The role of the fornix in spatial orientation, navigation and scene perception

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A Thesis submitted for the Degree of Doctor of Philosophy,

School of Psychology, Cardiff University (2017)
Declarations

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I would like to thank my supervisor, Kim Graham, for her support throughout these past 3 years, for her guidance and advice on research projects and my thesis. A special thank you goes to all members of our lab for their valuable feedback and support. I am grateful for the opportunity of being part of such a great team. Particular thanks to Carl, Rebecca, Alison, Matt, Beth and Hannah for their encouragement and precious help with my research and for being good friends despite the geographical distance.

Also, I couldn’t have achieved this important goal without my family that helped me to get through difficult times this year and that always supports me. Thanks to all my Italian friends with whom I share my biggest passion. Exploring and climbing the highest peaks of the Alps with them this summer has given me renewed motivation and determination not only to complete my PhD but also to start a new chapter of my life with a smile.
Thesis Summary

Spatial cognition is a multi-faceted domain that requires the support of a complex brain network. Moving beyond the role of the medial temporal lobe in memory, a growing body of literature suggested that the extended hippocampal network is critically involved in scene processing and in the formation of egocentric and allocentric references. Applying a combination of MRI techniques, the main goal of this thesis was to provide evidence about the specific contribution of the fornix and the hippocampus to different spatial skills. Different groups of healthy individuals were recruited for each study and underwent MRI scans. For each participant, diffusion MRI metrics of the fornix (fractional anisotropy, FA, and mean diffusivity, MD) were extracted. These were then correlated across subjects with the behavioural performance on spatial tasks.

Specifically, Chapters III and IV aimed to investigate the involvement of the fornix in spatial orientation and spatial learning. The results showed significant correlations with the performance on the Spatial Orientation task (SOT) and with the curve learning obtained from the Virtual Reality Maze task (VRMt) suggesting a critical role of this white-matter tract in orientation and navigation.

An fMRI repetition suppression paradigm was adopted in Chapter V to study the role of the HC in scene perception and the contribution of the fornix in viewpoint processing. However, the weak BOLD activity registered in the hippocampus and the lack of significant results suggested that further work is required to clarify the spatial representation formed by this area.
Overall, this thesis provides further evidence of the selective involvement of the fornix in spatial cognition supporting the theory of an extended brain network that underpins spatial processing in humans.
Chapter I: Introduction

1. Thesis overview

Spatial navigation represents one of the most important abilities for animals, ensuring they can find food and resources, safe environments away from predators and members of their own species. As discussed in Murray, Wise, & Graham (2017) mobile foraging was first adopted by the earliest vertebrates via the emergence of map-like representations in the hippocampus. This representation was composed of memory for objects, smells and other animals encountered during a journey, as well as the order in which they were experienced (a so-called cognitive map) (O’Keefe & Nadel, 1978; Ekstrom et al., 2014; Schiller et al., 2015). As animals evolved the function of this structure was refined, changing as animals increased in size, foraged in larger areas and made decisions about food choices at a distance (enabled via improved vision, including better colour discrimination). While spatial navigation is less critical to survival in humans, who also have access to modern tools such as maps, compasses and GPS (Global Positioning System), our spatial abilities reflect those that emerged in these early animal ancestors. Our hippocampi, therefore, remain critical for successful spatial navigation, such as encoding our location with an environment, and memory for the objects, people, food and building encountered during a trip, as well as the order in which these items are encountered (see Section 2.1.4 below).

In this thesis, I will focus on further understanding the contribution the hippocampus makes to our ability to orient and navigate within a spatial environment. Going beyond a focus on just the hippocampus, I will apply advanced brain imaging, in particular diffusion tensor imaging, to ask how a key white matter tract of the hippocampus –
the fornix – contributes to inter-individual variation in different forms of spatial processing, all of which are thought to be important for successful real-world navigation. My interest beyond spatial navigation per se stems from emerging work showing that the role of hippocampus in humans extends to complex scene discrimination (see Murray, Wise and Graham, 2017, as well as Section 2.1.4 below).

Prior to outlining my approach to these Chapters in detail (see Section 3), I provide some background about the role of the medial temporal lobe (MTL) in scene and object perception and memory.

2. The brain network underpinning spatial skills

2.1. A critical role for the medial temporal lobe in cognition

2.1.1. Medial temporal lobe and memory

In the past century, individuals suffering from medically intractable epilepsy commonly underwent surgical procedures, such as lobotomy, which involved resection of areas causing their epileptic seizures. Scoville and Milner (1957) noticed that patients with bilateral resection of the medial temporal lobe (MTL), an area of the brain that includes the hippocampus (HC), experienced severe memory loss, failing to remember events they had recently experienced as well as some loss of memories from the past. This was despite evidence of preserved intellectual abilities for other cognitive aspects, such as perception, reasoning and abstract thinking. Based on examination of a few cases, Scoville and Milner came to the conclusion that the hippocampal region represented the neural substrate necessary for memory and that

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the degree of memory impairment seen after surgery was dependent upon the extent of damage to this structure. Subsequently, Tulving and Donaldson (1972) proposed that human memory could be categorized into two memory systems: episodic and semantic memory. Memories that carry temporal-spatial and personal experience information about the content of events are part of the episodic memory system, whereas knowledge of facts about the world, objects and people was considered semantic. In the patients reported by Scoville and Milner, we see evidence of a loss of episodic memory with relative preservation of semantic knowledge.

Subsequent to these early studies, and informed by work in animals which failed to replicate the memory impairments seen in humans with hippocampal lesions (Correll & Scoville, 1965; Orbach, Milner, & Rasmussen, 1960), researchers discovered that other areas within the MTL also contributed to memory. Ablation of the perirhinal cortex and entorhinal cortex in macaques was found to affect memory (Mishkin, & Murray, 1986; Meunier, Bachevalier, Mishkin, & Murray, 1993; Murray, Suzuki, Zola-Morgan, Squire, & Amarap, 1993). Meunier et al. (1993), for example, tested lesioned monkeys at a delayed nonmatching-to-sample task (DNMS) that required the animals to compare two objects and choose the novel object in order to get a reward. For this task, trial-unique objects were used and the delay period between object presentations was 10 seconds. Once the object recognition learning phase was completed, the animals were divided in groups based on the specific surgical ablation: entorhinal cortex; perirhinal cortex or a combination of both (rhinal lesion). The postoperative performance showed that selective damage to the rhinal cortex, in particular to the perirhinal cortex, produced a severe deficit in visual recognition memory. After these studies, it became accepted that episodic memory was dependent upon a range of brain regions within the medial temporal lobe, including the HC,
perirhinal cortex and entorhinal cortex. Individuals with damage to these structures would have poor episodic memory, but preservation of other forms of memory not dependent upon them (e.g., semantic memory) (Squire, 1986; Squire, Stark, & Clark, 2004). For example, Squire and Zola-Morgan (1991), argued that, although the HC plays a central role in the memory system, other MTL structures (Figure 1), such as the hippocampal formation (which includes HC subcomponents, such as CA1, CA3, dentate gyrus, subiculum and entorhinal cortex), the perirhinal cortex (PrC) and parahippocampal cortex, also support to the mnemonic process.

![MTL Structures](image)

Figure 1. The image represents the anatomy MTL including the hippocampal formation, amygdala and parahippocampal cortex (figure from Raslau et al. (2015)).

2.1.2. MTL and perception

The vision of a unitary MTL system exclusively involved in mnemonic processes (Squire & Zola-Morgan, 1991) was challenged by subsequent findings, initially from animal studies, but subsequently also work in humans (Graham, Barense, & Lee, 2010;
Specifically, studies on rats showed that lesions to the rhinal cortex (including PrC) caused severe impairments in recognition memory judgment of objects (Mumby & Pinel, 1994; Wan, Aggleton, & Brown, 1999). More recently, Winters et al. (2004) conducted a study to compare the performance of groups of rats with either focal hippocampal or peri/postrhinal cortex lesion on spatial memory (using a radial maze) and object recognition tasks. The results showed that animals with PrC damage were significantly impaired in the choice phase of the recognition task in comparison to the hippocampal lesion and control groups, whereas the spatial accuracy at the radial maze task was significantly reduced for rats with HC damage relative to the other groups. Similarly, monkeys with hippocampal and PrC focal lesions showed selective deficits in the performance of spatial memory and object recognition tasks, respectively (Meunier et al., 1993; Suzuki et al., 1993; Murray & Mishkin, 1998). Moreover, the perceptual role of the PrC for the discrimination of complex objects was confirmed by the study of Buckley, Booth, Rolls, & Gaffan (2001) where an oddity paradigm (a visual odd-one-out task) was used to minimize the mnemonic demand. Overall, these findings suggest a clear functional distinction between these MTL areas, although anatomically highly interconnected, with the PrC supporting object recognition and the HC playing a critical part in forming spatial memories (Brown & Aggleton, 2001; Aggleton & Brown, 2005).

Based on these results from the animal literature, researchers became interested in whether human participants with damage to the MTL would show similar perceptual difficulties. To answer this question, amnesic patients with focal lesions to the HC or more extensive lesions including the HC and MTL areas were recruited to analyse the related deficits in higher-order perception. Evidence from a first study, (Lee, Bussey
et al., 2005) suggested a critical role of the MTL in the accurate discrimination of scenes, faces and objects, which raised the necessity to investigate the specific involvement of MTL structures in perceptual processing further. For this reason, subsequently, Lee, Buckley et al. (2005) used an oddity task in order to test the perceptual skills of patients with focal lesions to the HC (HC group) and with more extensive MTL damage, including PrC (MTL group). Specifically, this study was made of two different experiments, each one consisted of a series of oddity tasks adapted from a previous animal study (Buckley et al., 2001) and patients were instructed to detect the odd-one-out. In the first experiment faces, novel objects, size, colours and shapes were used as stimuli, whereas faces and scenes presented from the same or different viewpoints were used in the second experiment. The results showed that the MTL group was more impaired than the HC patients in the discrimination of faces (in both experiments) and novel objects (first experiment), and a deficit in both groups emerged for the condition where scenes were presented from different views (second experiment) indicating that MTL areas are crucial for the formation of coherent spatial representation. A further study by Barense et al. (2005) adopted a concurrent discrimination task to investigate how the degree of features overlap of different object categories (barcode bugs, beasts and blobs) affects the performance in these groups of patients. The authors found that amnesic patients with extensive MTL damage, including both the HC and PrC, were impaired in successfully discriminating highly similar items, but not items where there was no feature overlap, while individuals with hippocampal lesions performed normally on all conditions, regardless of the level of ambiguity of objects. On the contrary, the HC appears selectively involved in the processing of scene (Lee, Bussey, et al., 2005) as well as in virtual scene categorization and perceptual learning (Graham et al., 2006). Specifically,
Graham et al. (2006) tested the performance of patients with selective hippocampal damage at a categorization and perceptual learning task of faces and virtual scenes with three different levels of feature ambiguity. In this case, the level of ambiguity appeared not to have a negative impact on subsequent perceptual learning for both stimulus categories. However, in comparison to controls, patients were impaired on scene categorization and an altered pattern of reaction times also emerged for the discrimination of previously seen scenes (explained as inverse efficiency). This provided evidence for a selective role of the HC in the perceptual learning of virtual scenes and in the representation of conjunction of features that form this stimulus category.

Neuroimaging studies were subsequently conducted to verify whether similar conclusions about the MTL functional organization in memory and perception could be drawn for healthy human participants. In particular, Lee, Scahill, & Graham (2008) conducted a functional magnetic resonance (fMRI) experiment to investigate whether there was selective activation in the HC and PrC during the visual discrimination of similar scenes or faces without placing explicit demand on long-term memory. They adopted two oddity judgement tasks where faces and scenes were used separately. The paradigm consisted of the simultaneous presentation of 4 images, 3 of them depicting different views of the same stimulus and the participant was required to indicate the odd-one-out. To investigate functional activation of MTL areas during perceptual encoding and mnemonic processing separately, the same stimuli were used across all the experimental phases: during the first phase the stimuli were encoded as novel then they became familiar when repeated in phase 2 and 3. The results indicated a category-specialisation within the MTL with the HC, especially the posterior portion and the parahippocampal cortex, active during perception discrimination of scenes and the
PrC particularly engaged during discrimination of faces, as suggested by previous findings on patients (Lee, Buckley et al., 2005). Further evidence of the category-specificity of these brain areas in perceptual tasks came from a study by Taylor, Henson, & Graham (2007) that showed significant impairment of patients with focal hippocampal lesion in the recognition of pairs of scenes regardless of the viewpoint while having the performance for faces spared. Overall, these findings support the view that the MTL regions are crucial to bind multiple features together and ultimately form complex representations of scene and objects. This challenges previous theory that considered the MTL as exclusively involved in mnemonic functions (Squire & Zola-Morgan, 1991) and suggests, on the contrary, the role of MTL structures in perceptual processing of scenes and faces/objects supported by HC and PrC, respectively (Lee, Buckley et al., 2005; Lee, Scahill, & Graham, 2008). To test this hypothesis further, Barense et al. (2010) conducted an fMRI study to examine functional activation of these regions while the healthy participants identified the unique stimulus among three images belonging to the same category: scene, object or face. The fact that two of these items represented the same stimulus from different viewpoints, implied that the task could not be solved based on the identification of a single feature. The results were in line with the idea that the HC, especially the posterior portion, is essential for the correct judgment of scenes, with an increase in activation associated for different view compared to same view conditions. Activation of the PrC was associated with the discrimination of faces and objects, independent from the subsequent mnemonic retrieval of the same stimuli, and again influenced by viewpoint (different viewpoint resulting in greater activation than same viewpoint conditions).
From this converging evidence, new representational accounts of the MTL were proposed (Graham et al., 2010; Saksida & Bussey, 2010). For example, the representational-hierarchical account (Saksida & Bussey, 2010) stated that the PrC was critical for the formation of representations of objects that allow the individual to distinguish between highly similar stimuli based on the conjunctions of features. By contrast, spatial representations, crucial for the resolution of visual ambiguity of scenes, were proposed to be stored in the HC. Consistent with this view, the emergent memory account (EMA), proposed by Graham et al. (2010), claims that, beyond long-term memory function, the MTL regions actively contribute to the representation of conjunction of features that are used in both perceptual and mnemonic processing. Specifically, according to EMA, the HC and PrC selectively support scene and objects/faces features, respectively (Graham et al., 2006; Barense, Gaffan, & Graham, 2007). Furthermore, these representations are employed in both the perceptual and mnemonic domain, providing an explanation for patients with damage to the MTL who show category-sensitive impairments across perception, learning and episodic memory (Barense et al., 2005; Lee, Buckley et al., 2005; Lee, Bussey et al., 2005; Lee, Scahill, & Graham, 2008).

In conclusion, the literature provided crucial evidence about the specific role of MTL regions in perceptual processing, and the idea that these category representations within regions in the MTL might support episodic memory. As outlined at the beginning of this Introduction, the human HC has conserved functions present in our ancestors and its involvement in representing visual scenes, and the use of these to support successful spatial navigation fits well with this view. It is clear, however, that further research is required to clarify the exact nature of the scene representations stored within the HC. A key focus of this PhD thesis involved experiments aiming to
better understand how the HC network supports scene perception, spatial orientation and spatial navigation. The following sections, therefore, provide some background to work undertaken specifically in these spatial domains.

2.1.3. Allocentric and egocentric spatial representation

During navigation, a multitude of environmental information is processed in order to form cognitive maps that support the effective exploration of the surrounding (O’Keefe & Nadel, 1978; Murray et al., 2017). While navigating, the representation of the space requires continuous updating of the self-location as well as accurate processing of the relationships between external items. These processes are supported by two distinct coding systems: egocentric and allocentric (Galati et al., 2000). The egocentric system is dedicated to the processing of the relationships between the location of the animal or person and the objects in the space. It provides spatial representations that relate to viewer perspective. On the other hand, the location of the objects relative to one another is coded by the allocentric system which represents the space irrespective of the observers viewpoint (Wolbers & Wiener, 2014) (Figure 2).
In an fMRI study on healthy participants, Galati et al. (2000) used visual tasks to investigate the brain areas activation during the computation of allocentric and egocentric references. Specifically, the task consisted of a series of vertical lines intersected with horizontal planes and the participants were instructed to indicate whether the vertical bar was located on the left or right relatively to either their own midsagittal plane (egocentric condition) or to the horizontal line (allocentric condition). As a control condition, they were also required to determine the colour of the bar. The functional activation during each of the main experimental conditions was compared to the control condition and the results showed that the fronto-parietal system was engaged during the egocentric computation, including precuneus, superior parietal lobule, intraparietal sulcus, inferior temporal lobule and parieto-temporal junction, were more active during the egocentric condition. On the other hand, less extended activation of the parietal and frontal premotor cortices were found during the object-based condition (allocentric condition) and, when the activation for the allocentric condition was directly compared with the activation during the performance of the egocentric condition, hippocampal and parahippocampal activity was found. The role of HC in allocentric computation was further investigated in
patients with focal damage to this MTL region (Finke et al., 2011). In this study, the performance on memory-guided saccade tasks was examined using egocentric, allocentric and egocentric/allocentric experimental conditions. Patients were found to have impaired saccade accuracy for the allocentric condition only, when they were asked to remember the spatial relationship between the target object and the allocentric reference indicating the position of the target regardless from the misplacement of the cue. This result provided evidence of hippocampal contribution to the formation of accurate allocentric memories. However, a recent fMRI study on healthy subjects (Gomez, Cerles, Rousset, Rémy, & Baciu, 2014) reported right HC activation during the encoding phase indicating that this region is involved in the integration of self-location relatively to the environment which refers to the egocentric rather than the allocentric spatial representation. For this reason, further research is needed to clarify whether the HC in humans is exclusively dedicated to allocentric computation of spatial relations.

In summary, neuroimaging evidence suggests that the egocentric reference frame is supported by motor and parietal cortices, which carry out the integration of motor and sensory information and compute goal-directed movement, such as orienting towards the target object and navigating in the space to reach it (Byrne et al., 2007). On the other hand, the HC (see Section 2.1.4 below for more details) appears critical for representing allocentric information about environmental objects and their spatial relationships (Ekstrom et al., 2003) although further investigation is necessary to specify its contribution to the formation of different spatial reference frames. The allocentric reference frame is particularly important for successful spatial navigation because it allows the orientation of individuals in the space based on the external landmarks (Klatzky, 1998). Despite being considered as two distinct coding systems
that process spatial relations independently from each other, it is likely egocentric and allocentric references are both necessary to form accurate map-like representations of the space and that the integration of these two spatial reference frames is crucial for the implementation of navigation strategies (Ekstrom et al., 2014).

2.1.4. The role of the HC in spatial orientation and navigation

The first evidence of engagement of the HC in spatial cognition was provided by animal research which enabled the study of the behavioural consequences of selective brain lesions using invasive techniques. For example, O’Keefe and Dostrovsky (1971) evidenced “place cells” in the HC by implanting electrodes in the hippocampal substructures, CA1 and dentate gyrus and recording neural activity in response to the animal’s specific position within the environment. This line of research was followed by Morris et al. (Morris, 1981; Morris et al., 1982; Morris, 1984) who devised a new behavioural procedure to study the involvement of the HC in spatial navigation in rats. The water-maze task consisted of a circular pool of water where a platform was placed in a specific location and allocentric cues were provided to facilitate the orientation. Either the transparency of the water or the colour of the platform itself was altered to render the platform invisible. The rats were required to swim to the platform from different quadrants of the pool with time and distance to reach the hidden platform recorded. The group with selective hippocampal lesions failed to show an improvement in way-finding over a series of trials, even when the position remained the same (Morris et al., 1982). Spatial-selective cells have also been found in macaque monkeys HC but, unlike the place cells of rodents that respond to specific locations, these neurons showed a view-responsive activation (Rolls et al., 1998). Specifically, in this study Rolls et al., primates were left free to actively explore or make eye
movements within a room while the neural activity of neurons showing spatial view-selective responses was recorded. The analysis of the neural firing revealed the presence of hippocampal neuronal populations supporting the processing of spatial location. The hippocampal cells in primates, therefore, would contribute to the formation of spatial memories and the effective navigation of the environment (Rolls, 1999). Although animal species can differ in the hippocampal neural response, with rats showing location-selective activity (O’Keefe & Dostrovsky, 1971) and primates representing places regardless of specific location (Rolls, 1999), the HC appears to be crucial for learning and representation of spatial relations that support navigation across species (Murray, Wise & Graham, 2017).

Based on the evidence provided by animal literature, subsequent research on patients aimed to clarify the role of HC in navigation. For example, Astur et al. (2002) used a virtual version of the Morris water-maze task used with rats (Morris et al., 1982) to test spatial learning skills in a group of amnesic patients, who underwent unilateral hippocampal removal. In this task, participants could explore a 3D room in a first-person perspective and were instructed to find the hidden platform using external landmarks to orient themselves. After the training phase, the platform was removed (probe trials) and the time spent in the quadrant where the target used to be located was examined. The performance on probe trials was considered an indicator of spatial learning and a measure of allocentric representation memory. In line with the animal findings (Morris et al., 1982), these patients showed severe deficits in learning the most effective trajectory taking more time to find the platform during the training phase and spending significantly less time in the correct quadrant on probe trials.
Moreover, in order to test whether human hippocampal cells code for specific location, as shown for rats (O’Keefe & Dostrovsky, 1971), or for the subject’s view, as seen in monkey (Rolls et al., 1998), Ekstrom et al. (2003) conducted a single-unit recording study on epileptic patients to investigate the neuronal response to different aspects of the environment of MTL cells (HC, parahippocampal region, amygdala). Specifically, participants performed a virtual navigation game playing as taxi drivers who were required to deliver passengers to target shops. During the performance of this task, the neural responsiveness to participant’s location, view and task goal was recorded. Goal-responsive cells were found in the HC whereas the neurons in the parahippocampal areas responded to the individual’s view. Based on these results, Ekstrom et al. concluded that the HC and parahippocampal regions contribute, respectively, to the formation of goal-dependent and view-dependent representations of the environment and that both are necessary for spatial navigation and goal-directed actions.

Other neuroimaging methods, such as PET (positron emission tomography) and MRI, have been adopted to investigate the specific role HC in navigation in healthy populations as well. Maguire, Frackowiak, & Frith (1996), for example, conducted a PET study where the cerebral blood flow was measured while the participants passively viewed a film and were required to memorize the navigation through two different routes of a real-world town and the functional brain activity was compared to the conditions where either no large-scale navigation or explicit mnemonic demand were implicated. The cerebral blood findings in the hippocampal and parahippocampal regions during the topographical learning phase suggested a crucial role of the HC involved in the encoding of large-scale environments. The role of this MTL structure in navigation was subsequently confirmed by an investigation of the influence of expertise on hippocampal volume (Maguire et al., 2000). Specifically, research on
professional drivers showed that grey matter volume is modulated by the expertise in driving taxis, with the volume of right posterior HC increasing and the anterior HC decreasing with years of taxi driving (Maguire, Woollett, & Spiers, 2006).

The functional activity of the HC was investigated in a fMRI study by Zhang & Ekstrom (2013) where participants underwent functional imaging while moving within a virtual-reality environment. Subjects were required to direct their movement towards hidden target locations, previously learnt from aerial view, using external landmarks or store locations to orient themselves. The results revealed a complex brain network activated during the main experimental conditions. Specifically, precuneus, parahippocampal, retrosplenial and superior parietal cortices showed functional activation during rigid allocentric retrieval that required the use of external landmarks to navigate. On the other hand, parahippocampal cortex and HC were found to be active during the store-reference condition that involved a more flexible utilization of the allocentric reference, which represented spatial relationships among stores in the virtual environment. Findings from research on animals (O’Keefe & Dostrovsky, 1971; Morris, 1982; Rolls, 1999) and humans (Maguire et al., 1996; Maguire et al., 2000; Maguire et al., 2006; Ekstrom et al., 2003; Zhang & Ekstrom, 2013) suggest an important function of the HC in the processing of visuospatial information, as part of the parieto-medial temporal lobe pathway, which provides connection from the inferior parietal lobule to the MTL through posterior cingulate and retrosplenial cortices (Kravitz, Saleem, Baker, & Mishkin, 2011).

However, the contribution of this MTL area to the processing of different coordinate references requires further investigation. This issue will be addressed in this thesis through the examination of the association between hippocampal volume and behavioural performance at a Spatial Orientation (Chapter III) and Virtual-reality
maze tasks (Chapter IV) which require egocentric and allocentric coding, respectively, as well as its functional activation to changes in scene perspective (Chapter V).

2.2. The extended network underpinning spatial navigation and orientation

As the literature suggests, the HC plays a central role in the complex neural network that underpins the processing of the spatial information (Burgess et al., 2001; Banta Lavenex et al., 2014) and forms coherent and generalised scene representation, independent from the viewer perspective (Lee, Yeung, & Barense, 2012; Fidalgo & Martin, 2016) suggesting that the HC is critical for allocentric spatial processing (Burgess, Maguire, & O’Keefe, 2002). Nevertheless other areas, such as the retrosplenial cortex (RSC), parahippocampal cortex and prefrontal cortex have been found to contribute to the computation of spatial references (egocentric and allocentric) and processing of the space (Spiers & Maguire, 2006; Kravitz et al., 2011; Zhang & Ekstrom, 2013; Sulpizio et al., 2014). In particular, the parahippocampal cortex contributes to the formation of egocentric representation and carries out viewpoint-dependent processing of scenes (Epstein et al., 2003), while previous literature suggests that the HC integrates multiple viewpoints forming a global representation of the scene (Lee et al., 2006). However, the exact role of the HC in the processing of different perspectives is still unclear and this issue will be specifically addressed in Chapter V through the investigation of fMRI adaptation effect in response to different magnitude of viewpoint change.

A growing number of studies have also focused on the contribution of white matter connections (see Section below 2.2.1) to this cognitive domain examining the exact
support provided to the representation of the space. Specifically, the fornix microstructure was found to affect scene discrimination in humans (Postans et al., 2014; Hodgetts et al., 2015) and in navigation in animals (Aggleton et al., 1995; Buckley et al., 2004). However, the role of the fornix in the formation of spatial references in still unclear, especially in healthy humans. For this reason, we used diffusion imaging to investigate whether fornix microstructure is associated with spatial orientation accuracy (Chapter III), navigation (Chapter IV) and fMRI adaptation effect for viewpoint processing (Chapter V).

2.2.1. The fornix and the inferior longitudinal fasciculus (ILF)

The HC plays a key role in the network that supports the processing of scene representations (Hodgetts et al., 2016). The HC is highly interconnected with other MTL and diencephalic regions that are also involved in this cognitive process. The main hippocampal connection runs via the fornix, which constitutes the major information output to the thalamic nuclei. The efferent fibres of the fornix bundle originate from the subiculum and entorinal cortex. The fibers that arise from the bilateral hippocampal surface form the fimbria, which extends medially and projects to the contralateral HC. Medially, the fornix crus are created by the convergence of the fimbria and the fibers join beneath the splenium to form the body of the tract (see Figure 3 below) that connects the HC to cortical and subcortical regions (Saunders & Aggleton, 2007). Specifically, this tract projects to medial diencephalic regions, such as the anterior thalamic nuclei (ATN) and hypothalamic nuclei and the mamillary bodies (MB) (Aggleton, 2008) forming the extended HC-fornix-ATN-MB network that is thought to be important for episodic memory (Aggleton & Brown, 1999). As proposed in the model by Aggleton & Brown (1999), the hippocampal-diencephalic
connections, such as the fornix, support learning and mnemonic processing (especially recollection) carried out by the MTL structures (HC) and not in general recognition memory (as also showed by studies described below, e.g. Rudebeck et al., 2009).

As the fornix is a major output bundle from the HC, focal lesions of this white-matter tract in animals can help investigate the contribution of the fornix to navigation and spatial learning. For example, selective transection of the fornix in rats has been shown to cause significant impairments in spatial discrimination and navigation tasks (Aggleton et al., 1995). In particular, Warburton and Aggleton (1998) investigated the impact of fornix and thalamic lesions on object recognition and navigation skills. Lesioned rats were tested on three different behavioural tests: object recognition task where they had to discriminate between familiar and novel objects; T-maze, where rats had to perform forced choice to reach the reward and Morris water maze (MWM)
task that required good navigation ability to swim towards the platform (see Chapter III, Section 1 for full description of this experimental paradigm). Performance on the object recognition task was similar between the lesioned groups whereas some differences emerged for the other tests. In comparison to controls, both lesioned groups appeared impaired on the spatial tasks showing slower escape latencies and longer distance swam to reach the platform during the acquisition phase of the MWM and a deficit on the forced alternation in the T-maze. However, unlike rats with thalamic lesions, the fornix lesioned group performed normally on probe trials of MWM indicating that some aspects of spatial learning were partially preserved while thalamic (especially anterior) lesion produced severe impairment in allocentric memory.

Subsequent studies on monkeys addressed this issue providing further evidence of the engagement of the fornix in spatial learning (Buckley et al., 2004). In this study, Buckley et al. used a concurrent discrimination task to verify the involvement of the fornix in the formation of spatial memories. Specifically, tadpoles were used as stimuli and were defined by a series of spatial features, such as the position of the direction and length of the tail, and monkeys with bilateral fornix transection had to discriminate between these items. The results showed that the lesion produced severe impairment in the performance of this task, indicating that the fornix is critical for the representation of conjunction of spatial features that allow the successful discrimination of highly similar items. Wilson, Baxter, Easton and Gaffan (2008) conducted a study on lesioned monkeys using an object-in-place discrimination task that required the animals to choose the correct foreground object in each scene presented. Successful performance implied intact spatial learning and memory. Animals were subdivided in groups based on three different lesions: frontal-lobe
ablation, frontal-inferotemporal disconnection and frontal-inferotemporal disconnection with fornix transection. The results showed that additional lesion of the fornix produced increased impairment in the performance of the task, confirming the crucial involvement of this white-matter connection in scene learning in monkeys (Buckley, Wilson, & Gaffan, 2008). Overall, results from animal studies provide clear evidence of the engagement of the fornix in spatial learning and navigation (Warburton and Aggleton, 1998; Buckley, Wilson, & Gaffan, 2008; Wilson et al., 2008) as part of an extend network that, with the HC, underpin different aspects of spatial cognition (Gaffan, 1994; Aggleton & Brown, 1999).

Through the analysis of the association between white-matter microstructural variability and the performance at behavioural tests, recent studies have investigated the function of the fornix in humans as well. As major hippocampal projection, Rudebeck et al. (2009) used a diffusion-weighted imaging technique (see Chapter II, Section 2 for a detailed description) to test the hypothesis according to which this tract would be involved in mnemonic functions supported by the HC. Specifically, after an encoding phase of the stimuli, healthy participants were instructed to perform scene and object recognition tasks detecting (using a confidence scale) familiar items, previously seen in the encoding phase, among a series of similar stimuli. The receiver operating characteristics (ROC) curves were analysed and correlated with the fornix MR diffusion measure (fractional anisotropy, FA, see Chapter II section 2.1). Whole fornix FA was found to be significantly associated (positive correlation) with scene but not object discrimination supporting the theory that this hippocampal connection plays specific role in recollection (Aggleton & Brown, 1999). To further investigate the selective role of the fornix in the processing of spatial stimuli, two studies have been recently conducted in our laboratory using diffusion tensor imaging (DTI) and
behavioural paradigms. In particular, Postans, Hodgetts, Mundy, Jones Lawrence and Graham, 2014 used visual discrimination tasks where pairs of stimuli (scenes and faces) were presented sequentially and participants were asked to make same/different judgment. The accuracy of the discrimination was then correlated with the MR diffusion values (FA and mean diffusivity, MD) of the fornix and inferior longitudinal fasciculus (ILF). Inter-individual variation in fornix microstructure was found to be selectively associated with the accurate perceptual judgment of highly similar scenes (positive correlation with FA and negative correlation with MD), whereas accurate discrimination of faces was related to higher ILF FA values. This striking double-dissociation emerged also in a subsequent study (Hodgetts et al., 2015) that showed that the role of the fornix is not confined to the mnemonic domain as suggested by Aggleton & Brown (1999) but it is critical for perceptual processing as well. Specifically, Hodgetts, Postans, Shine, Jones, Lawrence and Graham (2015) adopted an oddity task paradigm and investigated the association between the same tracts and behavioural accuracy as well as the functional activation in HC and PrC during the perception of different stimulus categories (scene, face and size). Specifically, three items were presented simultaneously on the screen, two of them represented the same stimulus from different viewpoints whereas the third one depicted a completely different stimulus. The participants were instructed to indicate the odd-one-out and the number of correct responses was used as indicator of discrimination accuracy. As previously found by Postans et al. (2014), the results (see Figure 4 below) showed that fornix MD significantly correlated with the accuracy of the discrimination of scenes in comparison to faces. By contrast, inter-individual variation of the ILF MD was associated (negative correlation) with the accuracy in discriminate between faces, but not scenes. Overall, these findings support the theory that the fornix and ILF are part
of the substrates that underpin the processing of scenes and faces, respectively. On one hand, the ILF constitutes a pathway between the occipital visual regions and the anterior part of the temporal lobe, including the PrC (Gschwind et al., 2012). The fibers originate from the occipital pole, cuneus and lateral occipital cortex and project to the fusiform and inferior temporal gyri (Catani et al., 2002). Potentially connecting the fusiform face area (FFA), the occipital face area and inferior temporal lobe, the ILF is believed to be involved in higher-order visual perception and part of the extended face and object network. In fact, ILF microstructure was found to be associated with accurate discrimination of faces (Postans et al., 2014; Hodgetts et al., 2015) and, for this reason, the ILF was chosen as a control tract to verify the selective engagement of the fornix in spatial processing in Chapters III, IV and V. On the other hand, evidence from animals and human research has shown the contribution of the fornix not only in memory (Aggleton & Brown, 1999) and recollection (Rudebeck et al., 2009) but also in perceptual processing of spatial information (Postans et al., 2014; Hodgetts et al., 2015). However, further studies on humans are needed to extend previous model about the role of hippocampal network in memory (Aggleton & Brown, 1999) and perception and clarify the specific role of this hippocampal connection in egocentric and allocentric encoding that support orientation, navigation and viewpoint processing. These issues will be addressed in Chapters III, IV, V, respectively.
Figure 4. The image shows the double-dissociation observed by Hodgetts et al. (2015) that indicates on one hand the association between fornix MD and the scene discrimination accuracy, and on the other hand the significant relationship between the ILF MD and the accuracy in the oddity-judgment of faces.

3. Aims of this thesis

The aim of this thesis is to provide valuable new insights about how the human brain supports aspects of scene processing and spatial navigation, via consideration of the extended hippocampal network. As suggested by the literature outlined in the early sections of this chapter, the HC extended network provides critical support to different aspects of spatial processing but some questions remain about the circumstances under which these emerge. The studies conducted for this thesis focused on clarifying the role that the fornix and HC play in performing spatial transformations, forming allocentric representations able to guide navigation and processing of scene
viewpoints. A combination of MRI techniques was used including (a) diffusion tensor imaging to enable assessment of fornix microstructure as predictors of accuracy in spatial orientation (Chapter III) and spatial learning and navigation (Chapter IV) and (b) fMRI and diffusion tensor imaging to investigate scene viewpoint processing in the HC and fornix (Chapter V).

In the first two chapters (Chapter III and Chapter IV) the contribution of the fornix to the formation of egocentric and allocentric representations of environmental space was investigated. In Chapter III, this was tested by studying how fornix microstructure was associated with accurate performance on the Spatial Orientation task (Hegarty et al., 2008) and in Chapter IV, via use of a virtual-reality maze task akin to the MWM (Kolarik et al., 2016). The functional and structural specialisation of the extended hippocampal network in scene processing was further examined in the third experiment (Chapter V) where an fMRI adaptation paradigm was adopted to investigate sensitivity to changes in scene viewpoint. The overarching goal of these studies was to provide crucial evidence of the involvement of the fornix in the formation of a coherent representation of the environment. The specific aims and experimental procedures will be described in the following Chapters. All data for experiments described in Chapter IV and V were acquired during my Ph. D. whereas data for Chapter III were available on lab database.
Chapter II: Neuroimaging techniques: fMRI and DTI method

1. Introduction

As outlined in the Introduction, the key aim of this thesis is to examine whether inter-individual differences in fornix microstructure and hippocampal volume predict the performance at different spatial tasks as well as investigating the functional response of the HC to changes in scene viewpoint. In order to quantify white matter properties in vivo, I applied a magnetic resonance imaging technique known as diffusion magnetic resonance imaging (dMRI). As this method will be used across all experimental chapters, I will describe this technique in detail. Initially, I will describe briefly how dMRI allows the investigation of white matter pathways, before outlining two prominent dMRI analysis approaches that will be employed throughout, diffusion Tensor Imaging (DTI) and Tract-based Spatial Statistics (TBSS). fMRI and volumetric analysis were conducted on the HC to verify functional and structural associations with the behavioural measures of spatial orientation (Chapter III), spatial learning (Chapter IV) and scene-viewpoint processing (Chapter V).

2. dMRI

Diffusion MRI is a neuroimaging technique that exploits the diffusion properties of water molecules in the human brain to get an insight into white matter pathways in vivo. Using this technique, it is possible to virtually “dissect” – and indeed quantify -
the major white matter fasciculi in the human brain, including those major tracts converging on MTL structures, such as the hippocampus.

Specifically, dMRI is used to carry out in-vivo investigation of white-matter that carries electrical impulses throughout the brain and provides indirect measure of fibers microstructure through the analysis of water diffusion (Assaf, Johansen-Berg, & Thiebaut de Schotten, 2017). The local movement of the molecules is constrained within and along the axons and their diffusion (thermally driven random motion) is influenced by physical properties of tissue. The application of the diffusion tensor model to the dMRI data allows the derivation information about microstructure. This method extracts measures of water molecule diffusion and orientation (e.g. principle eigenvector) within each voxel, such as fractional anisotropy (FA) and mean diffusivity (MD), and provides a voxel-wise maps of white-matter organization (Jones, 2010). A key limitation of this model is that the dMRI measures are not specifically associated with any microstructural features of the tissue and the actual motion of molecules is oversimplified, therefore FA and MD values can reflect changes in fibers microstructure, such as cell size and density (Assaf et al., 2017) (see Section 2.1. below for further details).

However, in the past few decades, this technique has offered the opportunity to conduct studies on humans aiming at investigating white-matter contribution to cognitive abilities. For example, Metzler-Baddeley et al. (2011) used dMRI and found that fornix FA was significantly associated with performance on different episodic memory tasks, such as the associative visual object-location (PAL) that tests accurate recollection and the Free and Cued Selective Reminding Test (FCSRT) that assesses verbal learning, immediate and delayed memory, supporting the theory that this tract is critically involved in memory skills (Rudebeck et al., 2009). Moreover, as described
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in Chapter I (Section 2.2.1), recent evidence shows that fornix and ILF microstructure are associated with scene and faces discrimination respectively (Postans et al., 2014; Hodgetts et al., 2015) providing striking evidence of the category-selective contribution of these tracts to perceptual processing. These findings show that dMRI is an effective method to investigate the association between the microstructure of white-matter pathways and cognition.

2.1. dMRI metrics

The dMRI measures that are commonly extracted from the tract of interest are FA and MD, which convey information about tissue density and fiber directionality (Pierpaoli & Basser, 1996). These indices reflect the translation of water molecules through the fibers, which are affected by several factors, such as the anatomical configuration and axonal properties (e.g. myelination, membrane integrity, axon diameter, and density) (Jones, Knösche, & Turner, 2013). For example, the layer of myelin not only ensures the action potential to rapidly flow but it also constitute a barrier that prevents the water molecule from moving perpendicularly to the axon (Beaulieu, 2002).

In this thesis, individual measures of FA and MD were calculated by averaging the values across all the voxels of the tract. Specifically, the FA represents a scalar index of the diffusion of water molecules within a voxel and indicates anisotropy of the diffusion in different directions regardless from fiber orientation (0 indicated free diffusion of the molecules, whereas 1 indicates that the diffusion follows a single axis) (Pierpaoli & Basser, 1996). On the other hand, the MD refers to the average of magnitude of water diffusion (average of axial and radial diffusion) and it is often reported alongside the FA (Metzler-Baddeley et al., 2011; Metzler-Baddeley et al., 2012). Both indices are influenced by many white matter properties, such as
myelination, diameter and alignment that may have an influence on the effective transmission of the information through the axons (Jones, 2010). Recent evidence suggests that FA and MD values could be differently influenced by specific microstructural features. For example, as suggested by a recent post-mortem research on human brain, low density myelin and diffuse histological orientation would increase MD values, whereas high myelin density and histological orientation would be associated with higher FA (Seehaus et al., 2015). However, evidence suggests strong association between FA and MD values in different white-matter tracts, such as the cingulum and the fornix (Jones et al., 2013; Postans et al., 2014). Therefore, it is still unclear whether these dMRI diffusion indices provide distinct information about microstructure of individual tracts.

2.2. dMRI acquisition and data processing

Whole-brain diffusion-weighted MRI data were acquired using a 3T GE HDx Signa scanner with an eight-channel head coil at the Cardiff University Brain Research Imaging Centre (CUBRIC). Images were acquired using a diffusion-weighted single-shot spin-echo echo-planar imaging pulse sequence (with TE = 87ms, field of view = 23 x 23 cm², 96 x 96 acquisition matrix, 60 contiguous slices acquired along an oblique-axial plane with 2.4mm thickness and no gap), and the scans were cardiac-gated using a peripheral pulse oximeter. Gradients were applied along 30 isotropically-distributed orientations with b = 1200 s/mm² (Jones et al., 1999). Three non-diffusion-weighted images with b = 0 s/mm² were also acquired. T1-weighted 3D FSPGR sequence was also acquired for every participant using TR= 7.8ms, TE= 3ms, TI= 450ms, flip angle= 20°, FOV= 256 mm×192mm×172mm, 1mm isotropic resolution.
Using ExploreDTI (Version 4.8.3; Leemans, Jeurissen, Sijbers, & Jones, 2009), whole brain tractography was performed in each participant native diffusion-space to maximize the anatomical specificity. In order to obtain an accurate three-dimensional reconstruction of the tracts of interest, we used the deterministic tractography algorithm based on constrained spherical deconvolution (CSD) that, for each voxel, determines the fiber orientational density function (Jeurissen et al., 2011; Tournier et al., 2008). A step size of 0.5 mm and an angle threshold of 30° were set in order to prevent the reconstruction of anatomically implausible fibers. At each seed point, the main diffusion orientation was calculated at each step by the deterministic algorithm. The pathway followed the direction of the principal orientation until a direction change of 30° occurred. Moreover, a multiple region-of-interest (ROI) approach was adopted to define bilateral fornix and ILF drawing ROIs manually as shown in Figure 1 below (Wakana et al., 2004; Catani & de Schotten, 2008). Specifically, based on Metzler-Baddeley et al. (2011), the following ROIs were used to reconstruct the fornix: an AND (green line in Figure 1, left side) was drawn on the axial plane, at the level of the splenium of the corpus callosum, to include the bilateral crus of the tract. More anteriorly, on the coronal plane, a SEED ROI (see Figure 1 below on the left, blue line) was placed medially, below the corpus callosum, to encompass the body of the fornix. Finally, 2 NOT points (Figure 1 on the left, red lines) were drawn on coronal plane (anteriorly to the fornix pillars and posteriorly to the fornix crus and corpus callosum) and one on the axial plane above the corpus callosum in order to exclude fibers from the corticospinal tract and corpus callosum. On the other hand, the reconstruction of the unilateral ILF was carried out following the two-ROI procedure adopted by Wakana et al. (2007). On the coronal plane, a SEED ROI (Figure 1 on the right, blue line) was drawn to include the whole hemisphere and, more rostral, an AND
ROI was placed to encompass the temporal lobe and the fibers of the tract. Additional NOT ROIs were drawn to remove implausible fibers. Custom MATLAB scripts were then used to perform a post-hoc correction (free-water-elimination procedure) on the whole-brain images before extracting the dMRI indices in order to correct the values for partial volume errors related to free-water contamination (e.g. cerebral fluid) (Pasternak et al., 2009). The free-water-corrected images were then intersected with the individual-subject tractography and the mean FA and MD were computed for each reconstructed tract (Jones et al., 2005).

Figure 1. The figure represents the gates (NOT, AND and SEED) used in the tractography procedure used to extract the tracts of interest, the fornix and the ILF (modified image from Hodgetts et al., 2015).

3. Voxel-wise approach and Tract-based Spatial Statistics

In order to further investigate the presence of any voxel-wise correlations between the behavioural measure of orientation and spatial learning and diffusion-MRI metrics of white-matter microstructure, Tract-based Spatial Statistics (TBSS) was applied. In general, this voxel-wise approach may be more sensitive to local variation rather than that averaged over a whole tract. TBSS creates mean skeleton mask of FA and this method was used in all studies of this thesis to investigate both whole-brain and fornix
microstructure. This analysis was carried out with the threshold-free cluster enhancement (TFCE) approach developed by Smith & Nichols (2009) and allows one to detect significant clusters without needing to define at arbitrary threshold. The masks for each tract of interest were created by thresholding (20%) and binarizing the probabilistic masks of the fornix from the Juelich Histological Atlas. Fornix mask was then intersected with the skeletonized mean FA image. In order to obtain a more accurate mask of this ROI, a binarized mask of the corpus callosum (also provided by the Juelich Histological Atlas) thresholded at 30% was subtracted to the former one (Postans et al., 2014). As a result, we obtained a binary mask of the bilateral fornix, for which the exact number of voxels is specified in the method sections of the following chapters. Before voxel-wise cross-subject statistics were computed, nonlinear transformations were applied to register individual FA images on the FMRIB58-FA standard space image. Using a threshold FA value of 0.2, the resulting FA image was projected onto a skeletonised mean-FA white matter mask which corresponds to the centre of all tracts common to all subjects. General linear model and contrasts were then created in order to identify small areas within the skeletonised mean-FA mask in which a certain dMRI measure (FA and MD) was more predictive of the behavioural measures of orientation accuracy (SOT) (see Chapter III), spatial learning (VRMt) (see Chapter IV) and adaptation effect in the HC (see Chapter V). For the whole-brain TBSS, clusters were extracted using TFCE with a corrected threshold of $p = 0.05$, whereas uncorrected threshold of $p = 0.005$ was used for the ROI analysis of the fornix.
4. fMRI acquisition

Scanning was performed at the Cardiff University Brain Research Imaging Centre (CUBRIC) using a 3 T GE HDx Signa scanner with an eight-channel head coil. For functional imaging, a T2*-weighted gradient-echo, echoplanar imaging (EPI) sequence sensitive to blood-oxygenation-level-dependent (BOLD) contrast were acquired. 42 slices were collected per image volume covering the whole brain, prescribed 30 degrees inclined from the AC-PC plane to maximize signal coverage in the MTL (Weiskopf et al., 2006). Slice thickness was 2.4 mm (1 mm gap between the slices). Visual stimuli were rear projected onto a screen at the head of the scanner and the responses were registered using a button-pad system. The participants were instructed to use only the right index finger during both the repetition suppression and localiser tasks. Scanning parameters were set as follows: TR/TE, 2700/35 ms; flip angle, 90 degrees; slice thickness, 2.4 mm (1 mm gap between the slices); acquisition matrix GE-EPI, 64 × 64; ASSET (acceleration factor), 2; and HOS (acquired after the spatial discrimination task was completed). The HOS is a procedure that allows the scanner to (partially) correct for variations in the magnetic field that arise once a participant is placed in the scanner by adjusting shims inside the gradient coils according to a low-resolution magnetic field map. Additional high-resolution field maps were also acquired for every participant enabling the EPI datasets to be undistorted during imaging preprocessing. After the localiser task was completed, a structural scan was obtained for each participant using a T1-weighted sequence (3D FSPGR). Scanning parameters were as follows TR= 7.8ms, TE= 3ms, TI= 450ms, flip angle= 20°, FOV= 256 mm×192mm×172mm, 1 mm isotropic resolution.
Pre-processing on the fMRI data and specific analysis related to the adaptation task will be described in detail in Chapter V (Section 2.4) after the introduction to the experimental design.

5. Grey matter analysis

The registration/segmentation tool of FSL, FMRIB’s Integrated Registration and Segmentation tool (FIRST, Patenaude et al., 2011), was used to perform volumetric segmentation of the T1-weighted images and obtain a series of subcortical structures for each subject. FIRST is a model based-tool (Bayesian Appearance Model), which analyses the geometry and location of the brain structures, applies the variation of the most probable shape and provides labels for a series of cortical areas. Applying this method, we obtained unilateral hippocampal masks for each subject then the bilateral volume was calculated. SIENAX (Smith et al., 2004), another FSL tool, was used to normalize subjects head size and estimate the total brain tissue volume. Specifically, in order to ensure that the changes in the hippocampal volume did not reflect general changes in the grey matter, we used the scaling factor obtained through SIENAX (Vscaling score) to normalise the individual brain volume and correct the values for intracranial volume.
Chapter III: Investigating the role of the fornix in spatial orientation ability

1. Introduction

1.1. The neural basis of the egocentric representation

Accurate and flexible navigation depends on the successful combination of egocentric and allocentric representations that ultimately produces map-like representations of the environment, also called “cognitive maps” (O’Keefe & Nadel, 1978; Murray, Wise, & Graham, 2017) (see Chapter I, Section 2.1.3). As discussed in Chapter I (Section 2.1.3), spatial coordinates are processed by egocentric and allocentric coding systems that produce distinct representations of the space (Galati et al., 2000). The relationship between a landmark’s location and the viewer are processed by the egocentric system that provides a representation dependent upon the individual’s perspective and guides the goal-directed movement. On the other hand, the spatial relationships between items in the space, independent of viewer location, are processed by the allocentric system (Wolbers & Wiener, 2014). Previous findings indicate that parietal and motor cortices underlie egocentric processing, while hippocampal and parahippocampal areas appear to contribute to the formation of allocentric representations (Ekstrom et al., 2003; Byrne, Becker, & Burgess, 2007; Gomez, Cerles, Rousset, Rémy, & Baciu, 2014). However, the neural mechanisms underpinning these computations are still a matter of debate.
Neuroimaging studies in humans revealed that a fronto-parietal and premotor circuit underlies spatial transformation of visual information. In particular, Galati et al. (2000) carried out an fMRI experiment to investigate brain activation during visuo-spatial tasks that required both egocentric and allocentric references. The cognitive task used in this study consisted of two experimental conditions to investigate these spatial representations separately. The participants were presented with a series of vertical lines intersected with horizontal planes. For the egocentric condition, subjects were asked to indicate whether the vertical bar was located on the left or right relative to either their own midsagittal plane, whereas in the allocentric condition they had to determine whether the vertical bar was placed on the left or right of the horizontal line.

The fMRI results indicated that the egocentric condition (i.e. the position of the line relative to the body-position) elicited activity in a wide set of premotor and parietal areas that extended from the precuneus, superior parietal and intraparietal sulcus to the inferior parietal lobule and parieto-temporal junction. Interestingly, the authors found that part of this fronto-parietal network was also implicated in the allocentric coding condition. This result could indicate that, despite being separate processes, the neural substrates that support the formation of egocentric and allocentric reference frames could require the functional activation of similar circuits or that separate circuits could have overlapping neural architecture that supports the integration of both spatial representations.

FMRI has also been used to study the ability to imagine taking different perspectives in space (e.g., imagined self-rotation, Creem et al., 2001). In this study, before the scanning session, participants were required to memorize the location of an array of pictures (representing different objects, such as car, teapot and hammer) then underwent an fMRI scan and performed a rotation task. This task required the
individual to perform a mental rotation of 90°, 180° or 270° and indicate which object of the previously learnt array of stimuli was on the left or right side. In most subjects, this egocentric transformation was found to significantly activate the parietal lobule and the precuneus but also motor areas, especially premotor area and supplementary motor area. Thus, in line with previous findings, these results confirm the critical role played by the parietal-motor circuit in egocentric transformations (Galati et al., 2000), including perspective-taking tasks.

Expanding the findings on the neural substrate involved in spatial coordinates transformation, converging evidence indicates the contribution of the MTL structures, especially the HC, in spatial computation. Zacks, Rypma, Gabrieli, Tversky, & Glover (1999) conducted an fMRI study on healthy subjects to investigate the functional activity of brain areas specifically involved in egocentric transformation and mental rotation. Human figures (front or back facing) were used as stimuli and the task consisted of two different experimental conditions based on the spatial transformation adopted. Generally, participants were asked to indicate whether a black ball was held in the left or right hand. In the egocentric condition, subjects were required to take the figure’s perspective (presented upright) performing an egocentric transformation. By contrast, in the object-based condition, the figures were presented upside-down and a more complex egocentric computation was necessary to make the correct judgment. In general, longer time response times were recorded during the performance of these two experimental conditions in comparison to the control task, where participants were not required to make any spatial transformations. During the implementation of egocentric transformations, a set of brain areas in the occipital, parietal and temporal showed significant activation, such as occipital and lingual gyri, cuneus and precuneus, left superior parietal lobule as well as frontal and middle temporal gyri.
Overall, these results suggest that a wide circuit, that includes also medial temporal regions, is involved in spatial transformations that imply egocentric processing, such as mental rotation and perspective taking. However, the exact role of MTL structures in these processes is still unclear as shown by the findings on patients with unilateral temporal lobectomy in a study conducted by Feigenbaum & Morris (2004). Specifically, patients performed an analogue version of the Morris Water Maze task (MWM) under two different conditions. In the egocentric condition the external cues were moved although maintained the same spatial relationship between each other so the subjects had to rely on their egocentric representation from intra-maze cues to reach the hidden platform. In the allocentric condition the cues of the task remained in the same positions while the patients had to move around and base their orientation on the spatial relationships between self and landmarks location to reach the hidden platform. The results showed similar learning effect in both patient and control groups in the egocentric condition. For the allocentric condition, only patients with right lobectomy showed a significant impairment in comparison to controls. In order to clarify the differential neural basis of the allocentric and egocentric reference system, Gramann, Müller, Schönebeck, & Debus (2006) conducted an electrophysiological study, using the EEG, on healthy humans using a virtual navigation task. In this task, tunnels that were designed to virtually guide the navigator to a target location where no external cues were provided. Once the target was reached, participants were then required to indicate the position of the target location relative to their starting point. Based on the orientation strategy imposed by the experimenters, participants were divided into two groups: the “turners” adopted an egocentric reference and had to update their sense of direction after the turning point, whereas the “non-turners” referred to an allocentric representation of the tunnel configuration and their spatial
reference did not change with turns. The electrophysiological recording of the brain activity after the turning point showed fronto-parietal, including precuneus and postcentral gyrus, and temporal activation, such as middle and superior temporal gyri. On the other hand, activation of the occipito-temporal regions and anterior cingulate cortex was found in the “non-turners” group. Overall, these results suggest that these distinct spatial references are underpinned by extended brain networks that could be recruited in parallel while exploring a virtual environment (Gramann et al., 2006). In particular, the HC appears to be crucial for the integration of multiple spatial relationships to form a coherent representation of the surroundings and translate viewer-dependent representations into an allocentric reference (Wolbers & Büchel, 2005; Gramann et al., 2006). The HC appears to provide crucial support not only for navigation but also spatial imagery and related skills (Schacter & Addis, 2007). In particular, the hippocampal activation has been found to relate to scene construction, defined as the ability to imagine and maintain coherent representation of a scene (Zeidman, Mullally, & Maguire, 2015). This process could be comparable to the initial phase of having to imagine oneself taking a specific position in space and for this reason we investigated the association between hippocampal volume and a behavioural measure of spatial orientation.

Extending the investigation to hippocampal connection, a few studies have focused on the impact of fornix damage to egocentric computation. For example, Mogensen et al. (2005) tested the spatial orientation of rodents that underwent a fimbria-fornix transection. In this experiment, they used a water-maze paradigm without external cues so the platform could be reached using egocentric strategies only. The animals showed a significant impairment in the initial orientation towards the target although, after several sessions, their final performance was comparable to the performance of
the control group. The results clearly indicated a role of the fornix in facilitating the development of egocentric orientation at the initial stage that could be subsequently compensated for by the complex network that underlies spatial orientation. The significant association between the fornix and the accuracy in spatial orientation found in this study supports the hypothesis of a wider network that underlies egocentric transformations carried out mentally.

To date, only a few studies have investigated the contribution of the fornix in navigation and spatial orientation in humans. As discussed in Chapter I (Section 2.2.1) recent research has provided consistent evidence of the selective contribution of the fornix in perceptual discrimination of scenes in comparison to faces (Postans et al., 2014; Hodgetts et al., 2015). In line with the theory that the fornix is critically involved in spatial skills, the study described in this Chapter was conducted to clarify its specific role in spatial orientation and egocentric computation. The orientation of an individual in space requires the combined representation of self-location and the spatial configuration of objects within the environment, which relies on the egocentric system. The egocentric representation depends on the viewer’s perspective and it is necessary to support their accurate orientation in the space as well as guiding the action towards a target location. In this Chapter, the specific contribution of key components of the MTL, HC and fornix, to spatial orientation will be investigated in healthy human participants.

1.2. Aims and hypothesis

This study followed on from prior work looking at the role of the fornix in scene discrimination (Postans et al., 2014; Hodgetts et al., 2015). Specifically, I wished to test whether fornix microstructure would also be a good predictor of accuracy on a
simple spatial orientation task. For the following reasons, the Spatial Orientation Task (SOT) (Hegarty & Waller, 2004) was adopted for this experiment. First, the task provides a measure of egocentric spatial processing, involving identification of the orientation of targets in the environment. Second, gradations of orientation error can be calculated for pointing decisions providing a relatively subtle measure of inter-individual variation in performance. Third, the SOT does not place any demand on memory, allowing me to investigate spatial information about the environment without additional mnemonic burden. Prior to outlining the key hypotheses, I provide here a brief background of the task (expanded in the Methods section below) and a description of some relevant literature based on use of the task.

The SOT used in this study is a revised version of the Object Perspective Task, which was originally developed by Kozhevnikov & Hegarty (2001). The original study by Kozhevnikov & Hegarty (2001) was to test whether perspective-taking and object mental rotation could be considered as two distinct processes. For this reason, a range of tests was used to investigate these abilities separately. Card rotation task, Cube comparison and Paper Folding tests were used to evaluate the ability of mentally manipulating objects, whereas the Object Perspective Task and Map Perspective Test assessed the ability of imagining taking different perspectives in the space. The authors were particularly interested in testing the reliability of the measure provided by the newly developed Object Perspective Task and verifying the type of strategy adopted to solve it. This task was a pen-and-paper task consisting of an array of objects placed in circle and participants were required to mentally take the position of one of these objects without physically rotating the task paper. The rotation was manipulated in order to be greater or smaller than 90°. The results showed that participants used a perspective-taking strategy (imagining rotating their own orientation) only when the
perspective changes were greater than 90°. When this condition was met, the Object Perspective Task was found to provide a measure of spatial orientation. In a subsequent study, Hegarty & Waller (2004) used a modified version of this task including only perspective changes of least 90° in order to ensure that participants adopted a perspective-taking and not an object mental rotation strategy (see detailed description of the task in Section 2 below).

Therefore, the behavioural measure obtained with the SOT was considered a valid indicator of the ability of participants to orient in space. I predicted that this measure would be associated with fornix microstructure, but not with the microstructure of a control tract, the inferior longitudinal fasciculus (ILF) to provide evidence of the selective involvement of the hippocampal connection to egocentric computation and spatial orientation. As the behavioural measure of the SOT indicated the orientation error (see Section 2.2 below), I predicted to find negative correlation with fornix FA and positive correlation with fornix MD. Complementary TBSS analysis was also conducted to investigate whether any potential voxels outside the tract of interest were predictive of the performance on the SOT. According to our hypothesis, we expected to find a significant correlation between clusters in the fornix and the SOT as further support to the results from the DTI analysis.

Furthermore, as hippocampal volume has been shown to be associated with navigational expertise (Maguire et al., 1996; Maguire et al., 2010), I also hypothesised to find a significant association (positive correlation) between spatial orientation accuracy and inter-individual variation in hippocampal volume. This result would confirm the role of the HC in spatial processing indicating its involvement not only in navigation but also in the formation of egocentric representation of the environment.
2. Method: Spatial Orientation Task (SOT)

2.1. Participants

Thirty undergraduate students of the Cardiff University (School of Psychology) were recruited for this experiment (average age= 19, SD= 0.93; 2 males). Ethical agreement was given by the Cardiff University School of Psychology Ethics Committee, and all participants gave informed consent and were debriefed after the experiment. As well as performing the SOT, participants underwent an MRI scan to acquire structural and DTI data (see Chapter 2 for methods).

2.2. SOT experimental design

The SOT version adopted for this study was devised by Hegarty and Waller (2004). As discussed in the Introduction, and shown in Figure 1, in the SOT seven objects are displayed in a circle in the top-half of a piece of paper. On each of the twelve trials included in the test, participants were asked to imagine taking one of the object position and to face a specific direction, e.g. “imagine you are standing at the house facing the flower then point at the cat”. To test the participant’s ability to orient in space, a line had to be drawn to indicate the direction of a target item. This line was drawn by the participant in the circle presented in the bottom-half of the same sheet, which also provided information about where the participant was standing and their orientation towards an object. In the SOT, the change of perspective was always at least 90° and the direction of the target object was systematically changed by dividing the circle into four quadrants (quadrant 1: 0° to 90°; quadrant 2: 90° to 180°; quadrant 3: 180° to 270° and quadrant 4: 270° to 360°). The task sheets could not be rotated by
participants to facilitate the completion of the task. This meant that participants had to rely on their ability to perform imagined spatial transformations aligned to the location of objects with respect to themselves. The outcome measure on this task was absolute directional error. The absolute directional error was measured as the absolute deviation between the angle of the line drawn by the participants and the correct direction of the target (reported in Hegarty et al. (2008)).

For each trial, the ideal angle was subtracted from the angle formed between the line drawn by the participant and the line towards the target object (e.g., as shown in Figure 2, for trial 2 the ideal angle was 237° and the measured angle of the participant was 180° therefore the resulting absolute directional error was 57°). If the response in a trial was at chance a score of 90° was assigned to indicate that the participant did not point to any target direction (Kozhevnikov & Hegarty, 2001); this occurred in 10% of all the observations within this experiment. The average of the absolute directional error obtained for each item was calculated for each participant over the 12 trials, and considered the main indicator of pointing accuracy.
2.3. Neuroimaging data: DTI, TBSS and volumetric analysis

The protocol used to acquire neuroimaging data is described in Chapter II as well as the procedure that we followed to extract information about the microstructure of the tracts of interest and HC volume. In summary, for all participants we acquired...
diffusion tensor imaging (DTI) data to investigate white-matter microstructure and structural images to extract HC volume. Specifically, tractography approach (see Chapter II, Section 2) was used to define fornix and ILF for each subject then dMRI metrics, fractional anisotropy (FA) and mean diffusivity (MD), were extracted to investigate the association between white-matter microstructure and SOT performance. A further insight into white-matter microstructure was also provided by Tract-based Spatial Statistics (TBSS) (see Chapter II, Section 3), which was conducted on whole-brain. This analysis allows us to find any potential voxels, also outside the tract of interest, that correlate with the absolute directional error obtained with the SOT. Furthermore, the HC volume was calculated using FMRIB’s Integrated Registration and Segmentation tool (FIRST), following the procedure described in Chapter II, Section 5. This method provided a measure of the HC volume that was correlated with the performance at the SOT to investigate the involvement of this structure in egocentric processing.
3. Results

3.1. Behavioural results

For each individual trial and in each participant, two independent assessors (another lab member and myself) measured the angles formed between the black arrow that represented the direction faced by the individual and the line drawn by the participant to indicate where they thought the target object was located (see Figure 2 below). All participants (N = 30) were included in the following analysis. For each of the 12 trials, the directional error was calculated by subtracting the assessed angle from the correct direction of the target. The absolute value of the resulting directional error was considered in the following analysis (absolute directional error). Specifically, if a participant was highly accurate in their pointing this value should be very low; where a participant was not accurate, this value would be greater (reflecting the waywardness of the pointing).
**Instruction**: imagine you are standing at the cat and facing the tree. Point to the car.

![Diagram](image)

Figure 2. In this example, the participant was asked to imagine standing at the cat location facing the tree (black line) and was required to draw a line that pointed at the car (blue line). The angle between the ideal trajectory (green dotted line) and the line drawn by the participant represented the directional error (in yellow). In this case, the ideal angle (between the black line and the green dotted line) was $237^\circ$ and the measured angle (between the black line and the blue line) was $180^\circ$ therefore the resulting absolute directional error was $237^\circ - 180^\circ = 57^\circ$.

A two-way mixed intraclass correlation (ICC) was run to test for rater agreement on the average of absolute directional errors obtained on the sample (see Table 1 and Figure 3 below). The ICC was extremely high ($ICC = .969$ with the 95% CI = [.913, .987]) implying a high interrelated reliability between the two assessors’ measurements thus the measures that I obtained (Rater 2 only) were considered reliable and were used in the following analysis.
Table 1. Mean and standard deviation (SD) of the average absolute directional error (degrees) are reported for both raters.

<table>
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<tr>
<th>Rater</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Rater 1</td>
<td>30.53</td>
<td>17.42</td>
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<tr>
<td>Rater 2</td>
<td>33.93</td>
<td>21.81</td>
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Figure 3. The chart above represents the raters agreement on the absolute directional error (y axis) for each participant (x axis) with relative error bar representing the standard error (SE). Rater 1 average absolute directional error: M= 30.53, SD= 17.42; Rater 2 average absolute directional error: M= 33.93, SD= 21.81.

3.2. Relationship between white-matter and orientation accuracy

None of the participants were excluded from the analysis therefore the following results refer to the whole sample (N= 30). To determine whether there was a
relationship between inter-individual variation on the SOT and microstructural measures (FA and MD, see detailed description in Chapter 2, Section 2.1) obtained from the fornix and ILF, one-tailed Pearson correlations were conducted (see Table 2 and Figure 4 below). The results show a negative correlation between fornix FA and SOT measure \( r = -0.522, p = 0.002; \text{CI } 95\% = [-0.758, -0.261] \), which survives the Bonferroni correction \( p = 0.0125 \). By contrast, ILF microstructure was found not to correlate with the SOT confirming that this tract is not critically involved in spatial processing \( \text{ILF FA: } r = -0.090, p = 0.318; \text{ILF MD: } r = 0.025, p = 0.449 \).

The difference between the correlation between SOT and fornix/ILF FA was tested using the calculator Quantpsy (Lee & Preacher, 2013), which adopts Steiger's (1980) equation, and the result shows that the correlations are significantly different from each other (one-tailed \( p = 0.019 \)). The same analysis was carried out to test the difference between fornix and ILF MD correlations with the SOT and these resulted not to be significantly different (one-tailed \( p = 0.188 \)).

<table>
<thead>
<tr>
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<th>FA</th>
<th>MD</th>
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<tr>
<td><strong>Fornix</strong></td>
<td>( r = -0.522, p = 0.002^* )</td>
<td>( r = 0.270, p = 0.074 )</td>
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<tr>
<td></td>
<td>CI 95% = [-0.758, -0.261]</td>
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<tr>
<td><strong>ILF</strong></td>
<td>( r = -0.090, p = 0.318 )</td>
<td>( r = 0.025, p = 0.449 )</td>
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Table 2. One-tailed correlations between the absolute directional error and the diffusion MRI measures of the fornix and ILF. The fornix FA negatively correlates with the SOT measure \( r = -0.522, p = 0.002 \) and resists the Bonferroni correction \( p = 0.0125 \). No significant correlation with ILF diffusion measures was found.
Figure 4. The graphs show the linear relationships between the average directional error and the FA and MD values of the fornix (graphs on top) and the ILF (graphs at the bottom). The correlation between SOT and fornix FA was significant ($r = -0.522$, $p = 0.002$, CI 95% = [-0.758, -0.261]). No significant correlation was found with either fornix MD nor ILF diffusion measures.

Two-tailed Pearson correlation was also conducted to verify the association between the MR diffusion measures of FA and MD in each of the ILF and fornix, separately. This analysis was important to test whether FA and MD are independent from each other therefore convey distinct information about the tract of interest. As outlined in Chapter II (Section 2.1), previous literature shows that fornix FA and MD are correlated. Our results show that no significant correlation was found between ILF FA and MD ($r = -0.274$, $p = 0.143$), whereas a strong significant correlation was found between fornix FA and MD ($r = -0.422$, $p = 0.020$, CI 95% = [-0.637, -0.166]).
3.3. TBSS

Whole-brain TBSS analysis (for further details, see method description in Chapter II, Section 3) was conducted to investigate whether there were any associations between SOT performance and white-matter voxels outside the main tracts of interest. The whole-brain TBSS analysis for both FA and MD did not show any significantly correlations \( (p = 0.05) \) between voxels outside the fornix and the directional error. At a corrected \( p < 0.05 \) threshold, there were no significant clusters at the whole brain level, and similarly, when using the fornix as a mask, there were no voxels that survived \( p < 0.05 \) corrected for the mask volume. Therefore, TBSS analysis does not allow to draw clear conclusion about the specificity of the fornix response.

3.4. Investigating the HC volume

The total HC volume was correlated with the measure of spatial orientation accuracy. I expected to find the volume to negatively correlate with the error in pointing towards the target. For this purpose, we used a one-tailed Pearson correlation. This detected no significant relationship between total HC volume and SOT accuracy \( (r = .194, p = .156) \).
4. Discussion

This study investigated whether inter-individual variation in microstructure measures of the fornix, compared to the ILF, would be associated with individual performance on the SOT. Previous work has focused on the relationship between fornix microstructure with perceptual learning of scenes and scene discrimination (Rudebeck et al., 2009; Postans et al., 2014; Hodgetts et al., 2015), but not considered tasks involving egocentric orientation in a spatial environment. Specifically, the SOT requires participants to point towards an identified visual target from an imagined location within a scene. To achieve a high degree of accuracy, participants need to perform a mental transformation enabling a different perspective from the one they face currently. This relies upon the formation of an accurate egocentric reference frame that correctly represents the location of objects in relation to the current self-position (Galati et al., 2000; Zacks et al., 2000; Creem et al., 2001). In fact, an accurate egocentric representation is crucial for the ability of mentally taking different perspectives in the space and the SOT provides a reliable measure of this skill (Hegarty & Waller, 2004).

My results on white matter microstructure suggest that some aspects of spatial processing required to successfully perform the SOT may require involvement of a wider network of brain regions, including the key white matter input and output pathway to the hippocampus. Although previous literature suggests a strong negative association between scene perceptual processing and fornix MD (Postans et al., 2014; Hodgetts et al., 2015), no significant relationship with this specific MR diffusion measure emerged in this study. However, as previously found by Postans et al. (2014), we observed a strong relationship between fornix FA and MD which could be
explained by the fact that are both influenced by common axonal properties, such as axon diameter, myelination, density and configuration, that are not possible to isolate with the imaging technique used in this study (Jones, Christiansen, Chapman, & Aggleton, 2013). Therefore, no clear conclusion can be drawn regarding the differential contribution of these MR diffusion measures to spatial orientation. However, the analysis revealed a significant negative correlation between fornix FA and absolute directional performance on the SOT measure. This suggests that participants with higher fornix FA typically show better accuracy on the SOT (smaller directional error). Moreover, this was not a general effect of white matter, as no such pattern was evident when considering microstructural measures from the ILF. Based on this result fornix FA can be considered a stronger predictor of spatial orientation ability. Consistent with previous research on rodents (Mogensen et al., 2005), these results suggest that the fornix supports updating of the egocentric reference frame and linking scene feature conjunctions in HC with (simulated as in virtual reality tasks) head direction information along medial diencephalon (with potential scene representation updating via parietal networks). This could provide an explanation to the fact that this study shows significant correlation with the fornix but not with the HC volume. By contrast, the results provide further evidence about the non-contribution of the ILF to the processing of spatial information, consistent with other work in our laboratory where ILF was associated with success on perceptual discrimination for faces, but not scene stimuli. Taken together, these studies suggest that the ILF is not part of the extended brain network that supports the implementation of spatial skills, on the contrary it appears to be a critical part of the pathway that underpins the perceptual processing of faces (Postans et al., 2014; Hodgetts et al., 2015; Gomez et al., 2015).
In this study no significant relationship between HC and the performance on the SOT was found. This could indicate that this structure may not support egocentric computation. This is consistent with previous literature that has claimed that, across species, the formation of egocentric reference frames is supported by a posterior parietal and frontal circuit (Galati et al., 2000; Creem et al., 2001). The HC instead, as previously suggested, may be exclusively involved in the network that processes allocentric spatial relationships and navigation (Byrne et al. 2007; Gomez et al., 2014).

A limitation of testing whole hippocampal volume however could be that substructures may have more subtle roles and may contribute differently to allocentric and egocentric processing. Future research could investigate the differential contribution provided by the HC substructures, such as the subiculum, dentate gyrus, CA1 and CA3, by applying high-resolution 7T fMRI technique and conduct a more refine study also on their association with pre and postcommisural fornix.

Previous studies have found gender differences in spatial skills on both behavioural performance and brain activity. For example, using a virtual-reality maze task, Grön et al. (2000) found that males were generally faster in finding the way out of the maze. Furthermore, during the exploration of a virtual reality environment, a differential pattern of activation in specific brain areas emerged between the groups, with males showing left hippocampal and parahippocampal activation and females showing significant activity in the left superior frontal and right medial frontal gyri. Significantly better performance of males at the Mental Rotation Test and Map-Reading Test was also found by Zacks et al. (2000) suggesting that gender influences the ability of navigate and orient in the space. In this study, we did not carry out any analysis to investigate gender difference in spatial orientation because of the unbalanced numbers of participants for each group (28 females and 2 males). Future
studies could, however, address this issue by comparing the directional error obtained from male and female participants in order to test potential difference in the accuracy of orientation in the space, as well as the neural substrates for success on the SOT.

The main finding of this Chapter supports the growing body of evidence that the white-matter HC connectivity is critical for the formation of coherent representations of visual scenes (Postans et al., 2014; Hodgetts et al., 2015). Further investigation is necessary, however, to clarify the exact functional contribution the fornix makes in spatial processing. The next study, reported in Chapter IV, therefore, aimed to determine whether variation in fornix microstructure would be associated with inter-individual differences in spatial navigation, studied using a task akin to the MWM. As suggested in Chapter I (Section 2.1.3), allocentric encoding is required to successfully reach a target in the environment, based on processing of the relationships between external landmarks. If inter-individual variation in the fornix is also associated with behavioural performance in a spatial navigation task, as well as the SOT, this would further support the conclusion that the fornix is necessary for higher-order perception and also more complex spatial skills, such as orientation and navigation in environments supporting both egocentric and allocentric representations. On the other hand, if HC volume correlates with the measure of spatial learning and navigation obtained with the virtual reality task (see Chapter IV), this would indicate that this MTL structure provides a more specific contribution to the formation of an allocentric reference of space and a complex representation of visual scene (Chapter V).
Chapter IV: The role of the fornix in spatial navigation within a virtual room

1. Introduction

In Chapter III, I asked whether inter-individual variation in the microstructural properties of the fornix would be associated with performance on an egocentric spatial orientation task. In the spatial orientation paradigm, participants were required to indicate the location of objects within an environment presented within a circle on paper. I found that FA of the fornix, but not the ILF, was correlated with pointing accuracy: specifically, participants who had a greater FA measure in the fornix made less error in their pointing to objects in the circle. In this chapter, I aimed to extend this work by going beyond a simple egocentric pointing task, asking whether inter-individual variation in microstructural properties of the fornix would be associated with a task involving allocentric spatial processing. Specifically, I investigated navigation to a hidden platform within a virtual-reality room, a form of spatial navigation known to be affected in animals after hippocampal lesions (often tested using the MWM (Morris et al., 1982), see below for more details).

As discussed in Chapter I (Section 2.1.4), research on animals shows that the HC is essential for encoding the relations between objects and forming allocentric representations of space (Ekstrom et al., 2003; Zhang & Ekstrom, 2013). One of the paradigms most extensively used to investigate navigational skills and its underlying neural substrate, especially in animal literature, is the MWM which has been proven to be sensitive to hippocampal dysfunction (Morris et al., 1982; Morris, 1984).
Specifically, using the classical MWM or its variations, such as a T-maze, studies in rodents found that focal hippocampal damage caused severe impairment in using visual cues, such as distal cues outside the maze, to guide movement towards the platform effectively (Warburton & Aggleton, 1998).

A similar paradigm has been used also with monkeys. Specifically, Banta Lavenex et al. (2006) tested a group of monkeys with hippocampal lesions in an experimental arena with 18 cups in order to investigate the role of the HC in allocentric processing. The animals had to explore the space and find locations which had hidden food and they started the exploration from distinct points. In the local cue condition, monkeys were provided with cues (e.g., coloured cups) and food remained in the same location. By contrast, in the spatial relation condition, no local cues were provided and monkeys were required to find food based on allocentric memory of the space by remembering the location of cups with food in relation to the other cups. Results showed that lesioned monkeys had severe difficulty in finding the food when they had to rely on allocentric representation (spatial relation condition), exclusively. This evidence provided further support to the theory that the HC is critical for the processing of spatial relationships that form allocentric reference of the environment.

To assess navigation and spatial learning in humans, and enable comparison with work in animals, virtual versions of the MWM (vMWM) have been designed (Astur et al., 1998). In general, humans show considerable inter-individual variation in their navigational ability (Wolbers & Hegarty, 2010). Numerous studies on patients with MTL damage have used different versions of this paradigm to investigate the contribution of the HC in spatial learning and navigation but the findings are controversial. For example, Astur et al. (2002) tested patients with unilateral hippocampal damage on a vMWM task. Starting from different locations, patients
could navigate in a first-person perspective using a joystick and were required to find the hidden platform which was placed in two different locations. For each location, patients performed a 20 trial training phase then the platform was removed and a probe trial present (where the participant was required to navigate to the place where the platform had been). The performance on the two probe trials was analysed in terms of distance within the quadrant where the platform was located (correct quadrant). The results showed that patients were significantly slower in reaching the platform during the training and spent less time in the correct quadrant during the first probe trial, indicating that hippocampal damage produces severe spatial learning impairment. Similarly, Goodrich-Hunsaker et al. (2010) used a different version of the vMWM to investigate the impact of hippocampal lesions on navigation skills. In this study, amnesic patients, who had significant hippocampal atrophy, performed a vMWM, called Arena Maze, that consisted of a circular area within a square room and, similar to the paradigm used by Astur et al. (2002), participants were asked to explore and find the hidden platform. Consistent with previous research (Astur et al., 2002), patients took significantly more time and navigated a longer distance to reach the platform on probe trials. They also showed difficulties in reporting the spatial configuration (room recollection task) of the virtual space immediately after completion of the task. In line with animal research (Morris et al., 1982; Morris, 1984), these findings suggest that the HC is a key structure for spatial learning and effective navigation in humans as well. Some studies with amnesic patients have not, however, been consistent with these conclusions. Specifically, Bohbot & Corkin (2007) adopted a vMWM called the Invisible Sensor task (IST) to investigate a range of spatial skills in the famous patient (H. M.), who underwent bilateral temporal medial resection for intractable epilepsy. The path to reach two different target locations was analysed and
the results were not straightforward to interpret. In comparison to controls, H. M.’s performance was unimpaired for the first location but significantly impaired for the second one. He seemed to show partly spared spatial learning ability. In contrast with previous results (Astur et al., 2002; Goodrich-Hunsaker et al., 2010), the authors concluded that HC might not be the only crucial structure for allocentric processing and that other MTL areas, such as the parahippocampal cortices, could support the computation of spatial relations (Bohbot et al., 1998). It is worth noting, however, that the impairment observed for the second location could be an interference effect of the spatial memory referred to first location.

The lack of consistency in findings from previous studies has raised the necessity of improving existing measures of spatial navigation obtained from the vMWM as well as developing new and more accurate versions to study the exact role of the HC and hippocampal network in spatial learning. Kolarik et al. (2016) designed a new version of the vMWM modelled on the paradigm used by Astur et al. (2002). They called this task the Virtual Reality Maze task (VRMt), in order to investigate not only spatial learning but also perceptual precision of navigation around the hidden platform. The authors used this task on an amnesic patient with brain damage restricted to the HC. The patient was a highly-educated 29-year-old female who showed hippocampal volume loss following a car accident. The task consisted of a training phase followed by a probe trial. The same procedure was followed for the second (different) platform location. The paths taken on probe trials were analyzed in terms of the time spent in the correct quadrant and direction accuracy towards the target location. The authors’ analysis focused on the correct quadrant produced comparable results to that of Bohbot & Corkin (2007), showing significantly impaired performance in the patient on second probe trial only. Using precision windows drawn around the platform area, the authors
then analysed the accuracy of the patient’s trajectory towards the target calculating the
time spent within each precision window. According to the initial hypothesis, the
patient was expected to show impaired navigation accuracy, spending less time than
controls in the precision windows closest to the platform area. The results confirmed
this hypothesis for both probe trials, although the time spent in the larger precision
windows was comparable to controls. Overall, these findings suggest that, despite
severe damage to the HC, spatial learning and navigation skills can be preserved in
some patients.

Moving beyond grey matter, studies on animals suggest that the fornix, as a major
white matter bundle that connects hippocampal structures to various cortical and
subcortical structures (Aggleton et al., 2015), makes a crucial contribution to spatial
processing (see Chapter I, Section 2.2.1). Specifically, research on rodents has shown
that lesions to the fornix cause severe impairment on navigation (Aggleton et al.,
1995). As shown by Warburton & Aggleton (1998), selective transection of the
fimbria/fornix produces impairment in the learning phase of the MWM task and,
although other areas, such as the anterior thalamic nuclei, support the acquisition of
route-learning through additional training, rats exhibit decreased accuracy in
swimming towards the platform. In comparison to lesions to the medial prefrontal
cortex, damage to fimbria-fornix connections has been shown to produce a specific
deficit in location learning, especially when an allocentric reference is required (Bruin
et al., 2001). Further confirmation of the contribution of the fornix to allocentric
navigation has also been provided by research on monkeys, where deficits in scene-
learning and impaired spatial learning after fornix transections have been evidenced
(Buckley et al., 2008; Wilson et al., 2008) (for further details see Chapter I, Section
2.2.1). Overall, findings from the animal literature show that the efferent connections
from the HC, supported by the fornix, provide crucial support to spatial learning abilities, such as route-learning and spatial orientation. As discussed in Chapter I (Section 2.2.1), recent studies on humans have found that fornix microstructure is selectively associated with the successful discrimination of spatial features and scene discrimination but not with face processing (Postans et al., 2014; Hodgetts et al., 2015). In the previous chapter, I demonstrated further that microstructure of the fornix is also associated with spatial orientation, on an egocentric spatial paradigm. The main purpose of the study outlined in this chapter was to test whether inter-individual differences in fornix microstructure and hippocampal volume would be associated with spatial navigation abilities, going beyond the egocentric spatial processing task discussed in Chapter III. To test this hypothesis, I used the VRMt (Kolarik et al., 2016) to study spatial navigation (to a platform) of healthy young participants in a virtual environment. My analysis approach will focus on learning (as measured by analysis of learning curves obtained over repeated trials requiring navigation to the platform). Similar diffusion MRI techniques (DTI and TBSS, see Chapter II, Sections 2 and 3) were used to extract information about white-matter microstructure (dMRI metrics, FA and MD, described in Chapter II, Section 2.1). Based on the previous findings outlined above, I hypothesized that fornix microstructure would be a good predictor of learning ability on the virtual reality task. TBSS was applied to identify potential clusters outside the fornix, noting that while the tractography analysis in Chapter III was significant, the TBSS analysis was less sensitive to fornix. I also looked at how HC volumes were associated with learning performance, predicting to find a correlation between HC volume and spatial learning in the VRMt.
2. Method

2.1. Participants

33 participants underwent structural (T1) and DTI scanning prior to taking part in a behavioural session involving the VRMt. Eighteen females and fifteen males (average age = 24.12 years; SD = 3.54 years) participated in these data collection sessions. The Cardiff University School of Psychology Ethics Committee approved the experiment, and all participants were provided with an information sheet, signed a consent form and were debriefed at the end of the experiment. As well as scanning and the VRMt, participants also completed the Santa Barbara Sense of Direction Scale (SBSOD) at the end of the experiment. The SBSOD consists of 15 statements about orientation and spatial skills (e.g. “I am very good at giving directions”, “I enjoy reading maps”) and participants are required to indicated on a rating scale to what extent they agree with the statement (rating scale from 1, strongly agree, to 7, strongly disagree) (Hegarty, 2002). This standardized self-reported measure of navigational ability has been shown to correlate with real-world wayfinding performance (Janzen et al., 2008; Wegman & Janzen, 2011). Here, the questionnaire was administered to test for an association between self-awareness of spatial skills, as measured with the SBSOD, and how quickly participants learnt to navigate to the hidden platform in the VRMt.

2.2. Neuroimaging data

As described in Chapter II, neuroimaging data were acquired using a 3T GE HDxSigna scanner with an eight-channel head coil at Cardiff University Brain Research Imaging Centre (CUBRIC). Participants underwent a whole-brain diffusion-weighted MRI
scan (DWI). The scans were cardiac-gated using a sensor device (peripheral oximeter) placed on the subject’s fingertips. DWI scans were conducted using a diffusion-weighted single-shot spin-echo echo-planar imaging pulse sequence (30 directions, TE= 87ms; 60 continuous slices acquired along an oblique-axial plane with 2.4 mm thickness and no gap). T1-weighted 3D FSPGR sequence was also acquired for each subject using TR= 7.8ms, TE= 3ms, TI= 450ms, flip angle= 20°, FOV= 256 mm×192mm×172mm, 1mm isotropic resolution.

2.3. Virtual Reality Maze task (VRMt)

The VMRt was designed by Kolarik et al. (2016) using Unity 3D (Unity Technologies, San Francisco) and based on the task by Astur et al. (2002) (see Figure 1 below). Participants are required to explore a virtual reality room using 4 arrow keys to find a hidden sensor (a 0.4 x 0.4 virtual meter platform occupying 0.25% of the total room area). The room is 8 x 8 virtual meters with 4 different paintings hung on each wall. These paintings can be used as external landmarks to find the sensor. Once the participant has walked over the hidden platform, the platform becomes visible. A note, ‘You found the hidden sensor’, is displayed on the screen and the trial is ended after 10 seconds. The total duration of the trial can be up to 60 seconds; when a participant does not find the target location within this set time the platform is automatically made visible. After a trial, an inter-trial window appears and the participant clicks on the button to start the next trial.

The experiment consisted of two main experimental blocks: the first involved 20 training trials for target location A followed by a probe trial (see below for details). The second block had the same trial structure (20 trials plus a probe trial) but for a new target location (target location B). This order (training for location A followed by
location B) was the same for all participants. The training trials were split into 5 mini-blocks of 4 trials each, in which the participant would start the navigation from one of the 4 different sides of the room (order NSEW). During the probe trials the participants had only 30 seconds to navigate in the room and, unlike the training trials, the platform did not become visible even when the participants walked over the correct location. On probe trials, the starting point was randomly chosen.

Figure 1. On the top left: snapshot of the virtual reality room that the participants explore during the task and a view of the room as the participant navigates. On the bottom-right: screenshot of the moment when the participant finds the sensor during the training trials and the countdown of 10 seconds begins as displayed on the top left corner of the image. Modified image from Kolarik et al. (2016).
3. Results

3.1. Curve fitting approach

The analyses conducted here aimed to examine the relationship between spatial learning within a more realistic spatial environment (a virtual reality room) and fornix tissue microstructure. Specifically, for each training trial, I was interested in the time to reach the hidden sensor, and how that improved over repeated trials. In looking at the data obtained from the paradigm, it was clear that participants often showed quite variable performance after rapid learning, and that this was particularly evident in Location B, after the participants had already undertaken learning of Location A. In the analyses reported below, therefore, I focus only on Location A, supporting my decision to exclude Location B via application of a curve-fitting model and relative cut-off approach (described below and in Section 3.2). With this approach, 10 subjects were excluded from the Location B analysis, which was then considered too small a sample for robust statistical analysis. The variability of participants learning across training trials for Location A was then analysed as well as the individual learning curves. This approach also allowed the definition of a cut-off and exclude participants that showed poor model fit focusing the analysis on learners only. Correlational analysis was then conducted to verify the association between inter-individual variability of fornix microstructure and spatial learning (see Section 3.2 below). Analyses and results will be described in detail in the following paragraphs.

As can be seen in Figure 2, there was a high degree of inter-individual variability across participants in their spatial learning, with subjects varying in both speed of their learning and the robustness of their individual learning curves. Given this, and to
increase sensitivity to individual-level performance across training trials, I adopted a curve fitting approach. An ‘ideal’ learning curve was defined as a negative power function. Thus, the goodness of fit (defined as R-square) of a power curve at the individual level provides information about the speed and shape of learning in individual subjects.

Figure 2. Individual learning plots (n = 33) across trials to where participants were navigating to Location A. Y-axes represent the time to reach the target platform in seconds while the number of trials is shown on the x-axes. Subjects excluded from the analysis were numbers 10, 15, 18 and 21 (see justification below).

A notable aspect of performance on this task was that some subjects displayed very fast and steep learning (often plateauing before trial 10), before then displaying quite poor and variable performance in the later trials (see participants 9, 13, and 20). This
pattern of performance presents a challenge for my planned curve fitting approach (and potentially counterintuitive results), as some of the faster learners will actually show the poorest model fits. For instance, both subjects 9 and 16 display an initial steep learning curve and an early plateau (Figure 2), but a power model fit to all trials only provides a good fit for a participant who sustains performance until the final training trial (see Figure 3 below which illustrates this analytical problem).

Figure 3. Individual learning plots for the VRMt. Y-axes represent the time to reach the target platform in seconds. The number of trials is shown on the x-axis. The linear regression line reflects the power model fit of the log transformed data.

To address this issue, I adopted a data-driven approach to determine a cut-off in individual subjects. Specifically, a second degree polynomial model was applied to all trials obtained in each subject. The cut-off for an individual participant was defined as the trough of this curve, which is the point where the first derivative of the second degree polynomial crosses zero (see Figure 4a below). Trials up to and including this cut-off were then modelled using a power function (see Figure 4b below). Two measures of learning were derived from this modelling: the b parameter (b) and the goodness-of-fit (R-squared). The overall R-squared mean = 0.27, standard deviation = 0.2, range = 0 - 0.77). The b parameter provides a measure of slope curvilinearity in each subject, with more concave curvilinear fitting reflecting fast learning. Values
closer to zero reflect more linear fits. The R-squared indicates how well the power function fits the observed data and is an approximate measure of learning “consistency”.

Figure 4. (a) The method for determining the number of learning trials to-be-modelled. The first derivative of the second degree polynomial was used to define a cut-off at the point it crossed zero. This is shown for an example subject. (b) A power model was fit to the number trials determined by the first derivative method shown in (a).

3.2. Correlating model fits with tract microstructure

Directional correlations were conducted between learning for sensor location A (i.e., individual-level R-square values) and free water corrected MD and FA values for the fornix and ILF. Bivariate Pearson’s correlations between these DTI values showed that fornix FA and MD values are strongly associated ($r = -0.568, p = 0.020$) as well as ILF FA and MD ($r = -0.436, p = 0.002$). Pearson’s correlations between DTI measures and learning were Bonferroni-corrected by dividing $\alpha = 0.05$ by the number of statistical comparisons for each DTI metric (i.e., 0.05/2 = 0.025) (see Hodgetts et al., 2015, 2017). I also conducted one-tailed Pearson correlation analyses using SPSS and report confidence intervals (CI) for the correlations. One subject was removed from the analysis of Location A as they had a fornix MD value that was lower than two
standard deviations below the group mean. To exclude participants who were not engaging with the VRMt paradigm, a bootstrapping approach was applied where individual-level data was shuffled over 1000 permutations and confidence intervals derived. Therefore, participants with an $R$-squared that fell outside the CI of their individually defined random distribution were excluded (in this case participants number 10, 15, 17, 18 and 21). This resulted in a total sample of 28 participants for subsequent analyses. The following results refer to location A only, based on exclusion of ten participants for Location B using the bootstrapping approach, and insufficient participants for a correlational analysis of microstructure and learning (see earlier discussion).

As shown in Figure 5 below, we found a significant positive correlation between the $b$ parameter (a measure of slope curvilinearity) and fornix MD. This suggests that those subjects with higher fornix MD had steeper learning slopes ($r = -0.44, p = 0.01, 95\% \text{ CI } [0.08, 0.09]$; Figure 5). There was no significant relationship between the $b$ parameter and ILF MD ($r = 0.05; p = 0.059, 95\% \text{ CI } [0.03, 0.01]$). A directional Steiger Z-test (Steiger, 1980) confirmed that the correlation between the $b$ parameter and fornix MD was significantly different to that obtained between the $b$ parameter and ILF MD ($z = 2.18, p = 0.015$).
A moderate negative trend was observed between fornix FA and learning speed (b parameter) but was not significant at our experiment-wise significance level ($r = -0.33$, $p = 0.04$, 95% CI [-0.61, -0.04]). There was no evidence of a relationship between ILF FA and spatial learning ($r = -0.17$, $p = 0.2$, 95% CI [-0.51, -0.01]). These correlations were not significantly different ($z = 0.74$, $p = 0.23$).

My correlation analyses also revealed a significant association between the power model R-squared and fornix MD, such that individuals with lower fornix MD displayed more consistent learning ($r = -0.42$, $p = 0.01$, 95% CI [-0.66, -0.88], Figure 4). No significant relationship was found between the model R-squared and ILF MD ($r = 0.01$, $p = 0.62$, 95% CI [-0.34, 0]). A directional Steiger Z-test (Steiger, 1980) showed that the association with R-squared was significantly greater for fornix relative to ILF MD ($z = -1.95$, $p = 0.026$).
Regarding fornix FA, as for the b parameter, I found only a small to moderate non-significant trend with R-squared values ($r = 0.17, p = 0.19, 95\% \text{ CI} [0.01, 0.51]$). There was no evidence of a relationship between ILF FA and spatial learning ($r = -0.02, p = 0.46, 95\% \text{ CI} [0, 0.41]$). These correlations did not differ significantly ($z = 0.63, p = 0.26$).

3.3. Association with self-reported navigational ability

To explore the relationship between the objective measures of spatial learning obtained from the VRMt and self-reported navigational ability, I correlated the values obtained from the individual-level power model fits with scores obtained from participants on the Santa Barbara Sense of Direction (SBSOD) scale (Hegarty, Richardson, Montello, Lovelace, & Subbiah, 2002) (see Figure 6 below). A significant positive association was evident between power model R-squared and SBSOD scores ($r = 0.39, p = 0.02, 95\% \text{ CI} [0.06 0.65]$). There was a non-significant negative trend between the b parameter and SBSOD ($r = -0.19, p = 0.17, 95\% \text{ CI} [0, 0.31]$).
3.4. Association with HC volume

Segmentation of T1-weighted images and extraction of bilateral hippocampus were carried out using FIRST (Patenaude et al., 2011) (see Figure 7 below). One-tailed Pearson’s correlation was carried out to investigate the association between HC volume and VRMt, with the expectation that larger HC volumes would be associated with better (more rapid and consistent) learning in the VRMt. Contrary to this prediction, there was no significant correlation between these measures (R-squared: $r^2 = 0.03487$, $p = 0.271$; $r = 0.121$, $p = 0.271$; $b: r = 0.109$, $p = 0.295$).
3.5. Probe trials

In addition to the investigation of learning curves obtained in individual participants for Location A, performance on probe trials was also analysed, similar to Kolarik et al. (2016). For this analysis, all participants were included, and performance on both probe trials (for both location A and B) was investigated. Specifically, we measured the time spent in the correct quadrant, where the platform was located for both Location A and B. As noted above, the platform does not appear in probe trials when participants are on top of it. As shown in Figure 7, performance was significantly above chance (25%) for both locations (Location A: \( t (32) = 6.174, p < .001 \); Location B: \( t (32) = 5.335, p < .001 \)). That said, significantly less time was spent in the correct quadrant for Location B in comparison to Location A \( t (32) = 2.064, p = .047 \). One-tailed Pearson correlations (see Table 1 below) revealed that performance (time spent in the correct quadrant) was not significantly associated with DTI measures of either of my tracts of interest (fornix and ILF). Despite being the trials of main interest in Kolarik et al. (2016), it could be argued that performance on probes is not a good indicator of spatial learning in healthy individuals because they occur after a long training session (20 trials) during which many of the participants had already
developed rapid and effective trajectories to reach the target. For this reason, we believe that analysis of the individual curve learning reported in the previous sections is probably a more reliable indicator of spatial learning in this study (see Section 3.1 and 3.2).
Figure 7. The graph above represents the accuracy of spatial navigation during the duration of the probe trials. Each column displays the percentage of time that participants spent on average in the correct quadrant. For probe 1 mean = 58.22% of time, SD= 30.91%; for probe 2 mean = 45.26% of time, SD= 21.82%. The red line represents chance level (25%). The error bars represent the standard error.

Table 1. One-tailed Pearson correlations between accuracy in spatial navigation on probe trials and the diffusion MRI measures (FA and MD) for both fornix and ILF. None of the reported correlations were significant after multiple comparison correction of the p-value (p = 0.05/4).

<table>
<thead>
<tr>
<th>Accuracy in probe trials &amp; tracts of interest</th>
<th>Fornix</th>
<th>ILF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA</td>
<td>MD</td>
</tr>
<tr>
<td>Probe 1</td>
<td>r = -.191 p = .144</td>
<td>r = .015 p = .466</td>
</tr>
<tr>
<td>Probe 2</td>
<td>r = -.211 p = .120</td>
<td>r = -.106 p = .279</td>
</tr>
</tbody>
</table>

3.6. TBSS

To verify whether spatial learning correlated with white-matter outside the fornix, we conducted a whole-brain TBSS analysis (see method description in Chapter II, Section 3). Whole-brain analysis for FA and MD showed no significant correlations (p = 0.05) between voxels outside the ROI and learning curve (R-squared and b parameter) therefore no cluster coordinates can be provided here.
4. Discussion

Virtual versions of the MWM task have been widely used to assess spatial memory skills in humans, especially with amnesic patients who have suffered from MTL and hippocampal damage (Astur et al., 2002; Goodrich-Hunsaker et al., 2010). One of the latest virtual versions (VRMt) was used in a single-patient study by Kolarik et al. (2016) to investigate the effect of hippocampal damage on navigation skills and spatial memory. The VRMt was adopted in this study to verify whether this task could provide reliable metrics of spatial learning in a young healthy group as well as facilitating analysis of the contribution of white matter tracts connected to the hippocampus in virtual reality spatial navigation. The VRMt required the participant to learn the most effective trajectory to a hidden platform in the arena based on the processing of external cues that remained in the same location throughout the task. Despite a change of starting point in each trial, the representation of different viewpoints could potentially be condensed into the same reference frame and formed with little movement (Wolbers & Wiener, 2014). The amnesic patient tested by Kolarik et al. (2016) necessitated a long set of training trials to be able to form an accurate allocentric representation of the environment and learn the most effective route to reach the target. The use of 20 training trials for each platform location was designed to ensure a good level of familiarity with the environment and maximize the performance of the patient on probe trials, which were thoroughly analyzed in terms of accuracy of navigation. In a healthy young cohort, however, the use of such an extensive set of training trials resulted in less reliable information about spatial learning since highly functioning subjects needed only a few trials to rapidly orient in the space and develop accurate trajectories towards the platform. Towards the end of their training session, in fact, many participants showed a drop or fluctuation of
performance (taking longer) which impacted on analysis of their learning curves. This was particularly problematic in Location B, after the participants had already undertaken the set of trials for Location A.

To circumvent this problem, my analysis focused on the training trials of location A during which the initial representation of the virtual space was formed, and where the participants had less familiarity about the environment. A curve fitting approach was used which allowed me to investigate the relationship between inter-individual variability in learning consistency (R-squared) and learning curve (b parameter) and fornix / ILF microstructure. In line with my hypothesis, although the whole-brain TBSS analysis failed to provide confirmation of any relationship between white matter and spatial learning performance, the ROI tractography analysis confirmed a significant association between fornix microstructure and both b parameter / R-squared. Specifically, participants who showed better learning, as measured by their consistency and the curve of their slope, also had lower fornix MD values. No such association emerged with ILF. Consistent with previous studies (Postans et al., 2014; Hodgetts et al., 2015), these results suggest that fornix MD is a good predictor of successful spatial learning and navigational abilities (see Chapter I, Section 2.2.1). No significant relationship with FA values was found, although there was a non-significant trend in the expected direction. As noted previously it is not clear what different forms of microstructural properties are differentially associated with FA and MD, and why one measure may be more or less sensitive to behavioural performance (see Chapter II, Section 2.1).

A positive relationship was also evident between performance on the VMRt and SBSOD questionnaire; this indicates that individual subjective judgement of spatial navigational ability is correlated with objective measures of effective navigational
skills in virtual environments. In line with previous literature, this result provides further support for the view that SBSOD can be used as a quick and reliable subjective assessment of individual navigational ability in spatial environments.

Regarding the task design, it could be argued that the dimension of the room used in the VRMt is not comparable to the more sizeable scale of real-world environments, which humans are more used to navigating in. Specifically, the spatial representation formed for the “vista space” might not be considered analogous to the allocentric reference frame created during natural navigation (Wolbers & Wiener, 2014). Despite the restricted size of the virtual room, however, the results suggest that the representation of small-scale space requires a form of spatial processing that it is underpinned by the extended hippocampal network (see Introduction, Section 2.2).

The significant association between fornix microstructure and speed and consistency of spatial learning supports the theory that a widespread network of structures connected to the HC contribute to the processing of layout and geometry of both small-scale and large-scale environments, and ultimately to the formation of allocentric representations of external space (where location of external landmarks and the relationship between them guides successful navigation in humans, see Introduction, Section 2.2.1). The present study complements evidence of the role of the fornix in navigation and spatial learning in animals (Warburton and Aggleton, 1998; Buckley et al., 2004; Wilson et al., 2008).

Previous research suggested that showed that HC is associated with accuracy in pointing accuracy in an offsite direction estimation task suggesting that the HC is critically involved in the formation of map-like representations of the environment that guide navigation (Schinazi, Nardi, Newcombe, Shipley, & Epstein, 2013). By contrast, in this study, no significant association between the measures of spatial
learning obtained from the VMRt (R-squared and b) and inter-individual variability in participants HC volume was found. This result could indicate that the HC and the fornix make differential contributions to the formation of allocentric representations needed to undertake effective navigation in a virtual environment. Further research would be required to address this remaining question, particularly where more fine-grained assessment of hippocampal substructures can be undertaken.

I noted above that the duration of the training is potentially excessive for highly-functioning subjects who can quickly develop an accurate representation of the virtual room. Future studies should also consider modifying the task by reducing the number of training trials and analysing spatial learning for a range of different target locations using learning curve method. Moreover, room layout / size of the platform could be changed across each session in order to test the ability to form new allocentric representations of the room rapidly, which might improve the ecological validity of the task, noting that in real-world situations environmental settings continuously change as the individual moves in the environment.

In order to further investigate the brain network underpinnig spatial processing and the spatial representation formed by the HC, I conducted an experiment using a combination of fMRI and DTI techniques (see Chapter V). Specifically, an fMRI repetition suppression paradigm was used to study the functional response of the HC to changes in scene viewpoint aiming to test whether this area creates viewpoint-invariant spatial representation. This study also aimed to extend previous findings about the role of the fornix in scene perception investigating the involvement of this white-matter tract in viewpoint processing.
Chapter V: fMRI repetition suppression and the role of the fornix in scene perception

1. fMRI repetition suppression

An fMRI repetition suppression paradigm was employed as a powerful method to study the response of category-sensitive areas to small changes in stimuli presented repeatedly (see Section 2.2 in this Chapter for a detailed description of the experimental paradigm adopted in this study). The sequential presentation of either identical or highly similar images has been widely used as a paradigm in fMRI experiments to investigate the sensitivity of specific brain areas to small changes in presented stimuli. Repeating the same stimulus can lead to either an enhancement or a suppression of the neural response measured by the BOLD signal (Segaert et al., 2013) and has been interpreted as evidence that the corresponding brain region stores representations of that item. The suppression of signal associated with repetition of a stimulus is considered an improvement in performance at lowered metabolic cost (Gotts, 2016). Benefitting from this neural property, the fMRI repetition suppression paradigm was devised as a non-invasive and reliable method to investigate the nature representations stored within specific brain areas and, for this reason, it has been extensively used in recent neuroimaging research (Grill-Spector & Malach, 2001; Grill-Spector et al., 2006; Barron et al., 2016). For example, Epstein, Parker, & Feiler (2008) adopted an fMRI repetition suppression to investigate the effect of time intervals in the response of the parahippocampal place area (PPA), which is is known to form viewpoint-specific representation (Epstein, Graham, & Downing, 2003).
During the scanning phase of this study, participants were presented with pairs of images and they had to determine whether the image represented a location outside or within the campus of University of Pennsylvania. Each trial consisted of two images that could represent: the identical location within the campus, the same location within the campus but from different viewpoints, different locations of the campus or one image of the campus and the other one of a location outside. For the short-lag condition, the images of the trial were presented in sequence during the scanning phase, whereas for the long-lag condition the repeated images were presented between the study (pre-scanning) phase and the scanning phase. The results showed that the duration of the interval affected the repetition suppression occurring in the PPA. Specifically, when scenes were presented sequentially, the PPA showed strong repetition suppression for the identical condition in comparison to the different-view condition, whereas a similar repetition suppression was found for both these conditions for long intervals. These results suggested that scene representation in the PPA is underpinned by distinct neural mechanisms that are sensitive to the duration of time-interval, effectively forming viewpoint-specific representations when the interval is short and viewpoint-invariant representations when the interval is longer.

The mechanisms underlying repetition suppression can be explained by three main neural models (Figure 1):

1) *facilitation model*: decrease in firing rate could underpin early termination of neural activity and shorter duration of neural response;

2) *fatigue model*: this model argues that the repetitive presentation of similar stimuli leads to a considerable reduction in neural network activation, which is proportional to the initial response;
3) *sharpening model*: according to this model, the repetition suppression reflects fewer and sparser activation of some of the neurons initially activated by the first presentation of the stimulus.

![Figure 1](image.png)

Figure 1. Representation of the three models explaining the underlying neural response to repetitive presentation of the same or highly similar stimuli (image from Grill-Spector et al., 2006).

As a novel approach to investigate scene representations in the HC (see Section 2.2), the current study adopted an fMRI repetition suppression paradigm to verify the sensitivity of this MTL region to different degrees of change in viewpoint, but predominantly to investigate the association between individual variation in repetition suppression and microstructure of the fornix (following on from the previous Chapters). Specifically, I asked whether, in comparison to ILF, inter-individual variation in fornix microstructure would predict variation in the degree of repetition suppression occurring in the HC across different viewpoint changes. I also hypothesised that there would be no white-matter voxels outside the fornix significantly associated with individual variation in HC repetition suppression (see
TBSS results in this Chapter, Section 3.3). Previous studies on patients with hippocampal damage suggest that the HC stores viewpoint-invariant representations (Barense et al., 2010, see Chapter I, Section 2.1.2). For this reason, I expected to find similar levels of HC repetition suppression across all viewpoint conditions included in this study, compared to presentation of a different scene (novel item). If I also demonstrated a significant association with fornix microstructure and repetition suppression across these conditions this would be further evidence that the extended hippocampal network is crucially involved not only in spatial navigation and orientation (as demonstrated earlier in this thesis) but also in perception of complex scenes (see Chapter I, Section 2.2).
2. Method

2.1. Participants

Thirty-six healthy participants took part in the study, which consisted of both fMRI and DTI data acquisition. All participants had normal or corrected-to-normal vision. Seven participants were excluded from the analysis due to considerable head movement (> 3 mm); the final sample, therefore, consisted of 29 subjects (28 right-handed, 21 females, mean age = 22.90 years, SD = 4.29 years). The School of Psychology Ethics Committee, Cardiff University approved the experimental design and study. Participants were recruited through the online EMS system of the School of Psychology and were given detailed information sheets prior to signing the consent form. They were provided with a debrief explaining the experiment at the end.

2.2. Repetition suppression task: stimuli and paradigm

For this task, 301 3-D virtual reality scenes used in a previous study by Lee, Brodersen, & Rudebeck (2013) were modified using a computer game (Deus Ex; Ion Storm, Austin, TX) and a freeware editor (Deus Ex SDK v1112f). One of the scene was randomly chosen as the target (see Figure 2 below). Participants were instructed to detect the target scene in a sequential presented stream of scenes, pressing the response button when it appeared. The target occurred 30 times during the experiment, which was 17 minutes in duration, and it was included in the task in order to ensure participants maintained an adequate level of attention. Attention is considered fundamental to support neural activity in the brain regions I was interested in studying (Corbetta & Shulman, 2002). Trials with target presentation were subsequently
removed from the analysis. Trials consisted of the first presentation of a scene (400 ms) followed by a jittered ISI (between 100 and 1100 ms) and then the second presentation of a scene (400 ms). Scenes were trial-unique and the experiment consisted of 4 conditions relating to the second presentation of the scene (aligned to my interest in repetition suppression):

1) identical repeated scene (60 trials);
2) same scene presented but with a 15° (small) viewpoint change from the original scene (60 trials);
3) same scene presented with a 30° (large) viewpoint change from the original scene (60 trials);
4) novel scene condition where a completely new scene (compared to the prior item) was presented (60 trials).

There were also 30 additional trials during which a fixation cross was presented for between 1000 to 5000 ms. This was designed to be used as a baseline condition, in addition to the ISI between scenes, in the analyses described below. The trial-conditions were counterbalanced during the task across each of the 4 MRI runs therefore each condition was present in equal number of trials but in different order.
Figure 2. The figure above outlines the repetition suppression paradigm adopted in the study. The scene with the yellow frame represents the target that the participants were required to detect.

2.3. Localiser task: stimuli and paradigm

To identify the a priori chosen ROIs for the investigation of repetition suppression in this experiment we utilised an independent localiser task (Hodgetts, Shine, Lawrence, Downing, & Graham, 2016). The localiser was a one-back task (duration: approximately 10 minutes) during which the participants were required to respond every time they saw the same picture repeated twice in a row. Three greyscale stimulus-categories were used: objects, real-world scenes and scrambled pictures. The order of the stimulus-category within a block could be: 1) objects, scenes and scrambled pictures; 2) scrambled pictures, scenes and objects; 3) scenes, objects and scrambled pictures. These three type of stimulus-order did not change during the task. Each block order was repeated four times and 12 sets of blocks were repeated three times during the task. This resulted in 36 blocks experimental blocks. Each stimulus was presented for 200 ms with an ISI of 800 ms. In the study, the majority of
participants were found to have greater activity for the “scenes vs scrambled pictures” whole-brain contrast. Therefore this contrast was used to define the ROIs (HC scene-sensitive voxels) for the analysis on the repetition suppression task. I chose this contrast in comparison to the others because it revealed the highest number of active voxels, and largest clusters, in the HC.

2.4. fMRI data analysis

2.4.1. Localiser data pre-processing

The pre-processing of the localiser data was carried out using FEAT (fMRI Expert Analysis Tool) part of the software library of the Oxford Centre for Functional MRI of the Brain (FMRIB). The original fMRI data of both tasks was pre-processed applying motion correction (MCFLIRT); non-brain removal using BET; spatial smoothing using a Gaussian kernel of FWHM 4 mm; mean-based intensity normalization of all volumes; high-pass temporal filtering at 40 seconds (Mundy et al., 2013) and un-distorting the EPI data to correct for magnetic field distortions using individual field maps. Registration to high-resolution 3D anatomical T1 scans (per participant) and to a standard MNI template image (for group average analyses) was performed using FLIRT.

The three stimulus categories (objects, scenes and scrambled pictures) were modelled in the General linear model (GLM) and the hemodynamic response was implemented as a double-gamma convolution; an uncorrected threshold \( p = .001 \) was applied. The functional mask for the HC was obtained by co-registering an high-resolution anatomical MR image (probabilistic mask thresholded at 50\%, overlapping with the unilateral HC based on the Harvard-Oxford subcortical structural atlas (Desikan et al.,
2006) with the native data of each participant. With this procedure, functional masks of the HC were identified in 20 of the 29 participants.

### 2.4.2. Repetition Suppression task data pre-processing

Pre-processing of the main experimental fMRI data was carried out using FEAT. Data from fMRI tasks were pre-processed applying motion correction (MCFLIRT); non-brain removal using BET; spatial smoothing using a Gaussian kernel of FWHM 4 mm; mean-based intensity normalization of all volumes. FLIRT motion correction was applied and the EPI data was undistorted to correct for magnetic field distortions using individual field maps. Registration to high-resolution 3D anatomical T1 scans (per participant) and to a standard MNI template image (for assessment of group averages) was performed using FLIRT. The GLM was created by modelling separately each trial condition and each presentation within each condition to provide a measure of repetition suppression and activation for each presentation, respectively. Targets and responses to these were excluded from the analyses, whereas the activation during the fixation trials and the interval between 2 consecutive trials were combined into a baseline condition based on previous studies in which similar experimental paradigms have been used (Kourtzi & Kanwisher, 2001; Andresen et al., 2009). Pairs of scenes consisted of either of (a) repeated scenes, (b) scenes where there was a viewpoint change from the original- 15° or 30° viewpoint change and (c) novel scenes. The following variables were added into the GLM and the hemodynamic response was implemented as a double-gamma convolution applying an uncorrected threshold of \( p = .001 \): 1) first presentation, repeated condition; 2) second presentation, repeated condition; 3) first presentation, 15° viewpoint change; 4) second presentation, 15° viewpoint change; 5) first presentation, 30° viewpoint change; 6) second presentation,
30° viewpoint change; 7) first presentation, novel; 8) second presentation, novel; 9) target presentation, correct responses to the target and false alarms (responses without target presentation) to exclude the trials with any movement related to the response (button press).

Finally,Featquery was used to extract the percent signal change for each parameter estimate of the repetition suppression task from the functionally predefined areas of interest. These data were subsequently analysed by conducting repeated measures ANOVAs to investigate potential differences in the repetition suppression across conditions in the HC, as well as bivariate correlations with the DTI measures obtained from the analysis of the fornix and ILF (the latter used as control tract consistent with the other Chapters in this thesis).
3. Results

3.1. Repetition suppression in the HC

As a first step in our analysis, I successfully identified category-sensitive voxels within the HC based on the functional activation of “scene vs scrambled pictures” contrast from the localiser. Functional masks of the HC were successfully defined on 20 participants which represent the sample included in the following analyses. First, using Featquery, I extracted the percent signal change from the HC in response to each presentation of scenes (see Figure 3 and 4 below). A repeated measures ANOVA including viewpoint (0° change, 15° change, 30° change, novel) x presentation (first, second) showed no significant main effects nor interaction between viewpoint change and presentation ($p > .05$).

![Average activation: first and second presentation for each condition](image)

Figure 3. The graph represents the % signal change extracted from the HC for each condition, first and second presentation separately. The error bars represent standard error.
Chapter V

Figure 4. Inter-individual variability of the percent signal change (y-axis) extracted from bilateral HC for each condition, first and second presentation represented separately on x-axis. Participants are represented by coloured dots (N=20).

Repetition suppression was calculated by subtracting the percent signal change of the first presentation to the percent signal change of the second presentation (Epstein et al., 2008). The resulting values represent the size of any difference in BOLD between the presentations (whether an enhancement or suppression from the first presentation).

A repeated measures ANOVA including hemisphere (left, right) x repetition suppression (0° change, 15° change, 30° change, novel) showed no significant difference between the hemispheres (F (1, 19) = 1.187, p = .290). Given this, the HC was analysed bilaterally in the analysis described below. The one-way ANOVA was not significant (F (3, 57) = 1.531, p=.216) (see Figure 5 below) suggesting that the repetition suppression was not significantly different across viewpoint conditions.
However, further analysis was conducted in order to verify whether the repetition suppression for each condition was significantly different from zero. Post-hoc tests showed that only repetition suppression occurring during the 15° viewpoint change condition was significantly different from zero (t (19) = 2.395, p= .027). This result indicates that BOLD signal is significantly reduced for this condition, but not for other conditions.

Figure 5. The bar chart illustrates the change in BOLD signal in the HC after repetition of stimuli, calculated by subtracting the % signal change of the second presentation to the % signal change of the first presentation so values above zero represent the repetition suppression. The error bars represent the standard errors for each condition.

3.2. DTI and fMRI repetition suppression

To study how inter-individual variation in fornix microstructure was related to change in activity between the first and second presentation of stimuli in the HC, I conducted 1-tailed Pearson correlations to test the relationship between the microstructural metrics (FA and MD) and HC activity linked to repeated presentation for each
condition. Based on previous studies, I predicted a negative correlation between fornix MD and degree of BOLD change and a positive correlation with fornix FA (Hodgetts et al., 2015; Postans et al., 2014).

Based on these analyses, there was no evidence that the degree of BOLD change in the HC from the first and second presentation was significantly associated with either fornix or ILF microstructure (see Table 1 below).

<table>
<thead>
<tr>
<th></th>
<th>Fornix</th>
<th>ILF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA</td>
<td>MD</td>
</tr>
<tr>
<td>0° viewpoint change</td>
<td>r = -.042, p = .430</td>
<td>r = .301, p = .099</td>
</tr>
<tr>
<td>15° viewpoint change</td>
<td>r = -.207, p = .191</td>
<td>r = -.028, p = .454</td>
</tr>
<tr>
<td>30° viewpoint change</td>
<td>r = .118, p = .310</td>
<td>r = .415, p = .034</td>
</tr>
<tr>
<td>Novel</td>
<td>r = .071, p = .384</td>
<td>r = .007, p = .488</td>
</tr>
</tbody>
</table>

Table 1. 1-tailed Pearson correlations between DTI measures (FA and MD) of the fornix and ILF and the HC repetition suppression for each viewpoint condition. The reported p-values are not corrected for multiple comparisons (α = 0.05/8 = 0.006). None of the correlations reported in this table resulted to be significant after multiple comparison correction.

3.3. TBSS

Whole-brain TBSS analysis was also carried out in order to verify the activation in the HC correlated with white-matter outside the fornix (see Chapter II, Section 3). Clusters were extracted using Threshold-Free Cluster Enhancement (Smith & Nichols, 2009) and the results for both FA and MD showed no significant correlations (applying a
FWE-corrected threshold of $p = 0.05$) between voxels outside the fornix ROI and HC response across viewpoint conditions.
4. Discussion

Using a combination of neuroimaging techniques, the current study aimed to provide valuable insights into the nature of scene representations within the HC, and how the fornix may be associated with repetition suppression (where the BOLD signal in a brain region is decreased after repeating an identical or highly similar stimulus). Specifically, the experiment focused on analysis of fMRI repetition suppression in the HC in relation to scene viewpoint changes as well as investigating potential associations between inter-individual differences in repetition suppression elicited during this task and white-matter microstructure of the fornix (compared to that of the ILF, consistent with the approach taken in the previous chapters).

Although there was little evidence of significant repetition suppression in the HC, despite looking at an ROI identified from a comparison of scenes versus scrambled pictures in the 1-back localiser, post-hoc analyses revealed that when the viewpoint change between the first and second presented scene was relatively small (15°), there was some repetition suppression. This difference was found to be significantly different from zero, suggesting that the scene is perceived as the same, and BOLD signal is reduced, despite the viewpoint change. As a general assumption for the adopted paradigm, the repeated presentation of an identical stimulus would be expected to generate the greatest amount of repetition suppression, supporting the idea of faster processing as suggested by the facilitation model (Grill-Spector et al., 2006). According to my hypothesis, I expected to find significant repetition suppression for the 0° viewpoint-change. The results, however, failed to show significant repetition suppression for this condition and consequently no clear conclusion can be drawn.
about the nature of viewpoint-invariant representations that the HC may form (Barense et al., 2010).

In this study, the identified ROI corresponded to the voxels within the HC that responded preferentially to scenes over scrambled pictures in the independent localiser task. This task was previously used by Hodgetts et al. (2016) to identify scene-sensitive voxels in the HC, parahippocampal gyrus, retrosplenial cortex and transverse occipital sulcus. Therefore, it seems plausible that the general low signal detected in the HC on the primary task could indicate that fMRI repetition suppression task is not an effective paradigm to investigate the involvement of this area in scene processing given that only scene-sensitive voxels of the HC were analysed. Alternatively, it could be argued that the duration of the stimulus presentation was not long enough or that a single repetition of the same scene might not be sufficient to observe significant signal attenuation in this area. In line with this hypothesis, previous research suggests that the number of stimulus-repetitions affects the neural responsiveness of category-sensitive areas. For example, Müller, Strumpf, Scholz, Baier, & Melloni (2013) showed that lower PPA activity was particularly evident after the sixth presentation of a repeated scene and continued to decline until the ninth presentation. For this reason, future studies may wish to adopt a similar paradigm to Müller et al. (2013) to investigate the HC response across multiple repetitions of the same scene to verify whether more than two presentations are necessary to observe significant HC repetition suppression.

In addition to the relatively small sample size used here, another potential limitation of this design could relate to the configuration of the virtual scenes used as stimuli. Specifically, the scenes used in the fMRI repetition suppression task were virtually created whereas the stimuli used in the Localiser task consisted of real-world pictures.
The difference in the stimuli used in these tasks could explain the discrepancy of BOLD activation registered in the HC. Moreover, although in the repetition suppression task all scenes were different from each other (trial-unique), the stimuli shared some similar features, such as the wall textures and layout (columns, stairs, pools). Further investigation could be carried out comparing the response to real-world and virtual scenes or creating families of similar scenes to verify whether repetition suppression occurs with stimuli that have a high degree of feature overlap.

The lack of a significant correlation between the fornix and the HC could indicate that the fornix is less engaged in viewpoint processing than the perceptual discrimination of spatial features (Postans et al., 2014; Hodgetts et al., 2015). On the other hand, it is important to consider a potential methodological caveat of the procedure adopted for this study. The fMRI repetition suppression was adopted here as an effective method to infer sensitivity to relatively small changes in the stimuli presented, however the observed effects in the HC were not as clear as predicted and it is possible that these results did not provide the necessary power to successfully investigate the relationship between HC BOLD signal and fornix microstructure. Although a strong association with the HC activation was not found in this experiment, the involvement of the fornix in spatial processing has been demonstrated in numerous studies on animals and humans (Buckley, Wilson, & Gaffan, 2008; Rudebeck et al., 2009; Postans et al., 2014; Hodgetts et al., 2015), and evidenced strongly in my prior chapters.

Further work is, therefore, required to investigate responsiveness to complex scene viewpoint changes.
Chapter VI: Discussion

1. Thesis overview

In this thesis, I investigated how microstructural properties of white-matter tracts in the human brain would be associated with inter-individual differences in spatial cognition. Specifically, I examined the contribution of the fornix to behavioural performance in spatial orientation, navigation around a virtual reality room and scene processing across three different studies using a combination of neuroimaging techniques (DTI and fMRI).

As outlined in Chapter I (see Section 2.1.2), a growing body of evidence has suggested that MTL structures, such as the HC, underpin not only declarative memory (Squire & Zola-Morgan, 1991; Squire, et al. 2004) but spatial representations that support both mnemonic and higher-level perceptual processing, as proposed by the emergent memory account amongst others (Gaffan, 2002; Lee, Bussey et al., 2005; Graham et al., 2006; Graham et al., 2010). Specifically, previous findings in animals and humans have shown that the HC is involved in spatial memory and learning as well as in spatial perception (Bird & Burgess, 2008; Graham et al., 2010). In particular, the HC is central in the neural network that underpins spatial abilities and scene representation (Burgess et al., 2001; Hodgetts et al., 2016), especially allocentric representation (see Chapter I, Section 2.1.3 and 2.1.4). As part of the extended hippocampal network (see Chapter I, Section 2.2), the fornix constitutes the main bundle of fibers that connects the HC to the thalamic nuclei and previous findings suggest that this tract contributes to different aspects of spatial cognition (Gaffan, 1994). Research on lesioned animals provided consistent evidence of fornix role in navigation and scene learning.
Chapter VI

(Warburton and Aggleton, 1998; Buckley et al., 2004; Buckley, Wilson, & Gaffan, 2008). The development of dMRI techniques, such as DTI, has allowed us to extract information about white-matter microstructure and expand research in humans as well. This technique allows us to verify the relationship between white matter and cognitive domains in humans, and can be applied both in patients and healthy individuals (Jones, 2010; Metzler-Baddeley et al., 2011). In line with findings in animals, studies on humans have shown that fornix microstructure is specifically associated with scene discrimination and spatial processing (Rudebeck et al., 2009; Postans, Hodgetts, Mundy, Jones Lawrence and Graham, 2014; Hodgetts, Postans, Shine, Jones, Lawrence and Graham; 2015). However, the role of the fornix in navigation and spatial skills in healthy humans is still unclear. This thesis therefore aimed to provide evidence about the role of this white-matter tract in spatial cognition using dMRI. Specifically, three different paradigms (SOT, VRMt and fMRI repetition suppression), tapping distinct components of spatial cognition (spatial orientation, navigation and scene perception, respectively) were adopted. I asked whether there was a significant association between fornix microstructure (FA and MD) and performance to these tasks, which would be evidence of a contribution of this white matter to spatial cognition in humans. In this discussion, I will summarize and review the main findings of these studies, outline methodological considerations and limitations, and then discuss direction for future work.
2. Main findings

2.1. The role of the fornix in spatial orientation

The processing of egocentric coordinates is essential to allow us to accurately orient in space and requires processing of self-position in relation to external landmarks. This ability is fundamental not only to effectively move towards, but also to mentally orient, towards a target. The aim of this first experimental study was to investigate the contribution of the fornix and HC to spatial orientation using a simple pen and paper task, the SOT (Hegarty and Waller, 2004). The SOT requires participants to imagine different perspectives and indicate where a target is located by drawing a line on a piece of paper. The absolute directional error of the participant’s pointing can then be calculated (e.g., the difference between the ideal angle and the one obtained from the participant’s line) providing a marker of pointing (orientation) accuracy. In this study, I asked whether the resulting value was correlated with dMRI metrics of the fornix in each participant, with comparison of an association with the ILF as a control. The ILF is a large white matter tract which links the occipital and temporal lobes, and which has previously been shown to be associated with semantic knowledge (Hodgetts, Shine, Lawrence, Downing, & Graham, 2016). My prediction was that fornix, but not ILF, microstructure would predict orientation accuracy, as a proxy for egocentric processing. I found a significant negative correlation between the absolute directional error and fornix FA indicating that higher fornix FA is typically linked with greater pointing accuracy on the SOT. This pattern was not seen for ILF. These findings suggest that fornix microstructure can be considered a good predictor of spatial orientation ability, whereas the lack of any significant associations with ILF diffusion
measures confirms that this tract is potentially not involved in the brain network that supports this aspect of spatial processing.

In line with previous research conducted in our laboratory (Postans et al., 2014), the results from this study indicate that the fornix provides specific contribution to spatial processing. Specifically, these findings provide crucial evidence to support the theory of an extended network underpinning not only episodic memory as suggested by Aggleton and Brown (1999) but also spatial orientation in humans. Significant correlations found between behavioural measure and variability of fornix microstructure but not HC volume may indicate that fornix and HC contribute differently to scene processing. As previously suggested by Hodgetts et al. (2015), spatial cognition is a multi-faceted function and the fornix may mediate some aspects, such as spatial orientation and navigation, independently from the HC.

2.2. The fornix and spatial navigation within a virtual environment

Extending previous findings about the role of the hippocampal network in accurate navigation, my second experimental chapter investigated the association between measures of spatial learning and fornix microstructure. Specifically, based on previous findings that showed the selective contribution of the fornix to scene processing (Hodgetts et al., 2015; Postans et al., 2014), fornix microstructure was hypothesized to correlate with better (more efficient) spatial navigation whereas no association was expected to be found with the ILF (control tract). Going beyond the egocentric spatial processing task discussed in Chapter III, the main purpose of this study was to test whether inter-individual differences in fornix microstructure and hippocampal volume would be associated with spatial navigation abilities. The VRMt (Kolarik et al., 2016) was used to investigate spatial learning of healthy young participants in a virtual
environment. In general, all subjects resulted accurate on probe trials spending most of the time in the quadrant where the invisible platform. Performance on these trials was therefore not informative of participants learning variability therefore the analysis undertaken in the Chapter focused on training trials. Specifically, a curve fitting approach was adopted to investigate the learning curve of the participants throughout the training in the first session (where the participants navigated to Location A). R-squared and b parameter were extracted from the modelling and correlated with dMRI metrics (FA and MD) of the fornix and ILF. Consistent with the findings of the first experiment (see Section 2.2 above), a significant relationship was evident between the measure of spatial learning and fornix microstructure. Specifically, a negative correlation between spatial learning and fornix MD was found. This finding suggests that fornix microstructure is predictive of individual ability to rapidly learn an effective spatial trajectory within a virtual-reality space. In line with previous animal models (Buckley et al., 2004; Buckley, Wilson, & Gaffan, 2008), this study provides further evidence of the critical involvement of the fornix in navigation, which is also dependent upon allocentric processing.

2.3. The role of the fornix in scene perception: an fMRI repetition suppression study

The lowered fMRI signal registered after repeated presentation of the same or highly similar stimuli is commonly defined as repetition suppression. This marker has been used as an effective method to investigate the activation of category-sensitive brain areas to small changes in presented stimuli (Grill-Spector & Malach, 2001; Andresen, Vinberg, & Grill-Spector, 2009; Segaert, Weber, de Lange, Petersson, & Hagoort, 2013). Going beyond the current published literature, this experimental procedure was
adopted to study the role of the HC in the processing of different viewpoint changes (0°, 15° and 30° viewpoint changes). I also asked whether there would be an association between HC functional response during these repetitions (individual sensitivity to repetition suppression) and fornix microstructure allowing me to expand on my earlier studies looking at the fornix (see Chapter I, Section 2.2). Specifically, the association between the HC response and DTI measures of the fornix was investigated in order to test whether this hippocampal connection significantly contributes to the processing of scene viewpoint and to the formation of scene representation. The results showed no significant association between inter-individual variation in fornix microstructure and HC activity. The lack of significant results could be related to the generally weak activity of the HC registered during the repetition suppression task. Notably, in this study there was no evidence of significant repetition suppression in the HC, even in the 0° viewpoint change condition where the identical scene was re-presented although scene-sensitive voxels were identified with an independent localiser task. This may suggest that other experimental factors could have undermined the power of this paradigm, such as the restricted number of participants and the number of scene repetition. Further considerations about the methodological limitations of this study will be outlined in section 3.4 below.

3. Methodological limitations

3.1. Diffusion MRI measures

The results from Chapter III and Chapter IV revealed an association between inter-individual variation in spatial ability and fornix (but not ILF) microstructure.
Specifically, there was a significant correlation between FA and spatial orientation and between fornix MD and spatial learning. It is perhaps surprising that two different measures of white matter microstructure were associated with performance in each of the studies, and it is worth touching upon this difference. In both these experiments, FA and MD were found to be strongly correlated with each other, which suggests that they are not completely independence markers of different aspects of white matter. A strong association between these two DTI metrics was also found in previous studies in our laboratory by Postans et al. (2014), which limits distinct conclusions about their specific relationship with behavioural performance on the two tasks used in Chapters III and IV. The fact that FA and MD depend on similar white-matter properties, such as myelination, density and axon diameter (Jones et al., 2013), could explain the strong association found between these measures, but it remains unclear why – in different tasks – FA and MD might be more sensitive. In order to test whether associations with different dMRI measures existed in the same participants, further studies could test the same sample on different spatial tasks to investigate the association with fornix diffusion measures and also understand under what circumstances FA and MD might be associated.

As described in the above section (see Section 2.1 and 2.2) the selective associations found between fornix microstructure and spatial orientation and navigation support the theory of a distributed brain network that underpins spatial processing in humans (see Chapter I, Section 2.2.1). On the other hand, consistently with previous research (Postans et al., 2014), the ILF appears to provide no significant contribution to these cognitive skills. This result is not due to noise related to the tractography as tractography of this tract has been shown to be highly reproducible and reliable (Wakana et al, 2007). Overall, these findings extend previous literature and provide
evidence of the important contribution of the fornix in spatial cognition in healthy humans. It is still not clear, however, what diffusion measure can be considered a stronger predictor of orientation accuracy and spatial learning, and this should be a focus for future research.

3.2. Volumetric analysis

The relationship between hippocampal volume and spatial skills was investigated in Chapter III and Chapter IV. Specifically, I investigated whether SOT performance was related to HC volume, but found no significant association. This finding suggests that the fornix but not the HC may be crucial for the egocentric processing, which requires the processing of the self-position relatively to the objects in the scene. It could be argued that the fornix plays a critical role in the processing of spatial information from egocentric to more complex and flexible representations rather than in the use of complex spatial representations dependent upon the HC. The HC might be more involved in navigation that requires effective allocentric processing (Maguire, Woollett, & Spiers, 2006; Schinazi, Nardi, Newcombe, Shipley, & Epstein, 2013).

Going beyond the analysis of HC volume, future studies could focus on the HC BOLD response, during an oddity task for example, as a measure of HC function during complex spatial processing, and verify whether that is then associated with performance on the SOT.

Volumetric analysis was also conducted in Chapter IV in order to determine whether HC volume was significantly associated with spatial learning. In contrast with previous literature that support the critical role of this MTL structure in allocentric processing (Klatzky, 1998; Schinazi, Nardi, Newcombe, Shipley, & Epstein, 2013),
in this study no significant relationship emerged between HC volume and the measures of spatial learning obtained from the VRMt.

A lack of significant relationship between HC volume and behavioural measure was also found by Hodgetts et al. (2017). Specifically, in this study authors found that variation in fornix microstructure but not HC volume could predict individual differences in autobiographical memory. This results are consistent with the idea there is a complex neural network, rather than specific brain regions, that underpins and contributes to individual differences.

A potential reason for the lack of significant results may be related to the method used to extract the HC volume. In this study, FIRST was used as registration and segmentation tool to extract individual hippocampal masks (see Chapter II, Section 5) however other segmentation packages, such as FreeSurfer used by Schinazi et al. (2013), may also be adopted to extract anatomical measures of various brain regions. Both these tools generate automated volumetric segmentation from T1 structural images however the resulting hippocampal masks may be different (Morey et al., 2009). Differences in volume could therefore justify inconsistency of findings across studies.

The HC values reported in this thesis were corrected for the intracranial volume as correlating absolute HC volumes with behavioural measures would undermine any clear conclusions about the role of this MTL structure. It could be argued that a unique value for the whole HC is too broad. It would therefore be necessary to subdivide this region and extract more specific measures that reflect volumes of hippocampal substructures, which could be differentially implicated in cognitive functions such as spatial navigation.
3.3. VRMt

Previous studies that have used the vMWM in humans have obtained quite different results resulting in discrepancies in the literature regarding the contribution of the HC to allocentric processing, and in turn spatial navigation. Potential reasons for these discrepancies are the heterogeneity of participants tested across studies, including with regard to the number, age and degree of brain damage, as well as application of various different versions of the task. For example, Goodrich-Hunsaker et al. (2010) tested a group of participants that had significant abnormalities localized in the HC using an Arena Maze task. Probe trials occurred after 10 trials where the platform was invisible. Patients were found to spend significantly less time in the correct quadrant during probe trials. On the other hand, Bohbot & Corkin (2007) tested the ability to acquire spatial memories of a single patient, 72 year-old H. M. who had undergone bilateral temporal medial resection. They analysed his performance on the Invisible Sensor Task (IST), considered a comparable version of the MWM (Morris et al., 1987). Specifically, the patient was required to follow the most direct path to reach 2 different sensor locations. The path that the patient followed to reach the sensor was found to be impaired in one of the 2 sensor locations only, on the second one in particular. These findings suggested that the HC was not significantly involved in spatial learning since patients performance resulted unimpaired during the navigation towards the first sensor location. Moreover, the authors argued that an intact parahippocampal gyrus might be sufficient to compensate and support spatial learning even when the HC is lesioned.

Using a virtual version of the MWM, the VRMt, Kolarik et al. (2016) tested a 29 year-old amnesic patient that reported hippocampal damage following an accident. The
patient showed significant impairment on both probe trials spending significantly less time in the correct quadrant in comparison to controls. The aim of Chapter IV was to extend this work by using the VRMt to investigate involvement of the fornix and HC in spatial learning on healthy humans. I found that tract microstructure predicted inter-individual markers from the learning curves evident during training. Development of a novel method of analysis of spatial navigation was one outcome from this experiment, as well as confirmation that the inter-individual variation in the fornix (but not ILF) is associated with better spatial navigation in a virtual-reality version of the MWM.

Previous research used different versions of MWM mainly to test the performance of patients with localized brain lesions on probe trials (Astur et al., 1998; Astur et al., 2002; Bohbot & Corkin, 2007; Goodrich-Hunsaker et al., 2010). Results from the current study showed that probe trials failed to provide significant information about spatial learning in healthy participants. A potential reason for this is that probe trials occurred after 20 training trials and it is likely that subjects became overly familiar with the virtual room and learnt the most effective routes to reach the hidden platform within the first training trials. A new version of the task could reduce the duration of the training and include different environments (with different locations of the platform in the room) in order to increase the power of the experiment. For example, a set of virtual rooms could be designed to control for familiarization with the environment. This would make the task more difficult, but also ensure that participant’s needed to maintain their motivation and attention when performing that task. Both learning curves during the training and performance on probe trials for each location could then be analysed to investigate spatial learning and memory in healthy population.
3.4. fMRI repetition suppression paradigm

As a novel approach, fMRI repetition suppression was adopted here for the first time to investigate HC sensitivity to scene viewpoint changes (Chapter V). Unfortunately, repetition suppression in the HC was not strong, especially for the 0° viewpoint change (identical repeated scene). This may reflect limitations in the experimental design. For example, to observe significant activation and repetition suppression in the HC it may be necessary to provide more repeated presentations of the same stimulus. For this reason, further studies could adopt a different experimental design where the identical stimulus is repeatedly presented for 3 or more times. For example, Park & Chun (2009) analysed the functional response of the PPA and retrosplenial cortex across 3 identical or panoramic presentation of a scene. The results showed a decline in the response of both regions across repetitions of the identical scene and significant attenuation was found between the first and third presentation.

In a refinement of my experiment, use of a similar method would allow me to investigate hippocampal response to a larger set of repetitions of scenes. The analysis of the response attenuation across multiple presentations of the same stimulus would allow to define the minimum number of repetitions that is necessary to observe a significant decline in the HC activity. This would then allow to identify the best way in which to design an experiment to look at changes in repetition level in the HC, and how to use this data to identify the best marker of repetition suppression to investigate the association between repetition suppression and fornix microstructure.

HC activity could be investigated further by analysing not only signal reduction to repeated stimuli but also a different phenomenon called enhancement, that could underlie a different cognitive mechanism (Segaert et al., 2013). Enhancement and
suppression are effects that occur after the repetitive presentation of a stimulus and can coexist (Barron, Garvert, & Behrens, 2016). Enhancement reflects BOLD signal increases that can occur after the repetition of the same stimulus or highly similar stimuli. A possible explanation for this effect is that enhancement indicates additional cognitive processing (Henson, 2003). In this case, similar activation was observed during the first and second presentation could be related to the fact that the HC is processing every single scene presented. It would therefore be interesting to verify whether the number of scene repetition affects the HC activity and when suppression starts to occur, if it does at all. Functional connectivity studies could also allow investigation of suppression and enhancement of activity within the hippocampal network, and how that might be associated with inter-individual variation in structural connectivity.

As discussed in Chapter V (see Section 4), scene-sensitive voxels within the HC were identified through the independent localiser task based on the activity registered for the contrast “scene vs scrambled picture”. Given the sensitivity of these voxels for the scene category, significant activity was expected during the repetition suppression task but the activation of the HC resulted generally low and no repetition suppression was found in this region. The independent localiser task however was used in previous studies and it was shown to be an effective tool to identify scene-sensitive voxels in MTL areas, such as the parahippocampal gyrus, transverse occipital sulus, retrosplenial cortex and the HC (Hodgetts, Shine, Lawrence, Downing, & Graham, 2016). For this reason, this task was used to define the ROI (scene-sensitive voxels in the HC) for each participant and investigate the functional ROI activation during the fMRI task. Despite this procedure, the functional activity registered in the HC resulted to be quite weak and no significant effect was found.
A further possibility is that a small sample size (N = 20) could have affected my power to detect a strong effect in this study. In order to enhance the power of this research, future studies could address the same question on a larger group of participants, as well as make some of the methodological changes outlined above.

4. Outstanding questions and future directions

Further research could be conducted testing the same participants in a combination of spatial tasks, such as the SOT and VRMt, in order to test whether the performance on each of these relate to each other and if the orientation and spatial learning correlate with the same dMRI measures of the fornix. A new version of the VRMt could also be designed reducing the duration of training, and developing new measures of spatial learning and navigation accuracy. Future studies could also adopt a multi-modal cognitive imaging approach, including dMRI and fMRI, to investigate further the relationship between white-matter tracts and the functional response of the structures that form the extended network. Our findings suggest that spatial processing recruits a large-scale distributed network, the exact contribution of each structure to the formation of spatial representation, however, still needs to be clarified. For example, evidence from animal research suggested that the cingulum bundle, which connects diencephalic regions to the temporal lobe (Aggleton & Brown, 1999), might be critical for spatial learning (Warburton and Aggleton, 1998). Specifically, future studies could focus on the parahippocampal cingulum (Jones et al., 2013) and investigate whether there are associations with performance on the SOT and VRMt tasks in comparison to the relationships found with the fornix. Furthermore, more sophisticated imaging approaches could be applied to look at the biological mechanisms underpinning these
effects. For example, quantitative Magnetisation Transfer (qMT) and multi-component Driven Equilibrium Single Pulse Observation of T1 and T2 (McDespot) could be used to obtain measures of tracts myelin and investigate the relationship with behavioural performance at spatial tasks. The development of these techniques will allow to clarify which measure of tracts microstructure is a reliable predictor of performance at specific cognitive tasks.

5. General conclusions

This thesis provides further evidence about the critical role of white-matter in cognitive function showing that inter-individual variation in fornix microstructure is strongly associated with individual performance on spatial learning and orientation in healthy young participants. These findings support the theory of an extended brain network that underpins spatial processing and extends the growing body of literature demonstrating the contribution of MTL structures, and their key white matter connections, to non-mnemonic spatial tasks (Yonelinas, 2013). Specifically, this thesis showed that fornix microstructure could be considered a reliable predictor of orientation accuracy, which is based on egocentric processing, as well as spatial learning and navigation, which depends on allocentric spatial reference. By contrast, the involvement of the HC in these spatial skills requires further investigation, given a lack of association with HC volume and performance. This will help clarify the contribution of key brain regions, as well as their broader connectivity within the brain, in the formation and storage of different spatial representations.
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