experiment. Other experimenters such as D.H., E.B., Z.A. and V.R. were also involved.

A.L. and E.H. tested about half of the control participants (VR only), while D.H., E.B., Z.A. and V.R. tested the rest.

Z.A. and E.B. administered the neuropsychological battery to control participants.

D.H. scheduled the MRI scans for aMCI patients and controls.

Lumbar punctures for CSF testing were performed by E.B.

A.L. performed manual segmentation of the entorhinal cortex for aMCI patients and controls, along with D.H. and Z.A.

D.H. extracted the hippocampal subfields using a Freesurfer automated protocol.