UNIVERSITY OF CALIFORNIA, SAN DIEGO

Spatial Navigation and Memory: The Role of the Hippocampus and Neocortical Structures

A Dissertation submitted in partial satisfaction of the requirement for the degree Doctor of Philosophy

in

Neurosciences

by

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DEDICATION

I dedicate this dissertation to my family and friends. To my mom, dad, and sister Jordy- nothing that I have accomplished in my life would be possible without your love, support, guidance, and unwavering belief in me. A mi familia extendida- muchisimas gracias por todo su amor y apoyo por toda mi vida. To my friends across the country and around the world- thank you for your humor, support, and advice. To Sarah- thank you for all the music, books, and deep friendship you have brought to my life over the years. To Becca, Carrie, and Rachel- thank you for being my person(s) regardless of distance and time. To TRAMPS- thank you for being my support system and the best friends I could have imagined in graduate school. To Reidan, Jenny, and Sunil- thank you for being the people I could turn to during times both awesome and terrible. To my wide and wonderful support network, both online and IRL- thank you for helping me through the toughest and best times. To Dr. Griffin- thank you for giving me the experience and tools to point me on the path to this dissertation. To Amber and Marta- thank you for being a sounding board and a source of unwavering support in the lab. To Laura- thank you for welcoming me into the lab with open arms and being a rock throughout my time there. To Jena- thank you for being a mentor in every sense of the word, and for all you have taught me about being a better scientist, teacher, and person. To Bob- thank you for taking a chance on me and for standing by me through times both good and bad. You have taught me so much about science, mentorship, and how to push myself to be better.

EPIGRAPH

Nothing in neuroscience makes sense except in the light of behavior
~Gordon Shepherd

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ABSTRACT OF THE DISSERTATION

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by

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Doctor of Philosophy in Neurosciences

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Professor Robert E. Clark, Chair
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The hippocampus- the aptly named seahorse-shaped structure located in the medial temporal lobe of the human brain- has been central to the study of memory for over fifty years. Over this time period, memory research in humans and rodents has focused on the hippocampus' role in memory differently, with the human work focused on declarative memories for events and facts while rodent work has emphasized the spatial nature of memories. While these two views are not necessarily incompatible, there are still many open questions as to why the rodent hippocampus appears to rely upon spatial aspects of memory far more than its human counterpart.

This dissertation was designed to bridge these two traditions using a spatial navigation task, path integration, which was first described in both humans and animals by Charles Darwin. We approached this first by studying whether working memory could support path integration. Working memory is independent of the hippocampus; however, its capacity is limited by the amount and complexity of information needed for recall. If the paths the subjects needed to recall were short and simple, presumably working memory could support them. Indeed, we found that patients with hippocampal damage were able to path integrate as well as normal subjects. However, rats with hippocampal damage were impaired relative to controls regardless of path complexity.

This finding may reflect the limited capacity of rodent working memory; it may not be possible for rodents to path integrate without using long-term memory. Rats with lesions of the hippocampus and the medial prefrontal cortex (the region commonly associated with working memory) were tested on a variety of spatial and nonspatial memory tasks. We concluded that the hippocampus is needed for spatial memory in rodents not because it is dedicated to spatial processing, but because spatial tasks inherently exceed the capacity of rodent working memory.

These studies, in addition to a third study on extrahippocampal regions involved in the path integration, help to connect the declarative and spatial memory views of the hippocampus and provide a cross-species interpretation of how the brain helps us remember.

CHAPTER 1:

INTRODUCTION

More than almost any other cognitive process, memory is fundamental to who we are as human beings. Our memories of our past experiences form our sense of self and allow us to learn from our mistakes to enable our future survival. While memory is most commonly thought of as our conscious recall of these past events and experiences, it also is central to the processes of daily life, including such basic but critical activities as navigating to and from our home each day.

This work focuses on this most fundamental kind of memory, the ability to recall where we have been and how to return to a place of safety each day. Without this spatial memory, any creature, human or otherwise, would find it difficult to know where to find food, where to escape from a predator, or even where to safely sleep during the evening. There are still many open questions about the structures of the brain involved in spatial memory and how they work in concert with each other to form and recall a map of the surrounding environment.

The goal of this dissertation is to explore the relationship of the brain structures involved in a specialized kind of spatial memory, called path integration (or dead reckoning). The experiments use lesions of these brain regions and a variety of spatial and nonspatial tasks to determine under what conditions these

regions are necessary for successful spatial navigation, and when an animal can compensate using other intact brain structures. This work explores a new interpretation of how spatial memory is supported by the rodent brain, one that bridges two traditional but separate views of the relationship between space, the brain, and memory.

Fundamental divisions of memory

When memory is spoken of in common vernacular, it is usually in reference to what is known to scientists as "declarative memory", or memory for events and facts. Declarative memory has been associated with the medial temporal lobe (MTL) region of the brain since a patient know as H.M. underwent a resection of the MTL to mitigate his epileptic seizures. The procedure was successful in treating the patient's epilepsy, but left him with severe amnesia (Scoville and Milner 1957). H.M. was unable to form new memories for facts or events that came after the surgery (anterograde amnesia) and could not recall memories that happened in the time just before the surgery (retrograde amnesia). This characteristic disruption of both the formation and recall of declarative memories has become strongly linked to the functioning of the MTL and the hippocampal formation in particular (Squire et al 2004; Squire and Wixted 2011).

Despite his profound amnesia, H.M. still retained his personality, intellect, and some specific kinds of memory. He remained capable of performing routine functions that are now commonly classified as habit memory, and could learn new

motor skills with extensive practice, even if he could not recall ever performing these skills before (Squire et al 2004). These skill and habit memories fall under the category of "non-declarative memory", with the fundamental distinction that they are not impaired by injury of or disruption to the functioning of the MTL (Squire and Wixted 2011). The experiments in this dissertation focus on the anatomical and behavioral correlates of declarative memory in rodents. All subsequent discussions of memory are based upon the relationship between declarative memory and the MTL.

Working and long-term memory

Declarative memory itself is often divided into two further categorizations, working memory and long-term memory. Working memory (sometimes known as short-term memory) refers to information that is held in recall through active maintenance and effort (Squire and Wixted 2011). While it is commonly thought that the number of stimuli is what determines whether something can be recalled with working memory (most commonly cited as "7 +/- 2" objects), more recent work has demonstrated that the capacity of working memory is dependent upon the complexity of the stimuli involved (Unsworth and Engle 2007; Jeneson et al 2010). For simple stimuli such as numbers or words, working memory falls within the 7-9 object range; however, once the stimuli become more complex, working memory capacity falls to a range of 1-4 objects (Drachman and Arbit 1966; Eng et al 2005; Jeneson et al 2010; Jeneson and Squire 2012).

When the capacity of working memory is exceeded, long-term memory must be engaged for successful recall. Thus, long-term memory is required not just for memories separated by long periods of time but for those tasks or events that have a memory load too large for working memory to maintain on its own (Squire and Wixted 2011; Jeneson et al 2011). Similar to declarative and non-declarative memory, long-term and working memory can be additionally distinguished by their relationship to the structures of the MTL. Working memory is largely independent of the MTL and remains intact in patients with damage similar to that of H.M (Squire 2009; Jeneson and Squire 2012). By contrast, long-term memory is primarily reliant upon the MTL, particularly the hippocampal formation, and is disrupted in these patients (Squire 2009). Thus, the characteristic anterograde and retrograde amnesia seen in patients with MTL damage result from disruption of the processes that underlie the formation and recall of long-term, as opposed to working, memory.

Rodent studies of memory

In parallel to the study of the MTL and its role in declarative memory in patient populations is a line of research interested in how the hippocampus and surrounding structures process spatial information, particularly in rodents. This concept of spatial processing is based upon the discovery of place cells in the hippocampus of freely moving rats (O'Keefe and Conway 1978). These cells show a preference for specific locations in a testing environment, indicated by a significant increase in firing rate as the rodent passes through the location (O'Keefe and

Dostrovsky 1971). Many other spatially selective cell types, the most notable of which is the grid cell, have joined place cells as the fundamental units of spatial representation in the rodent brain. Grid cells are found not in the hippocampus but in the medial entorhinal cortex (MEC) which serves as the primary input to the dorsal hippocampus (Hafting et al 2005). Grid cells fire at multiple locations in the testing environment forming a hexagonal structure that repeats in a triangular pattern that tiles the testing arena (Moser et al 2008). The spacing and size of the grid cell field is organized based on the cell's location in the MEC. As cell recordings move from the dorsal to ventral regions of the MEC, the spacing and field size of grid cells increases, providing a decrease in specificity about the rodent's spatial location (Hafting et al 2005).

As the electrophysiological properties of these and other spatially selective cells have become more widely studied, the behavioral function of these cells has become a question of increasing focus. Of particular interest is the role spatially selective cells play in tasks of spatial cognition and memory. It has been proposed that these cells work in concert to provide the rodent with the knowledge of its specific location in space and of the path it has taken to reach that location (McNaughton et al 2006). While the firing of these cells often becomes linked with external locational cues present in the testing environment, place and grid cells can and do fire when only internally generated self-motion cues are available (Moser et al 2008). Thus, it may be that spatially selective cells in the rodent are fundamental to the process of spatial navigation, particularly when environmental cues are

absent. This process of using only internally generated self-motion to navigate through an environment is referred to as path integration; the experiments in this dissertation will primarily focus on the behavioral expression of this path integration process in the rodent.

Path integration

Path integration, or dead reckoning, was first suggested by Charles Darwin in 1873. Darwin described the ability of birds, insects, and humans to navigate complex routes with "neither a compass, nor the north star, nor any other such sign" to guide them (Darwin, 1873). Path integration behavior was initially observed experimentally in invertebrates, but was later found to be present in mammals as well (Mittelstaedt and Mittelstaedt 1980). It has since been well established that the hippocampus is critical for successful path integration in rodents (Whishaw et al 1997; Maaswinkel et al 1999; Save et al 2001). However, these studies have focused on the role of the hippocampus as a structure that processes space rather than as one critical for the function of long-term memory, leaving open the question of which of these roles is key for successful path integration.

The experiments in this dissertation were intended to answer this and other outstanding questions regarding the involvement of long-term and working memory in path integration, and which brain regions play a role in this task. The experiments were also designed to unify the two divergent views of the role of the MTL; the human literature that traditionally studies its role in formation and recall

of memory, and the rodent literature that primarily studies its role in spatial navigation and cognition.

Working memory, long-term memory and path integration

The first study of this dissertation, presented in Chapter 2, examined the performance of human and rodent subjects on a path integration paradigm. Previous path integration literature assumed that rats with lesions of the hippocampus are impaired at this task due to the structure's critical role in spatial cognition and processing, an idea supported by computational models that suggest that place cells are essential for normal spatial navigation. The performance of the animals on this task was usually measured based on the vector of the rat's return path after finding the food reward, and all trials were analyzed together regardless of length or complexity.

The lack of analysis based on trial difficulty in these previous experiments leaves open the question of whether the hippocampus is required for path integration because of its role in spatial navigation or its role in long-term memory. Given a trial that takes a very short time to complete and involves no turns, it could be possible that a rat with a hippocampal lesion could successfully path integrate using working memory, which remains intact despite the lesion. However, once the trials become longer and more complex, working memory capacity would be exceeded and long-term memory would be required to support performance, leading to impaired performance by the hippocampal lesion animals.

The idea that working memory could support path integration has been tested once before in our lab using patients with damage to the MTL. Subjects were blindfolded and navigated along a path of predetermined length and complexity. Once they arrived at the end of the path, they were asked to point towards their original starting location. Subjects with lesions of the hippocampus and the full MTL performed as well as age matched controls on this task, regardless of path complexity, suggesting that humans are capable of path integration using working memory (Shraeger et al 2008). However, this task differs substantially from the paradigm usually used to study path integration performance in rodents, in which the subject, rather than the experimenter, determines the path length.

The study in Chapter 2 examines the performance of both humans and rodents on analogous path integration tasks to determine if either species can path integrate successfully within the confines of working memory. The path integration paradigm used with human and rodent subjects were designed to be as functionally similar as possible to allow for direct comparison of performance on the tasks. The paradigms were also designed such that the outward paths that the subjects took before returning to the start location could be closely tracked and measured. We were then able to analyze trials based on the distance (meters), complexity (90° turns), and amount of time (seconds) taken on this outward path. If working memory is sufficient to support path integration performance, patients and rodents with hippocampal damage would presumably perform as well as controls on paths that are short (<1 m), quick (0-3 sec), and straight (0 turns). This study confirmed

the findings from Shraeger et al., namely that humans are capable of path integrating as well as controls despite hippocampal damage, but rodents are impaired on path integration regardless of the simplicity of the outward path. We suggest that this is may be because rodent working memory capacity is more limited than that of humans, and cannot support the information needed for successful path integration, thus requiring the assistance of the hippocampus and long-term memory.

Medial prefrontal cortex and spatial working memory

The second study of this dissertation, presented in Chapter 3, was designed to follow-up on the conclusions from the experiments in Chapter 2; namely, whether the information needed to path integrate successfully will always exceed working memory capacity. This study involved a series of experiments examining the role of the medial prefrontal cortex and hippocampus in spatial and nonspatial memory tasks.

Studies of the role of the prefrontal cortex and its role in working memory were first done in non-human primates using visuospatial delayed response tasks (Goldman-Rakic 1980). The dorsolateral region of the prefrontal cortex (DLPFC) has been of particular interest, as inactivation of this subregion has produced similar impairments in visuospatial working memory tasks to those seen in earlier, larger lesions of the prefrontal cortex (Goldman-Rakic 1980). Recordings of single neurons in the DLPFC have found that a large proportion of neurons show an increased firing

rate during the delay period of the task. This delay-period response is seen only on trials in which the animal chooses correctly. On trials in which it makes an error, the DLPFC neurons show no increased firing during the delay period (Funahashi 2006).

The anatomy of the rodent prefrontal cortex is not entirely analogous to that of primates. One cannot assume that the dorsolateral region of the rodent prefrontal cortex will also be crucial for the maintenance of information during working memory. In fact, anatomical and behavioral studies have found that the medial prefrontal cortex (mPFC) of the rat, particularly the pre- and infralimbic regions, is closest in structure and function to the primate DLPFC (Kesner 2000). Rats with lesions of the mPFC show a profound impairment on several spatial working memory tasks, of which the most commonly used is spatial alternation- a task in which animals must alternate between entering the left and right arms of a T or radial arm maze (Granon and Poucet 1995; Kesner et al 1996; Raggozino et al 1998). While many neurons in the rodent mPFC show increased firing rates during delay periods, only a small percentage of these are reflective of correct-incorrect responses (Chang et al, 2002). Instead, ensembles of neurons show increased firing rates during this delay period and can be decoded to predict the goal choices of the rodent during a working memory task (Baeg et al, 2003).

The first set of experiments in Chapter 3 examines the performance of animals with lesions of the mPFC on path integration. These animals were also tested on the spatial alternation task in order to confirm the presence of a working memory deficit. These tests allowed us to further examine whether long-term

memory is indeed being used to support path integration performance, even at the shortest delays.

Medial prefrontal cortex, hippocampus, and nonspatial working memory

In the primate, nonspatial working memory has been well studied, and damage to the inferior aspect of the DLPFC has been associated with impairment of object working memory without a spatial component (Levy and Goldman-Rakic 2000). By contrast, the vast majority of rodent working memory tasks have a spatial component. Comparatively few studies have been done investigating nonspatial working memory tasks and the rodent mPFC. A few studies have found an impairment in mPFC lesion rats on a delayed non-matching to sample (DNMS) task that required animals to remember visual rather than spatial information within a time frame that could be supported by working memory (Kolb et al 1994, Kesner et al 1996). Another study used an odor-guided continuous DNMS design to test the effect of orbitofrontal cortex lesions on nonspatial working memory, although this task design has not been repeated using animals with mPFC lesions (Otto and Eichenbaum 1992). However, the DNMS task has considerable procedural differences as compared to spatial alternation that makes it more difficult to compare the two tasks. The most notable difference is the inherent difficulty of learning the DNMS task (Kinnavane et al 2015).

The role of the hippocampus in nonspatial working memory has been better documented in the literature. Despite robust impairment on spatial working

memory tasks (such as spatial alternation), rodents with lesions of the hippocampus are capable of using working memory to perform nonspatial tasks, including object and odor DNMS (Aggleton et al 1986, Dudchenko et al 2000, Clark et al 2001). Interestingly, the Dudchenko study included a test of both odor and spatial span in which the experimenters determined how many odors or locations a normal rodent could recall when presented sequentially. While normal rodents could recall an average of 8 sequential odors, they could only successfully recall an average sequence of fewer than 5 spatial locations. Thus even with access to long-term memory, the successful recall of spatial information is limited compared to that of nonspatial information. Accordingly, it would stand to reason that working memory capacity for spatial information would be similarly reduced.

While it has generally been assumed that all spatial working memory tasks require the hippocampus, this may not be entirely accurate. One study used two tasks, testing working memory for either spatial response or spatial location, to compare and contrast the effects of hippocampal and mPFC lesions (Kesner et al 1996). In the spatial response task, animals needed to recall whether they had made a left or right turn into the open arm during the sample phase and repeat that turn response, regardless of the location of the arms used within the radial arm maze. In the spatial location task, animals needed to reenter the same arm used during the sample phase, which requires a highly specific memory of that arm's location within the maze. Animals with lesions of the mPFC were impaired on both of these tasks, but those with hippocampal lesions were impaired only on the spatial location task.

On the spatial response task, which requires the maintenance of less complex information to complete successfully when compared to the spatial location task, animals with full hippocampal lesions performed as well as controls. Thus, it may not be that the hippocampus is required for spatial working memory tasks because it is an inherently spatial task, but because the amount of spatial information that must be maintained to successfully complete these tasks exceeds rodent working memory capacity.

The second half of the experiments in Chapter 3 were designed to explore the role of the mPFC and hippocampus in a nonspatial working memory task. Rather than using the DNMS paradigm as in earlier studies, we created a novel nonspatial working memory task ("odor alternation") that more closely mimics the spatial alternation task. Rather than alternating between selecting left and right turns in a maze, rats learned to alternate digging in cups of sand scented with cocoa or cinnamon to receive a food reward. Both scents were presented simultaneously, and the side that the reward was presented on was pseudorandomnly determined. By presenting the scent sets in three locations spaced throughout a circular arena, rats were required to rely on their memory of the scent that was rewarded on the previous trial to be successful on the task, rather than using the spatial location of the reward. By creating a nonspatial working memory task that closely mimics spatial alternation, we were able to directly compare the working memory deficits following mPFC and hippocampus lesions. While lesions of the mPFC impaired performance on both spatial and odor alternation, rats with lesions of the

hippocampus performed as well as controls on odor alternation, despite a robust impairment on the spatial alternation task. We suggest that this is not because the hippocampus is a structure primarily concerned with the processing of spatial information, but because the amount of information needed for successful performance on spatial tasks exceeds rodent working memory capacity, while that needed for nonspatial tasks does not. Thus, the rodent hippocampus may be critical for spatial memory because it must always be supported by long-term memory.

Medial entorhinal cortex and spatial navigation

The third study of this dissertation, presented in Chapter 4, examined the role of two extrahippocampal regions, the medial entorhinal cortex (MEC) and parietal cortex, that are believed to play a role in the path integration network. The first of these, the MEC, is not far removed from the hippocampus; indeed, the MEC is a division of the entorhinal cortex, which serves as the primary input area for the hippocampus (Eichenbaum and Lipton 2008). The MEC is of particular interest for studies of spatial navigation as it is the region where grid cells (the previously mentioned spatially selective cells that fire in a regular and distinct pattern across an environmental space) are primarily located (Hafting et al 2005). The presence of grid cells and other spatially selective cells (including head direction and border cells) in the MEC have marked it as a primary candidate for the role of the "path integrator"; that is, the theoretical structure which integrates angular and self-

motion cues to provide the animal with continual updates about its previous path and current location in space (Etienne and Jeffery 2004; Moser et al 2008).

Despite the centrality of the MEC for theoretical and electrophysiological studies of spatial processing, very few studies have examined the specific role of the MEC in spatial memory and cognition. Early experiments found that lesions of the full entorhinal cortex had little to no effect on tasks of spatial memory, which was in marked contrast to the severe impairment on these tasks after lesions of the hippocampus (Pouzet et al 1999; Bannerman et al 2001; Jarrard et al 2004). These results are puzzling given that any sensory information used to complete these memory tasks must be transmitted to the hippocampus via the entorhinal cortex, accordingly, entorhinal lesion would deprive the hippocampus of almost all the input needed to form a memory. However, these early lesions appeared to primarily damage the ventral aspect of the entorhinal cortex while leaving much of the dorsalcaudal aspect of the MEC intact (Van Cauter et al 2012). This distinction is critical, as this dorsocaudal region is where the most precise grid cells are found. Thus, the hippocampus of the animals in these studies could receive considerable spatial information from this spared region. Indeed, animals exhibit a spatial memory deficit comparable to that seen with hippocampal lesions when they receive lesions targeted to the grid cell areas of the MEC (Hales et al 2014).

Parietal cortex and spatial navigation

The second extra-hippocampal region of interest in the third study, the parietal cortex, is among the neocortical regions known to provide spatial sensory information to the MEC, particularly the posterior aspect of the association cortex (Eichenbaum and Lipton 2008; Save and Poucet 2009). Originating from studies of the deleterious effects of posterior parietal lesions on spatial vision in non-human primates, the role of the parietal cortex in spatial cognition and its relationship with the structures of the MTL has become a topic of increasing interest in the rodent literature. Despite this increased attention, the specific aspects of spatial navigation controlled by the posterior parietal cortex (PPC) are still relatively unclear.

Studies of PPC lesions in the watermaze, the benchmark task of spatial memory, have generated mixed results. Several studies have found severe impairments in watermaze performance, though whether this impairment is found regardless of pre-surgical training (DiMattia and Kesner 1988) or limited to when acquisition of the task occurs post-lesion (Hoh et al 2003) is still unclear. Others have suggested that watermaze performance in animals with parietal lesions is dependent upon the location of the environmental cues available during the task. Some groups have reported that when only proximal landmark cues are available, animals with parietal lesions are impaired on the watermaze relative to controls; by contrast, performance for these animals is only mildly affected when distal cues can be used in the task (Kolb and Walkey 1987; Save and Poucet 2000).

The few experiments that have looked at parietal cortex involvement in path integration have found that lesions of the parietal cortex impair the accuracy of the

animal's return path, though this impairment is milder than those found in hippocampus lesion animals on this task (Save et al 2001). Interestingly, though parietal lesion animals have been reported to use an inefficient "looping" search strategy when looking for the hidden platform in the watermaze, it has not been reported whether this search strategy is seen in the return paths of parietal lesion animals in the path integration task (Kolb and Walkey 1987).

The experiments in Chapter 4 were designed to confirm and compare the effects of lesions of the MEC and the parietal cortex on the path integration task. Additionally, a MATLAB script was designed to determine whether these lesions affected the efficiency of the animals' return paths, even when the task was performed successfully.

Summary

Although the critical role of the hippocampus and its surrounding structures in memory has been well known for several decades, there has been a divide in how the human and rodent literature has characterized hippocampus-dependent memory. While the human literature traditionally focuses on the importance of the hippocampus for the encoding and recall of long-term declarative memories, the rodent literature has emphasized the hippocampus' role in spatial processing and cognition. The studies in this dissertation were designed to try to connect these two traditions, as well as to better understand the role of extra-hippocampal regions in spatial navigation under different conditions. The findings have suggested that the

rodent hippocampus is necessary in spatial tasks not because of their spatial nature, but because of the limited capacity of rodent working memory. This interpretation is consistent with the understanding of the nature of declarative memory in the human hippocampus, as well as with increasing evidence that the rodent hippocampus is critical for processing all spatial and nonspatial aspects of memory events.

CHAPTER 2:

CONTRASTING EFFECTS ON PATH INTEGRATION AFTER HIPPOCAMPAL DAMAGE IN HUMANS AND RATS

Abstract

The hippocampus and other medial temporal lobe structures have been linked to both memory and spatial cognition, but it has been unclear how these ideas are connected. We carried out parallel studies of path integration in patients with medial temporal lobe lesions and rats with hippocampal lesions. Subjects entered a circular arena without vision, searched for a target, and then attempted to return to the start location. Patients performed accurately, and as well as controls, so long as the outward path was relatively direct and the target was found within 20 sec. In sharp contrast, rats with hippocampal lesions were impaired, even when the outward path was shorter than 1 m, involved no turns, and the target was found within 3 sec. We suggest that patients succeeded because performance could be supported by working memory and that patients and rats differ after hippocampal lesions in their ability to construct a coherent working memory of spatial environments.

Introduction

Two ideas have been central to recent discussions about the function of the hippocampus and other medial temporal lobe (MTL) structures. One idea emphasizes the role of these structures in memory (Squire and Wixted, 2011; Squire, 1992; Eichenbaum and Cohen, 2001), and the other emphasizes their role in spatial cognition, including spatial navigation and path integration (O'Keefe and Nadel, 1978; Whitlock et al, 2008; McNaughton et al, 2006). Path integration refers to the ability to use self-motion cues as one moves through space in order to keep track of a reference location (Etienne and Jeffery, 2004; Benhamou, 1997). These two ideas are compatible with each other to a large extent, because path integration requires memory, but there is potential mismatch as well and it has been unclear how the two ideas relate to each other.

Discussion of the MTL and memory typically draws a fundamental distinction between working memory and long-term memory. Working memory (the limited amount of information that can be held in mind by active maintenance) is thought to be independent of the MTL and spared after MTL damage (Drachman and Arbit, 1966; Jeneson and Squire, 2012; Baddeley and Warrington, 1970; Milner, 1972), whereas long-term memory is impaired (Squire et al, 2004). One might therefore expect that path integration should be intact after MTL damage whenever performance can be managed within working memory. In one study (Shrager et al, 2008a), memory-impaired patients with bilateral damage to the hippocampus or

adjacent MTL structures were able to path integrate as well as controls in conditions when working memory likely supported performance (i.e., for paths involving only one or two turns and trial durations shorter than 35 sec). In this study, however, the procedure was quite different from the standard methods traditionally used to test path integration in experimental animals.

Discussions about path integration in rodents emphasize the possible role of hippocampal place cells and entorhinal grid cells in computing information about spatial location (Whitlock et al, 2008; McNaughton et al, 2006). If MTL structures are needed to carry out the computations needed for path integration, then MTL damage should impair path integration even in the case of short paths and short trial durations. That is, in the case of path integration, the distinction between working memory and long-term memory might be irrelevant. Most studies of path integration after hippocampal or entorhinal damage in rats have found impairment (Maaswinkel et al, 1999; Whishaw et al, 2001; Save et al, 2001; Parron and Save, 2004; but see Alyan and McNaughton, 1999). Yet, it is notable that none of these studies reported how long it took to complete the trials. Accordingly, it remains possible that the animals in these studies might have performed well whenever trials were accomplished quickly, because in those instances performance might have been supported by working memory.

To address these issues, we carried out parallel experiments of path integration in humans and rodents. In both experiments, subjects searched for a target in a circular arena in the absence of vision and then tried to return to the start

location. We assessed the accuracy of path integration as a function of three different measures: the distance traveled on the outward path, the time needed to find the target, and the number of turns taken on the outward path.

Materials and Methods

Experiment 1

Participants

Five memory-impaired patients participated (Table 1), four with bilateral lesions thought to be limited to the hippocampus (CA fields, dentate gyrus, and subicular complex) and one with larger medial temporal lobe lesions. Patients GW and DA became amnesic in 2001 and 2011, respectively, following a drug overdose and associated respiratory failure. Patient KE became amnesic in 2004 after an episode of ischemia associated with kidney failure and toxic shock syndrome. Patient LJ (the only female) became amnesic in 1988 during a 6-month period with no known precipitating event. Her memory impairment has been stable since that time. Estimates of medial temporal lobe damage were based on quantitative analysis of magnetic resonance (MR) images from 19 age-matched, healthy males for GW, KE, and GP, 8 younger healthy males for DA, and 11 age-matched, healthy females for patient LJ (Gold and Squire, 2005). GW, KE, LJ, and DA have an average bilateral reduction in hippocampal volume of 48%, 49%, 46%, and 35%, respectively. All values are more than 2.9 SDs from the control mean. On the basis of

two patients (LM and WH) with similar bilateral volume loss in the hippocampus for whom detailed postmortem neurohistological information was obtained (Rempel-Clower et al, 1996), the degree of volume loss in these four patients likely reflects nearly complete loss of hippocampal neurons. The volume of the parahippocampal gyrus includes temporopolar, perirhinal, entorhinal, and parahippocampal cortices. GW, KE, LJ, and DA have an average bilateral reduction in the volume of parahippocampal gyrus by 10%, 11%, -17%, and -5%, respectively (all values within 2 SDs of the control mean). The volumes for parahippocampal gyrus differ a little from the volumes reported previously for these patients and are based on newly published, more detailed guidelines for identifying the caudal border of the gyrus (Frankó et al, 2012).

One patient (GP) has severe memory impairment resulting from viral encephalitis. GP has demonstrated virtually no new learning since the onset of his amnesia, and during repeated testing over many weeks he does not recognize that he has been tested before (Bayley et al, 2005). GP has an average bilateral reduction in hippocampal volume of 96%. The volume of the parahippocampal gyrus is reduced by 94%. Eight coronal magnetic resonance images from each of the five patients are available as supporting information (Figure S2).

Eleven healthy volunteers also participated (three females; mean age, 61.3 years; range = 25-76 years; mean education, 14.8 years). All procedures were approved by the Institutional Review Board at the University of California at San Diego, and participants gave written informed consent prior to participation.

Apparatus

The experiment was carried out in an indoor circular space (4 m diameter). A string was laid out on the floor and marked every 5 degrees to describe the perimeter of the circle (arc length = 17.4 cm). Participants wore a blindfold and noise-cancelling earphones, and a walker was provided for safety.

Path Integration

The task was to start from one of eight equidistant locations on the perimeter of the circle, find a square tile (19 cm) placed on the floor within the circle, and return to the start location. On each trial, the tile was equally likely to be within one of the six 45° segments of the circle that were most remote from the start location. In addition, the tile could be in any of four positions along a radius within a segment: near the origin, 0.75 m from the origin, 1.5 m from the origin, and near the perimeter. Of these 24 possible tile locations, 16 different locations were selected for each session.

Participants could detect the tile with their feet or with the wheels of the walker. If the participant reached the perimeter of the circle while searching for the tile, he or she was guided back into the circle. If the participant could not locate the tile within 5 min (on 2.3% of the trials), he or she was guided to the tile and then allowed to return to the start point. Participants were instructed to actively maintain the start location in mind as they proceeded, so that they could be successful at returning to the start point. Immediately after completing each trial,

participants provided a rating (1 to 5) to indicate their confidence that they had returned to a point within one arm's length of where they had started from.

Two practice trials were given, first without the blindfold and then with the blindfold. Sixteen trials (two from each start location) were then given in which the blindfolded participant searched for the tile and then tried to return to the start location. Controls were tested in a single session. Patients were given two sessions separated by1-10 weeks (2 practice trials, 16 test trials, and confidence ratings). To confirm that participants were indeed relying only on self-motion cues rather than using external cues beyond experimental control, four rotation trials were also given after the first session (14). For the rotation condition, participants were led from a start location directly to a platform without making any turns (average duration, 5.3 sec). After stepping onto the platform, participants were slowly rotated 190° by a remotely controlled motor (~14°/sec) and then tried to return to the start point. If participants were relying only on self-motion cues and were unable to use external cues, their performance should be disrupted by the rotation. After the rotation condition, participants were asked 10 factual questions (4 free recall, 6 true-false) to assess their memory for the entire test session. The ten factual questions about the testing session yielded a score from 0 to 10. Chance performance was estimated to be 35 percent.

During testing, one experimenter followed the participant with a measuring wheel to record the distance traveled on the outward path. Another experimenter traced onto a map of the arena the path taken by the participant and also recorded

the time taken to find the tile. Two raters independently recorded the point on the perimeter to which participants returned (mean inter-rater error = 1.9°). In addition, the number of turns taken on the outward path (changes in heading direction ≥ 90°) was later counted by two raters, based on the drawings (mean inter-rater error = 0.3 turns).

Data Analysis

The accuracy of the return path (absolute difference in degrees between the return location and the start location) was measured as a function of the distance traveled on the outward path (0-2, 2-4, 4-6, 6-8, 8-10, > 10 m), the time needed to find the tile (0-10, 10-20, 20-30, 30-40, 40-50, > 50 sec), and the number of turns taken on the outward path (0, 1, 2, 3, 4, > 4 turns). Participants distributed their trials rather evenly across these values, and a minimum of 7 observations contributed to each of the 18 bins (3 measures × 6 bins). Also, of the 11 controls, 9.4 on average contributed scores to each of the 18 bins. Of the 5 patients, 4.8 on average contributed scores to each of the 18 bins.

Experiment 2

Subjects

Subjects were 18 male Long Evans rats weighing between 300-350g at the beginning of the study. Rats were individually housed and maintained on a 12:12 hr light:dark cycle and tested in the light phase of the cycle. Six rats were used to verify

that visual cues could not be used to guide performance (Vision Test). Five rats were prepared with complete hippocampal lesions (H group), and 7 rats served as controls (CON). All procedures were approved by the University of California, San Diego IACUC.

Apparatus

The apparatus was a 2 m diameter circular Plexiglas table painted white and elevated 64 cm above the floor. Eight (12 cm diameter) holes were placed equidistant around the perimeter with centers 13 cm from the edge. Start boxes attached below each of the holes were filled with used rodent bedding to distribute odor cues. A movable mesh screen could block access to and from the boxes. The apparatus was mounted on a central bearing that allowed it to be rotated. In addition, a fixed central platform (45.5 cm in diameter) was mounted flush to the table surface. In this way, the main table could be rotated while a rat on the central platform remained stationary. The apparatus could be illuminated by fluorescent lights and could also be insulated from visible light. A camera mounted above the center of the table and attached to a video tracking system (Smart Tracking, San Diego Instruments) allowed animals to be tracked in the dark by an infrared camera with the aid of infrared lights.

Path Integration

Preoperative Training

Pretraining began after rats were food deprived to approximately 80% of their free-feeding weight. First, rats explored the illuminated table for 10 min with no food present and all boxes blocked. After two days of exploration, five food pellets (750 mg rodent pellets, Bio-Serv, Frenchtown, NJ) were placed on the table, and rats were given 10 min to eat three or more pellets. After a rat had done this on two consecutive days, similar trials began with the rat inside a start box. After a rat completed three trials within 10 min for two consecutive days (exit start box, return with food to start box), training then continued with the lights off. After two successful days in the dark (three or more pellets eaten within the time limit), the final phase of training was introduced. In this phase (4 trials/day), the rat was required to exit a start box in the dark, locate a single pellet on the table, and return to the same box (all other box entrances were blocked). Preoperative training was complete when a rat successfully completed 4 trials in a day on two consecutive days (5-min time limit). On average, pretraining required 18 days.

Surgery

Surgical methods for removing the hippocampus bilaterally are described in *SI Materials and Methods.*

Post-Operative Testing

Rats were given four standard trials (see below) and one odor probe trial each day until they accumulated 50 standard trials. All trials were conducted with the lights off and with a 5-min time limit. Trials were discarded if the rat consumed the food on the testing table rather than returning to the start box (this occurred on fewer than 5% of the trials). The food pellet could be located in any of 12 locations distributed across the table. The order in which these locations were used was determined pseudorandomly. In addition, each start box was equally likely to be used each day. No box was repeated until all eight boxes were used. The table was rotated between trials, and each start box was equally likely to be placed in each of 8 possible locations in the testing room.

Standard Trials

For the first 4 trials of each day, rats were placed in a start box with a food pellet placed on the table. The trial began when the rat left the start box and ended when the rat located the food and returned to the open start box. The rat was allowed to eat the pellet in the box before being removed.

Data Analysis

Performance was measured by how accurately the rat returned to the start box after locating the food. The animal could return to the start box itself (a score of zero), one of the boxes 45° on either side of the start box (a score of 1), one of the boxes 90° on either side of the start box (a score of 2), one of the boxes 135° on either side of the start box (a score of 3), or the box that was 180° from the start box

(a score of 4). We also included a second performance measure (Percent Correct). This measure referred to the proportion of trials in which the rat returned to the start box before visiting any other boxes.

Standard Trials

Performance accuracy (score of 0-4) was recorded as a function of the distance traveled on the outward path (0-1, 1-2, 2-3, 3-4, 4-5, > 5 m), the time needed to find the food (0-3, 3-6, 6-9, 9-12, 12-15, > 15 sec), and the number of turns taken on the outward path (0, 1, 2, 3, 4, > 4 turns). Rats distributed their trials across these values, and a minimum of 20 observations contributed to each of the 18 bins (3 measures × 6 bins). Also, all 7 control rats and all 5 rats with hippocampal lesions contributed scores to each of the 18 bins.

Results

Experiment 1: Path Integration in Humans

Overall performance across all trials was similar for the two groups (controls = 51.6 ± 4.2° error; patients = 57.8 ± 5.4° error; t [14] = 0.9, p > 0.1). Both scores were better than chance (90°)(ps < 0.05). To assess variability in individual performance, the standard deviation (SD) of each participant's return scores was also calculated, and the individual SDs were then averaged for each group. These

scores (68.6 ± 5.3° for controls and 77.2 ± 2.7° for patients) indicated that the two groups exhibited a similar dispersion in their return paths (t [14] = 1.0, $p > 0.1$).

For both groups, the accuracy of the return path was better and well above chance levels when the distance traveled on the outward path was short (Figure 1A), when the tile was found quickly (Figure 1B), and when only a small number of turns were taken on the outward path (Figure 1C). The two groups performed similarly according to each of the three measures and at every bin size (all ps > 0.15 with two exceptions; at 3 turns, Figure 1C, $p = 0.07$; at 20 sec, Figure 1B, $p = 0.08$). For both groups, the accuracy of the return path gradually declined to chance levels as participants had more difficulty finding the tile. Even for controls, performance approached (or reached) chance levels when the outward path was > 8 m, when > 30 sec was needed to find the tile, and when > 1 turn was taken on the outward path.

In the rotation condition, participants were unable to return accurately to the start location (Figure 1A). Because the perceived direction heading was shifted systematically by rotation, accuracy was even worse than chance levels (ps < 0.05). There was no difference between groups (t [14] = 0.9, $p > 0.1$). This result confirmed that participants were relying on self-motion cues to accomplish the task and did not have available other external cues.

Both groups demonstrated a gradual and similar decline in their confidence about the accuracy of the return path as the time taken to find the tile increased (Figure S1). The results were the same when confidence ratings were plotted as a

function of distance traveled or number of turns taken. For the ten factual questions asked at the end of the test session, controls averaged 8.0 correct answers. Patients performed more poorly than controls, averaging only 5.0 correct answers ($p = 0.01$). Thus, despite the fact that the patients performed as well as controls at path integration, their memory for the test session itself was markedly impaired.

Experiment 2: Path Integration in Rats

Behavioral Findings

Vision Test

Rats took substantially more time to locate the object during dark trials than during light trials (43.9 ± 3.2 sec vs. 8.1 ± 1.0 sec; t [5] =13.3, $p < 0.01$). These findings indicate that rats were unable to use visual cues to guide their performance.

Odor Test

Control rats tended to return to the original start box location more often than to the displaced start box and its associated odor trail and more often than to the box opposite the odor trail (18.3 ± 1.8% vs. 8.9 ± 1.6% and 10.0 ± 2.0%; ps < 0.05). This finding indicates that rats used path integration rather than odor trails to return to the open box. The H group did not discriminate among the three locations.

Standard Trials

The control group performed best when the distance traveled to the food was short (Figure 2A), when the food was found quickly (Figure 2B), and when only a small number of turns were taken on the outward path (Figure 2C). The accuracy of the return path declined as animals traveled further to find food, took more time, and made more turns. The animals with H lesions performed differently. For the H group, performance was poor even when rats traveled short distances to find food, when they found food quickly, and even when they made no turns. Specifically, compared to its control group, the H group was impaired when rats traveled 2 m or less on their outward path, when they took 6 sec or less to find food, and when they made zero or one turn (Figure 2; *t*s [10] > 2.5, *p*s < 0.05). For longer distances, longer times, and greater number of turns, both groups performed poorly. Note that an animal performing entirely randomly should be expected to achieve a score of 2 on this task.

We also calculated how often rats returned to the correct start box before visiting other boxes (percent correct). The H group was impaired relative to its control group for 7 comparisons (for 0-1 and 1-2 m; for 0-3, 3-6, and 6-9 sec; and for zero and one turn). For these 7 comparisons across all 3 measures, controls averaged 47.8% correct choices, and the H group averaged 12.9% correct choices (*t*s [10] > 2.4, *p*s < 0.05).

Neurohistological Findings

Figure 3 shows reconstructions of coronal sections through the hippocampus of the lesion group. Numbers (right) represent the distance posterior to Bregma. All lesioned rats sustained bilateral damage to all cell fields of the hippocampus. The damage included 85% to 97% of the hippocampus (mean = 93%). Sparing occurred most frequently to the most medial aspect of the dorsal dentate gyrus and the dorsomedial CA1 cell field. The ventral-most region of the hippocampus was also spared in some animals. In all rats, there was some damage to the cortex and to the fimbria overlying the dorsal hippocampus, which was associated with the placement of the syringe during surgery and with spread of neurotoxin up the needle track. Two rats had minor damage to the posterior aspect of the lateral entorhinal cortex and posterior subiculum. There was no evidence of damage to the amygdala or thalamus in any animal. Figure S3 shows histological images at three A-P levels for each rat.

Discussion

In two experiments, one with humans and one with rats, we assessed the capacity for path integration after bilateral damage to the hippocampus. In both studies, subjects entered a circular arena in the absence of vision, searched for a target, and then attempted to return to the start location at the perimeter of the arena. Experiment 1 demonstrated that patients with lesions to the hippocampus or larger MTL lesions returned to the start location accurately, and as well as controls,

so long as the distance traveled on the outward path was short, the target was found quickly, and when only a small number of turns were taken on the outward path (Figure 1). Patient and control groups also made similar confidence judgments about the accuracy of their returns (Figure S1). Performance of both groups approached chance levels as participants had more difficulty finding the target. A control condition, in which path integration was disrupted by rotation, confirmed that performance depended on self-motion cues and not on other cues beyond experimental control (Figure 1A). Lastly, despite the fact that path integration was intact when the path was short and direct, patients were impaired after the session at remembering facts about the tasks they had just completed.

It is often reported that controls outperform patients as a task becomes more difficult and as the material to be remembered comes to exceed what can be supported by working memory (see Figure 3 in Jeneson and Squire, 2012). In that situation, controls can draw on their long-term memory, but patients cannot. In the present case, however, controls never outperformed the patients. Instead their scores approached chance levels as the task became more difficult. It appeared that once participants traveled a sufficient distance and made a number of turns, they became lost. Working memory could support performance up to a point, but beyond that point it was not possible to transfer accurate information into long-term memory, presumably due to the interfering effects of additional distance, time, and turns, and the accumulation of errors. There is precedent for this idea that memory can be vulnerable to interference during the seconds after learning such that little

long-term memory is formed. When humans or monkeys tried to memorize the pitch of a single tone or a synthetic sound, recognition accuracy deteriorated rapidly (within seconds) when intervening sounds were presented (Deutsch, 1970; Scott et al, 2012).

In sharp contrast to the findings for humans, Experiment 2 demonstrated that rats with complete hippocampal lesions were impaired at path integration relative to controls even when the outward path was shorter than 1 m, even when the target was found within 3 sec, and even when animals made no turns on the outward path (Figure 2). Both groups performed poorly for longer distances, longer times, and greater number of turns. Control conditions ruled out the relevance of visual or olfactory cues.

In earlier studies, rats with hippocampal lesions also exhibited impaired path integration (Maaswinkel et al, 1999; Whishaw et al, 2001; Save et al, 2001). However, performance was not evaluated as a function of the time required to accomplish each trial (or as a function of distance traveled or number of turns taken). Accordingly, it remained possible that rats might succeed when trials were completed quickly and the paths to the target were short and direct. The present study, however, demonstrated impaired path integration after hippocampal lesions, even on trials when rats took short, direct paths to the target that required only a few seconds.

We have considered two possible ways to understand these contrasting findings for humans and rats. One possibility is that humans and rats used different

strategies to accomplish path integration. For example, rats may have used self-motion cues exclusively, and the impairment after hippocampal lesions then reflected the failure of the hippocampus to carry out computations necessary for spatial navigation. Perhaps humans found an alternative way to accomplish the same task that did not require the specific contribution to the task that is supported by the hippocampus. While it is difficult to exclude this possibility, we cannot identify any particular novel strategy that participants used. Most participants simply described trying to visualize the environment and keep track of where they were (i.e., as if they were using self-motion cues). A few participants reported trying to count their steps, but these participants performed no differently than those who did not report counting. In any case, it is unclear how counting steps could aid performance, inasmuch as what is important to good performance is not only keeping track of the distance traveled but also the angles through which one moves. No participant reported performing verbal calculations for the turns that were made.

A second possibility turns on the organization of working memory in humans and rats. In an earlier study of path integration in patients with MTL damage (Shrager et al, 2008a), performance was also intact when path lengths and trial times were short. We supposed that performance in that case reflected the successful maintenance of spatial information within working memory. First, just as in the present study, participants were encouraged to hold actively in mind the paths they took as they moved, so that they might later be able to point to their start

location. Second, performance of patients was disrupted when efforts were made to interfere with the maintenance of working memory by introducing distraction. In the present case, we suggest that patients also relied on working memory to accomplish path integration when the path lengths and trial times were short. Working memory in humans is independent of the MTL and intact after MTL damage (Drachman and Arbit, 1966; Jeneson and Squire, 2012; Beddeley and Warrington, 1970; Milner, 1972; Shrager et al, 2008b).

If working memory can support path integration in patients with MTL lesions (so long as the path is simple), what accounts for the inability of rats with hippocampal lesions to path integrate even under the simplest of conditions? One possibility, which has been given little attention, is that rats may be limited in their ability to construct a coherent working memory of spatial environments. Under conditions where spatial working memory is effective, it is thought to depend importantly on medial prefrontal cortex (Fuster, 2008; Horst and Laubach, 2009; Kesner et al, 1996; Kolb et al, 1974; Le Marec et al, 2002). A related idea is that the medial prefrontal cortex works in collaboration with the hippocampus to accomplish spatial working memory (Hyman et al, 2010; Jones and Wilson, 2005; Wang and Cai, 2006; Lee and Kesner, 2003; Siapas et al, 2005). Specifically, successful performance has been related to synchronous activity of prefrontal neurons and hippocampal theta oscillations (see Hyman et al, 2011 for a review).

Thus, there are two ways that the organization of working memory in rodents could account for the effect of hippocampal damage on path integration.

First, poor path integration after hippocampal lesions may reflect a need to depend on long-term memory (because spatial working memory capacity in the rodent is limited). The situation would be analogous to patients with hippocampal lesions who are impaired at recalling 10 word pairs immediately after learning (Manns et al, 2003), because in humans remembering 10 word pairs exceeds the capacity of working memory. The point is that performance can depend on long-term memory even when memory is tested within seconds of learning (also see Baddeley et al, 2010), and performance after hippocampal lesions will be impaired within seconds after learning whenever working memory capacity is exceeded. Indeed, several studies have reported impairments in rats performing spatial tasks at short delays after hippocampal lesions: spontaneous or forced-choice alternation at delays of 0-5 sec (Gross et al, 1968; Racine and Kimble, 1965) and delayed-matching-to-position at delays of 1-10 sec (Kesner et al, 1996; Steele and Morris, 1999). Note though that for object recognition tasks, rats with hippocampal lesions have exhibited intact performance at short delays (and impaired performance at longer delays) (Clark et al, 2001; Clark et al, 2000). In any case, impairments at short delays in spatial tasks could reflect a need to depend on long-term memory.

A second possible reason for impaired path integration after hippocampal lesions is that performance may reflect an impairment of working memory itself. For example, the rodent hippocampus could contribute to spatial working memory by providing essential spatial information to prefrontal cortex. A potentially important difference between humans and rodents is that the human hippocampus,

in comparison to rodent hippocampus, makes a relatively weak contribution to cortical theta, and hippocampal and cortical theta are not reliably synchronized (Hyman et al, 2011). Thus, the interaction between the hippocampus and mPFC in rats may be more critical for working memory than it is in humans. Specifically, a hippocampal lesion in rats might be expected to have a larger effect on mPFC function than a hippocampal lesion in humans. If so, spatial working memory and long-term memory may not be as sharply distinguished in the rodent as in humans.

In summary, in tests of path integration fundamentally different findings were obtained after hippocampal lesions in humans and rats. The findings for humans may be understood in terms of the historic distinction between working memory and long-term memory and the idea that working memory is independent of MTL function. Specifically, path integration succeeded when the outward path was simple and direct and when the task could presumably be managed within working memory. In contrast, rats with hippocampal lesions failed to path integrate even under the simplest conditions (when they traveled less than 1 m within 3 sec and made no turns). We considered two possible ways to understand these data. First, humans may have found an alternative strategy for path integration that did not depend exclusively on self-motion cues, or a strategy different in some way from the spatial strategy thought to support path integration in the rat (and depend on the hippocampus) (Whitlock et al, 2008; McNaughton et al, 2006). Second, we suggest that rats may have failed path integration because (unlike humans) they are limited in the kind of information that can be supported by working memory. Thus,

after hippocampal lesions rat prefrontal cortex may be unable to construct a coherent working memory for spatial environments, either because the capacity of working memory is exceeded or because prefrontal cortex does not have input that it needs from hippocampus. The first of these alternatives, that working memory capacity itself might limit path integration performance in rats, is novel to the best of our knowledge and calls out for further study.

Acknowledgments

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CHAPTER 3:

THE EFFECTS OF MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPAL LESIONS ON SPATIAL AND NONSPATIAL MEMORY

Abstract

Working memory, the information that can be held in the mind with active maintenance, is independent of the hippocampus and medial temporal lobe (MTL), and limited by the complexity of the information being held. A recent study (Kim, Sapiurka et al) found that humans with damage to the MTL were capable of using intact working memory to support performance on path integration, a spatial navigation task. In contrast, rodents with lesions of the hippocampus were impaired at path integration, even on the simplest trials. We suggest that this may be because path integration exceeds the capacity of rodent working memory and that intact long-term memory is required to successfully path integrate. To investigate this, we tested rats with medial prefrontal cortex (mPFC) lesions on the path integration task, as well as on spatial alternation, a task that tests spatial working memory. If path integration requires intact long-term memory, mPFC lesions should have no effect on performance; indeed, mPFC rats performed as well as controls despite impaired performance on spatial alternation. We then compared performance on

these tasks to that of rats with hippocampal lesions, as well as on a novel task of nonspatial working memory; odor alternation. Rats with hippocampal lesions were impaired on both the path integration and spatial alternation tasks; however, they performed as well as controls on odor alternation, while mPFC lesion animals performed no better than chance. We suggest that the information needed for spatial memory exceed rodent working memory capacity; thus, the hippocampus is required in these tasks due to its role in long-term memory formation, rather than the inherent spatial nature of that task.

Introduction

The formation of declarative memory depends on the integrity of the hippocampus and related medial temporal lobe (MTL) structures (Squire, 1992; Eichenbaum, 2001). These structures have also been associated with spatial cognition, including spatial navigation and path integration (O'Keefe and Nadel, 1978; Moser et al, 2008). These two ideas are not entirely compatible (Eichenbaum and Cohen, 2014; Buffalo, 2015). The issue centers on the historic distinction between short-term (working) memory and long-term memory. Working memory refers to the limited amount of information that can be held in mind by active maintenance for a short time after learning (sometimes characterized as 7 digits, 4 objects, or 1 face). Working memory has been thought to be independent of the MTL and intact after MTL damage (Milner, 1972; Baddeley and Warrington, 1970;

Jeneson and Squire, 2012). If so, tasks that can be managed within working memory, including spatial tasks, should be spared after MTL damage. Yet, if MTL structures support the computations needed for spatial tasks such as spatial navigation and path integration, then MTL damage should impair performance on these tasks regardless of the availability of working memory. Indeed, for spatial tasks the distinction between working memory and long-term memory might be irrelevant.

Several studies of patients with MTL damage have found intact performance on spatial tasks, including path integration, under conditions when working memory appears to support performance (Shrager et al, 2008a; Shrager et al, 2008b; Jeneson et al, 2010; Kim, Sapiurka et al, 2013). However, a recent study of path integration in rats with hippocampal lesions obtained different results (Kim, Sapiurka et al, 2013). In path integration, rats search for food in the dark and then attempt to return to their start location. The finding was that rats performed at chance even with the simplest paths, e.g., when their outward path was only 1m length, involved no turns, and was completed in 3s. If working memory can support successful path integration in humans with MTL damage, what might account for the inability of rats with hippocampal lesions to path integrate even in the simplest conditions?

In the present study we considered the possibility that rats might fail to successfully path integrate because rodent working memory is not sufficient to handle the amount of information necessary to construct a coherent spatial

representation of the path. By this view, successful path integration in normal rats must be represented in long-term memory (LTM) and should not be disrupted by damage to structures thought to be important for working memory. Accordingly, we tested the idea that lesions of the mPFC, the structure most commonly associated with supporting working memory (D'Esposito et al., 1995; Fuster, 2008; Euston et al., 2012), would leave path integration intact because long-term memory would be available to support performance. For comparison, we also tested animals with hippocampal lesions on path integration. To evaluate how mPFC or hippocampal lesions would impact tests designed to evaluate working memory, we trained rats on two versions of an alternation task. The first task was the classic T-maze spatial alternation task where the rat learned to alternate left and right turns. This task is thought to require working memory and is sensitive to lesions of the mPFC and hippocampus (Sánchez-Santed et al., 1997; Le Marec et al., 2002; Horst and Laubach, 2012). We also developed a nonspatial alternation task where the rat learned to alternate between different odor-scented cups.

Experiment 1: Medial prefrontal cortex lesions, T-maze alternation and Path integration

Materials and Methods

Subjects.

Male Long Evans rats (n=35) were prepared with bilateral lesions of the medial prefrontal cortex (n=18) or served as sham-operated controls (n=17). Cohort 1 (lesion = 12, control = 11) was tested on spatial alternation and then on path integration. Cohort 2 (lesion = 5, control = 6) was tested on path integration and then on spatial alternation. In cohort 2, one lesion animal did not complete path integration. In addition, two lesion animals and 1 control animal did not complete spatial alternation. These animals were excluded from the analysis of the relevant tasks. Rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego, Institutional Animal Care and Use Committee.

Spatial alternation apparatus

A T-maze was constructed from wood and painted flat black. The stem of the maze was 52 cm long with a 20 cm start box and 32 cm runway. The left and right arms were 35 cm long. All the alleys of the maze were 10 cm wide, and the maze itself was 18 cm high. Plastic doors could be inserted to confine animals to the start box and to block entrance to either arm. A black plastic food cup (5 cm diameter) was held in place by Velcro at the end of each arm. The cup was 1.5 cm tall, enough to block the reward from view as the rat entered the arm.

Spatial alternation pre-training

During pre-training rats were maintained at ~80% of their free-feeding weight. Pre-training occurred over a five- day period (six trials per day). On the first three days, the rat was placed in the right or left arms six different times. One half of a Froot Loop was placed in a food cup at the end of the arm, and the rat had five minutes to consume the food. On the final two days, one arm was open, while the other arm was blocked off (forced-choice trial). The animal had five minutes to travel from the start box into the open arm to consume the food. The arm that was open on each trial was varied randomly to ensure that the rat spent equal time in each arm but did not learn any specific rule.

Surgical procedure

Bilateral excitotoxic lesions of the medial prefrontal cortex were made using ibotenic acid (IBO; Biosearch Technologies). Isoflurane gas (delivered in O_2 at 1 L/min) was used to maintain anesthesia throughout the surgery and was varied from 0.8–2.0%. The rat was placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted until Bregma was level with Lambda. IBO dissolved in 0.1 M PBS (concentration: 10 mg/ml, pH 7.4) was injected using a 10µl Hamilton syringe attached to a Kopf microinjector (model 5000) mounted on the stereotax. The syringe was first lowered to the target depth and left in place for 1 minute. After injection at a rate of 0.1µl/min, the syringe stayed at depth for 2 minutes to prevent the IBO from moving up the needle. Lesions were made at multiple locations (all coordinates are in millimeters and relative to Bregma): anteroposterior (AP) +2.0,

mediolateral (ML) +.07, dorsoventral (DV) -3.0; AP +3.0, ML +0.7, DV -3.0, -4.0; AP +4.0, ML +.07, DV -3.0; AP +5.0, ML +.07, DV -3.0. At each site, 0.3 µl of IBO was injected.

Spatial alternation testing

Rats received seven trials each day for ten days. Every trial began with the rat confined to the start box for 10 seconds. The first trial of each day was a forced-choice trial with one arm blocked (left or right arm equally often). On the subsequent six trials, both arms were open to allow the rat a free choice of which arm to enter. The open arm on the first trial was baited with reward, and on subsequent trials the baited arm was always opposite to the arm entered on the previous trial. A choice of arms was counted when all four paws were within one of the arms. The door was then lowered, the rat was allowed 10 seconds to either consume the food or to find an empty food cup, and the rat was then returned to the start box.

Spatial alternation data analysis

Performance was measured as the percentage of trials on which a rat correctly alternated arm entrances (six possible alternations each day).

Path integration apparatus

The testing table was made of circular Plexiglas (2 m in diameter) elevated 64 cm above the floor and mounted on wheels around a fixed central platform that allowed the table to be easily rotated (Kim et al., 2013). Eight holes (12 cm diameter) were placed equidistantly around the table with plastic boxes mounted beneath them (Figure 1). Each box was filled with used rat bedding. Wire mesh screens could be inserted between the box and the table in order to block entrance to a given hole. Infrared lights and an infrared camera were mounted above the table to track the animal's movements in darkness.

Path integration pre-training

During pre-training and testing, rats were maintained at ~80% of their free-feeding weight. Pre-training began with two days of exploration of the table in lighted conditions with all holes blocked off. After this initial period of exploration, five pellets of food (750-mg rodent pellets, Bio-Serv) were placed on the table for the rat to consume. After 3 or more pellets were consumed for two consecutive days, a hole was opened and the rat was placed inside. Once the rat exited the hole and consumed 3 or more pellets on each of two days, the procedure was repeated in the dark. The final phase began when the rat consumed 3 or more pellets in the dark condition. In the final phase the rat left the hole, found a single pellet, and brought it back to the hole to consume. Four trials were given each day with a 5- minute time

limit per trial. Pre-training was complete when the rat successfully completed all four trials within the time limit on two consecutive days.

Path integration testing

Rats were given four trials per day with the lights off until they had successfully completed 50 trials. A successful trial was one in which the rat left the open hole, found the food pellet on the table, and brought it back to the open hole to consume, all within a total of 5 minutes. Only one of the eight holes was open on each trial, and the open hole changed after each trial based on a pseudorandom sequence. The table was rotated three different times during each test session to change the position of the holes relative to the room (after all animals had received the first trial, after all had received the second trial, and after all had received the third trial). No hole was re-used in a session until all the other holes had been used. The food was located at one of eight locations distributed across the table. Once the rat returned to the open hole with the food, he was allowed to consume it before being returned to the home cage. If the rat consumed the food on the table or dropped the food before returning to the open hole, the trial was discarded (less than 1% of trials). One animal in the lesion group would not bring the food back to the open hole on the majority of trials and was excluded from analysis for this task.

Path integration data analysis

Performance was measured by how accurately the rat returned to the open hole after finding the food. The animal could return to the start box itself (a score of zero), one of the boxes adjacent to the start box (a score of 1), one of the boxes 90° removed from the start box (a score of 2), one of the boxes 135° removed from the start box (a score of 3), or the box opposite from the start box (a score of 4) (Figure 4). The accuracy of the return path was recorded as a function of the amount of time taken to find the food (0-3, 4-6, >6 s), the number of 90° turns made on the outward path (0, 1, >1) or the length of the outward path (0-1, 1-2, >2 m).

Results

Neurohistological findings

All rats sustained bilateral damage to the prelimbic and infralimibic areas of the prefrontal cortex (Figure 5) that ranged from 85% to 100% (mean 97%). All rats also sustained damage to surrounding areas of the prefrontal cortex, in particular the medial orbital cortex (mean 93%), cingulate cortex (Area 1 mean: 68%, Area 2 mean: 47%) and the dorsal peduncular cortex (mean: 56%).

Spatial Alternation

The two cohorts of animals with mPFC lesion performed nearly the same, and were significantly impaired regardless of whether testing occurred before or after testing on path integration ($ts > 2.5$, $ps < 0.05$). Overall, the mPFC lesion group

was impaired relative to the CON group across the ten days of testing (mPFC mean =67.6 ±2.5%; CON mean=78.8±1.2%; $t(30)=4.10$, $p<0.001$; Figure 6) and both groups performed better than chance ($ts(15) > 7.1$, $ps < .001$).

Path integration

The two cohorts performed similarly on all measures, and their data were combined (Figure 7). Animals with medial prefrontal cortex lesions (mPFC) performed as well as control animals (CON) at path integration and were often numerically better. For the more complex outward paths that were longer and involved more turns, both mPFC and CON animals performed worse than they did on the simpler trials (all $ts>5$, all $ps<.05$). The marginal advantage of the mPFC group is likely attributable to poor performance of the control group than to a facilitation of the mPFC group (see Figure 6 for better control performance).

Experiment 2: Hippocampal lesions, T-maze alternation and Path Integration

Methods and Materials

Subjects

Two groups of male Long Evans rats were tested. For spatial alternation, 8 animals were prepared with bilateral lesions of the hippocampus, and 8 animals served as sham-operated controls. One control animal did not complete spatial

alternation. For path integration, 5 animals were prepared with bilateral lesions of the hippocampus, and 7 animals served as sham-operated controls. Data from these animals have been reported previously (Kim et al., 2013). Rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego, Institutional Animal Care and Use Committee.

Spatial alternation and Path Integration

Spatial alternation and path integration testing followed the same procedure as in Experiment 1.

Surgical procedure

Bilateral excitotoxic lesions of the entire hippocampus were performed using IBO under the same conditions as Experiment 1. Coordinates for this surgery can be found in Clark et al., (2000). Sham-operated control animals underwent the same surgical procedures up to the point of the craniotomy. Animals were given 14 days to recover after surgery before testing resumed.

Results

Neurohistological findings

Animals tested on spatial alternation sustained bilateral damage to all cell fields of the hippocampus. This damage included 82-100% of the hippocampus, with 96% mean damage. Animals tested on path integration sustained bilateral damage to all cell fields of the hippocampus. This damage included 85-97% of the hippocampus with 93% mean damage. Sparing occurred most frequently in the most medial aspects of the dorsal dentate gyrus and CA1 cell field, as well as the ventral-most region of the hippocampus. All rats had some damage to the cortex and fimbria overlying the dorsal hippocampus, which was associated with the placement of the syringe during surgery.

Spatial alternation

Animals with hippocampal lesions were impaired relative to CON animals across ten days of testing (H mean=55.2± 3.7%, control mean=77.2±2.9%, $t(13)=4.5$, $p<.01$) (Figure 8). The CON group performed better than chance ($t(6)=9.3$ $p<.001$), but the H group did not ($t(7)= 1.4$, $p >.1$).

Path integration

Animals with lesions of the hippocampus (H) were impaired relative to sham operated controls (CON) (Figure 9). This impairment was observed even in the simplest conditions: when the food was found within 3 seconds ($t(10)=3.8$, $p<.01$), when the outward path involved no turns ($t(10)=3.6$, $p<.01$), or when it was less than 1 meter in length ($t(10)=2.9$, $p<.05$). In addition, the H group performed no

better than chance in any condition (calculated as an accuracy score of 2; H: all *t*s < 1.5, all ps > .1). For more complex outward paths involving 4-6 seconds ($t(10)=3.6$, $p<.01$), one turn ($t(10)=3$, $p<.05$) or 1-2 meters ($t(10)=2.5$, $p<.05$), the H animals were also impaired relative to the control group and performed no better than chance (H: all *t*s < 1, all ps > .1; control: all *t*s >5, all ps<.01). As expected, the control group exhibited poorer performance as outward paths became more complex (repeated measures ANOVA; All $Fs(2,12) > 9.9$, all ps<.01).

Experiment 3: Hippocampal and mPFC lesions and odor alternation

Materials and Methods

Subjects

Male Long Evans rats (n=11) were prepared with bilateral lesions of the hippocampus (n=6) or served as sham-operated controls (n=5). The two groups were matched based on performance before surgery. Later, the control group underwent a second surgery and received bilateral lesions of the medial prefrontal cortex. Rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego, Institutional Animal Care and Use Committee.

Odor alternation apparatus

Testing took place on a platform (91 cm diameter at its widest point) with a 51 cm straight edge placed flush to a wall (Figure 4C). A black plastic wall (9.5 cm high) was attached to the circular portion of the platform's perimeter. Initial training occurred on the platform. For formal testing, a plastic circular insert (48 cm diameter; 35 cm tall) was placed in the center of the platform to create an annular corridor 24 cm wide (narrower next to the wall). Testing took place within the 24 cm-wide portion of the annulus to insure that the two stimuli were equally accessible as an animal approached them.

Two 1% mixtures of scented play sand were prepared (one with cocoa and one with cinnamon). The scented sand was placed into two different glass cups (150 g each). One cup was 6.5 cm tall and 6 cm in diameter, with a smooth exterior design. This cup was used exclusively to hold cinnamon-scented sand. The other cup was 7 cm tall and 7 cm in diameter with a rough, pitted exterior design. This cup was used exclusively to hold cocoa-scented sand. The two cups were presented on holders constructed of .25 cm acrylic Perspex. The base of each holder was 22 cm X 10 cm, and a divider (10 cm high X 10 cm wide) was centered on the base between the cups. The cups were attached to the holder using Velcro. Ten cups of each design and holders were constructed so that all the odor pairs needed for a single testing session were available at the beginning of the session.

Odor alternation pre-training

During pre-training and testing, rats were maintained at ~80% of their free-feeding weight. Pre-training began with 150 g of unscented sand in a clear glass cup (6 cm diameter, 8 cm tall) placed in a plastic holder. 12 Froot Loop halves were placed at three different depths (4 fully buried, 4 half buried, 4 on top). The holder was placed in the home cage, and rats were allowed to consume the Froot Loops. This procedure was repeated after one hour and twice more on the following day. Beginning on the third day, 6 trials were presented on the platform, with a single glass cup on either the left or right side of the holder. Froot Loop halves were buried at varying depths, and each trial ended when the rat had eaten two halves. As the rat learned to dig across the six trials, the depth of the buried food was increased until the reward was fully buried in the sand (1.5-2.0 cm deep). Trials were separated by 10 sec, and there was a 20-min time limit to complete the 6 trials. This phase of pre-training ended when the rat completed the 6 trials within the 20-min time limit.

The rats were then trained on the odor alternation task in the open field of the platform. Rats received one trial with a single cup followed by 10 trials with two cups. On the two-cup trials, both scented cups were presented together. To receive a reward, the rat had to select the scented cup that was not selected on the previous trial. Thus, if the rat selected the cocoa-scented cup on a two-cup trial (regardless of whether the choice was correct), the rat needed to select the cinnamon-scented cup on the following trial in order to receive a reward (odor alternation). A choice was scored when the animal began to dig in one of the cups. If the choice was correct, the

animal was allowed to find and consume the food reward. The holder was then removed. If the choice was incorrect, the holder was removed after the rat dug far enough to have obtained a reward had it been there. In either case, when the holder was removed, it was immediately replaced by another holder somewhere else along the corridor. The time between trials was determined by the rat's behavior and averaged about 10 sec. The left-right position of the correct cup was counterbalanced across trials.

Formal odor alternation testing was conducted within the annulus. On each trial a holder was placed within the annular corridor. Critically, spatial information was irrelevant to performance because the correct cup could appear at any location within the 24 cm-wide portions of the corridor. Between animals, the holders and platform were cleaned with 50% ethanol. After each day, all cups and holders were washed with water and cleaned with 50% ethanol. Scented-sand was remade weekly.

Surgical procedure

Bilateral excitotoxic lesions of the hippocampus were performed using IBO under the same conditions as Experiment 1. Following the first round of post-surgical testing, the 5 animals that previously served as sham-operated controls received bilateral lesions of the medial prefrontal cortex under the same conditions and using the same coordinates as in Experiment 1.

Odor alternation testing

Rats received one single-cup trial and ten two-cup trials per day until ten days of testing were completed.

Odor alternation analysis

Performance was measured as the percentage of trials on which a rat correctly alternated odor choices (ten possible alternations each day). Testing occurred during 10 days following recovery from surgery.

Results

Neurohistological Findings

Animals that received hippocampal lesions sustained damage to all cell fields of the hippocampus (Figure 10). This damage included 83-96% of the hippocampus, with 90% mean damage. Sparing occurred most frequently in the most medial aspects of the dorsal dentate gyrus and CA1 cell field, as well as the ventral-most region of the hippocampus. All rats had some damage to the cortex and fimbria overlying the dorsal hippocampus, which was associated with the placement of the syringe during surgery.

All rats that received mPFC lesions sustained bilateral damage to the prelimbic and infralimibic areas of the prefrontal cortex (Figure 10) that ranged from 70% to 97% (mean 87%). All rats also sustained damage to surrounding areas

of the prefrontal cortex, in particular the medial orbital cortex (mean 70%), Area 1 of the cingulate cortex (mean 46%) and the dorsal peduncular cortex (mean: 25%).

Odor Alternation Results

During the last 10 days of preoperative testing, the 11 rats performed at 62.5% correct (Figure 11A), which was well above chance ($t(10)=6.1$, $p<.001$). This group was then divided into two groups based on preoperative performance. This yielded a control group (n=5, mean=62.4±3.2%) and a group to be given hippocampal lesions (n=6, mean=62.6±3.0%; $t(9)=.042$, $p>.1$). After surgery (Figure 11B), the rats with hippocampal lesions performed as well as controls (H mean: 63.8±2.8%; control mean: 60.9±2.4%; $t(9)=.8$, $p>.1$), and both groups performed above chance (H: $t(5)=4.9$, $p<.01$; control: $t(4)=4.5$, $p<.05$). Subsequently, after testing was complete for the H and CON groups, the control group received bilateral lesions of the medial prefrontal cortex (mPFC). These animals performed at chance ($t(4)=1.4$, $p>0.10$). They were impaired relative to the H group (mPFC mean: 51.6±1.1%, $t(9)=3.8$, $p<.01$) and also relative to their own pre-lesion performance ($t(4)=4.9$, $p<.01$).

Discussion

In a set of three experiments, we investigated the role of the medial prefrontal cortex and hippocampus with two tests of spatial and one test of

nonspatial memory. In Experiment 1, rats with bilateral lesions of the mPFC were impaired relative to controls on the spatial alternation task, a task believed to require intact working memory. In contrast, both mPFC lesion and control animals performed well on path integration when the outward paths where quick, short and involved one turn or less. Performance declined in both groups as outward paths became longer and more complex. In Experiment 2, rats with bilateral lesions of the hippocampus were also tested on the spatial alternation and path integration tasks. Rats with lesions of the hippocampus performed no better than chance on spatial alternation and were robustly impaired relative to control animals. Lesions of the hippocampus also produced chance levels of performance on the path integration task; this was true even when a trial took less than 3 s and the outward path was less than 1 m and had no turns. In Experiment 3, we tested animals with bilateral lesions of the mPFC or hippocampus on odor alternation, a novel nonspatial working memory task that was designed to approximate the cognitive demands of the spatial alternation task without the spatial component. We found that rats with hippocampal lesions did not differ from controls on this task, with both groups performing above chance. However, once the control group received lesions of the mPFC their odor alternation performance was no better than chance, and they were impaired relative to both the hippocampal lesion group and their own pre-lesion performance. These results are consistent with the view that the mPFC is a structure critical for working memory and that working memory remains intact following hippocampal damage, as mPFC lesion animals were impaired on both the spatial and

odor alternation tasks while hippocampal lesions were not impaired on odor alternation. They also suggest that working memory does not support path integration performance, as mPFC lesion animals perform as well as controls on the path integration task. The key question then, is why lesions of the hippocampus impair performance on tasks of spatial working memory or even spatial memory in general? An important caveat to note here is that we are using the term "working memory" to refer to processes supported by the mPFC that are important for supporting some forms of memory performance. These processes could include working memory *per se*, but also other functions like attention, reduction of interference, or other executive functions.

One possibility that has not previously been evaluated is that the amount of information needed to guide performance on tasks that require spatial information exceeds the working memory capacity of the rodent. Our previous study of path integration showed that humans with damage to the MTL performed normally on a path integration task when the trials were short and simple (Kim, Sapiurka et al., 2013). Presumably these patients were using their intact working memory abilities to complete the task because their long-term memory was impaired. By contrast, rats with hippocampal lesions were unable to path integrate even under the shortest and simplest conditions (Kim, Sapiurka et al., 2013). We suggested that human working memory might be able to accommodate the necessary amount of information required to perform spatial tasks under the simplest conditions, while the more limited rodent working memory cannot. If correct, this idea would also

indicate that even normal rats must necessarily solve spatial tasks by relying on long-term memory rather than working memory. In other words, a spatial task like path integration will always require the hippocampus and long-term memory because the amount of information that must be maintained to perform a spatial memory task, exceeds work memory limits.

Studies with amnesic patients have shown how memory performance rapidly declines once working memory capacity has been exceeded. For example, an early study by Drachman and Arbit (1966) showed that working memory capacity was not impaired following damage that included the hippocampus, with both patients and controls able to recall a mean span of 7-8 digits. However, when asked to recall longer digit spans, controls were able to recall spans of 20 digits or greater, while patients were impaired on spans longer than 8 digits, indicating that they could not use their long-term memory to maintain information once the amount of information exceeded their working memory capacity. Further, the number of discrete items that can be maintained by human working memory decreases as item complexity increases; this has been shown with paired light associations (3 pairs; Drachman and Arbit 1966), object-location associations (3 sets; Jeneson et al., 2010), and faces (1; Eng et al., 2005). Thus, it is possible that the memory demand of the tasks used to study working memory in rodents may be an important factor; if the demand is such that rodent working memory cannot support it without the involvement of long-term memory, lesions of the hippocampus will necessarily compromise performance on those tasks.

A study comparing spatial and nonspatial memory in the rat is also instructive. Dudchenko et al. (2000) used an adapted version of the "memory span" task to determine the normal memory ability of rats for both spatial and nonspatial lists. Animals were tested on their recognition memory for an increasing number of familiar odors (in the nonspatial version) or locations (in the spatial version). The median span for the nonspatial task in normal animals was 8 odors, while for the spatial task it was fewer than 5 locations; an analysis of the two tasks revealed that the spatial span task was indeed more difficult for normal rats than the nonspatial span task. Thus, it appears that even in normal rodents, spatial information may be more difficult to store than nonspatial information. Further, a relevant study that also tested rats with hippocampal or mPFC lesion on spatial and nonspatial tasks reported similar findings to the present experiments (Kesner et al., 1996). Animals were tested on two variations of a working memory task in the radial arm maze; in the first, they were required only to repeat a directional response (left versus right turn, independent of the spatial location those responses took the rat, for example to the West or East arm), while in the second task they were required to select an arm based on its spatial location (West or East, independent of whether the animal turned left or right to get there). Lesions of the mPFC led to an impairment on both tasks, consistent with a generalized working memory deficit subsequent to mPFC damage. In contrast, lesions of the hippocampus only impaired performance on the spatial location version of the task; animals with hippocampal lesions performed as well as controls on the response version (Kesner et al., 1996). We suggest that

because the directional response task only required memory for the prior response, the amount of information required to successfully perform the task could be maintained in working memory. Thus, animals with hippocampal lesions performed normally. Whereas in the spatial version of the task, the animals had to maintain all of the distal spatial elements required to form a coherent representation of space in order to make the correct spatial response. This memory demand may have overwhelmed working memory. These findings are consistent with the present findings. Animals with hippocampal lesions performed normally on the nonspatial, odor alternation task, whereas the animals with mPFC lesions were impaired on both the spatial and nonspatial working memory tasks. We suggest then, that animals with hippocampal lesions fail spatial memory tests not because of some particular feature of spatial information that the hippocampus is uniquely able to resolve, but rather because constructing a coherent spatial representation in order to guide performance exceeds the capacity of rodent working memory. Thus, normal animals must use long-term memory to solve spatial memory tasks and animals with hippocampal lesions are impaired on spatial memory tasks because of their impaired long-term memory and not due to an impaired spatial processing ability.

The idea that spatial memory involves more information that than nonspatial memory is not novel. Others have suggested that spared performance following hippocampal lesions on tasks that require little or no spatial information may be due to spatial tasks requiring more information to support performance than nonspatial tasks. Specifically, the authors suggested that operant delayed matching

to position tasks, where the animal must select the right or left bar press lever require less information than spatial maze tasks (Lalonde, 1991; Mair et al., 1998; Sloan et al., 2006).

There is a question that arises from the idea that all spatial tasks require long-term memory in the rodent. That is, why are animals with mPFC lesions impaired on spatial working memory tasks if they still have intact long-term memory? In other words, why are rats with mPFC lesions unable to simply use their intact long-term memory abilities to solve the spatial alternation task the way they apparently do with the path integration task? We suggest that while spatial alternation and path integration may both require the use of long-term memory, the specific demands of these tasks are different in an important way. Path integration is a test of single trial learning; that is the information needed for an individual trial is independent from the trials before or after it and all of the paths are unique. Thus, there should be only minor interference from the experience of earlier trials in the path integration task. In contrast, each spatial alternation trial is dependent on information from the one previous to it. Further, each response is repeated multiple times each session. In this case, interference from the experience of earlier trials would certainly be higher than in path integration. A study by Granon et al. (1994) found that rats with mPFC lesions were impaired on working memory task and importantly, exhibited a detectable interference effect; that is, their performance declined across the testing session as they performed more trials. It may be that an intact mPFC is important during tasks with high interference and that working

memory is serving to manage the separation of multiple similar events to support the expression of the encoded long-term memory. It is notable that animals with mPFC lesions, while impaired, were still able to perform the spatial alternation task at a better than chance level. So while these spatial working memory tasks may require intact long-term memory, the processing provided by the mPFC may help to mitigate the interference of repeated responses during the task.

Work in humans established the idea that hippocampal damage impairs long-term memory while mPFC damage impairs processes that help support working memory. However, in the rodent literature, there is the suggestion that the mPFC and hippocampus work in concert to support successful working memory. This idea emerged because, as in this study, both hippocampal and mPFC lesions appear to impair performance on tests that are designed to measure working memory, with hippocampal lesions tending to produce more robust spatial memory impairments than mPFC lesions. Further, successful performance on tests designed to test spatial working memory appears to be related to the degree of physiological synchrony between the two structures (Gordon, 2011; Hyman et al., 2010). For example, it was reported that optogenetic inhibition of the hippocampus to mPFC projection during a spatial working memory task impaired performance (Spellman et al., 2015). Specifically, inhibition during the encoding phase of the task disrupted performance, while inhibition during maintenance or retrieval had no effect. This is unexpected for a working memory task, as generally an interruption or disruption during the maintenance phase of the task is sufficient to impair working memory performance

(Fuster et al., 1985; Baddeley et al., 1992, Clapp et al., 2009). Indeed, tasks dependent on long-term memory are usually more sensitive to disruption during encoding and retrieval and much less sensitive to disruption during maintenance (e.g., Riedel et al., 1999). Thus, it may be that the task used in the Spellman et al. study may in fact have relied on long-term rather than working memory and the inhibition result could be explained as impaired encoding of long-term spatial memory and not by a critical interaction of the hippocampus and mPFC to support spatial working memory. This underlines an important caveat for studies of working memory; it can be difficult to determine whether working or long-term memory is being used to support performance.

A related idea is that hippocampal lesions may specifically disrupt rodent "spatial" working memory. For example, the rodent hippocampus could provide essential spatial information to the mPFC in order to accomplish a spatial working memory task. A potentially important difference between humans and rodents is that the human hippocampus, in comparison with the rodent hippocampus, makes a relatively weak contribution to cortical theta, and hippocampal and cortical theta are not reliably synchronized (Hyman et. al., 2011). Thus in the rodent, hippocampal lesions may serve to disrupt mPFC functioning and the processes associated with spatial working memory.

In the rodent literature, the standard explanation for the spatial impairments that follow hippocampal damage is that rodent hippocampus is a structure that is primarily concerned with processing and encoding spatial environments (O'Keefe

and Nadel, 1978). This interpretation stems from the discovery of place cells in the rodent hippocampus; these cells preferentially fire when the rat is in a specific spatial location in the environment (O'Keefe and Dostrovsky, 1971). The discovery of place cells, in addition to other spatially selective cell types in the MTL, have suggested that the hippocampus primarily maps the spatial environment and continually updates this map as the animal moves through the environment (McNaughton et al., 2006; Moser et al., 2008; Hartley et al., 2014). We underscore that this interpretation is consistent with the results reported here; lesions of the hippocampus impaired performance on both of the spatial tasks (spatial alternation and path integration) and spared performance on the nonspatial task (odor alternation). Accordingly, our findings do not rule out the possibility that the spatial impairments observed after hippocampal lesions are due to spatial processing deficits.

However, what is now clear is that the rodent hippocampus is not dedicated to spatial processing. Animals with hippocampal lesions are impaired on a variety of nonspatial tasks including familiarity-based object recognition tasks (reviewed in Squire et al., 2007; Clark and Martin, 2005), trace eyeblink and fear conditioning paradigms (reviewed in Clark, 2011), social transmission of food preference (Clark et al., 2002; Winocur et al., 2001; Winocur, 1990; Alvarez et al., 2001; Ross and Eichenbaum, 2006), and odor tasks that require sequence memory or relational memory (reviewed in Eichenbaum and Cohen, 2014). Further, while hippocampal place cells are prominent in animals foraging in an open field, many other

hippocampal firing patterns are observed when rats are actively engaged in a motivated task. For example, hippocampal cells have been shown to encode for the order of events (Manns et al., 2007), encountered stimuli (Komorowski et al., 2009; Itskov et al., 2012), and hippocampal network properties have been proposed to track the passage of time (MacDonald et al., 2011; Mankin et al., 2012; Mankin et al., 2015). In goal directed tasks, hippocampal neurons appear to encode multiple, event related aspects of the task structure, even when these different events occur in the same physical location (e.g., Woods et al., 2000). Further, it now appears that there are a class of hippocampal neurons that code for highly specific time intervals ("time" cells), similar to the way place cells code for location (Eichenbaum, 2014). Thus, it appears that the rodent hippocampus is not a spatially selective structure, but rather that space is one of a number of features that the rodent hippocampus encodes. It has been suggested that a better way to understand hippocampal physiology and function is that it helps form "mental" rather than "spatial" maps and that these maps are organized in time and space and perhaps in higher conceptual dimensions (Eichenbaum 2004; Eichenbaum and Cohen 2014).

What typically differentiates the spatial impairments from the nonspatial impairments that follow hippocampal damage is that nonspatial memory impairments are often incomplete or dependent on a particular task parameter. For example, objection recognition memory is often unimpaired following hippocampal damage if the delay is short and impaired only when the delay is long (e.g. Clark et al., 2001). Similarly, simple odor recognition memory is often normal following

hippocampal damage and impaired when the sequence or relationship among the odors needs to be remembered (e.g., Eichenbaum and Cohen, 2014), whereas spatial memory impairments are complete and non-conditional. That is, spatial impairments are typically robust (i.e., behavior not different from chance performance) and occur under all circumstances following hippocampal damage (Martin and Clark, 2007). We suggest that the nonspatial memory impairments that follow hippocampal damage are conditional because under some circumstances, working memory can support performance. Whereas, the impairments on spatial memory tasks are non-conditional because spatial memory is too complex to be supported by rodent working memory or that rodent spatial working memory is disrupted by hippocampal lesions. Accordingly, animals with hippocampal lesions may be impaired on spatial memory tasks because of their impaired long-term memory and not due to an impaired ability to process spatial information.

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Chapter 3, in full, is currently being prepared for submission for publication of the material. Sapiurka, Maya; Squire, Larry R.; Clark, Robert E. The dissertation author was the primary investigator and author of this material

CHAPTER 4:

CONTRASTING EFFECTS OF PARIETAL CORTEX AND MEDIAL ENTORHINAL CORTEX LESIONS ON PATH EFFICIENCY IN THE PATH INTEGRATION TASK

Abstract

The hippocampus receives much of its neocortical input via the entorhinal cortex. The posterior parietal cortex (PPC) contributes spatial information to the hippocampus via the medial entorhinal cortex (MEC); as the role of the hippocampus in spatial navigation and memory has become of increasing interest, so to have the contributions of the PPC and MEC to spatial cognition. While electrophysiology studies have revealed much about how these regions process and encode spatial information, the behavioral roles of the PPC and MEC in tasks of spatial navigation and memory are less clear. We tested animals with lesions of the PPC and MEC on path integration, a spatial navigation task that relies on internally generated self-motion cues, to determine if and how these regions contribute to spatial navigation. Animals left a start box and searched for a piece of food on a testing table in complete darkness, and then returned to the start box to consume the food. While both MEC and PPC lesion animals were less accurate and efficient than control animals on outward paths that were 1 meter or shorter and took 3 seconds or less to complete, animals with PPC lesions had less efficient return paths

than either MEC or control animals even when they returned directly to the starting location. This suggests that the PPC plays a role in planning and implementing navigational routes, while the MEC is more involved with the memory aspect of spatial navigation task.

Introduction

Studies of spatial cognition in the rodent have focused on the hippocampal formation since the discovery of place cells more than 40 years ago (O'Keefe and Dostrovsky, 1971). Over the ensuing decades, the hippocampus has become the central figure in a complex spatial processing network, theoretically receiving sensory input from the neocortex and integrating it into a map of the environment (Moser et al., 2008). The hippocampus receives much of its neocortical spatial information from the posterior parietal cortex (PPC) via the medial aspect of the entorhinal cortex (MEC); this anatomical circuit has made the PPC and MEC key areas of interest for electrophysiological and behavioral studies of rodent spatial cognition.

The most well characterized spatially selective cell type in the MEC is the grid cell (Hafting et al., 2005). Grid cells fire in a regular hexagonal pattern as the animal moves through space, serving to tile and map the surrounding environment. Grid cells are often co-localized with head direction cells and conjunctive cells that encode for both position and direction; this network may be central to integrating

spatial information as the animal moves through space (Sargolini et al., 2006). Lesions of the MEC that eliminate the entirety of the grid cell area impair performance on the watermaze, a test of spatial navigation memory, but did not impair performance on other hippocampus dependent memory tasks (Hales et al., 2014).

Cells in the PPC also show selective properties; however, rather than showing preferential firing for specific locations or directions, parietal cortical cells appear to fire to specific epochs in navigational sequences and paths, and tune for the movement and acceleration of the animal along that path (Nitz 2006, Whitlock et al., 2012). The behavioral effects of PPC lesions on spatial memory tasks have been somewhat mixed. Impairments on the watermaze task have ranged from severe (Di Mattia and Kesner, 1988) to mild (Van Cauter et al., 2012), and may be dependent on whether the available environmental cues are proximal or distal (Save and Poucet, 2000). Additionally, animals with PPC lesions have been reported to display a characteristic and inefficient looping search pattern in the watermaze which affects their ability to find the escape platform (Kolb and Walkey, 1987; Save and Poucet, 2000).

Only a few studies have been done on the contributions of the MEC and PPC to path integration, a spatial navigation task in which animals must use internally generated motion cues to recall and return to their starting location. Lesions of the entorhinal cortex have been found to impair performance on path integration; path integration impairments have also been found subsequent to PPC lesions, though to

a lesser extent than entorhinal or hippocampal lesions (Save et al., 2001; Parron and Save, 2004, Van Cauter et al., 2012). However, it has not been reported if the looping search pattern seen in the watermaze has also been present in path integration. It is possible that PPC lesions affect not only the memory for the starting location, but also impair their ability to use the most efficient path to return to that location.

In the present study, we tested the performance of rodents on the path integration task following lesions of the posterior parietal and medial entorhinal cortex. We measured their performance relative to the distance, path complexity, and amount of time taken to find the food, as well as the accuracy and efficiency of the return path once the food had been retrieved.

Methods and Materials

Subjects

Male Long Evans rats (n=36) were prepared with bilateral lesions of the parietal cortex (n=6), medial entorhinal cortex (n=12) or served as sham-operated controls (n=18). All rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego Institutional Animal Care and Use Committee.

Surgical procedure

Bilateral excitotoxic lesions of the posterior parietal cortex were made using ibotenic acid (IBO; Biosearch Technologies). Isoflurane gas (delivered in O_2 at 1 L/min) was used to maintain anesthesia throughout surgery and was varied from 0.8-2.0%. The rat was placed in a Kopf stereotaxic instrument and the incisor bar was adjusted until bregma was level with lambda. IBO dissolved in 0.1 M PBS (concentration: 10 mg/ml, pH 7.4) was injected using a 10µl Hamilton syringe attached to a Kopf microinjector (model 5000) mounted on the stereotax. The syringe was first lowered until the opening of the needle fully punctured the cortex and left in place for 1 minute. After injecting the IBO at a rate of 0.1µl/min, the syringe stayed at depth for 2 minutes to prevent the IBO from moving up the needle track. Lesions were made at multiple locations (all coordinates are in millimeters and relative to bregma): anteroposterior (AP) -4.0, mediolateral (ML) +2.0,; AP -4.0, ML +3.0; AP -4.7, ML +3.5; AP -4.7, ML +5.0; AP -4.0, ML -2.0; AP -4.0, ML -3.0; AP -4.7, ML -3.5; AP -4.7, ML -5.0. At each site, 0.2 µl of IBO was injected.

Bilateral excitotoxic lesions of the medial entorhinal cortex were made under the same conditions using NMDA. The needle was pointed anterior at an angle of 22° and placed immediately anterior to the transverse sinus; it was then lowered at ML±4.6 mm and NMDA was injected into 8 sites along a single track across the dorsoventral axis (DV -5.2, -4.7, -4.2, -3.7, -3.2, -2.7, -2.2, -1.7).

Path integration apparatus

The testing table was made of circular (2 m diameter) Plexiglas that was elevated 64 cm above the floor and mounted on wheels around a fixed central platform that allowed the table to be rotated easily (Kim et al, 2013). Eight holes (12 cm diameter) were placed equidistantly around the edge of the table with plastic boxes mounted below them. Each box was filled with used rat bedding, and wire mesh screens could be inserted between the box and table in order to block the entrance to the hole. Infrared lights and an infrared camera were mounted above the table to track the animals' movements in the darkened room.

Path integration pre-training

During pre-training and testing rats were maintained at ~80% of their free-feeding weight. Pre-training began with two days of free exploration of the table in light conditions with all hole entrances blocked. After this initial exploratory period, five pellets of food (750-mg rodent pellets, Bio-Serv) were placed on the table for the rats to consume. After 3 or more pellets were consumed for two consecutive days, a hole was opened and the rat was placed inside. Once the rat exited the hole and consumed 3 or more pellets on two consecutive days, the procedure was repeated in the dark. The final phase began when the rat consumed 3 or more pellets in the dark condition. In this final phase the rat left the hole, found a single pellet, and brought it back to the hole to consume. Four trails were given each day with a 5- minute time limit per trial. Pre-training was complete when the rat successfully completed all four trials within the time limit on two days in succession.

Path integration testing

Rats were given four trials per day with the lights off until they had successfully completed 50 trials. A successful trial was one in which the rat left the open hole, found the food pellet on the table, and brought it back to the open hole consume within a total of 5 minutes. Only one of the eight holes was open on each trial, and the location of the open hole changed after every trial based on a pseudorandom sequence. The table was rotated three times during each testing session to change the position of the holes relative to the room. This was done after all animals had received their first, second and third trials. No hole was re-used in a session until all other holes had been used once. The food was placed in one of eight locations distributed across the table. Once the rat returned to the open hole with the food, he was allowed to consume it in the hole before being returned to the home cage. If the rat consumed the food on the table or dropped the food before returning to the open hole, the trial was discarded. This occurred in less than 1% of trials.

Path integration data analysis

Performance was measured by how accurately the rat returned to the open hole after finding the food. The animal could return to the start box itself (a score of zero), to one of the boxes adjacent to the start box (a score of 1), to one of the boxes 90° removed from the start box (a score of 2), to one of the boxes 135° removed

from the start box (a score of 3), or to the box opposite from the start box (a score of 4) (Figure 1). The accuracy of the return path was recorded as a function of the amount of time taken to find the food (0-3, 4-6, >6 s), the number of 90° turns made on the outward path (0, 1, >1) or the length of the outward path (0-1, 1-2, >2 m).

The efficiency of the return path was determined by measuring the tortuosity of that path (see Hollup et al., 2002). Tortuosity was determined by dividing the length of the return path by the length of the ideal path (the length of a straight line drawn between the point where the animal found the food and the start box). This was calculated using a MATLAB script that rotated each path so that the starting point (where the animal found the food) was at (0,0) and then calculated the ideal path length, actual path length, and tortuosity for each individual trial.

Results

Neurohistological findings

All rats sustained bilateral damage to the posterior parietal cortex (PPC) and medial entorhinal cortex (MEC) (Figure 12). The PPC damage ranged from 82% to 93% (mean: 85%), with the most sparing occurring at the most dorsal aspect of the parietal cortex. Some animals sustained minor damage to the most dorsal aspect of CA1. The MEC damage ranged from 67% to 100% (mean: 84%), with sparing occurring most frequently at the most lateral aspect of the MEC, particularly in the deep layers.

Behavioral findings

Path integration performance: Accuracy

Animals with PPC and MEC lesions were impaired relative to controls on trials that took less than 3 seconds (MEC: $t(28)=2.3$, $p<.05$; PPC: $t(22)=2.8$, $p<.05$) and performed no better than chance (MEC: $t(11)=1.7$, $p>.05$; PPC: $t(5)=.9$, $p>.05$) (Figure 13A). PPC animals were also impaired on trials less than 1 m ($t(22)=2.7$, $p<.05$) and with no turns ($t(22)=2.7$, $p<.05$), and performed no better than chance (all ts>1, all ps>.05), while MEC animals were marginally impaired (Distance: $t(28)=1.8$, $p=.081$; Turns: $t(28)=1.8$, $p=.076$) (Figure 13B and 13C). On trials that took 4-6 seconds to complete and had 1 turn, PPC animals were impaired relative to controls (Time: $t(16)=2.6$, $p<.05$; Turns: $t(16)=2.3$, $p<.05$) and performed no better than chance (all ts >.2, all ps>.05).

Path integration performance: Tortuosity

MEC lesion animals had more tortuous path than sham animals on trials that took 3 seconds or less ($t(28)=2.1$, $p<.05$) or were shorter than 1 m ($t(28)=2.1$, $p<.05$), and were marginally impaired on trials that involved 0 turns ($t(28)=1.9$, $p=.0746$) (Figure 14). PPC lesion animals, however, had more tortuous paths than

control animals on almost every measurement (all *t*s >2, all ps<.05); PPC animals were marginally impaired relative to controls only on trials with an outward path length of 1-2 m ($t(22)=2.1$, p=.0514). While these results may be reflective of less accurate (and therefore longer) paths, they are more robust than the accuracy measure and may be a more sensitive measure of path integration performance.

Accuracy and tortuosity

In order to determine if the decreased efficiency of return paths following MEC and PPC lesions was dependent upon memory, we analyzed the tortuosity of these paths relative to the accuracy score for each trial. Of particular interest were those trials in which animals were able to remember their starting location and returned directly to the start box, receiving an accuracy score of 0. While MEC and control animals showed no difference in tortuosity on these direct return paths ($t(28)=.3$, p>.05), the paths of PPC animals had higher tortuosity scores than either the MEC ($t(16)=2.7$, p<.05) or the control ($t(22)=2.6$, p<.05) animals despite their perfect accuracy score (Figure 15B). In fact, PPC animals' paths were more tortuous than those of the control group on trials with an accuracy score of 1 ($t(22)=2.3$), p<.05) and than both groups on trials with accuracy scores higher than 1 (all *t*s>5, all ps<.001) (Figure 15A). This suggests that even when animals with PPC lesions can recall their starting location and successfully path integrate, the paths they take are less efficient than those of control or MEC lesion animals.

Discussion

Using the path integration task, we investigated the contribution of the posterior parietal cortex (PPC) and medial entorhinal cortex (MEC) to spatial navigation and memory. Animals with lesions of the PPC and MEC were both impaired relative to controls when trials took less than 3 seconds; PPC animals were also impaired when trials were less than 1 m in length and involved no turns while MEC animals were marginally impaired under these same conditions. We also looked at the tortuosity of the return paths as a measure of how direct and efficient the animals are when path integrating. On successful trials, those in which the animal returned directly to the starting hole after finding the food, the paths of PPC animals were more tortuous than those of MEC or controls. Indeed, paths of PPC lesion animals were more tortuous than either group at all accuracy measures, suggesting that this inefficiency of navigation is independent of memory for the starting location. Thus, it may be that while lesions of the MEC impair path integration performance due to impairment in spatial memory, lesions of the PPC affect path integration due to impairment in route planning and efficiency.

This interpretation is consistent with electrophysiological work that suggests that the PPC is key for the planning and execution of navigational routes. A 1994 study by McNaughton et al. recorded the activity of single neurons in PPC during a simple spatial navigation task in the radial arm maze. They found that these neurons discriminated between a variety of movements in the maze, including left

and right turns, forward motion and stillness, and clockwise or counterclockwise movement. Further, many cells were responsive to particular movements only when preceded by a particular behavior, or in a specific location in the maze, suggesting that PPC neurons are responsive to the path the animal takes, rather than to the specific spatial and environmental aspects of the maze itself (McNaughton et al., 1994). Another study looked at the activity of PPC neurons while rats ran a series of paths between food sites in a maze. While single PPC neurons again showed altered firing rates related to movement direction, position, and behavior, they also showed a consistent selectivity to the rat's position in a route. This position was dictated by the order of behavior exhibited by the animal, rather than the location in the maze or direction of motion; thus, the routes encoded by these parietal cortical neurons may be independent of external environmental cues (Nitz, 2006).

Whitlock et al. (2012) further explored the relationship between the PPC and environmental cues. The activity of PPC neurons was recorded while the rat freely explored an open field. These neurons were tuned to the self-motion and acceleration of the rat as it explored the space independent of its spatial location. When these animals were then shifted from the free movement of the open field to a behaviorally constrained hairpin maze, PPC cells were responsive to discrete locations in the maze, firing just before or during turns, or in the straight alleys of the maze. While this initially suggests that the PPC neurons shifted to reflect spatial locations in the maze, when the animals were tested on a "virtual" version of the hairpin maze that lacked the spatial cues of the maze itself, the response of PPC

neurons was more similar to that seen in the actual hairpin maze than that seen in the open field. This suggests that the change in PPC neuron response from the open field to the hairpin maze was not because of the spatial and environmental aspects of the maze, but instead reflects a shift in navigational behavior.

Our data are consistent with this interpretation of the PPC's role in spatial navigation. Path integration requires the use of internally generated self-motion cues for navigation in the absence of visual and other environmental cues; if PPC neurons use this self-motion and acceleration information to plan or execute routes, one would expect lesions of the PPC to effect path integration performance regardless of the animals' memory of the starting location. Our data also suggest that the path integration impairment following MEC lesions is related to impairment in spatial memory, as the paths taken by these animals did not differ from those of controls on successful trials. This is consistent with previous studies of path integration impairment subsequent to entorhinal cortex lesions (Parron and Save, 2004; Van Cauter et al., 2012), as well as others reporting impaired watermaze performance following MEC lesions (Hales et al., 2014; Steffanach et al., 2005).

It is possible that the path integration impairment following these lesions is due to the disruption of the PPC-MEC connections that provide spatial information to the hippocampus. Some studies have suggested that cooperation between the parietal cortex and hippocampus (Rogers and Kesner, 2007) and entorhinal cortex and hippocampus (Parron et al., 2006) are necessary for spatial memory tasks. However, the tasks that are affected by these impairments require either the

acquisition of spatial memory over several days, or require the animal to associate objects with spatial locations, while path integration is a test of single trial learning. Additionally, Whitlock et al. (2012) reported that while grid cells in the MEC shift their firing fields when in different rooms, PPC neurons maintain the same response pattern. Thus, while the PPC and MEC are anatomically connected and require coordination with the hippocampus for some tasks of spatial memory, it is likely that this connection is not the primary cause of the impairment on path integration.

Acknowledgments

Chapter 4, in part, is currently being prepared for submission for publication of the material. Sapiurka, Maya; Squire, Larry R.; Clark, Robert E. The dissertation author was the primary investigator and author of this material.

CHAPTER 5:

CONCLUSIONS

The studies described in this dissertation examined the contribution of the hippocampus and related neocortical structures to spatial memory and navigation, and provided a more nuanced view of how these structures work together and in parallel to allow subjects t onavigate through the world. They serve to provide a model by which we can reconcile the hippocampus' often differentiated roles in supporting declarative memory in humans and processing spatial information in rodents. They suggested that the rodent hippocampus is needed for spatial memory tasks not because it is primarily a spatial structure but because the information needed to support these tasks exceeds their working memory capacity and must be encoded by long-term memory. The studies also suggested that other extrahippocampal structures, particularly the posterior parietal cortex (PPC) and medial entorhinal cortex (MEC), work in parallel with each other and support spatial navigation in complementary ways.

Chapter 2 presented the first study, in which we ran parallel path integration tasks in humans and rodents with hippocampal and medial temporal lobe (MTL) damage (Kim, Sapiurka et al., 2013). While previous studies had shown that the hippocampus was critical for successful path integration in rodents, it was unclear whether this was the case for simple trials that could presumably be supported by

intact working memory (as had been suggested to be the case in humans). While we found that patients with MTL damage were able to use working memory to path integrate as well as control subjects, rodents with lesions of the hippocampus were incapable of path integration, even when the paths were 1 meter or less in length, took 3 seconds or less to complete, and involved no turns. We concluded that these results may reflect a difference in working memory capacity between rodents and humans. While humans can support the information needed for path integration with working memory, rodent working memory capacity may be exceeded by this information, requiring the task to be supported by long-term memory and the hippocampus.

Chapter 3 presented the second study, which further investigated whether long-term memory was indeed required for path integration. We hypothesized that rodents with lesions of the medial prefrontal cortex (mPFC) would show no impairment on path integration, as these lesions cause a deficit in working memory while leaving long-term memory intact. Indeed, mPFC lesion animals performed as well as control animals on path integration, suggesting that long-term memory is used to support path integration. We then compared the performance of animals with mPFC and hippocampal lesions on spatial alternation (a task that requires spatial working memory), as well as a novel nonspatial working memory task (odor alternation). mPFC and hippocampal lesions both led to poor spatial alternation performance, but only animals with mPFC lesions were impaired on the odor alternation task. Hippocampal lesion animals performed as well as controls on odor

alternation. We concluded that while it is possible that the rodent hippocampus may be a structure primarily concerned with spatial information, it is may also be that the amount of information required for spatial memory tasks always exceeds rodent working memory capacity. Thus the rodent hippocampus is critical for these tasks because of its role in encoding long-term declarative memory.

Chapter 4 presented the third study, which investigated the contributions of the PPC and MEC to path integration as well as differentiated between how lesions of these areas affect performance. We measured both how accurate the animals' memory of their starting location was, as well as the efficiency of the path to the start box. While PPC and MEC lesions both impaired the accuracy measure, animals with PPC lesions had less efficient return paths than either MEC or control animals, even when they successfully returned directly to the starting location. This suggests that the PPC and MEC contribute to spatial navigation via different means. While the MEC appears to contribute to the animals' spatial memory of the path, the PPC contributes to the planning and execution of the most efficient routes independent of memory.

Together these studies present a model of spatial memory that is based around the hippocampus and its role in declarative memory. While the spatial nature of these memories and tasks is key, long-term memory is needed for the rodent to successfully navigate through the environment. The PPC and MEC serve to support this navigation both by planning efficient paths and routes and by supporting the recall of critical locations in the environment. They provide a

different view of the interaction of space and memory in the hippocampus, one that opens up new avenues of research in both rodents and humans.

Appendix: Figures and Tables

Table 1: Characteristics of memory-impaired patients

				WMS-R				
Patient	Age (yrs)	Education (yrs)	WAIS-III IQ	Attention	Verbal	Visual	General	Delay
D.A.	30	12	95	104	90	91	90	56
K.E.	70	13.5	108	114	64	84	72	55
L.J.	74	12	101	105	83	60	69	<50
G.W.	53	12	108	105	67	86	70	<50
G.P.	61	16	90	102	79	62	66	50

WAIS-III is the Wechsler Adult Intelligence Scale-III and the WMS-R is the Wechsler Memory Scale-Revised. The WMS-R does not provide numerical scores for individuals who score < 50. IQ score for D.A. is from the WAI

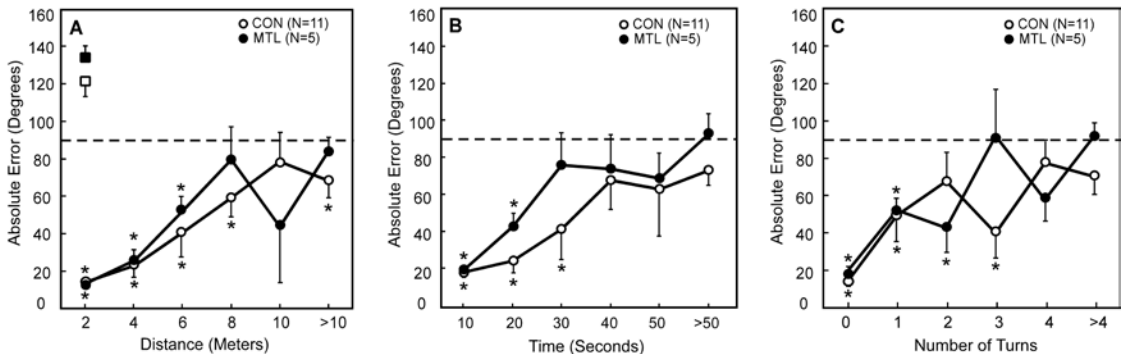

Figure 1: Experiment 1: Path integration by memory-impaired patients (MTL) and controls (CON). A. Performance as a function of the distance traveled to find the tile. When participants were disoriented by rotation (squares), they were no longer able to rely on self-motion cues and failed to path integrate. B. Performance as a function of the time taken to find the tile. C. Performance as a function of the number of turns made to find the tile. The dotted line indicates chance performance (90° error). * denotes above-chance performance. Brackets show S.E.M.

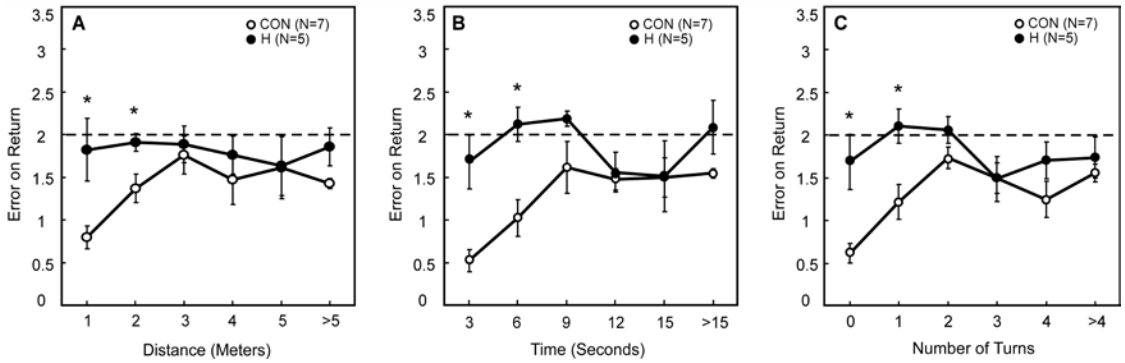

Figure 2: Experiment 2: Path integration by rats with complete hippocampal lesions (H) and controls (CON). The error in the return path was measured by which box the animal first returned to (start box = 0; the two boxes immediately adjacent to the start box = 1; the two boxes 90° removed from the start box = 2; the two boxes 135° removed from the start box = 3; the box 180° from the start box = 4). A. Performance as a function of the distance traveled to find the food. B. Performance as a function of the time taken to find the food. C. Performance as a function of the number of turns made to find the food. The dotted line indicates chance performance. * denotes group difference, $p < 0.05$. Brackets show S.E.M.

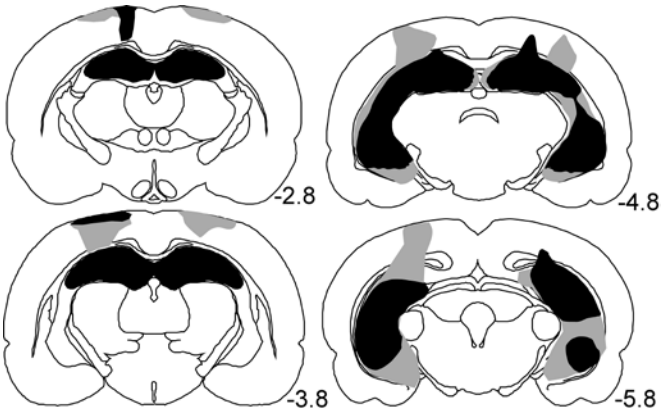

Figure 3: Reconstruction of coronal sections at four A-P levels through the hippocampus showing the smallest (black) and largest (gray) lesion. Numbers to the right of each section represent the distance (mm) posterior to Bregma. The upper left section is the most anterior section and the lower right section is the most posterior section.

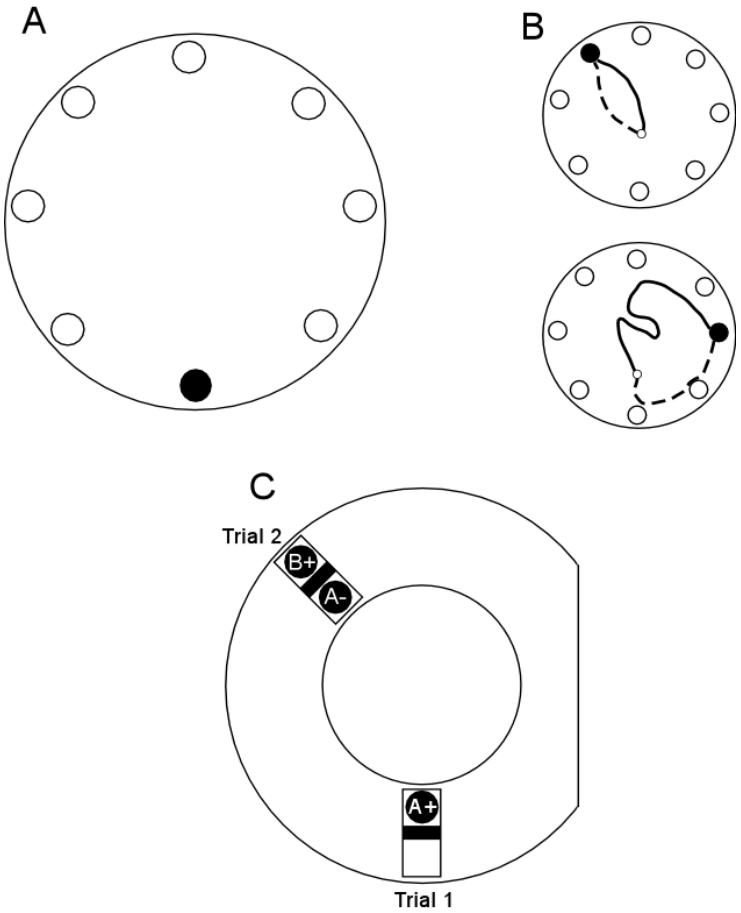

Figure 4: Path Integration and odor alternation. (A) The platform (2m diameter) used for path integration had eight escape holes, only one of which was open for any trial (indicated in black). The trial began when the rat left the escape hole to search for a food pellet and ended when the rat returned to the escape hole to eat the food. The accuracy of the return path was determined by the first hole that the rat visited after finding the food. A direct return to the open hole received a score of 0. A return to a hole adjacent to the open hole received a score of 1, and a return to holes further from the open hole received scores of 2, 3 or 4. (B) Two sample trials where the outward path to find the food is indicated in black, and the return path to the open hole is indicated by a dashed line (the food is represented by a white circle). The shorter path would receive a score of 0, and the longer path a score of 2. (C) The platform (91 cm diameter at widest point) used for odor alternation had a circular insert (48 cm diameter, 35 cm tall) in the middle, creating an annular corridor (24 cm wide). On trial 1, a holder with a baited cup of scent A was placed in the annulus. After the animal successfully dug to retrieve the food reward, the first holder was removed, and a second holder with cups of both scent A and B was placed in a second location in the corridor. On trial 2, the cup with scent B was baited, and the presentation side of the baited cup was counterbalanced across trials. The animal was recorded as making a choice when it began to dig in one of the cups.

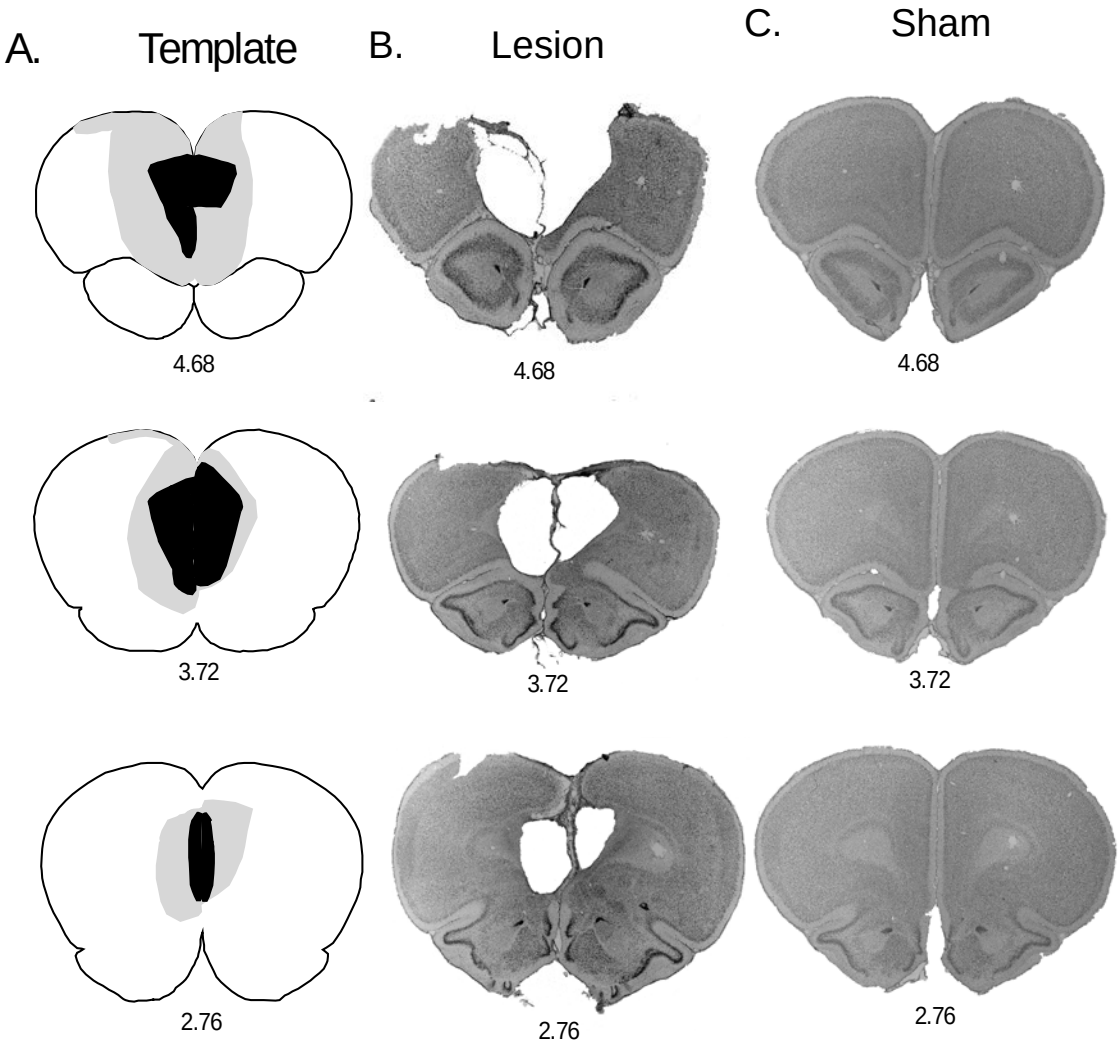

Figure 5: mPFC Histology. (A) A template showing the extent of the largest (grey) and smallest (black) lesions. Numbers below each template represent millimeters anterior to bregma. Damage to the prelimbic and infralimbic cortices ranged from 85%-100% (mean: 97%). There was also significant damage to the medial orbital cortex (mean: 93%), cingulate cortex (Area 1 mean: 68%, Area 2 mean: 47%), and the dorsal peduncular cortex (mean: 56%). (B). Representative histological sections from an animal with a medial prefrontal cortex lesion.

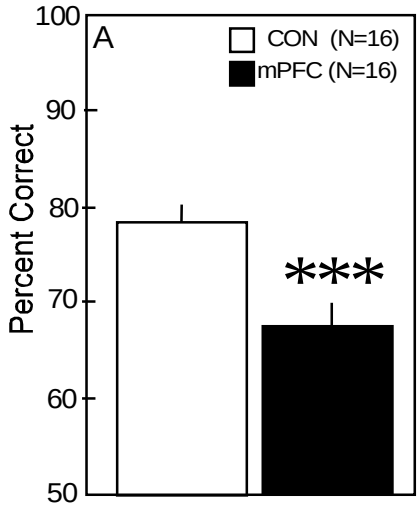

Figure 6: Spatial alternation after lesions of medial prefrontal cortex. Performance on the spatial alternation task was measured by the percentage of trials in which the animal correctly alternated arm entrances to receive food reward (percent correct). Animals received 7 trials a day for ten days. Animals with mPFC lesions were impaired relative to sham operated controls across ten days of testing. Error bars show the standard error of the mean (***p<.001)

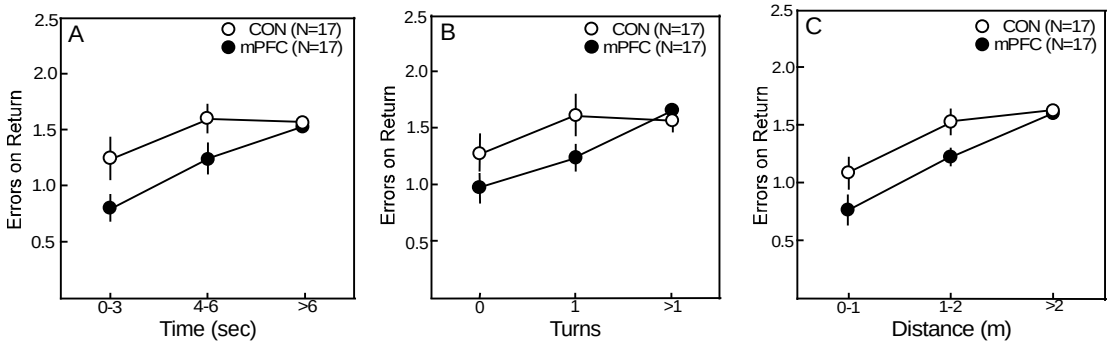

Figure 7: Path integration after lesions of medial prefrontal cortex. Trials were sorted according to the amount of time taken on the outward path (A), the number of turns taken on the outward path (B), and the distance traveled on the outward path (C). The accuracy of the return path (errors on return) was determined by the distance between the first hole visited and the open hole where the trial started. Lower scores indicate better performance. Animals with medial prefrontal cortex lesions (mPFC) performed as well as sham-operated controls (CON) in every condition and usually numerically better. Error bars show standard error of the mean.

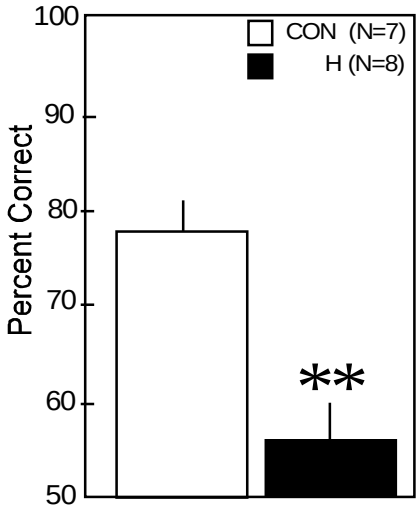

Figure 8: Spatial alternation after lesions of hippocampus. Performance on the spatial alternation task was measured by the percentage of trials in which the animal correctly alternated arm entrances to receive food reward (percent correct). Animals received 7 trials a day for ten days. Rats with hippocampal lesions were impaired relative to sham operated controls across the ten days of testing. Error bars show the standard error of the mean (**p<.01).

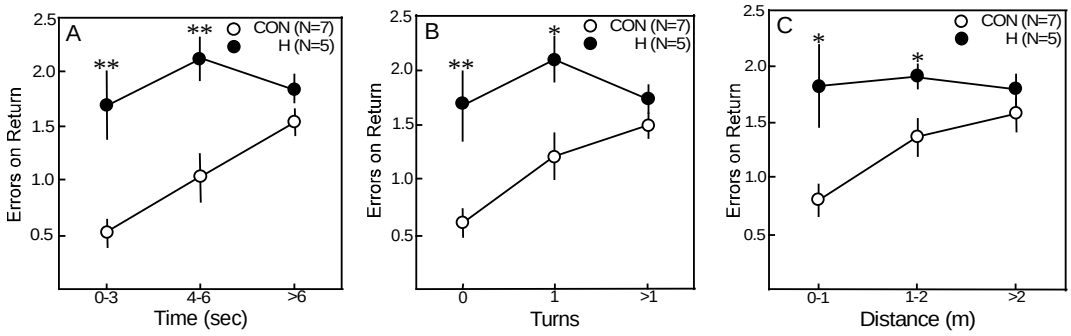

Figure 9: Path Integration after lesions of the hippocampus (from Kim, Sapiurka et al 2013). Trials were sorted according to the amount of time taken on the outward path (A), the number of turns taken on the outward path (B), and distance traveled on the outward path (C). The accuracy of the return path (errors on return) was determined by the distance between the first hole visited and the open hole where the trial started. Lower scores indicate better performance. Animals with hippocampal lesions (H) were impaired relative to sham operated controls (CON) on outward paths that took 6 seconds or less to complete (A), involved 0 or 1 90° turn (B), and were 2 meters or shorter (C). As trials become more complex, both groups performed poorly. Error bars show the standard error of the mean (** p<.01, *p<.05).

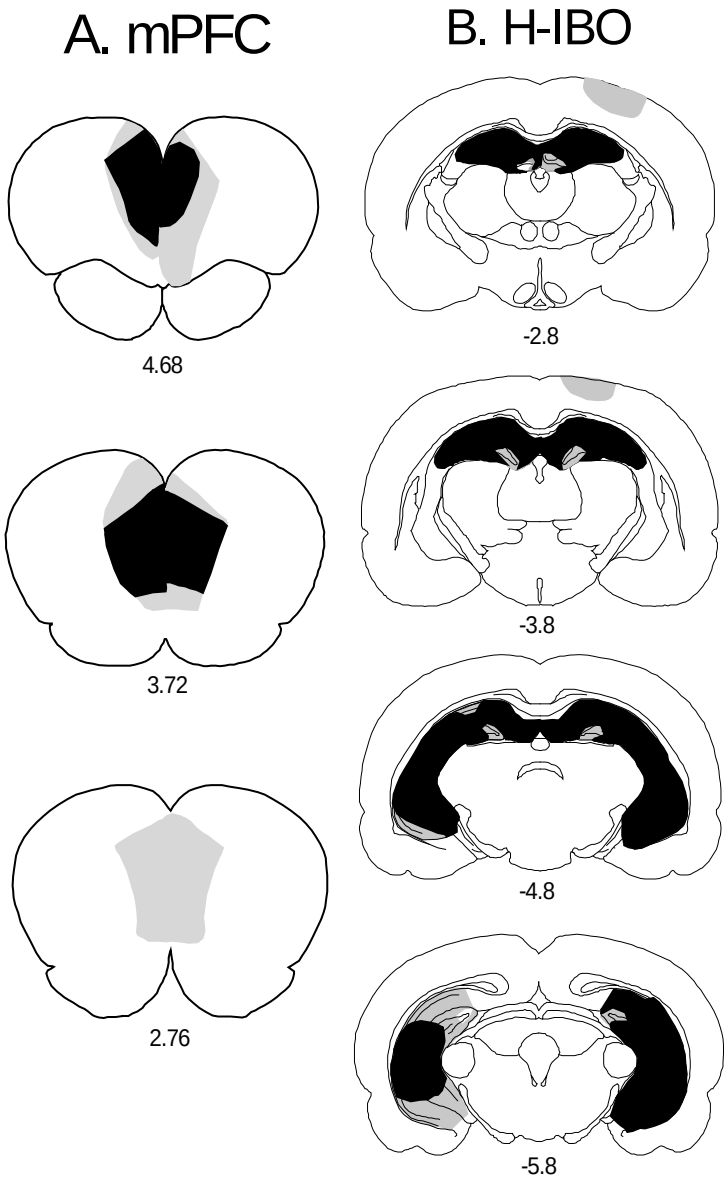

Figure 10: Odor alternation histology. (A) A template showing the extent of the largest (grey) and smallest (black) mPFC lesions. Numbers below each template represent millimeters anterior to bregma. Damage to the prelimbic and infralimbic cortices ranged from 70%-00% (mean: 87%). There was also significant damage to the medial orbital cortex (mean: 70%), area 1 of cingulated cortex (mean: 46%), and the dorsal peduncular cortex (mean: 25%). (B) A template showing the extent of the largest (grey) and smallest (black) H lesions. Numbers below each template represent millimeters anterior to bregma. Damage to the hippocampus ranged from 83-96% (mean: 90%); sparing occurred most frequently in the most medial aspects of the dorsal dentate gyrus and the ventral-most region of the hippocampus.

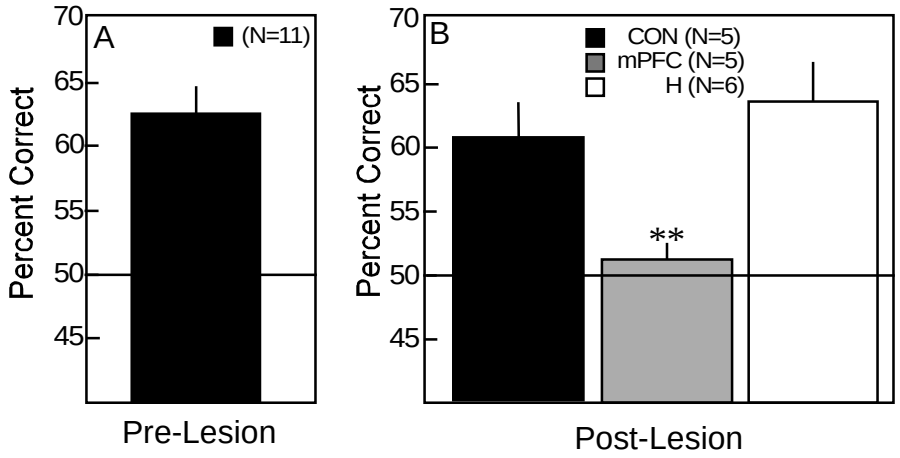

Figure 11: Odor alternation. Performance on the spatial alternation task was measured by the percentage of trials in which the animal correctly alternated digging in scented sand to receive food reward (percent correct). (A) The performance of all animals during the final two weeks of pretraining (mean=62.5%). Animals were performing better than chance (50%). They were subsequently split into two groups of matched performance and given either a bilateral hippocampal lesion (H) or sham surgery. The animals who received the sham surgery later received bilaterally lesions of the medial prefrontal cortex (mPFC) (B). The performance of the animals in the two weeks of testing post-surgery. H animals performed as well as sham controls after surgery and both performed better than chance. By contrast, the mPFC animals were impaired relative to both the H and sham groups, and did not perform better than chance. Error bars show standard error of the mean (** $p<.01$).

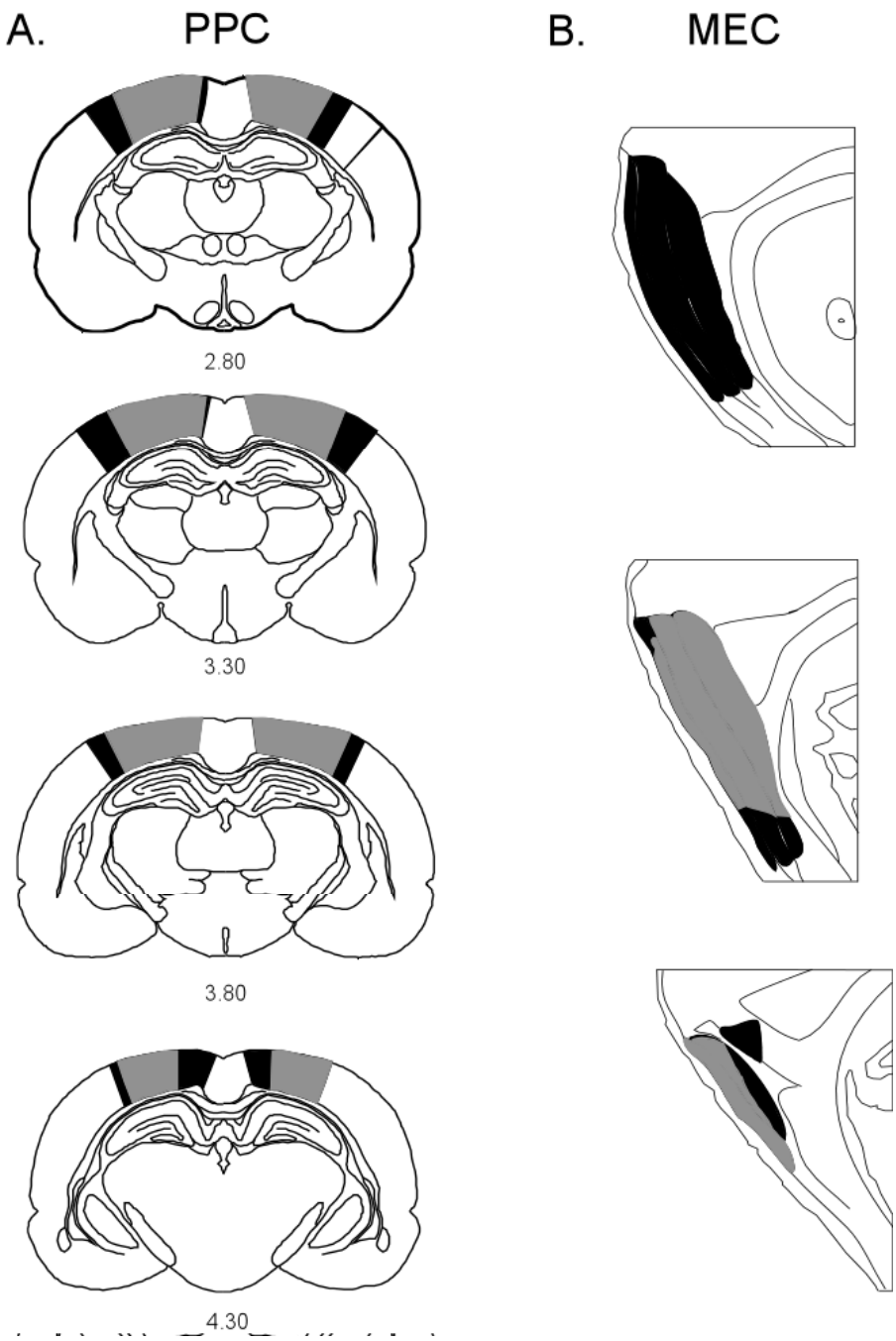

Figure 12: Histology. (A) A template showing the extent of the largest (black) and smallest (grey) posterior parietal cortex (PPC) lesions. Numbers below each template represent millimeters posterior to bregma. Damage ranged 82% to 93% (mean: 85%). (B) A lateral-medial template showing the extent of the largest (black) and smallest (grey) medial entorhinal cortex (MEC) lesions. Damage ranged from 67% to 100% (mean: 84%), with sparing occuring most frequently at the most lateral aspect of the MEC.

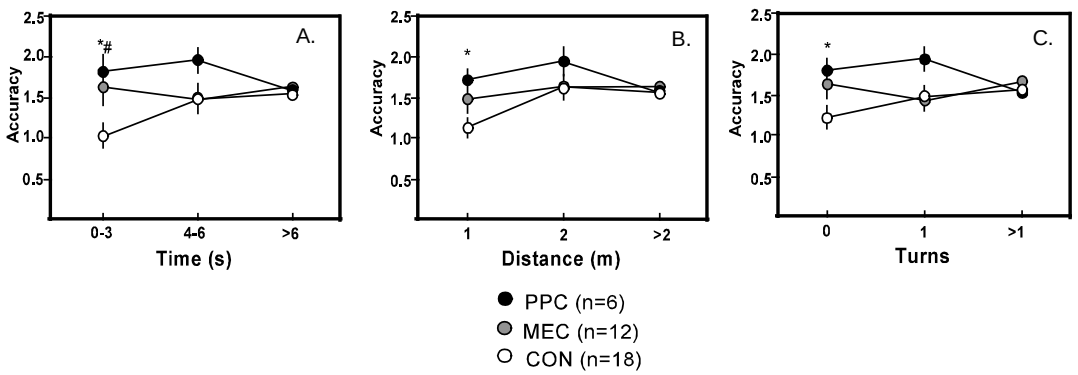

Figure 13: Path integration accuracy. Trials were sorted according to the amount of time (A), distance traveled (B), and number of turns taken (C) on the outward path. The accuracy of the return path was determined by the distance between the first hole visited and the starting location. Lower scores indicate better performance. Animals with MEC lesions were impaired relative to controls on paths completed in 3 seconds or less (A), while animals with PPC lesions were impaired on paths that took 3 seconds or less (A), were 1 meter or shorter (B), and involved no turns (C). Error bars show the standard error of the mean (MEC: #p<.05; PPC: *p<.05)

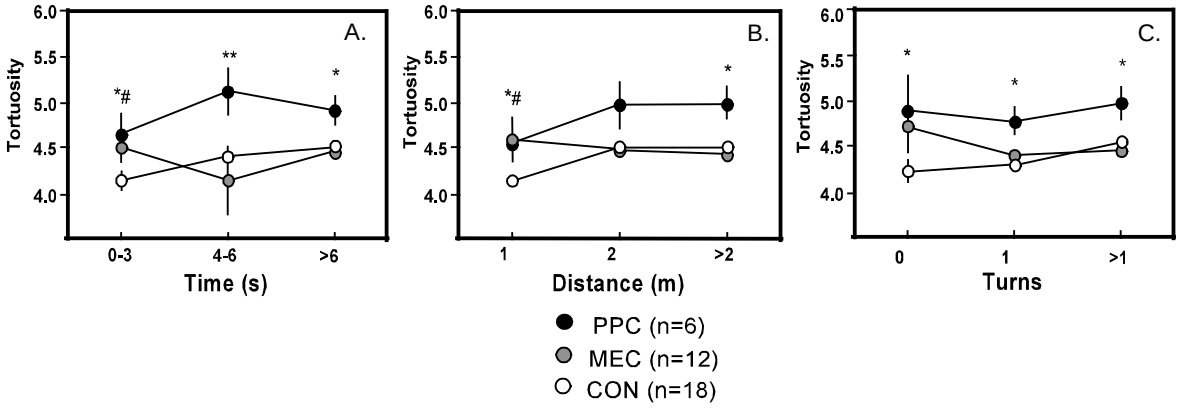

Figure 14: Path integration tortuosity. Paths were sorted in the same manner as in Figure 13. Performance was measured by the tortuosity, or efficiency, of the return path. This was calculated by dividing the length of the return path by the length of a direct path from the point where the food was found to the start box. Lower scores indicate better performance. Animals with MEC lesions showed more tortuous paths than controls on trials where the food was found in 3 seconds or less (A) or when the outward path was 1 meter or less in length (C). Animals with PPC lesions had more tortuous return paths than controls on almost every measure of the outward path, showing a robust effect of the lesion on navigational efficiency. Error bars represent standard error of the mean (MEC: #p<.05; PPC: *p<.05, **p<.01)

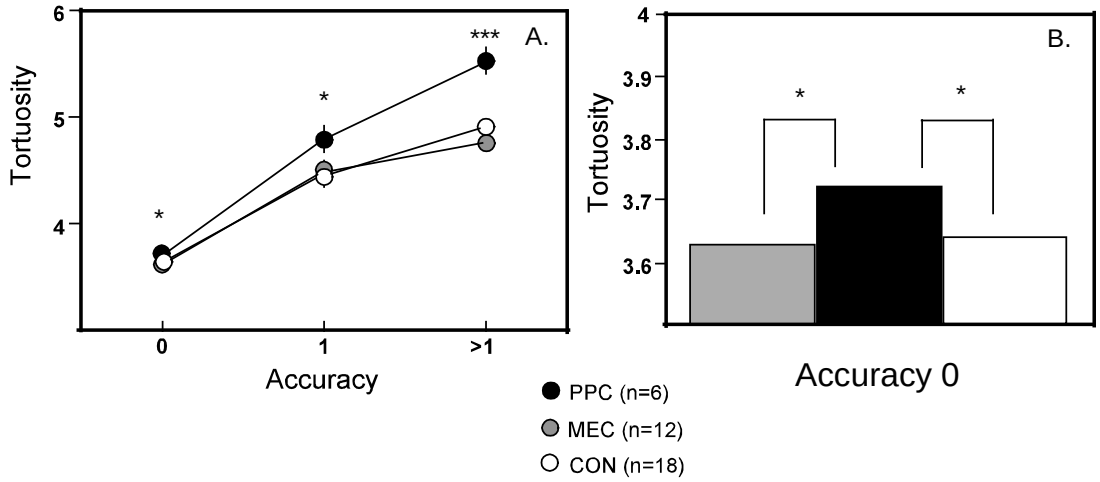

Figure 15: Accuracy and tortuosity. Trials were sorted based on the accuracy of the animal's return path, with 0 representing a direct return to the starting location and intact memory for that location. Tortuosity was used to measure the efficiency of this return path, with lower scores indicating better performance. Animals with PPC lesions had more tortuous and less efficient paths than MEC or control animals (A), even when they returned directly to the starting location (B). Error bars represent standard error of the mean (*p<.05, ***p<.001

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