An Investigation of the Postsubiculum's role in Spatial Cognition

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Abstract

The hippocampal formation has been implicated in spatial formation for many decades. The hippocampus proper has received the most attention but other regions of the hippocampal formation contribute largely to spatial cognition. This thesis concentrated on one such region, the postsubiculum. The postsubiculum is considered important because it contains head direction cells and because it thought to be a major input to the hippocampus, via the entorhinal cortex. This thesis aims to test the functional role of the rat postsubiculum under two types of situation: one where the rat must rely on idiothetic cues for navigation, and another where the rat has visual cues present and can rely on these for orientation. The thesis also investigates hippocampal place cells and their stability over time after short exposures to novel environments.

Chapter 3 of this thesis aimed to test whether the postsubiculum is necessary for path integration during a homing task. Rats were trained on a homing task on a circular platform maze. Once the task was acquired, rats were given lesions of the postsubiculum or sham lesions and then re-tested on the path integration task. The homing performance of rats with lesions of the postsubiculum was as good as that of the sham rats. A series of manipulations suggests that the rats were homing by path integration, confirmed by probe tests. The rats were then tested on a forced-choice delayed alternation T-maze task that revealed a significant impairment in alternation with delays of 5, 30, and 60 seconds. This suggests that the postsubiculum is not necessary for path integration in a homing task but is necessary for avoiding previously visited locations as is necessary in an alternation task.

The experiments in Chapters 4 and 5 of this thesis aimed to investigate the effects of postsubiculum pharmacological inactivation on hippocampal CA1 place cells when rats were introduced to a novel environment with visual cues. A necessary first step was to assess place cells without any manipulation of the postsubiculum (Chapter 4) and then use information gained from this in the design of experiments in Chapter 5.

Rats chronically implanted with recording electrodes in the CA1 region of the hippocampus were exposed to novel cue-rich environments whilst place fields were recorded. Following delays of 3, 6, or 24 hours, the same cells were recorded again in the same environment but with the cues rotated by 90°. Pixel-by-pixel correlations of

the place fields show that stability of the place fields was significantly lower at 24 hours than at 3 hours. Stability after 6 hours was not significantly different from 3 hours.

In the third set of experiments, rats were implanted with drug infusion cannulae in the postsubiculum and recording electrodes in CA1. Following infusions of either the AMPA receptor antagonist CXQX, the NMDA receptor antagonist D-AP5 or a control infusion of ACSF, place field stability was assessed as rats were exposed to a cylindrical environment with a single polarising cue card for 3 x 10 minute sessions and then again 6 hours later. There were no differences in place field correlations between the 3 drug conditions, although there was evidence of larger changes in spatial information content between cells in the CNQX and AP5 drug condition, but not the ACSF condition. The results suggest that, under the present testing conditions, place fields stability did not depend upon AMPA receptor-mediated transmission nor did it depend on NMDA receptor-mediated synaptic plasticity.

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Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

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Abbreviations

3D	3 dimensional
ACSF	Artificial cerebrospinal fluid
ADN	Anterior dorsal thalamic nucleus
AMPAR	Amino-3-hyroxy-5-methyl-isoxazole-4-propionate receptor
AP5	D-(-)-2-Amino-5-phosphonopentanoic acid
ATN	Anterior thalamic nucleus
CA	Cornu ammonis
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
DG	Dentate gyrus
DTN	Dorsal tegmental nucleus of Gudden
EC	Entorhinal cortex
EPSP	Excitatory post synaptic potential
fEPSP	field Excitatory post synaptic potential
LDN	Lateral dorsal thalamic nucleus
LMN	Lateral mammillary bodies
LTP	Long term potentiation
MEC	Medial entorhinal cortex
NMDAR	N-methyl-D-aspartate receptor
PaSUB	Parasubiculum
PER	Perirhinal cortex
PoS	Postsubiculum
PreS	Presubiculum
Rd	Retrosplenial dysgranular cortex
Rga	Retrosplenial granular a cortex
Rgb	Retrosplenial granular b cortex
RSPL	Retrosplenial cortex
RT	Reticular nucleus
ST	Striatum
VIS	Visual area

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Chapter 1: Overview

When animals navigate, they use a variety of sources or information to guide them. Although navigation is a fundamental cognitive ability, it is complex because information from different sensory modalities is used that needs to be integrated over time and space (Wolbers and Hegarty, 2010). Our ability to understand the neural circuits involved in navigation and how they interact would be a major benefit to neuroscience since navigation is one of the oldest forms of cognitive function, from an evolutionary viewpoint. Our understanding of the brain regions involved in navigation was greatly increased when place cells were discovered in the hippocampus of the rat (O'Keefe and Dostrovsky, 1971) and again when head direction cells were discovered in the rat postsubiculum, a structure in the hippocampal formation. Place cells are neurons in the hippocampus that fire in a circumscribed region of the animal's environment, whereas head direction cells are neurons that fire when an animal's head is oriented in a particular direction, irrespective of the animal's location. These two cell types are thought to give animals the ability to know their location in space (place cells) and the direction that they are facing (head direction cells).

How information from these cell types transfer to behaviour is not fully understood and experiments designed to relate neural physiology to behaviour are extremely important to our understanding of the neural underpinnings of navigation. Head direction cells are thought to support the location-specific firing of hippocampal place cells, but despite many important studies, exactly how this occurs and which brain regions are involved in this is still largely open for debate. The discovery of other cell types that are modulated by spatial information (Hafting, 2005; Solstad et al., 2008) adds to our knowledge but also the debate.

This thesis concentrates on one structure in the head direction cell system, the postsubiculum, and explores its involvement in two types of spatial cognitive function. The first is the ability to path integrate, that is, to keep track of direction and distance travelled using idiothetic (internally generated) cues. The other function is that of place cells to form associations with visual cues in the environment. Chapter 3 of this thesis explores path integration and chapters 4 and 5 investigate the postsubiculum's role in associating visual landmark information with internal representation of location as encoded by place cells in one sub-region of the hippocampus, the cornu amonis subfield 1 (CA1).

The postsubiculum is a brain region containing head direction cells and it has strong projections to the same layers of the entorhinal cortex that give rise to the inputs to the hippocampus. The postsubiculum is thought to influence the CA1 (Calton et al., 2003) and the anterior dorsal thalamic nuclei (ADN) (Goodridge and Taube, 1997) when visual landmarks are present. In addition, lesions to the postsubiculum are known to cause spatial memory impairments (Taube et al., 1992; Kesner and Giles, 1998).

However, it is not known if the postsubiculum is necessary for navigation that requires no visual information, but only idiothetic cues. The first experiment (Chapter 3) was designed to test this. Rats were trained on a food-collecting homing task that was designed to test path integration. Rats were then given lesions of the postsubiculum and tested on the path integration task and on another spatial task, delayed alternation on a T-maze.

The next two experimental chapters of this thesis form an investigation of the postsubiculum's involvement in forming CA1 place cells. A previous lesion study has reported that lesions of this structure impair CA1 place cells ability to *anchor* to

visual landmarks (Calton et al., 2003). A known property of place cells is that the location that they fire in can be strongly influenced by salient visual cues, such that when the cue is moved, the location that the cell fires in moves in register. In addition to the lesion study by Calton et al., several pieces of evidence suggest that this structure is a possible site for the convergence of internal representations of orientation and external representations of landmarks. One such piece of evidence is that CA1 place cells in novel environments with visual cues are affected by systemic administration of an N-methyl-D-aspartate (NMDA) receptor antagonist CPP (Kentros et al., 1998), suggesting that place cell formation and long-term stability requires synaptic plasticity that depends upon these receptors. However, since in this study the drug was injected systemically, the location(s) of the NMDA receptor-mediated plasticity is unknown. The hippocampus itself is unlikely to be such a location, because removal of the hippocampus does not abolish landmark learning in head direction cells (Golob & Taube, 1997; 1999). The premise that that the postsubiculum is a site of this plasticity is tested in this thesis.

Because the stability of CA1 place cells over time is required to test this premise, a study was conducted that investigated this over different time delays (Chapter 4). Place cells were recorded in novel environments with salient visual cues and then stability was tested following delays of 3, 6, and 24 hours. Information from this experiment was then factored into experiments designed to specifically test the postsubiculum's involvement in place cell stability in novel environments (Chapter 5). Place cells were recorded in CA1 in novel environments following infusion of an animo-3-hydroxy-5-methyl-isoxazole-4-proprionate (AMPA) receptor antagonist or an NMDA receptor antagonist directly into the postsubiculum. The same cells were recorded again after a suitable time delay. The AMPA antagonist was used to

determine if neural transmission in the postsubiculum is necessary for the association of landmark and internal representation of orientation and the NMDA receptor antagonist was used to determine whether NMDA receptor-mediated synaptic plasticity in the postsubiculum is necessary for such an association.

This thesis, therefore, aims to contribute towards current attempts to elucidate the roles of the various components of the hippocampal formation in spatial cognition in the hope that doing so will lead to a greater understanding of the neurobiological underpinnings of spatial memory.

Chapter 2: Introduction

The hippocampus is one of the most extensively researched brain regions with tens of thousands of studies relating to the region having been published to date. Since the late 1950s, the hippocampus has been closely linked with memory function and since the early 1970s it has also been linked to spatial cognition. This chapter will first of all introduce the hippocampal formation, of which the postsubiculum is a component, explaining why it is important and how it came to be associated with memory and spatial cognition. The chapter will then go on to describe the specific anatomical connections of the rat hippocampus before focusing on spatial aspects and the different types of neurons that encode spatial elements of the environment. Finally, the chapter will come to a close by outlining the aims of this thesis.

2.1 The hippocampal formation

The definition for the hippocampal formation used in this thesis will be the same as that described by Amaral and Lavenex (2007). Within the hippocampal formation is the hippocampus proper and also other nearby regions. The hippocampus proper includes the regions CA1, CA2 and CA3. The other regions of the hippocampal formation are the dentate gyrus, entorhinal cortex, subiculum, presubiculum (which includes the postsubiculum) and the parasubiculum. The subiculum, pre- and parasubiculum are collectively termed the subicular complex.

The hippocampus has been a structure of great interest since the brain was first studied (Amaral and Lavanex, 2007). It has been subject to several theories concerning its function over the years, ranging from involvement in the olfactory system to a key structure in the Papez circuit, once thought to be the seat of emotion (Papez, 1937). However, nowadays, the hippocampus is closely associated with

memory, a situation that arose mainly due to studies of a patient, Henry Molaison, known as H.M (Scoville and Milner, 1957). H.M had received surgery, which removed large portions of his medial temporal lobes, including approximately 2/3 of his hippocampus. The temporal lobes include the entorhinal cortex, which is the major input structure to the hippocampus. In H.M's case, these inputs to his hippocampus were also damaged almost completely, bilaterally, in addition to most of the amygdaloid complex, and the parahippocampal gyrus (Corkin et al., 1997). His surgery caused severe anterograde amnesia as well as a moderate retrograde amnesia. H.M's retrograde amnesia left him unable to remember events that occurred around one to two years prior to his surgery, although some memories as far back as 11 years were lost also. His anterograde amnesia left him unable to make long-term memories for new events or facts (declarative memories), and even unable to remember his caregivers' names after years of care (Cohen and Eichenbaum, 1993). He was, however, able to learn new procedural skills. This was demonstrated in a mirror drawing task (Corkin, 1968), in which the task was to view an object drawn on paper (e.g. a star) in a mirror, and trace around object carefully, without looking directly at the object. H.M's ability in this task improved over days, although he had no conscious recollection of ever having done it before. Aside from these major deficits, H.M. had intact working memory; he could remember information long enough for conversations, but if distracted he then forgot ever having the conversation, never mind what was said during it (Corkin, 2002).

The combination of H.M's amnesia and the fact that there was a known causal factor for it sparked many animal studies of the medial temporal lobe.

Early lesion studies

With H.M's amnesia in mind, researchers searched for an animal model of amnesia using lesion techniques to find memory impairments (Anderson et al., 2007). There were two main strands of research: monkey studies, in which the lesions made tended not to be exclusive to the hippocampus, and rat studies with hippocampal lesions. Orbach et al. (1960) studied the effects of combined amygdala and hippocampal lesions in monkeys and found that they were impaired on a delayed, spatial alternation task, where monkeys were required to alternate spatial responses following a delay period. However, no deficit was found when making a learned response following a delay period, which would have been expected considering H.M's deficits. Other studies around that time period also failed to produce an amnesic effect using a variety of different experimental procedures (Horel, 1978), including delayed response tasks (Orbach et al., 1960, Mahut and Cordeau, 1963) and matching to sample tasks (Correll and Scoville, 1965, Mishkin and Oubre, 1976). For the matching to sample type, typically, two objects were presented (out of reach) to the animal and one was baited with food in view of the animal. Then, after a time delay a test phase took place where the animal was presented with the two objects. The animal's task was to select the object that was baited. However, as mentioned above, animals (monkeys and rats) had relatively unimpaired performance, even after long delays. This led some researchers to explain the disparity of animal and human hippocampal lesions as simply a difference in species (Anderson et al., 2007). The delayed spatial alternation task was similar to the delayed matching to sample task and involved two objects, one on the left and one on the right. The experimenter would bait one covertly and the animal would then have to choose (guess). On the

following trial, the other object would be baited and the alternation between trials would continue, with a delay in between trials.

A key factor in explaining the animals' lack of impairment in the delayed response task was the practice of using same object pair repeatedly over trials that became common in the 1970s (Eichenbaum, 2002). When a new pair of objects was used in each trial, animals with hippocampal damage had time-dependent impairments: the impairment became more noticeable with longer delays, indicating that the hippocampus was involved in novel object memory.

In the 1970s experimental procedures for investigating lesioned animals improved markedly and several theories pertaining to different types of memory arose, which gave researchers a better understanding of the type of mnemic deficits that accompanied hippocampal damage. For example, a way of classifying memory dependent on context, such as autobiographical memories, as episodic memory in contrast to semantic memory (memory for facts) was proposed by Tulving (1972). In addition, the influential theory of declarative memories that include semantic and episodic memories in contrast to procedural memories (learned skills and habits) was introduced (Cohen and Squire, 1980, Squire, 1992). Around that same time, an important discovery was made involving the enhancement of long lasting synaptic efficacy within the hippocampus by electrically stimulation that led to theories of how the hippocampus actually forms and stores memories.

2.2 Long-term potentiation and learning

Donald Hebb, writing in the late 1940s, suggested that when organisms learn, there is a physical change that occurs in the brain (Hebb, 1949). Specifically, he stated that when two neurons fire at the same time, the strength of the connection between them becomes stronger. In the early 1970s, Bliss and Lomo (1973) discovered that

when two connected hippocampal neurons were stimulated, it led to a long lasting increase in the efficacy of the connection. This is typically accomplished by recording the EPSPs generated at postsynaptic neurons with an electrode, following some stimulus to a number of fibres of a particular neural pathway. These EPSPs are then compared to "baseline" EPSPs recorded, and plotted on a graph as percentage of the baseline. Two different phases of LTP are commonly differentiated: early phase LTP and late phase LTP. Early phase describes LTP occurring up to around one hour, whereas late phase LTP occurs after one hour (Malenka and Bear, 2004). There are different molecules that can trigger the process of LTP and different types of LTP result depending on the triggering molecule. In the CA1 hippocampal subregion, the most prominent type is N-methyl-D-aspartate (NMDA) receptor-dependent LTP. NMDA receptors are voltage-dependent ligand-gated ionotropic receptors that require two ligands to bind, glutamate and glycine, and are blocked by magnesium cations are resting membrane potential (D'Angelo et al., 1990). In CA1, NMDA receptor (NMDAR)-mediated LTP requires postsynaptic NMDAR activation that can occur with tetanic stimulation, which depolarises membrane potential of the postsynaptic neuron. The result is an influx of calcium cations through the NMDAR into the dentritic spines of the postsynaptic neuron. This then triggers LTP through mechanisms not fully understood and beyond the scope of this thesis. After the early phase of LTP, late phase then involves gene transcription and protein synthesis (Abraham and Williams, 2003, Pittenger and Kandel, 2003). Late phase LTP can last many hours and weeks.

LTP has long been thought to be a process by which new memories are made. The synaptic modification seemed to be what Hebb had theorised. The fact that the NMDARs can open only when the postsynaptic cell is depolarised means that

NMDARs functions as detectors of coincidental activity (Nakazawa et al., 2004) giving weight to Hebb's idea of synchronicity between cells. There have been thousands of studies investigating LTP and its biochemistry; however, evidence supporting its role in learning has been comparatively scarce.

In the early eighties, Collingridge (1983) discovered that a highly specific NMDAR antagonist, D-AP5, blocked the induction of LTP in CA1 *in vitro*. Morris and colleagues (1986) tested the effects of D-AP5 (both D and L enantiomers) pumped into the ventricles in rats' brains as they were trained on a spatial reference memory task in the Morris water maze. The group given the drug performed significantly poorer at finding the hidden platform than control animals, taking significantly more time to do so. Although this was evidence that the same specific NMDAR antagonist that blocks LTP in CA1 impairs spatial memory, it was not evidence of a causal role of LTP in learning. An important study by Whitlock and colleagues (2006) provided the first evidence of this causal relationship. They found that the same glutamatergic NMDAR changes occur in the hippocampus after one trial inhibitory avoidance learning that occurs during high frequency stimulation. They also recorded from dorsal hippocampus after learning and found that field EPSPS (fEPSPs) were elevated significantly compared to control animals. In addition, the learning masked the effects of high frequency stimulation delivered post-learning.

Studies with genetically altered mice have also given weight to the evidence supporting NMDAR-mediated plasticity as a mechanism for learning. Specifically, mice with the NR1 gene deleted specifically in the CA1 hippocampal subregion (CA1-NR1 knockout) have been shown to have impaired spatial memory as demonstrated on a hidden platform water maze task (Tsien et al., 1996) and also

impaired spatial tuning of CA1 place cells (McHugh et al., 1996). The NR1 subunit is one of seven identified NMDAR subunits that is necessary for functional NMDARs.

LTP is thought to be one of many forms of activity-dependant plasticity that contributes to learning and memory (Malenka and Bear, 2004). There are, however, studies that do not support the role of NMDAR-dependant plasticity as necessary for spatial learning. For example, when rats have had pre-training in the hidden platform water maze task, NMDAR pharmacological blockade does not impair new place learning (Bannerman et al., 1995).

2.3 The hippocampus and cognitive maps

In 1971, perhaps one of the most important and influential papers was published concerning the hippocampus. O'Keefe and Dostrovsky (1971) recorded hippocampal neurons in awake, moving rats and discovered that some of them fired as a function of the rat's location. These cells came to be known as *place cells*, which will be discussed in detail in section 2.5.1. The discovery of place cells in the hippocampus was a major factor that led to the influential book, "The hippocampus as a cognitive map" (O'Keefe and Nadel, 1978) some years later, in which the main idea posited was that the hippocampus forms an internal representation of the environment (analogous to a survey map), which allows the animal to make navigational short-cuts along a route when available. Their envisioned cognitive map is an *allocentric*, or world-based map. Allocentric refers to the relationship between landmarks in the environment, in contrast to egocentric, which refers to the relationship of landmarks in the environment and the navigator (rat). An allocentric map, therefore, functions independently of the position of the navigator.

The idea of a cognitive map comes from Tolman based on experiments carried out by himself and colleagues. The experiment from his laboratory most commonly

cited as evidence of the existence of cognitive maps involved a *sunburst* maze (Tolman et al., 1946). In this experiment, rats were trained on an alley-type maze (Figure 1.1) to retrieve a food reward at a goal location after following a single route. In a test of acquisition of a cognitive map, the training maze was adapted to a sunburst maze as shown. The learned route was blocked off and rats had to choose an alternative in order to reach the goal box. The result taken as evidence of a cognitive map is that 36% of rats chose the arm leading directly to the goal box. However, in this particular experiment, the light at position H may have been used as a beacon to the goal box, which negates the need for a cognitive map. In addition, the results could not be replicated (Gentry et al., 1947, Gentry et al., 1948). Olton (1979) points out that another flaw in this design was that, since the rats were originally trained to traverse a path with three 90° turns, it is hard to say what their correct response to the test *should* have been. For example, one may have expected them to choose an arm adjacent to the original arm C (say, arm 10) in an attempt to reach the original route at a point beyond the block.

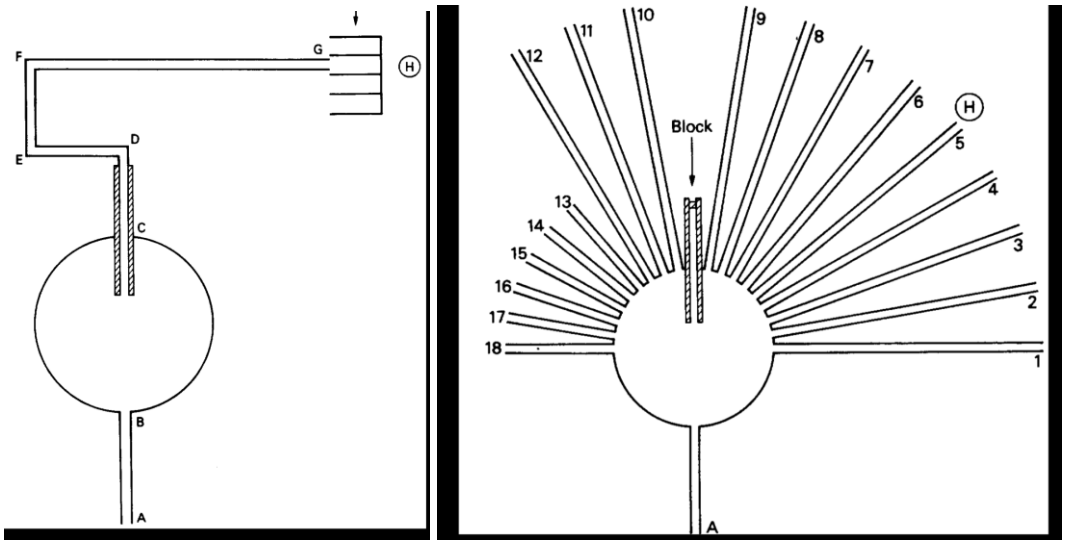

Figure 1.1. Training maze (left) and sunburst maze (right) used by Tolman et al. (1946). Start point indicated by A and goal box by G. H indicated the location of a light that shone down the F-G alley. Figure from O'Keefe and Nadel (1978).

However, there are many other experiments that do appear to support the idea of a cognitive map, or locale navigation strategy. Morris (1981) introduced his water maze in a task where, in one condition, there was a platform submerged below the water. The water was made opaque by adding milk so that the platform was hidden and no proximal cues were available but the room had a number of (distal) cues beyond the maze, for example, a window on one wall and some shelves on another. Rats managed to find the hidden platform when placed into the pool at different starting locations, suggesting that they were able to use the constellation of distal cues to form a cognitive map allowing for location of the hidden platform to be ascertained. When the location of the hidden platform was changed from trial to trial, performance was poor, suggesting that the rats could not see/smell the platform (or use any other possible local cues). Additional evidence supporting O'Keefe and Nadel's (1978) theory comes from Morris et al. (1982). In this study, rats with hippocampal lesions were tested on the reference memory water maze task. The lesioned rats were unable to find the hidden platform in contrast to control rats. The lesioned rats could, however, find the platform if it was cued. There is also evidence of impaired spatial cognition in humans with hippocampal damage (Maguire et al., 1996), which supports the view that the hippocampus contains a cognitive map.

One of the main problems that the *hippocampus as a cognitive map* theory has is that the hippocampus encodes not only space, but also many non-spatial aspects. For example, place cells have been recorded that respond to odours (Wood et al., 1999), goal locations (Breese et al., 1989, Wiener et al., 1989) and running speed (Muller et al., 1987). In addition, place cells tend not to fire when the animal is stationary. If the main function of the hippocampus is to store some type of maps for different environments, it is difficult to explain why it encodes all of these non-spatial

elements. Another problem arises when the animal lesion literature is compared to the human literature. Whilst both rats and humans with hippocampal lesions are impaired on spatial tasks, humans have impaired episodic memory and profound amnesia.

A final problem is that is often unclear exactly what is meant by a *cognitive map*, to the extent that the question is almost meaningless (Bennett, 1996) or at least, very difficult to answer (Jeffery, 2008).

2.4 Anatomy of the hippocampal formation

The hippocampal formation is strongly implicated with spatial cognition and so it is worthwhile outlining how it is organised anatomically and its known connections. This section is in two parts, the first covering important connections relating to the hippocampal formation and the second will include connectivity within the head direction cell system and how it is associated with the hippocampus. The head direction cell network is heavily implicated in navigation and will be discussed in section 2.5.3.

2.4.1 The hippocampal formation's connectivity

The hippocampus is unlike many neocortical brain areas in that many of its connections are unidirectional (Figure 1.2). The main input to the hippocampus is from the entorhinal cortex. Layer II neurons in the entorhinal cortex project to the dentate gyrus and the CA3 subfield along what is known as the perforant pathway (Masaoka et al., 1989). Neither projection is reciprocated. Granule cells in the dentate gyrus project via mossy fibres (axons) to CA3 unidirectionally, and CA3 neurons project to the CA1 along Schaffer collaterals (axons), also unidirectionally (Amaral and Lavanex, 2007). This pathway described so far is called the tri-synaptic loop. CA3 also projects contralaterally via hippocampal commissural conections to all

subregions of the hippocampus: CA1, CA2 and CA3. CA1 neurons then project unidirectionally to the subiculum (Witter, 1993, Amaral and Lavanex, 2007, van Strien et al., 2009). The CA1's main projection is to the deep layers of the entorhinal cortex, completing the tri-synaptic loop (Witter, 1993, Amaral and Lavenex, 2007, van Strien et al., 2009). The subiculum's main projection is also to the entorhinal cortex (Witter, 1993, Amaral and Lavenex, 2007, van Strien et al., 2009). It projects to all layers, but is strongest in deep layers, particularly layer V. The entorhinal cortex also sends a reciprocal connection to the subiculum, through the perforant pathway (Witter, 1993, Amaral and Lavenex, 2007, van Strien et al., 2009). The pathway was called so because it passed through (perforated) the subiculum, but evidence now confirms that some of these fibres terminate here. The subiculum also projects to layer I of the presubiculum, but layer II in the dorsal portion[1] (postsubiculum) (Witter, 1993, Amaral and Lavenex, 2007, van Strien et al., 2009). The subiculum also has strong unidirectional projections to the retrosplenial cortex (Wyss and Van Groen, 1992).

[1] The dorsal presubiculum is called the postsubiculum by many authors. This thesis will refer to it as the postsubiculum; however, there are cases when the literature does not differentiate between them.

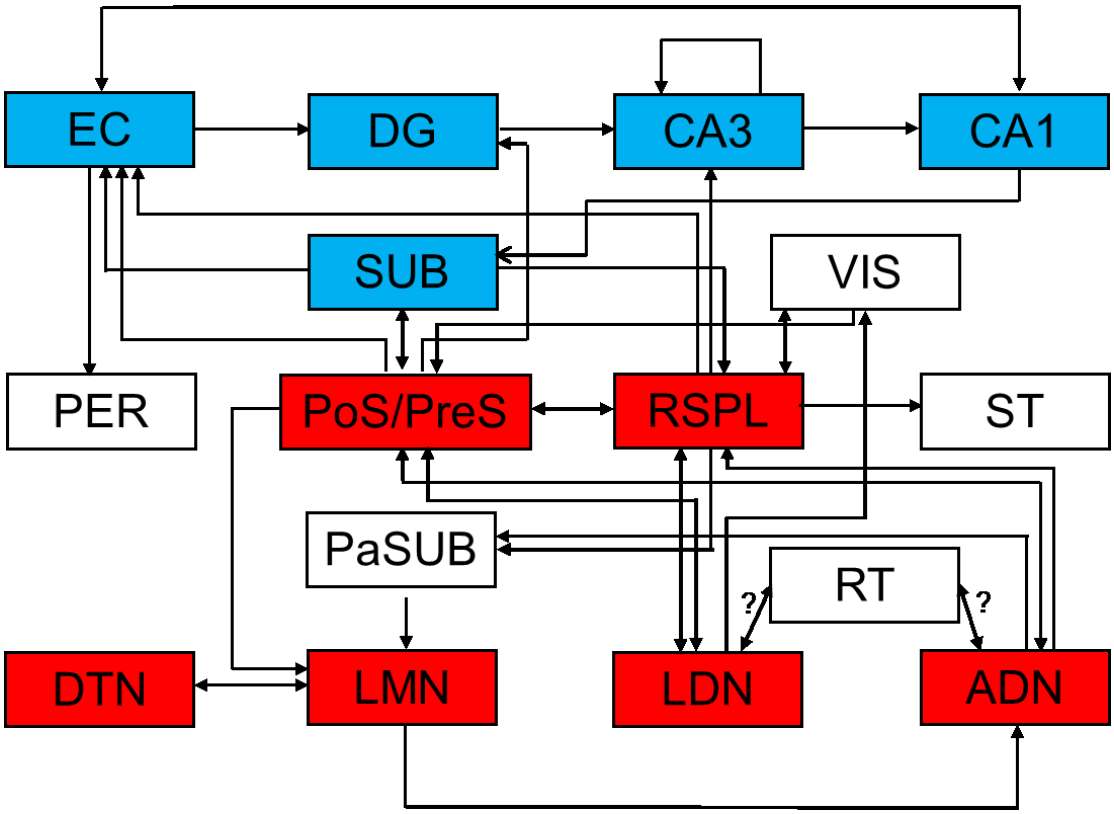

Figure 1.2. Schematic showing known connections relating to the hippocampal formation, the head direction cell system and other related areas. Regions in blue are known to contain place cells; regions in red are known to contain head direction cells, although there is overlap in some regions. (EC, Entorhinal cortex; DG, dentate gyrus; PER, perirhinal cortex; PoS/PreS, postsubiculum/presubiculum; RSPL. Retrosplenial cortex; ST, striatum; PaSUB, parasubiculum; VIS, visual area 18b; DTN, dorsal tegmental nucleus; LMN, lateral mammillary nucleus; LDN, lateral dorsal thalamus; ADN, anterior dorsal thalamus; RT, reticular nucleus.)

Although information is largely unidirectional through the hippocampus, there are mechanisms for feedback to itself. CA3 contains recurrent collateral fibres, which are intrinsic to CA3. In addition, the output from the subiculum is fed back through to the deep entorhinal cortex layers. CA3 also projects to the septum (Amaral and Witter, 1995), which can feedback to the CA3 via the subiculum and entorhinal cortex. The entorhinal cortex also has a direct projection from layer III to CA1. One of the entorhinal cortex's main outputs is the perirhinal cortex, which also has a reciprocal connection with the entorhinal cortex.

2.4.2 The Head Direction cell network's connectivity

Head direction cells have been found in numerous structures (see section 2.5.3), which include the postsubiculum, the anterior thalamic nucleus (ATN), the lateral dosal thalamic nucleus (LDN), the lateral mammillary bodies (LMN) and the dorsal tegmental nucleus of Gudden (DTN). In addition, the retrosplenial cortex will be considered part of this network also, since it contains neurons that are influenced by head direction, although there are important differences between these cells and those from other regions. Head direction cells will be fully described in section 2.5.3, but it is suffice here to say that they are neurons that encode an animal's head direction on the horizontal plane and are thought to be extremely important in spatial cognition.

The presubiculum and parasubiculum receive inputs from the anterior thalamic nucleus (Hopkins, 2005). The postsubiculum receives thalamic projections primarily from anterior dorsal (ADN) and anterior ventral portions of the ATN (van Groen, 1990b). There are also projections from the LDN to the postsubiculum (van Groen and Wyss, 1990b). The postsubiculum also has connections with the retrosplenial cortex (van Groen and Wyss, 1990a, 1992a, 2003); it projects lightly to both the dysgranular and the Rgb subregions but receives projections from all three main retrosplenial sub-regions (Rd, Rgb and Rga). The postsubiculum receives projections from layer V of visual area 18b to layers II and III (Vogt and Miller, 1983). The presubiculum (including postsubiculum) has a strong projection to layers I and III of the medial entorhinal cortex (less so to layer II), both ipsilaterally and contralaterally (van Haeften et al., 1997). The projections are arranged topographically, such that the postsubiculum projects to the dorsal medial entorhinal

cortex whereas the ventral presubiculum projects to ventral portions of the medial entorhinal cortex.

The LDN has strong projections to the retrosplenial dysgranular cortex, and also the pre- and parasubiculum, whilst it receives projections from the whole retrosplenial cortex in addition to both post- and presubiculum (Hopkins, 2005). The LDN also has a reciprocal connection with the postsubiculum (Thompson and Robertson, 1987b, van Groen and Wyss, 1992a). The thalamic nuclei are not known to have interneurons, but perhaps can interact via the reticular nucleus (Crabtree et al., 1998).

Like the LDN, the anterior dorsal thalamic nucleus (ADN) has strong projections to the postsubiculum and the retrosplenial cortex (Hopkins, 2005). But unlike the LDN, the retrosplenial projection back to the ADN is weak (Van Groen and Wyss, 2003). The presubiculum and parasubiculum have (at least) weak projections directly to the dentate gyrus (Kohler, 1985), through which information from the thalamic nuclei could reach the dentate gyrus. In addition, there is a prominent projection from the nucleus reuniens to the CA1 stratum lacunosum-moleculare where there is an overlap with the fibres originating from the entorhinal cortex (Amaral and Lavenex, 2007). Thus, there is a potential direct route for information from the thalamic nuclei to reach the hippocampus proper as well as two indirect routes (via retrosplenial cortex > CA3 and from thalamic nuclei > DG > CA3). This may be important as the thalamic nuclei are important structures that encode head directional information (see section 2.5.3). The nucleus reuniens also projects to the subiculum (Amaral and Lavenex, 2007), thereby providing yet another potential indirect source of information from the thalamus to the hippocampus (Nucleus reuniens > subiculum (also > postsubiculum) > entorhinal cortex > CA3).

The head direction cell signal is thought to originate in the DTN, which receives projections from the vestibular nuclei via the nucleus prepositus (Liu et al., 1984, McCrea and Baker, 1985). The DTN then projects to the LMN (Groenewegen and van Dijk, 1984, Shibata, 1987). The DTN also receives inputs from a region thought to be important for motor control and is a potential site for information pertaining to the animal's movements to enter into the head direction cell system (Clark and Taube, 2009). A series of lesion studies has revealed the directional flow of information along most of the head direction cell network. Goodridge and Taube (1997) lesioned either the ADN or the postsubiculum; no postsubicular head directions were found following ADN lesions yet head direction cells were found in the ADN following postsubiculum lesions. This indicates that information flows from the ADN to the postsubiculum. Head direction cells in the ADN are present after lesions of the LDN (Golob et al. 1998) and after lesions of the hippocampus (Golob and Taube, 1997). In addition, lesions to the retrosplenial cortex impaired the ability of ADN cells to anchor to landmarks (cue card), but did not abolish directional tuning of ADN cells.

Lesions of the LMN (Blair and Sharp, 1998) and the DTN (Bassett and Taube, 1999) both impair the directional tuning of cells in the ADN suggesting that the flow of information is from LMN to ADN or from the DTN to LMN. However, as there are no known connections between DTN and ADN, it is presumed that information flows from DTN > LMN > ADN, and the to the postsubiculum.

2.5 Neurons that encode spatial information

2.5.1 Place cells

One of the most important advances in the field of spatial cognition was the discovery of a class of cell in rats that encodes space (O'Keefe and Dostrovsky, 1971). Called place cells[2], they are pyramidal complex-spike neurons of the hippocampus (CA1 and CA3) that fire when the head of the rodent is in relatively small, circumscribed regions of the environment. The region in which a place cell will fire is termed its *place field* (Figure 1.3). Different place cells can have different place fields within the rodent's environment. In addition, a place cell can have a field in multiple environments. At present, it cannot be predicted (i) whether or not a given place cell will have a field a given environment, or (ii) where in the environment a cell's place field will likely be.

[2] They were referred to as "place units" in the seminal paper; the term place cell arose later.

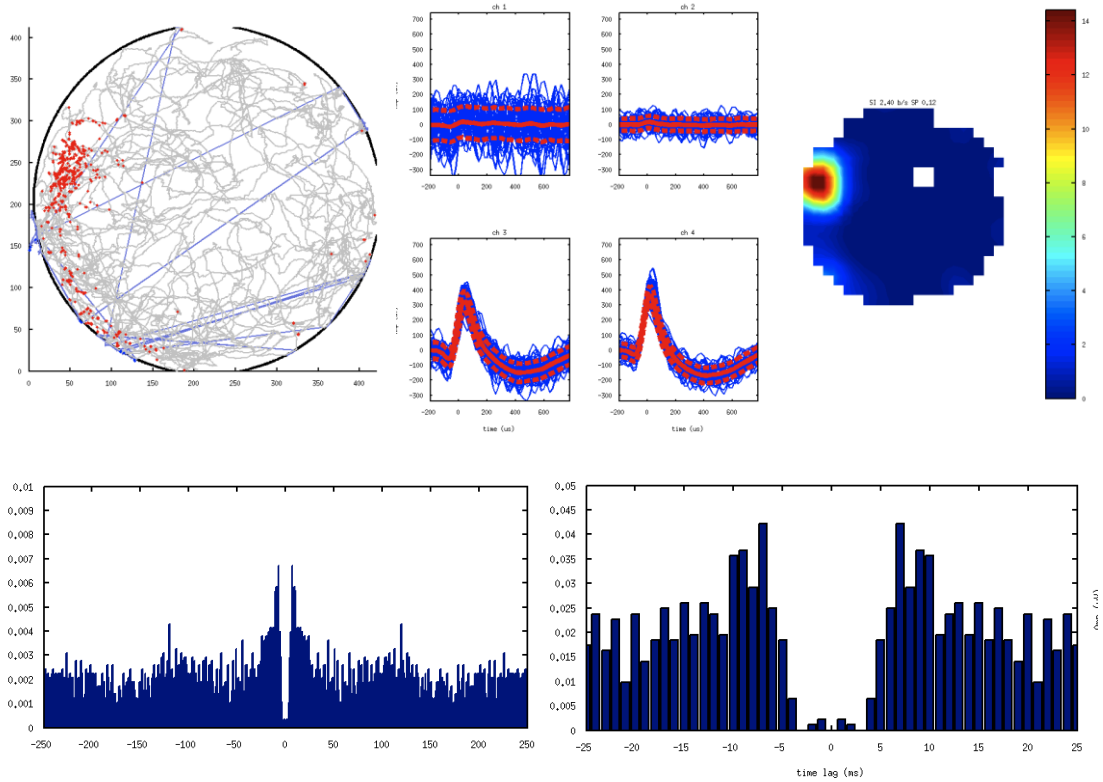

Figure 1.3. Example of a place cell. *Top left* Trajectory of rat (O'Keefe and Black) with unit spiking (red); *Top centre* Waveforms of the cluster on each separate wire on tetrodes. *Top right* Smoothed firing rate map of cluster. Coloured bar represents scale of firing rate where red colours represent higher firing than blue colours. *Bottom left* Autocorrelogram of cluster over 500 ms showing theta wave modulation. *Bottom right* Autocorrelogram over 5 ms showing a clear refractory period. (Bett, unpublished data)

One property of place cells is that their place fields are usually stable across multiple recording sessions within the same environment, even across long periods of time (Muller and Kubie, 1987, Thompson and Best, 1990). If small changes in the environment are made, place cells will tend to retain their place fields. If larger changes are made, place cells can alter their firing properties and either, (i) fire in a different location, (ii) fire in the same location but with a change in firing rate, or (iii) cease firing altogether (Bostock et al., 1991, Kubie and Muller, 1991). Scenario (i) and (iii) are termed global remapping, or complete remapping, whereas scenario (ii) is called rate remapping (Leutgeb et al., 2005). All this is suggestive of a population of place cells that map different environments, with different subsets of the population

being active in different environments. Approximately 50% of place cells are active in a given environment (Muller et al., 1996), although Thompson and Best (1989) report a lower rate of 36.8% in a thorough study investigating *silent* cells. So, what causes place cells to fire in specific locations? The answer, it turns out, is not straightforward.

What factors affect place cell firing?

Visual landmarks

One of the first known properties of place cells was that they are strongly influenced by environmental landmarks. O'Keefe and Conway (1978) recorded hippocampal place cells in freely moving rats on a T-maze, with four salient extra-maze stimuli. They reported that when the stimuli were rotated, place fields tended to rotate in a similar manner, as though the cells' firing were *anchored* to the distal stimuli.

In another study, Olton et al. (1978) investigated distal vs. proximal cues and reported that distal cues have more influence over place fields than proximal cues. However, these cues have to be stable if they are to exert influence.

Jeffery (Jeffery, 1998, Jeffery and O'Keefe, 1999), exposed rats to a square box environment with a polarising cue card and place fields were established. Then, the cue card was rotated with the rat either present or absent. When the rats were absent during rotations, place fields tended to rotate with the cues; however, when the rat was present during the cues being moved, place fields tended not to be anchored to the cues. In other words, when the rats were present during cue movement, their place cells were not "fooled" by the manipulation. This seemed not to be the case though, when there was only a small cue movement. In a study (Rotenberg and Muller, 1997) in which a salient cue card was rotated by 180° in the presence of the rats,

hippocampal place fields did not rotate; however, when the cue card was rotated only 45°, place fields tended to rotate also.

In experiments from another laboratory, Cressant and colleagues (1997, 1999) recorded from freely moving rats in a cylindrical environment, which had three proximal cues near the centre. Rotation of these cues had little effect on place fields. This was not the case when the same cues were moved to the periphery (or near the periphery) of the cylinder; in this case, rotation of the cues in the rats' absence led to similar rotations of place fields.

Proximal cues do, however, exert an influence on place fields. For example, some place cells that had fields near (proximal) cues responded strongly to removal of the cue by reducing their firing rates (Hetherington and Shapiro, 1997). In another study (Muller et al., 1987), a barrier was placed on top of place fields and the effect was that the fields tended to disappear (Breese et al., 1989). One study (Shapiro et al., 1997) used a double rotation, where they rotated distal cues in one direction and proximal cues in the opposite direction. The result was that some place fields were influenced by the distal cues and other fields by the proximal cues.

Idiothetic cues

It is, therefore, well established that visual landmarks can have a large influence on place cell firing. But what happens when there are no visual cues available? The first test of this was by O'Keefe (1976), who recorded from the CA1 area in freely moving rats when the room lights were switched off. He reported that 86% of place cells remained unchanged when the lights were switched off; although some cells lost some spatial information the first time this was done, they improved thereafter with experience. Thus, place cell firing is not only dependent on visual cues or specific configurations of visual stimuli. Muller and Kubie (1987) reported that

CA1 place fields in a cylinder remain relatively unchanged following removal of a polarising cue card (which was sufficient to anchor place fields) between recording sessions. Following removal, place fields lost some spatial coherence, or "crispness", and in some cases were found at random angular positions. When the cue card was replaced at the beginning of the next session, place fields tended to return to their original angular position. This highlights that, whilst visual landmarks can be sufficient to control place cell activity, they are not necessary. In some cells, angular position of fields was maintained between the cue card session and the no cue card session, although the fields followed the cue card after a between session rotation.

Hill and Best (1981) recorded from place cells in dorsal hippocampus of rats that were both blindfolded and deafened. Testing was done on a radial arm maze, and rotations of the maze in the absence of rats led to corresponding rotations of place fields. That is, place fields were anchored to proximal maze cues [in contrast to the distal cues in the Olton et al. (1978) study with rats that could both see and hear]. However, a number of cells recorded had fields that remained anchored to the actual room (i.e. did not rotate with the maze). This suggests that these cells were relying on idiothetic information to keep track of location. To disrupt this, the rats were disoriented by being spun around between the standard and rotated session, and when this was done, most cells then rotated with the maze. Another study (Save et al., 1998) looked at place fields in rats that had been blinded - this time shortly after birth. In a cylinder with 3 proximal cues (3D objects) located at the periphery, the blinded rats developed relatively normal place fields, which rotated with the cues. One difference from the sighted control rats was that these fields were not active until the animals approached at least one of the cues. This means that the rats used intrinsic features of the cues themselves (e.g. tactile and/or olfactory cues) to distinguish the object that

they first approached from the other two. Another noteworthy point in this study is that the blinded rats had place fields not only near the objects, but also between the objects. This means that when navigating between objects, rats likely had to rely on idiothetic cues to track their distance and direction travelled. So, although the rats had no vision, their place cells were influenced by salient landmarks and also by idiothetic cues.

Gothard and colleagues (1996) recorded CA1 place cells whilst rats ran the length of a linear track back and forth to receive food rewards. During the outward journey, the start box was moved up the track to one of five different locations. The authors found that place cells with fields near the start box always fired relative to the box, whereas those with fields further up the track were fixed to the some (unknown) room cues. This was taken as evidence that the fields near the start box are responding to path integration, the ability to navigate using only internally generated cues, since the box was behind the rat and the rat therefore could not see it. Gothard et al. (2001), in a similar experiment, found that in the dark, place fields further down the alley anchored to the start box compared to when conducted under normal illumination. This supports the path integration hypothesis since presumably, in lighted conditions there is visual information to compete with path integration. In addition, the 2001 study also recorded from cells that fire as a function of location, simultaneously from the dentate gyrus and found a similar effect, suggesting that path integration occurs upstream of the dentate gyrus.

Other factors that modulate place cell firing

Place cells, despite their name, encode more than just space. For example, McNaughton and colleagues (1983) recorded complex spike cells in CA1 and CA3 when rats were performing an 8-arm radial arm maze task. What they found was that

these place cells tended to fire selectively in one direction, in contrast to earlier studies (e.g. O'Keefe, 1976). In another study, involving a spatial memory task, Wiener and colleagues (1989) found that 124 of 179 CA1 complex-spike cells (69%) were significantly modulated by speed, 77% had significant directional modulation and 62% were modulated by angular movements. In addition, 49% were influenced by all three variables. Hirase et al. (1999) also reported that place cells were modulated by speed of running. They recorded CA1 place cells whilst rats ran on a stationary running wheel and observed that in addition to running speed, firing rate was also affected by direction of the rat's head.

Wood and colleagues (1999) tested the idea that CA1 and CA3 complex-spike cells encode more than just location in a task where rats were trained on an odour recognition task. The task involved rats being successively presented with different odours (mixed with sand in a cup) and the rats task was to dig and find a food reward whenever the odour presented did not match the previous odour. The location of the cup varied so that rats were doing the same task in different spatial locations on different trials. Of 127 cells recorded, 51 (40.2%) were correlated with non-spatial elements, such as odour, trial type and approach to cup. Many cells had a spatial correlate (31.5%), however, only 11% coded only location.

The firing of place cells that do encode location can be influenced by task. In one study (Markus et al., 1995), cells were recorded in a cylinder with a cue card as rats foraged for randomly scattered pellets. Then, the task was changed such that food rewards were only available at distinct locations within the cylinder. The result was that some cells changed the location of their firing fields whereas others developed an additional field in another location or lost their firing field altogether. Approximately one third of place cells made one of these changes with the change in task, and it

occurred rapidly. In another study, Breese et al. (1989) recorded CA1 place cells as rats traversed a square platform surrounded by black curtains to collect water rewards available randomly in the four corners and in the centre. They found that place cells had a tendency to change the location of their firing fields when the task was changed such that only one particular location was baited. They even observed one cell's field changing twice on different sessions where the fixed baited location changed, i.e. the same cell had three distinct and non-overlapping place fields within the same unchanged environment under different task conditions. Overall, 40 of 47 (85.1%) cells in eight rats had significant changes in place field location following the change in task. This is in contrast to Speakman and O'Keefe (1990), who recorded CA1 place cells on a plus maze in a cue rich environment. Recordings were made within a black curtained environment where cues and reward location were rotated by 90° every trial. After around 12 trials when the rats had 90% correct performance, the location of the food reward changed relative to the constellation of cues. The rats were then given enough trials so that the new location was learned. Place fields recorded in the first set of trials with those recorded when the food location was changed were compared, revealing that 17 of 19 (89.5%) of cells had place fields that did not change significantly. It is hard to reconcile this result with the Breese et al. (1989) result. One difference between the studies that may be important is the shape of the environments used. There is evidence that in rectangular shaped environments, rats (Cheng, 1986, Golob et al., 2001) and toddlers (Hermer and Spelke, 1994) can mistake diagonally opposite corners when disoriented. In addition, results from Golob et al. (2001) suggest that rats' head direction cells may be more influenced by idiothetic cues in a square shaped box with one polarising cue card. Another difference and a potential factor in the discordance may be that, in the Speakman and O'Keefe study, rats were

removed from the arena between each trial for up to 10 minutes. This may have the effect of encouraging cue use for orientation upon re-entry to environment.

In a study by Dupret et al. (2010), rats were trained to find food in three locations on an open field "cheeseboard" maze. The food locations were changed daily, but fixed throughout each 40 trial daily sessions. They found that over the course of the 40 trials, place cells in CA1 tended to get progressively closer to the food locations. Interestingly, this only occurred in CA1 place cells and not CA3 place cells. In a study by Hollup and colleagues (2001), CA1 place cells were recorded during a hidden platform *annular* water maze task. They reported that place fields were overly represented at the platform location, even although it was hidden from view. Also, it has been demonstrated that CA1 place fields on a continuous T-maze task gradually move closer to goal locations over the course of one session (Lee et al., 2006).

Another place cell phenomenon relating to cognitive demands of task was discovered by Wood et al. (2000). In this study, CA1 place cells were recorded during a continuous T-maze task, and found that, on the central stem of the T, some cells tended to fire more when the rat was about to turn left, and other cells fired more when the rat was about to turn right. This differential place cell firing was replicated by Lee et al (2006) on a continuous T-maze and has been reported in other studies (Frank et al., 2000, Ainge et al., 2007, Johnson et al., 2007, Pastalkova et al., 2008, Ainge et al., 2011).

Aside from landmarks, one part of the environment that has a large influence on place cell firing is barriers or boundaries. The Muller and Kubie (1987) study, mentioned above, demonstrated that place fields tended to disappear when a barrier was placed directly over the place field. This was also confirmed in the Breese et al.

(1989) study. This was the case even when the barrier was transparent. This hints that place cells may respond only to destinations that are presently available to the rat. A study by Foster et al. (1989) provides further evidence for this. In this study, place cell firing was recorded when rats were restrained and unable to move and also when rats were not moving, yet able to move. When rats were physically restrained, place cell firing ceased, yet would fire if the rats were motionless but unrestrained. O'Keefe and Burgess (1996) reported that place cells recorded in different rectangular environments tended to fire in similar relative positions within each, that is, they tended to fire a fixed distance away from two walls. When a square environment was changed to a rectangle between sessions, place fields tended to stretch out along the longer wall; similarly, when a small square was changed to a larger square, place fields would appear to stretch in both directions. Muller and Kubie, (1987) reported similar findings for only 36% of cells recorded when they changed the environment from a smaller to larger cylinder. In this case, the place fields stretched out when in the larger cylinder, and stayed at the same relative distance and direction as when in the smaller cylinder. However, most of the cells recorded (52%) did not do this; instead, they remapped.

It has also recently been suggested that place cells in the rat are modulated by attention between proximal and distal cues (Fenton et al., 2010). The authors' ideas were based on the observation that place cell firing is often "unreliable" and firing rates can vary from moment to moment within the same place fields. This fits the prediction that place cell firing switches states as rats' internal attentional states switch from a representation with reference to self-motion cues to a representation based on distal cues. Using decoding algorithms, they provided evidence that this was the case, and that rats' attention does change state on a second timescale.

Another recent study has shown that place cells are modulated by time as well as space (Macdonald et al., 2011). This study adds to the literature on the importance of the hippocampus on episodic memory by providing evidence that hippocampal principal cells encode episodic-like events. In this study, rats sampled one of two objects, then had to wait out a 10 second delay before being presented with one of two odours. The odours and objects were paired, such that if the sample object matched the odour, the rat was permitted to dig for a food reward. If the pair was mismatched, the rat could get a reward in a different location by *not* digging. This task ensured that the rats needed to keep track of the temporal sequence of events. The main finding was that, during the 10 second delay, which was in an maze alleyway, place cells tended to fire at discrete temporal intervals rather than simply fire in the same *place* field.

2.5.2 Location specific cells in the subiculum

Sharp and Green (1994) recorded from the hippocampus proper's main output region, the subiculum. They found location specific firing in many cells, although the firing locations were not as specific as place cells in CA1 or CA3. Subicular cells showing location specific behaviour tended to have higher firing rate across the whole environment in contrast to place cells, whose firing rates tend to drop dramatically when outside their place field. In addition, whereas hippocampal place cells have unique representations for individual environments, subicular cells tend to display similar firing over different environments (Sharp, 2006). For example, a subicular cell with a place field along the North wall of a rectangular environment will tend to have a similarly placed field in a larger rectangular environment along the North wall (Sharp, 1997, 2006).

2.5.3 Head Direction Cells

In 1984 a class of cells was discovered in rats that respond to their head direction (Ranck, 1984). These cells fire maximally when the head of the animal is facing a specific direction and firing rate quickly reclines to around zero as the head changes direction (Figure 1.4). The direction in which a given cell fires is termed the cell's *preferred firing direction*. These cells fire in their preferred direction irrespective of the rat's location within its environment, unlike place cells. The position of the rat's body does not affect head direction cell firing either, nor does it matter if the rat's head is facing down or up; firing rate depends only on the direction that the head is facing.

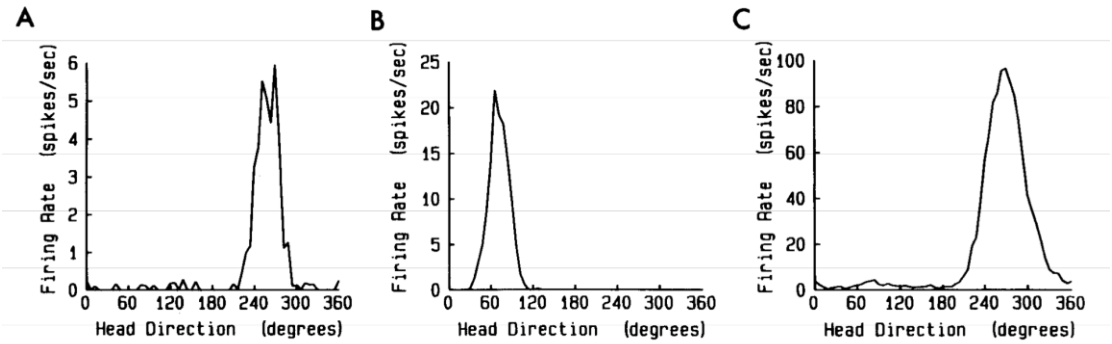

Figure 1.4. Firing rate of 3 representative head direction cells. Firing rates increase rapidly when head direction approaches the preferred direction, and falls sharply when the head turns away. The three examples demonstrate a large peak firing rate variation between different cells. From Taube et al. (1990).

Head direction cells were discovered accidently by Ranck, who was attempting to record from the subiculum. The recording electrodes, however, ended up in the postsubiculum, a nearby structure. Ranck discovered a neuron that fired in accordance with the rat's head direction, over a directional range of around 90°. He later recorded from more of these cells, in the same and other rats.

An interesting feature of head direction cells is that, in some regions they appear to be modulated not only by head direction, but also angular velocity of the head. Angular head velocity has been reported to modulate firing rate (e.g. Stackman

and Taube, 1998) and also preferred firing direction (Blair et al., 1998, Stackman and Taube, 1998) in multiple regions (see anatomy section 2.4 above). Angular head velocity modulation leads to differences in the preferred firing direction between clockwise and anticlockwise turns of the rat's head.

Anticipatory firing is an explanation of the difference in preferred firing direction between clockwise and anticlockwise head turns of the same cell (Figure 1.5). The actual time value of the anticipatory firing is calculated using a time-shift analysis where the spike times (times at which the putative cell fires) for firing during one direction of a putative cell are shifted to fit best with some waveform parameter(s) (such as preferred firing direction) of the spikes from the opposite direction.

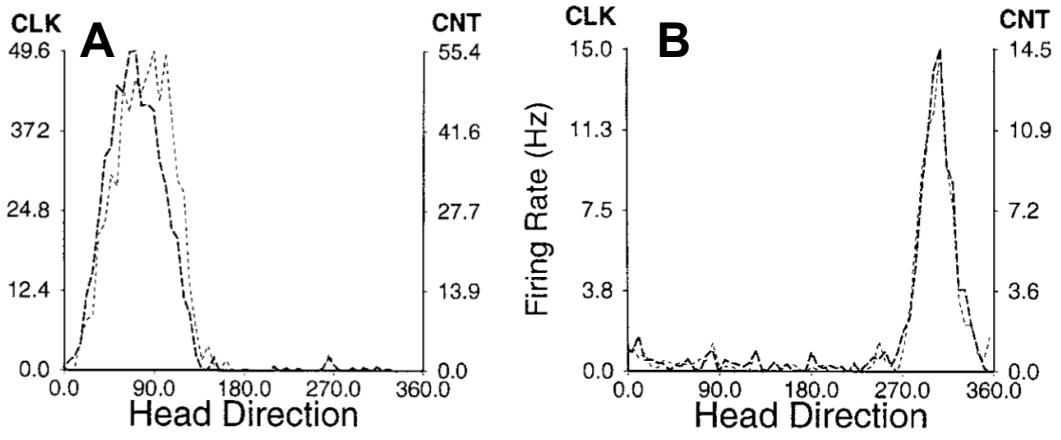

Figure 1.5. Head direction cells in the (A) anterior dorsal thalamic nuclei and the (B) postsubiculum. Thick dashed lines represent clockwise head turns (CLK) and thin dashed lines represent anticlockwise head turns (CNT). The two distributions on graph (A) are misaligned, whereas this is not the case for (B). In (A), since the two distributions are bimodal, the preferred direction of the cell depends upon the angular head velocity of the rat's head. Adapted from Blair and Sharp (1995).

2.5.3.1 Postsubiculum

The first published full description of head direction cells appeared some years later than the original discovery (Taube et al., 1990a). Experiments were conducted in a grey cylinder with a white polarising cue card and the preferred firing directions of 24 cells from 7 rats encompassed all 360° of the environment, with an equal distribution across this range. Of 269 cells recorded, 61 (23%) were classed as head direction cells. The average tuning curve width of postsubicular head direction cells is approximately 65°.

The postsubiculum has projections to the entorhinal cortex; thus, it is possible that head direction information is passed to the hippocampus through this route. The postsubiculum receives inputs from the retrosplenial cortex, the lateral dorsal and the anterior dorsal thalamic nuclei, and also the subiculum. The latter structure is known to contain cells that respond the location (Burgess and O'Keefe, 1996), similar to hippocampal place cells, whilst the others are known to have cells that are sensitive to direction.

Since their discovery, head direction cells have been found in numerous brain regions, including the lateral dorsal thalamic nucleus (Mizumori and Williams, 1993), the anterior thalamic nuclei, striatum, (Wiener, 1993, Taube and Burton, 1995), the lateral mammillary nucleus, (Blair et al., 1998, Stackman and Taube, 1998); the dorsal tegmental nucleus of Gudden, (Bassett and Taube, 2001), and the retrosplenial cortex, (Cho and Sharp, 2001). The following sections deal with each of these regions separately.

2.5.3.2 Lateral Dorsal Thalamic Nucleus

Mizumori and Willams (1993) reported head direction cells in the lateral dorsal thalamic nucleus (LDN), an area that both receives projections from and

projects to the postsubiculum (Thompson and Robertson, 1987a, van Groen and Wyss, 1992b), whilst rats performed an 8-arm radial arm maze task. Approximately 30% of cells recorded in the region were sensitive to direction. When lights were then switched off, head direction cells' preferred firing directions changed after 2-3 minutes, although the specificity of these preferred firing directions was unchanged. When room lights were switched back on, the cells' preferred firing direction reverted back to their original direction.

2.5.3.3 Anterior Thalamic Nuclei

Head direction cells have also been found in the anterior thalamic nuclei (ATN) (Taube, 1995). In this region, 60 of 107 (56.1%) recorded cells were modulated by head direction, and most head direction cells in this study were found in the anterior dorsal thalamus (ADN). Like postsubicular head direction cells, the directions represented by these cells also ranged equally over 360°, with each individual cell having only one maximum preferred firing direction. The average tuning curve width is around 57°. In addition, these head direction cells were found to be influenced by the rate of head turning through the preferred firing direction; that is, the angular head velocity. The higher the angular velocity was, the higher the firing rate was. The firing of these cells anticipated head direction by around 25 ms.

2.5.3.4 Lateral Mammillary Nucleus

Another area in which head direction cells have been found is the lateral mammillary nucleus (LMN). Stackman and Taube (1998) reported that 17 of 87 (20%) recorded cells in this region were head direction cells. They described the cells as being very similar to those head direction cells recorded in the postsubiculum, and that each direction in 360° was equally represented by the sample of cells recorded.

The average tuning curve width is around 80°. Similar to head direction cells in the ADN, head direction cells in the LMN were modulated by angular head velocity. Another feature of these LMN cells that was not found in the postsubiculum or the ADN was that cells' firing rates were higher for either a clockwise or anti clockwise approach to their preferred firing direction. In addition, this was dependent upon which side of the brain the cell was recorded: cells in the right hemisphere had higher firing rates on clockwise turns into their preferred firing directions, whereas, conversely, cells on the left hemisphere had higher firing rates when rats approached the cells preferred firing direction from an anti-clockwise direction. Head direction cells in this region preceded (anticipated) head direction by around 66 ms, compared to only around 25 ms in the ADN. This value is higher than that reported by Blair and Sharp (1995), who reported 37 ms, around half of Stackman and Taube's value.

2.5.3.5 Dorsal Tegmental Nucleus of Gudden

Cells modulated by head direction were found in this region by Bassett and Taube (2001). However, these cells were unlike head direction cells found elsewhere (e.g. postsubiculum or ADN) in that they fired over a much wider angular range (~240°) (i.e. had a larger tuning curve) and had much lower firing rates. In addition, only 5 of 44 recorded cells (11%) were modulated by head direction, and these were also modulated by angular head velocity. The interesting thing about this study was that most of the cells recorded (75%) were angular head velocity cells. That is, they fired as a function of angular head direction.

Another study, however, published the same year (Sharp et al., 2001), found head direction cells in this region that were more like the classic head direction cells. Six of 48 recorded cells (12.5%) were classified as head direction cells, the rest being

angular head direction cells. The average width of their tuning curves was 109° and their firing rates were closer to those from the postsubiculum and ADN.

2.5.3.6 Retrosplenial cortex

The first reports of head direction cells in this region were by Chen and colleagues (1994). They observed that 9% of cells recorded in this region were head direction cells, with properties similar to those reported in the postsubiculum. However, these cells were recorded in rats on an 8-arm radial arm maze, which constrained the directions that the rats travelled and likely also their head movement, unlike the open field experiments used in other studies (e.g. Taube et al., 1990a, b).

Cho and Sharp (2001) recorded cells in the retrosplenial cortex using an open field environment and found, like Chen et al. above, that 10% of the cells recorded were classic head direction cells. That is, 10% had one clear preferred firing direction (single peaked Gaussian tuning curve) and fired at zero or close to zero for all other directions. The average tuning curve width is around 44°. These cells were modulated by angular head velocity and anticipated actual head direction by ~25 ms. Cells recorded in this region were also modulated by running speed and movement.

2.5.3.7 Striatum

Cells that are modulated by head direction have also been found in the striatum (Wiener, 1993). These cells, however, are not like the classical head direction cells found in other regions (e.g. the postsubiculum). The experiments in the Wiener (1993) study involved rats traversing a square shaped environment in which there were liquid rewards available at the four corners and at the centre. This could perhaps have limited the sampling of the rats' head directions, which may have been a factor in the reporting of these cells.

In addition, cells from this region recorded simultaneously tended to fire in register with either proximal or distal cues, that is, not coherently (Wiener, 1993, Shibata et al., 2001). This suggests that the cells were not firing based on an allocentric direction. Also, recent work from the Redish laboratory has shown that cells the dorsal striatum encode spatial information only when it is required to solve a task (Schmitzer-Torbert and Redish, 2008) and similarly, cells in the ventral striatum encode spatial information during expected reward sites on a maze and also a high choice decision point on which reward depends (van der Meer and Redish, 2009). This suggests that cells in the striatum can encode spatial information, but tend to do so only when necessary in solving a task.

2.5.3.8 Interpeduncular nucleus

Another region is worth mentioning at this stage. The interpeduncular nucleus, whilst it is not known to contain head direction cells, is thought to be important in the generation of its signal. It has reciprocal connections to the DTN (Contestabile and Flumerfelt, 1981, Liu et al., 1984) and receives projections from the entopeduncular nucleus (van der Kooy and Carter, 1981), an area thought to be involved in motor function.

A recent study investigated the effects of lesions of the interpedunculus nucleus on head direction cells in the ADN (Clark et al., 2009). They found that following cue card rotations in the absence of the rats that head direction cells in the ADN were mildly impaired in anchoring to the cue card. They also reported several other properties that were significantly different from control cells in the ADN; these included a larger tuning width, reduced peak firing rate and decreased anticipation of future directional heading. In addition, whilst rats were randomly foraging in darkness,

ADN cells had larger drifts in preferred firing direction following lesions of the interpedunculuar nucleus than cells from control rats. The authors contribute a broad role to the interpeduncular nucleus, stemming from the transfer of idiothetic motor cues to the head direction cell system.

2.5.3.9 What controls head direction cells' preferred firing directions?

Like place cells, head direction cells are strongly influenced by visual landmarks. Taube et al. (1990b) recorded head direction cells in a grey cylinder, and, following rotation of the cue card by 90°, the preferred firing direction of the cells changed by a similar amount. The change in firing direction was not exactly 90°, however, indicating influence from other environmental cues. In the same study, the effects of removal of the cue card in the absence of the rats were assessed. Between session removal led to a non-predictable change in the preferred firing direction. This suggests that cues other than the cue card itself were being used by the head direction cells, of which the most likely sources are idiothetic cues. In addition, Taube et al. (1990b) also conducted cue card rotations in four steps of 90°, without removing the rat from the cylinder. The cells' preferred firing directions closely followed the rotations, but shifted less than 90° consistently, indicating competing influence by other cues, presumably idiothetic.

Head direction activity is also maintained in darkness (Goodridge et al., 1998), which further indicates a dependence of the cells on idiothetic information. Reliance on idiothetic cues has also been eloquently demonstrated by Taube and Burton (1995), who recorded head direction cells in rats as they walked from a familiar cylindrical environment to a novel rectangular-shaped environment via a narrow U-shaped passageway. The preferred firing direction of the recorded cells was maintained from leaving the familiar environment, which had a polarising cue card, to entering the new

environment. Since there were no familiar landmarks in the novel environment to anchor the cells' firing directions, idiothetic cues must have been utilised for the maintenance of firing direction through a path integration process.

However, visual landmarks do exert more of an influence on the preferred firing direction of head direction cells than path integration. Goodridge and Taube (1995) tested for this in an experiment where rats were familiar with a cylinder with a cue card to the extent that their head direction cells had a stable preferred firing direction. Then, the cue card was removed between sessions and if, on the next session, the rat's cell's preferred firing direction shifted, the cue card was reintroduced. The cue card then had the effect of exerting control over the preferred firing direction, which reverted back to that of the first session. Another study tested the relative strengths of landmark control and idiothetic control over preferred firing direction of head direction cells (Dudchenko and Zinyuk, 2005). In this clever study, head direction cells were recorded in the ADN of rats in two distinct novel box environments (A and C). Then, rats walked from A to a novel box B. As expected, the preferred firing directions were maintained by path integration. Then, in a test of path integration vs. landmark control, rats walked from box B to box C. It was found that the landmarks of the familiar box C exerted control of the preferred firing direction.

An important study (Zugaro et al., 2001) showed that distal cues have much greater influence on the preferred firing direction of ADN head direction cells than proximal cues. In a clever experiment, based on that of Cressant et al. (1997), cells were recorded from rats inside a cylinder with three 3D objects serving as cues placed around the periphery. Rotation of the objects together by 120° in the absence of the rats led to reliable control of the cells' preferred firing directions. Then, the cylinder walls were removed revealing pleated black curtains surrounding the apparatus, at a

distance of around 1 m. This time, when the cues were rotated, preferred firing direction did NOT follow the cues. It appeared that the presence of the cues in a more distal position than the three cues served to anchor the cells.

2.5.4 Grid cells

Twenty two years after the discovery of head direction cells, a remarkable study was published reporting yet another cell type that encodes spatial information (Hafting et al., 2005). These cells were found in the medial entorhinal cortex and are similar to place cells in that they fire in distinct circumscribed locations within the animals' environment. However, these cells have multiple firing fields in a regular hexagonal (or triangular) tessellating pattern spanning the extent of the environment. This firing pattern is referred to as a grid. The grids from different cells recorded from the same area within the same rat have identical spacing between the grid fields but the grids are offset from each other. Another feature of grid cells is that there is a gradient of increasing field spacing of the grids from dorsal regions to more ventral regions of the medial entorhinal cortex (MEC). This parallels the increase in place cell field size from the septal to temporal pole of the hippocampus (Jung et al., 1994, Brun et al., 2008). Indeed, dorsal MEC projects more to the septal pole of hippocampus, whereas more ventral regions project more to the temporal pole.

Before the discovery of grid cells, electrophysiological recordings had been carried out in the entorhinal cortex (Mizumori et al., 1992, Quirk et al., 1992, Fyhn et al., 2004). Cells were reported that are location specific and tend not to change much between different environments. The Fyhn et al. study even reported multiple peaks. One reason that grid cells were not discovered previously was that the recording environments were not large enough that the clear hexagonal pattern could be seen. This was coupled with the fact that the grid spacing increases the more ventral the

recording electrodes are, up to about 3 m spacing at the most ventral portions of the medial entorhinal cortex (Brun et al. 2008). Earlier recordings were not taken from the most dorsocaudal region, where grids may have been visible on the apparatuses used (Savelli et al., 2008).

Grid cells have garnered a lot of interest since their initial discovery. They have now been found in the postsubiculum and parasubiculum (Boccara et al., 2010), both input structures to the entorhinal cortex. Grid cell firing is thought to be important for path integration (McNaughton et al., 2006, Burak and Fiete, 2009) and the stability and remapping of hippocampal place cells (Fyhn et al., 2007). Fyhn et al. recorded simultaneously from entorhinal grid cells and hippocampal place cells and found that global remapping in CA1 place cells was accompanied by shifts and rotations of the firing vertices of grid cells. This was not found during CA1 rate remapping.

2.5.5 Border cells

Yet another class of spatially modulated cell was found in the hippocampal region recently (Savelli et al., 2008, Solstad et al., 2008). Solstad et al. recorded from principle cells in the medial entorhinal cortex and adjacent parasubiculum and discovered that approximately 10% of cells fire along one or more walls or edges in a square shaped environment. When the walled environment was elongated, these border cells likewise increased their size along at least one wall. Savelli et al. (2008) recorded cells from the most dorsocaudal region of the medial entorhinal cortex in a square shaped environment and, after six or seven minutes, removed the walls to reveal a larger square shaped environment to the rats. They reported that a proportion of cells that had fields near the borders of the small box then changed their firing location in the larger box such that place field positions were relatively similar. For

example, a cell that had been firing along the middle of the North wall in the small box would also have a firing field in the middle of the North wall in the larger box.

The existence of these boundary cells lend support to a model by Burgess and colleagues (Burgess and O'Keefe, 1996, O'Keefe and Burgess, 1996) in which place cells in a square environment result from the combined inputs from at least two walls. This was later updated to account for environments in general, not only square shaped ones, by Hartley et al. (2000) in a "boundary vector" model of place cell firing. In this model, place cell firing location is derived from inputs to the hippocampus with information pertaining to distance and direction of boundaries, exactly the type of information that border cells may input.

2.6 Brief overview of the rodent navigational system

Navigation is a process that most people do each day, perhaps with little conscious thought. Different types of navigation require different computations in the brain. Travelling along a familiar route likely involves following distinct landmarks from memory, and perhaps the knowledge that, say, a right turn is required at a particular location and a left turn at another (Wolbers and Hegarty, 2010). Calculating an unknown route towards a known distant landmark may require a simpler strategy, such as heading straight for the landmark, whereas retracing your footsteps in an unknown terrain would require a different strategy, involving the ability to remember where you had been previously. This last example becomes more difficult if visual cues are obscured or if there is darkness. Animals likely employ a variety of navigational strategies during typical laboratory tasks and behaviours in the wild. Because different navigational strategies require different levels of information processing in the brain, and likely use a combination of different anatomical brain regions, researchers have attempted to break these down into individual component

strategies. There have been a number of different taxonomies used by different researchers (O'Keefe and Nadel, 1978, Gallistel, 1990), which share certain features, but here I shall outline that of Redish (1999) due to its conciseness. According to Redish (1999), there are five strategies an animal can use to navigate to a goal: Random navigation, Taxon navigation, Praxic navigation, Route navigation and Locale navigation.

2.6.1 Random navigation

This type of strategy would likely be used in situations where the animal does not know where its goal is and thus has to search for it. The goal may be food, water or an escape route. Random navigation, or exploration, may also be used if the animal is disoriented and does not have current location information. Another situation in which a random strategy may be used is if the animal is in a novel environment. In novel environments, information about current location isn't known so exploration is necessary. However, if the novel environment has an open space, rodents typically stay close to the borders, or edges, of the environment in a behaviour called thigmotaxis[3]. Thus, in novel environments, exploration is perhaps not truly random. In addition, if a rodent was placed into a familiar environment, but from a different starting place than usual, some random navigation may be required until the animal finds some familiar cue. One more situation that rodents may use a random navigational strategy in is in a familiar environment in which, on different trials, the goal is moved. Thus, random navigation would be necessary until the goal is found. In practise, however, this is unlikely to be truly random as rodents would tend not to visit the same place on multiple occasions when searching for the goal.

[3] Using O'Keefe and Nadel's (1978) taxonomy, this would be classified as an orientation strategy under their route following taxon.

2.6.2 Taxon navigation

Taxon navigation is when an animal uses a landmark to aid navigation towards a goal, such as the use of a landmark as a beacon. This strategy doesn't involve any spatial knowledge or processing to accomplish, and is simply a stimulus-response strategy. Thigmotaxic behaviours utilise taxon navigation, as does following an odour trail. In a water maze task where the platform is visible, a rodent can use taxon navigation to reach the platform.

2.6.3 Praxic navigation

Praxic navigation is where an animal uses a series of motor sequences to get from A to B. Praxic navigation can be used in situations where a fixed route between a fixed starting point and fixed goal is viable. For example, a praxic strategy can be used to solve a T-maze task where one arm is constantly rewarded over trials, if the animal always starts from the same starting location. The rat can reach the goal by using a praxic strategy involving a fixed sequence of motor responses, for example, running up the stem of the T once released, and then making a turn (left or right) when it reaches the T-junction. It is not essential that the rat know anything of the spatial layout of the maze or the room or the relationship between the goal location and the start location. Early experiments using elaborate alley mazes, such as the Hampton Court maze (Small, 1901) demonstrated praxic navigation (Carr and Watson, 1908). Carr and Watson trained blinded rats to navigate the maze successfully, suggesting that the rats were using a sequence of motor responses to navigate. In one experiment, they shortened the length of the maze after rats had learned it well. When this was done, even rats with normal vision ran at full speed into the walls of the now shortened alleyway.

Path Integration This is a special case of praxic navigation. Path integration is the ability to use internally generated cues (e.g. no visual, olfactory, tactile cues, etc.) to keep track of a journey and generate a direct route back to the start point. Path integration will be discussed in more detail in section 2.7.

2.6.4 Route navigation

Route strategies can be a combination of both taxon and praxic strategies. In fact, taxon and praxic strategies are special cases of the more general route navigation (Redish, 1999). Which strategy will be used would depend upon the information available to the animal. For example, if there is a lack of sensory cues in the environment during a homing task, then path integration may be the only viable strategy, other than a random one. Fairly complex routes can be accomplished using route strategies by linking separate sub-routes together, each of which could involve taxon or praxic strategies.

2.6.5 Locale navigation

Locale navigation is different from all of the aforementioned strategies in that it is the only one that requires knowledge of space and relationships between cues in the environment. It is the only strategy that requires a cognitive map, such as that of Tolman, (1948) and O'Keefe and Nadel (1978). Locale navigation, and thus a cognitive map, allows the animal to generate shortcuts in an environment. A short cut is essentially a novel route between two locations in the environment and by definition, must be shorter and therefore more direct than any previous route used. According to Redish (1999), one feature that separates locale navigation from route strategies is that it is an all-or-nothing ability in that the map is either known or it is not. Evidence of this comes from Barnes et al. (1997) who found that the path length

of routes taken by rats in a hidden platform water maze task had a bimodal distribution: path lengths were either short or long. However, after training, the distribution became weighted in favour of short path length as a cognitive map of the environment was learned.

2.7 Path integration

Path integration, mentioned above, is a special case of praxic navigation. Path integration is the ability of an animal to use internal cues to keep track of its distance and direction travelled and use this information to generate a direct route back to the start point at any given time throughout the journey. This requires constant updating of direction and distance and some sort of integration of the two. In addition, it is the ability to do so using only idiothetic cues (those internally generated by self-movement). Idiothetic cues include linear and radial optic flow, vestibular acceleration (translational and rotational), copies of locomotor commands (motor efference copies[4]) and proprioceptive feedback from muscles and joints (Etienne and Jeffery, 2004). This means that path integration can occur when there are no external cues; indeed, this is an important factor to consider when demonstrating it.

Path integration has been demonstrated in a variety of different and diverse species ranging from desert ants (Wehner and Srinivasan, 1981) and honeybees (Collett and Collett, 2000), to rodents (Mittelstaedt and Mittelstaedt, 1980) and humans (Beritashvili, 1965). Path integration can be difficult to demonstrate, however. In order to demonstrate path integration, all external references must be ruled out as explanations of animal behaviour (Etienne and Jeffery, 2004). This can be achieved by (i) eliminating or masking all distal and proximal cues in the environment, (ii)

[4] Motor efference copy is an internal copy of motor commands used for bio-feedback.

designing experiments such that path integration conflicts with external cues so that one can observe which of the two cue types is used by the animal (Etienne et al., 1996, Etienne et al., 2000) or (iii) manipulating information contributing towards path integration, for example, rotating animals very slowly so that acceleration is not picked up by the vestibular system, thereby "fooling" the directional system of the animal (e.g. Mittelstaedt & Mittelstaedt, 1980).

Path integration does not require a cognitive map of the environment since it is computed continually in the absence of any reference cues that such a map might have. However, path integration leads to accumulative errors (Etienne and Jeffery, 2004), which means that long excursions using only path integration will be inaccurate.

Path integration has been reported on numerous occasions in rodents. For example, Mittelstaedt and Mittelstaedt (1980) demonstrated that gerbils use an internal rather than external reference frame to guide homing behaviour following slow rotation. Gerbils were rotated slowly in a cup following a journey from a home location. The gerbils, once released from the cup, then made errors in homing which corresponded to the amount they were rotated. The explanation for this was that the rotation was at an acceleration below that which the vestibular system can detect, thus, in a sense, fooling the gerbil's sense of direction. Path integration has also been demonstrated in hamsters (Etienne, 1986, Siegrist et al., 2003). They found that, in a circular arena with a home location on the periphery, hamsters found their way home using internal cues. Rats have also been used to demonstrate path integration. Maaswinkel et al. (1999) provided evidence for path integration in rats in a homing task on a circular arena.

Path integration and selective brain lesions

Whishaw and Maaswinkel (1998) demonstrated the necessity of the fimbria fornix in path integration in a circular arena homing task; likewise, studies from Whishaw's laboratory have found that hippocampectomised rats, but not control rats, were impaired on path integration (Maaswinkel et al., 1999, Wallace and Whishaw, 2003). Results from the fimbria fornix lesion study may not necessarily indicate hippocampal involvement, as Redish (1999) pointed out, as fimbria fornix lesions include disconnecting the subiculum from the nucleus accumbens, disconnecting the postsubiculum projections to the thalamic nuclei (Witter and Groenewegen, 1990), and disconnecting the hippocampal formation connections to the mammilary bodies, thereby disrupting the head direction cell system. In addition, Redish (1999) interprets the Maaswinkel et al. data not as an impairment in path integration, but as an impairment in path integration error correction, since the lesioned rats could still do the task. There is also conflicting behavioural evidence from Alyan and McNaughton, (1999) who found no impairment on a circular arena homing task with hippocampectomised rats.

Path integration abilities have also been implicated with other brain regions. For example, lesions to the retrosplenial cortex led to impaired path integration abilities in a circular arena homing task (Whishaw et al., 2000), and lesions to either parietal or entorhinal cortices impaired path integration on a similar task (Save and Moghaddam, 1996, Parron et al., 2001, Parron and Save, 2004). Frodhardt et al. (2006) found that rats with lesions of either the DTN or ADN were impaired on a food-carrying task in a circular arena. Both groups were impaired under normal illumination and blindfolded, although rats with DTN lesions had greater impairment.

It is likely then, that multiple brain areas are involved in path integration but it is not clear in which region(s) path integration takes place. In addition to the above

brain regions implicated in path integration due to results of lesion studies, the entorhinal cortex has also been implicated due to the presence of both grid cells and conjunctive grid x head direction cells, which have properties that fit with path integration models (McNaughton et al., 2006).

2.8 Thesis aims

The literature review above gives a brief introduction to the hippocampal formation and the properties of its spatially modulated cells. A combination of lesion, behavioural and single unit recording studies has highlighted the importance of the hippocampal formation in spatial cognition. This thesis is intended to add to this literature, using lesion, single unit recording and pharmacological inactivation techniques.

1. The first aim is to explore path integration in the rat and determine the effects of bilateral lesions of the postsubiculum on this ability. A homing task was developed first of all that employs path integration, and a group of rats with postsubiculum lesions was compared to control rats on this task and its variations. The same rats were also compared on a T-maze alternation task.

2. Single unit recording was utilised to compare the stability of place fields in novel environments between initial exposure and various time delays prior to a test session. The memory of place cells following exposure to novel environments has never systematically been studied in this way. Analysis will determine if stability decreases over time. In addition, results from this chapter are vital for the design of the experiments in the following chapter.

3. A combination of single unit recording and intracranial drug infusions was utilised to determine the effects of AMPAR and NMDAR blockade in the postsubiculum on the long-term stability of place fields in novel environments. The

length of time delay between exposure to novel environments and test of place cell stability will depend upon results from the previous chapter. Analysis of place fields in these experiments will help determine the postsubiculum's possible role in integrating visual landmarks with internal representations of environments as measured by hippocampal place cells.

Chapter 3: The postsubiculum and homing by path integration

A manuscript based on this chapter has been published online (Bett et al., 2012).

3.1 Introduction

The postsubiculum (dorsal presubiculum) is a brain structure of considerable interest in spatial cognition. This is due to its connectivity, the distinctive properties of its cells, and its putative role in spatial cognition. It serves as an input structure to the hippocampus due to its projections to the entorhinal cortex (Amaral & Witter, 1995). In addition, it receives inputs from the lateral and anterior dorsal thalamus, the subiculum, and the retrosplenial cortex, in addition to cortical inputs from visual areas 17 and 18b (Vogt and Miller, 1983, van Groen and Wyss, 1990a). The postsubiculum also feeds back into the head direction cell system via connections with the LMN (Allen and Hopkins, 1989, Shibata, 1989) and the retrosplenial cortex (van Groen & Wyss 1990b). The postsubiculum also contains cells that encode both direction and location simultaneously (Cacucci et al., 2004). Recent work has shown that it possesses grid cells, neurons that show regularly spaced firing fields, and also border cells, neurons that are tuned to environmental boundaries (Boccara et al., 2010).

3.1.1 Head direction cells

The postsubiculum was the structure in which head direction cells were first discovered and characterised (Ranck, 1985, Taube et al., 1990b, a). Head direction (HD) cells are neurons whose firing is tuned to specific directions in the animal's environment, with different cell populations representing different directions encompassing all 360º in the horizontal plane. Since their discovery in the postsubiculum, head direction cells have been found in multiple brain areas including

lateral dorsal thalamic nuclei (Mizumori and Williams, 1993), the striatum, (Wiener, 1993), anterior thalamic nuclei (Taube, 1995), lateral mammillary nucleus, (Blair et al, 1998; Stackman and Taube, 1998); dorsal tegmental nucleus of Gudden, (Bassett and Taube, 2001), retrosplenial cortex, (Chen et al., 1994). See section 2.4 of this thesis for a detailed overview of anatomical connections between these areas.

Head direction cells have been studied for more than 20 years now and yet their function(s) remain elusive. Head direction cells are thought to underlie one's sense of direction (Taube, 1998, Dudchenko, 2010) . However, experimental evidence demonstrating this is sparse. A study by Dudchenko and Taube (1997) investigated head direction cells in the ADN whilst rats were performing in an eight-arm radial arm maze task in which there was one constantly reinforced arm. Room cues were masked and a single white cue card was used. When the task was learned, results from probe trials in which the cue card was rotated indicated that the preferred firing direction of cells tended to correlate with behaviour. That is, when the preferred firing direction was anchored to the cue card during rotations, the rat typically chose the maze arm that corresponded to the amount of rotation. In contrast to this, a study from the same laboratory recorded ADN head direction cells in a square box where rats were trained to find a reward in the same corner on each trial (Golob et al., 2001). The corner could be identified by its relationship with a white cue card. Probe trials were conducted where the square box was replaced by a similar, but rectangular box. The reward was given at the equivalent corner, indicated by the cue card. However, when the rats were put into the rectangular environment, shifts in head direction were typical although the rats located the correct corner for the reward. Thus, head direction cell information and overt behaviour are not always coupled. Golob et al. (2001) reported that the preferred firing directions of cells were less likely to follow

cue card rotations when in the square environment, as opposed to a cylinder. One difference in a cylinder with a cue card (and in the 8-arm radial arm maze) is that the cue card is the only salient polarising visual cue, whereas in a square box there is four-fold symmetry. Golob et al. reported that occasional shifts in preferred firing direction between trials (without cue card rotations) tended to occur in multiples of 90º, indicating that the information guiding head direction cell firing was likely from the geometry of the environment rather than the cue card.

Another study from the same laboratory, Frohardt et al. (2002), recorded ADN head direction cells in a circular arena that had a polarising cue curtain and found that preferred firing direction and behaviour were not always coupled. In this task, rats started from one of eight home nests on the periphery of the arena and collected food from the centre and then carried it home. However, when rats made homing errors, the preferred firing direction of cells did not change to *agree* with the choice. There was also a condition where the rats had to navigate in darkness and in this condition, there was more of an agreement between preferred firing directions and behaviour, indicating that perhaps head direction cells were useful during path integration. Path integration is the ability to use internally generated cues to keep track of one's direction and distance travelled and is described in detail in section 2.7.

Neural processes that are thought to underlie path integration have been investigated at the level of individual head direction cells (e.g. Taube and Burton, 1995). The ability of head direction cells to maintain their preferred firing direction in the dark, or when walking from a familiar to a novel environment, and thus relying on idiothetic cues is thought to contribute to path integration. One important finding relating to the head direction cell system is that it is sensitive to the angular velocity of the animal's head. Indeed, some head direction cell regions contain a network of

cells whose primary correlate is not to head direction but angular velocity (Sharp, 1996, Stackman and Taube, 1998, Bassett and Taube, 2001). Angular velocity is detected by the vestibular system and vestibular lesions lead to loss of directional specific firing of ADN head direction cells (Stackman and Taube, 1997) and postsubicular head direction cells (Stackman et al., 2002). The vestibular system detects both angular velocity and linear direction speed and is therefore likely used by animals to keep track of their movements in the absence of external cues such as visual input, that is, path integration. In a study by Blair and Sharp (1996), the floor of a cylindrical maze apparatus was rotated with a rat inside at either an acceleration and velocity that was large enough that their vestibular system detected it, or at a much slower acceleration and velocity. Head direction cells in the ADN did not change their preferred firing direction during fast rotations. In contrast, during slow rotations, the preferred firing directions changed by around the same amount. This indicates that during the fast rotation, the vestibular system detected the angular acceleration, whereas, with the slower rotation the head direction cells continued to fire in the same preferred direction relative to the floor. Rotations of an animal below its vestibular detection threshold have been demonstrated behaviourally also. Mittelstaedt and Mittelstaedt (1980) experimented with manipulating vestibular input to mice in a homing task. The task involved a circular arena where the mice started their journey from a nest site at the periphery to find their missing pups that the experimenter had placed on the maze. During these journeys, mice were slowly rotated inside a cup after the outward component of their journeys. When released, they tended to return not to their original start point, but to the point in space corresponding to the amount of rotation inside the cup, as if the rotation had not been noticed. These two findings suggest that vestibular input is necessary for normal firing of head direction cells and

for behaviour that requires the animal to keep track of direction travelled using internally generated cues. van der Meer et al. (2010) demonstrated that head direction cell firing in the ADN and behaviour were closely related in a homing task following slow 90º rotations between outward and homeward journeys. The evidence presented above suggests that a good way of investigating possible head direction cell function would be to combine a path integration based task involving rotations below the vestibular detection threshold with some manipulation of the head direction system.

3.1.2 Postsubiculum

Evidence suggests that the postsubiculum is a site where internal representations of orientation and external representations of landmarks converge. For example, removal of the postsubiculum causes between session instability in the place fields of hippocampal place cells when a polarising cue card is either rotated by 90° or removed completely between sessions (Calton et al., 2003), and combined lesions of the presubiculum (including the postsubiculum) and parasubiculum weaken the spatial tuning of place cells (Liu et al., 2004). Postsubiculum lesions also weaken the stimulus control of a landmark over the preferred firing directions of head direction cells in the anterior thalamus (Goodridge and Taube, 1997).

Surprisingly, few studies have examined the behavioural function of the postsubiculum directly. Taube et al. (1992) showed that lesions of the postsubiculum impair performance on a working memory version of the radial arm maze, and on a reference memory version of the Morris water maze. However, on both of these spatial tasks, the performance of the animals without a postsubiculum improved over the training period. On a probe test in the water maze, the lesioned animals showed less preference for the platform location compared to control animals, yet swam in

this location more than other locations in the maze. Thus, removal of the postsubiculum impaired spatial performance, but did not abolish it.

Combined electrolytic damage to the presubiculum and the parasubiculum produced a deficit in the short-term memory for previously visited arms of a 12-arm radial maze (Kesner and Giles, 1998). Lesions of either the lateral or medial entorhinal cortices did not produce a deficit in this same study. As in the Taube et al. experiment, however, there was evidence that the performance of the lesioned animals improved with additional training.

Liu et al. (2001) found that combined ibotenic acid lesions of the parasubiculum and presubiculum (including the postsubiculum) produced impairments in a number of spatial memory tasks. On the traditional water maze task, the lesioned animals took longer to learn the location of the hidden platform than control animals. After training with a visible platform, however, the lesioned animals showed spatial learning that was comparable to control animals. Animals with the combined lesions were impaired in object recognition, and object-place recognition. The lesioned animals also showed a clear, delay-dependent impairment in T-maze, with unimpaired performance at a 5 s delay between the sample and choice runs, but progressively larger impairments at delays of 30, 90, and 180 s.

In addition, results from an Immediate Early Gene (IEG) study reported on the activity of the postsubiculum during a standard working memory task on an 8-arm radial arm maze (Vann et al., 2000). In this study, the expression of the protein Fos, an indicator of neuronal activity, was compared in multiple brain regions as rats performed either an 8-arm radial arm maze task or simply ran up and down one arm of the maze. Fos expression levels in the postsubiculum were significantly higher

during the 8-arm radial arm maze task, indicating that this region was active during the task.

Together, these studies suggest that the postsubiculum is necessary for spatial learning and memory in traditional behavioural paradigms, although deficits following removal of this brain region can be transient. However, what has yet to be assessed is whether this brain region is essential for the maintenance of orientation in the absence of external landmarks, or path integration. Such tasks may be particularly sensitive to disruption of the postsubiculum, as it contains both head direction and grid cells, both of which have been implicated in the updating of orientation by self-motion (Taube & Burton, 1995; McNaughton et al., 2006).

3.1.3 Path integration and lesion studies

Path integration has been reported on numerous occasions in rodents (see Etienne & Jeffery, 2004). For example, path integration has been demonstrated in hamsters (Etienne et al., 1985, Siegrist et al., 2003). They found that, in a circular arena with a home location on the periphery, hamsters found their way home using internally generated cues. Rats have also been used to demonstrate path integration; for example, Maaswinkel, Jarrard and Wishaw (1999) provided evidence for path integration in rats in a homing task on a circular arena similar to that used in the Etienne laboratory. In addition, they found that hippocampectomised rats, but not control rats, were impaired on the food-collecting task that depends upon path integration (Whishaw et al., 2001, Wallace and Whishaw, 2003). (But also see Alyan & McNaughton, 1999). In addition, in a task that should involve path integration, walking from a familiar to an unfamiliar environment through a series of novel alleyways, the preferred firing direction of ADN head direction cells were found to be unstable in rats with hippocampal lesions (Golob and Taube, 1999). Another study

from the same laboratory (Frohardt et al., 2006) tested path integration abilities in a food-collecting task on a circular maze in rats with lesions of either ADN or DTN. They reported path integration (homing) impairments in both groups of rats, but only mild impairments in the ADN lesion group compared to the DTN lesion group. This is interesting, because if path integration involves the head direction cell system and the hippocampus, information is likely required to pass from the postsubiculum to the hippocampus (via the entorhinal cortex) because the postsubiculum projects strongly in this direction.

It not as yet known whether or not the postsubiculum is necessary for path integration. The primary aim of the present study was to determine if the postsubiculum is necessary for path integration by demonstrating path integration in rats, and then creating lesions in the postsubiculum and re-testing path integration abilities and comparing with control rats. The underlying assumption is that the head direction cells in the postsubiculum would be responsible for any resulting effect. A variation of a homing task in which it has recently been shown that head direction cells correlate with behaviour (van der Meer et al., 2010) was used in this study. The parameters of this homing task were explored, and attempts were also made to replicate the deficits previously observed on the T-maze.

3.2 Method

3.2.1 Overview

Rats were trained on a food-gathering task on a circular platform. The task involved the rats leaving a nest site on the periphery of the platform and retrieving food from the centre. After acquisition of task, delay where the rats were confined to the maze centre and slow rotations of the rat on the maze centre were included to test

path integration. Rats were then given either sham lesions or lesions to the postsubiculum before being re-tested on the path integration task and various task modifications. Rats were then trained on a delayed forced alternation T-maze task to further test spatial memory.

3.2.2 Subjects

12 male Lister Hooded rats (Charles River Laboratories, UK), weighing 250-300g at the start of the experiment, served as subjects for this study. The rats were housed 4 to a cage in a 12 hour light/dark cycle environment. During the experiment, all rats were food restricted to ≥ 85% of their free feeding weight and allowed free access to water. All procedures were compliant the UK Animals Scientific Procedures Act, (1986) and with the European Communities Council Directive of 24 November 1986 (86/609/EEC) legislation governing the maintenance of laboratory animals and their use in scientific experiments.

3.2.3 Apparatus

Path integration maze. The rats were trained on a path integration maze (Figure 3.1), which was a circular table (height: 190 cm, diameter: 180 cm) with a white plastic laminate surface. Eight equidistant slots were cut out of the table periphery, into which "home" boxes (30 x 25 x 8 cm) could be inserted. These boxes rested below the table surface when inserted, which meant that the rats needed to climb out of the box to reach the maze surface. During experimentation, 7 of these boxes, containing animal bedding, were covered with a transparent Perspex lid. A home box, identical to the other boxes except that it was not covered, was inserted into the free slot. Its location could be changed by swapping with one of the 7 covered boxes. On the centre of the maze was a circular central platform (diameter 25

cm) that could be rotated in the horizontal plane independently. Surrounding the central platform was a dark grey tube that could be elevated from below the maze to a height of 40 cm above; this tube rotated along with the central platform. The rotation and tube elevation was made possible by a motor attached to the central platform, and was controlled by a nearby switch. Surrounding the maze were black curtains on a circular rail (diameter approximately 220 cm), which allowed 4 possible entry points to the maze. These entry points were closed during experimentation. A radio was positioned directly beneath the maze and was used to generate noise to mask potential auditory cues.

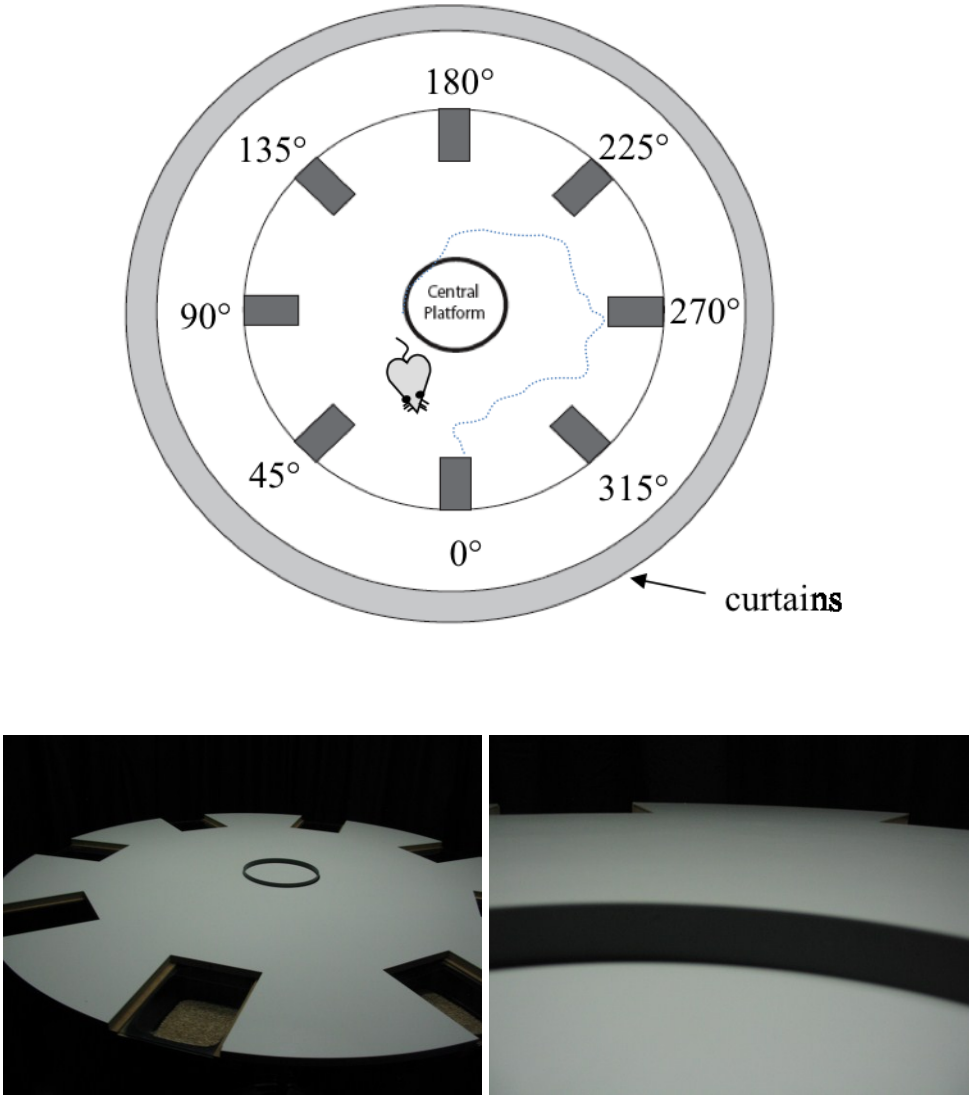

Figure 3.1 *Top:* Schematic of the path integration maze. Rats ran from a home box on the maze periphery to a central platform, where they found a food reward. They typically gathered this reward, and returned directly to the home box to consume it. *Bottom left:* Photograph of maze. *Bottom right:* Photograph of maze taken from the central platform at approximately 6 cm height demonstrating a section of the rat's viewpoint showing 2 adjacent box locations.

T-maze The T-maze had a wooden floor (12 cm wide), which was painted black, and transparent Perspex walls (16 cm). The stem of the T was 81.5 cm in length and each arm of the T was 68.5 cm in length. A small circular dish was placed at the end of each arm of the T, and at the beginning of each trial a food reward (half chocolate Weetos cereal loop, Weetabix, UK) was placed in each of these. The maze was positioned on top of the circular path integration maze.

3.2.4 Procedure

Habituation to the path integration maze Prior to surgery, rats were trained on a homing task on the path integration maze. In this task, the rat left its home box on the periphery of the circular table, and moved to the centre of the table where it gathered a food reward. The animal then returned, with its reward, directly to the home box.

A subsidiary aim of the experiment was to assess the nature of this homing behaviour. To do so, three manipulations of the task were assessed prior to surgery. These included varying the home box location, adding a memory delay between the outbound and the homeward bound journeys, and slowly rotating the animal prior its homebound journey.

Varying the home box location Rats were allocated to one of two groups for the initial training phase (habituation and acquisition); the *same start* group started each trial in a session from the same home box location (n=6) whilst the *different start* group started from a different home box location (n=6) on each trial within a session. The rationale for this was as follows: if homing trips are anchored solely to the departure site (the home box), then the accuracy of return trips should be equivalent whether the animals return to a single home box location repeatedly or whether it returns to different home box locations. If, however, homing trips are more accurate for the single box location, it would suggest that uncontrolled cues external to the home box (i.e., cues on the maze or in the experimental room) help to guide the animal's homing behaviour.

Rats were each handled for 5 min/day for 3 days before they were introduced to the maze. Following initial handling, the rats were then habituated to the maze for 10 min/day for 10-18 days. For each of these sessions, rats were individually

transported, passively in an opaque box, from a holding room to the experiment room and placed on the maze by entering the curtains from one of the 4 entrances. The point of entry and exit through curtains was varied daily. Passive transportation has been shown sufficient to disrupt the maintenance of preferred firing direction of postsubicular head direction cells from a familiar to a novel environment (Stackman et al., 2003), and so this passive journey from the holding room would likely have been sufficient to disrupt the rats' maintaining their orientation, or sense of direction. During the habituation phase, rats were shaped to fetch a food reward (1/2 Wotsit cheese puff, Walkers snacks, UK) from the central platform and then return home. During this phase, the tube surrounding the central platform was kept at a height of ~ 3 cm. Rats in both groups were interrupted at 5 min, removed along with their home box, and placed in the transport box whilst the maze was wiped down with a detergent soaked cloth. Animals in the *same start* group were then put back to the same home box location, whereas those in the *different start* group were returned to the maze location 180° from the original location. The starting home box location was varied unsystematically each day. After each rat made ≥10 fetches from the central platform per session on 2 consecutive days, it was then moved on to the next stage of training.

Training The next stage consisted of eight trials per session. Each trial started with the experimenter removing the home box, with the rat inside, from the transport box and slotting it into the maze. The central platform was baited prior to this with food. As the rat emerged from the home box and fetched the food, the experimenter moved to different positions around the maze periphery so as not to serve as a cue to the position of the home box. After retrieving the food reward from the central platform, rats typically returned to one of the boxes on the periphery of the maze. A home box choice was scored if a rat approached a box and placed its head over it. A

choice was also recorded if the animal ran towards a box directly from the central platform to within approximately 10 cm of the box before abruptly changing direction. Such an abrupt change could reflect the detection of the clear Perspex lid, and by including these responses as choices we hoped to capture the rat's initial homing choice.

When the rat returned to its home box, it was taken out from the maze and placed back inside the transport box. Once the central platform and exposed section of the tube was wiped with a detergent soaked cloth, the transport box containing the rat was placed on top of the tube and the rest of the maze surface wiped down. During each session, the *same start* group was placed back in the same home box location for each trial of the session, whereas the *different start* group's home box location was unsystematically moved to a different location for each successive trial.

Memory delay trials To test the accuracy of the rat's memory for the home box location, trials were run where a delay was introduced between each rat's outbound and return journey. To achieve this, the motorised tube that surrounded the centre of the maze was used to constrain the rats. Rats were initially habituated to confinement in the centre of the maze by introducing a mock-tube containing four curtained doorways. Within this mock-tube the motorised tube could be raised. However, as the rats' tails were occasionally caught by this configuration, the mock-tube was eventually abandoned, and rats were habituated to the motorised tube by raising it in small increments across training days.

Once the rats habituated to a 60 s delay in the confinement tube, they were tested with delays of 30 s, 60 s, 120 s and 240 s. Each delay session consisted of 8 trials; 4 no-delay trials and 4 delay trials. No-delay trials were included so that the rats' fetching behaviour was not extinguished. Delay trials were always preceded by a

no-delay trial and were run in the same manner as ordinary trials, although, to facilitate homing behaviour, a food reward was placed on the central platform at the end of the delay. During the delay period, while the animal was in the confinement tube, the experimenter wiped the maze surface with a detergent-soaked cloth to minimize potential odour trails. Rats' choices of box locations were recorded for the homeward journey; if the correct box was not chosen on the first attempt then the second and third choices were recorded also. If the rats could not locate the correct home box within 3 box choices, then they were picked up and placed into their home box. Oftentimes, rats would find the correct home box only after the second or third choice of location.

Slow rotation trials The next stage involved slowly rotating the rats on the central platform whilst the tube was raised. If rats rely on an internal sense of orientation to return to the home site, then rotation of the animal below its vestibular threshold should cause it to home in the incorrect direction (as has been shown for desert mice; Mittelstaedt & Mittelstaedt, 1980). Rats were given additional habituation trials to the 60 s delays within the confinement tube, to establish a baseline for the rotation trials.

The rotation trials were carried out in the same way as the delay sessions described above. During each session there were 4 trials: one 90° rotation over 60 s (either clockwise or anti-clockwise), one 60 s delay trial with no rotation, and 2 no-delay trials. Based on good performance on the 60 s delay trials during the previous stage of the experiment, 7 rats were selected for the slow rotations and 10 sessions were carried out for each; 5 anticlockwise rotation sessions followed by 5 clockwise rotation sessions.

Cue training Following the rotation trials, rats were also trained on a cued version of the task, but as this was not pursued post surgery, this section is included as Appendix I.

Pre-surgery baseline Four sessions were then conducted to generate a baseline performance of the basic homing task, prior to surgery. These sessions contained 5 trials where the experimenter was inside the curtained maze area, and no intra-maze landmarks were available. Here, the food reward was held back for 5 s before the experimenter threw it onto the central platform. This was to rule out the possibility of rats using a simple turning strategy where they would run to the central platform, collect the food and immediately turn 180° and head home. Rats typically moved about within the central platform during this delay and this thus prevented a simple turning strategy.

At this point, rats were given either postsubiculum or sham lesions, as described below in section 3.2.5

Post-surgery standard task. Ten sessions of 10 homing trials were conducted following the recovery period. These sessions were run in the same manner as the pre-surgery baseline trials except that there were 10 trials in each session.

Delay trials Six sessions were conducted in which rats were delayed by either 60 s, 120 s or 240 s on the central platform. During these trials, the experimenter exited the curtained environment when the motorised tube was lowered. This was to rule out the possibility that the experimenter was in some way imparting information to the rats to guide their homing behaviour.

Trials under darkness Trials were conducted with room lights switched off to minimize the possibility that uncontrolled visual cues could be used to identify the location of the home box. During these trials, the experimenter delayed the placing of

the food reward on the central platform by either 5 s, 10 s or 20 s. This delaying of food meant that it was not necessary to constrain the rats by raising the tube. The experimenter used a night vision video camera with an infra-red light during these sessions (Sony NightShot CCD-TR84OE PAL, Sony Corporation, Japan) so that behaviour could be observed. Two additional sessions were run under darkness where the motor was left on continuously, rotating the central platform at 2.25°/s. Reward delivery was delayed by 20 s, and this ensured that the rat was slowly rotated by 45° before returning home.

Control tests A series of probe tests were conducted under dark conditions (and with no bedding in any box) in order to test that the rats were using path integration. The first was to rule out the possibility that the rats were using cues from their home box. For this test, 4 trials were run as normal with a 5 s delay of food. At the end of the 4th trial, rats were put in a new home box and put into the transport box while the old home box was moved to the 180° position and covered. On the 5th trial, rats were started off from the same location as before. Once the rats made their way onto the central platform, the Perspex lid covering the old home box was removed. If the rats used found the home box based on odour cues, they should select the old home box (where, presumably there were stronger odours) more frequently than the new box.

A second probe session sought to test path integration by starting the rats from a different home box and location each trial. During these trials, there was a 10 s delay on the central platform. If rats use uncontrolled extra- or intra-maze cues to find the home box, their performance should be disrupted when the home box location changes on each trial. Conversely, accurate homing in this situation would suggest that the animal maintains its orientation based on its starting point on each trial.

A third probe session tested whether the rats would use an odour trail to find the correct home box location. To allow an odour trail to build up, 4 trials were run from a single location without cleaning the maze. After the 4th trial, the rats were put in the transport box and placed on a nearby table. The home box was moved to a 90° location and fresh home boxes were opened at the original location and at the 180° location. The rat was then placed directly on to the central platform, where a food reward was available. As the rats had not made the outbound journey from the home box to the central platform, they could not use path integration to return to home the home box. The probe thus tested whether rats would use any odour cues left by the preceding trials. The box at 180° was opened to test whether rats were able to somehow detect and home to any open box.

A final probe session was conducted to test whether use of confinement tube itself disrupted homing. This session consisted of 3 trials with a short 25 s tube raise once the rats reached the central platform interspersed with trials with a 10 s delay and no tube raises.

Forced choice delayed alternation T-maze task. Following testing on the path integration maze, rats were trained on a delayed alternation task in a T-maze. One 10 min habituation session was run where both arms were baited. Rats were placed in the starting arm and were returned to the start approximately 10 s after they found the food at the end of either of the terminal arms.

Following habituation, trials were run as follows. One half Weeto (Weetabix, UK) was placed in a cup at the end of both arms of the T-maze. During a sample phase, one of these arms was blocked and the rat was placed on the maze at base of the T, and confined there by a wooden block. This block was removed at the start of the trial and the rat would run up the stem and into the open T arm to get the Weeto

reward. Then, the rat was picked up and placed inside a cardboard box with air holes for 30 s during which time the block was removed from the opposite arm and the start block put back in place. Then the rat was placed back onto the starting area for the choice phase. The start block was then removed and the rat would be rewarded for entering the alternative arm from that of the sample phase. If the rat chose the wrong arm, it was blocked into that arm of the maze for 10 s. Sessions consisted of 6 trials; 21 sessions were conducted with a 30 s delay between sample and choice phases followed by a further 6 sessions with a 5 s delay and 3 sessions with a 60 s delay.

3.2.5 Surgery

Eleven rats (1 animal was withdrawn from the experiment during the delay training due to illness, unrelated to the experiment) were allocated to either the bilateral postsubiculum lesion group (n=6) or the sham surgery group (n=5). Animals were anaesthetised with isoflurane (Abbott, UK) and placed in a stereotaxic frame (Kopf Instruments, USA). Anaesthesia was maintained via an inhalation nose cone affixed to the mouth bar on the frame. Under sterile conditions, a midline incision was made and the skull exposed. For the sham animals (n=5), holes were drilled into the skull bilaterally over the postsubiculum. The dura was then pierced several times on each hemisphere and the animals' skin sutured up. For the lesion animals (n=6), holes were drilled bilaterally over the postsubiculum using coordinates derived from pilot experiments. Ibotenic acid hydrate (Biotechnology, CA) dissolved in phosphate buffered saline to 10mg/ml (pH 7.4) was injected at 7 sites in each hemisphere (Figure 3.2). At each site, 0.04 ml was injected.

A-P	1.3	1.7	1.7	2.2	2.2	2.7	2.7
M-L	3.5	3.3	3.5	3.2	3.5	3.5	3.9
D-V	-3.6	-3.8	-4.2	-3.8	-3.8	-3.8	-4.8

Figure 3.2 Table to show coordinates of ibotenic acid infusions for postsubiculum lesions. All values are shown in mm; A-P values are with respect to the transverse sinus, M-L with repect to the midline between bregma and lambda, and DV with respect to the brain surface.

After surgery, all animals were then given analgesic (Rimadyl) in their drinking water for 7 days (0.04 ml/L) and a given 14 days to recover before returning to the experiment (the first 7 days on free-food and then 7 days on restricted food).

3.2.6 Histology

At the completion of testing, rats were given an overdose of pentobarbital solution (Euthatal; Merial Animal Health, Harlow, UK), and transcardially perfused with saline, followed by a 4% formalin solution. The brains were removed and stored in 4% formalin before being egg-embedded. The egg embedding involved beating an egg yolk (no white) and pouring it over and around the brain inside a small plastic dish. The brains were then enclosed in a receptacle containing 4% formalin (but not submerged) and incubated at 40°C for another 24 hours. Subsequently 30 μm brain slices were obtained in a cryostat. One in every three sections from the postsubiculum were mounted on slides, dried, stained with a standard Nissl stain procedure, and coverslipped. The lesion extent was measured using software to measure the area of the spared postsubiculum, which was compared to the mean area for the control rats. Images of the sections were captured on a PC running Image Pro Plus (version 6.2; Media Cybernetics, USA) using a microscope (Leica DMRB, Germany) with a 2.5× objective equipped with a QICAM camera (QImaging, Canada). For each image, the area around the postsubiculum was drawn manually and the area calculated by the

software. Damage to the postsubiculum was calculated for each animal by comparing the mean values obtained to the mean values for the control animals.

3.3 Results

Rats home equally well from the same home box location or from different home box locations Following habituation to the maze, each animal was given 16 sessions of 8 trials each, either starting from the same home box location on each trial, or starting from a different home box location on each trial. Across the 16 sessions on the maze, rats returned to the box from which they had departed, the home box, more than any other box (Figure 3.3A). To quantify this, a circular statistic, the V-test, was conducted (Batschelet, 1981). A significant value on the test statistic, u, indicates that the observed values cluster around a specific value, and the value used in our comparison was the angle of the home box (0^o). This test confirmed that the distribution of home box choices was centred on the home box ($u = 35.9$; $p < 0.001$). A repeated measures ANOVA revealed that animals from the *different start* group homed as accurately as those in the *same start* group [$F(1, 10) = 0.7$, $p = 0.424$; Figure 3.3B] across the 16 testing days. Performance did not differ across days [$F(15, 150) = 0.69$, $p = 0.78$], and the two groups did not differ on different days [$F(15, 150) = 0.59$, $p = 0.87$].

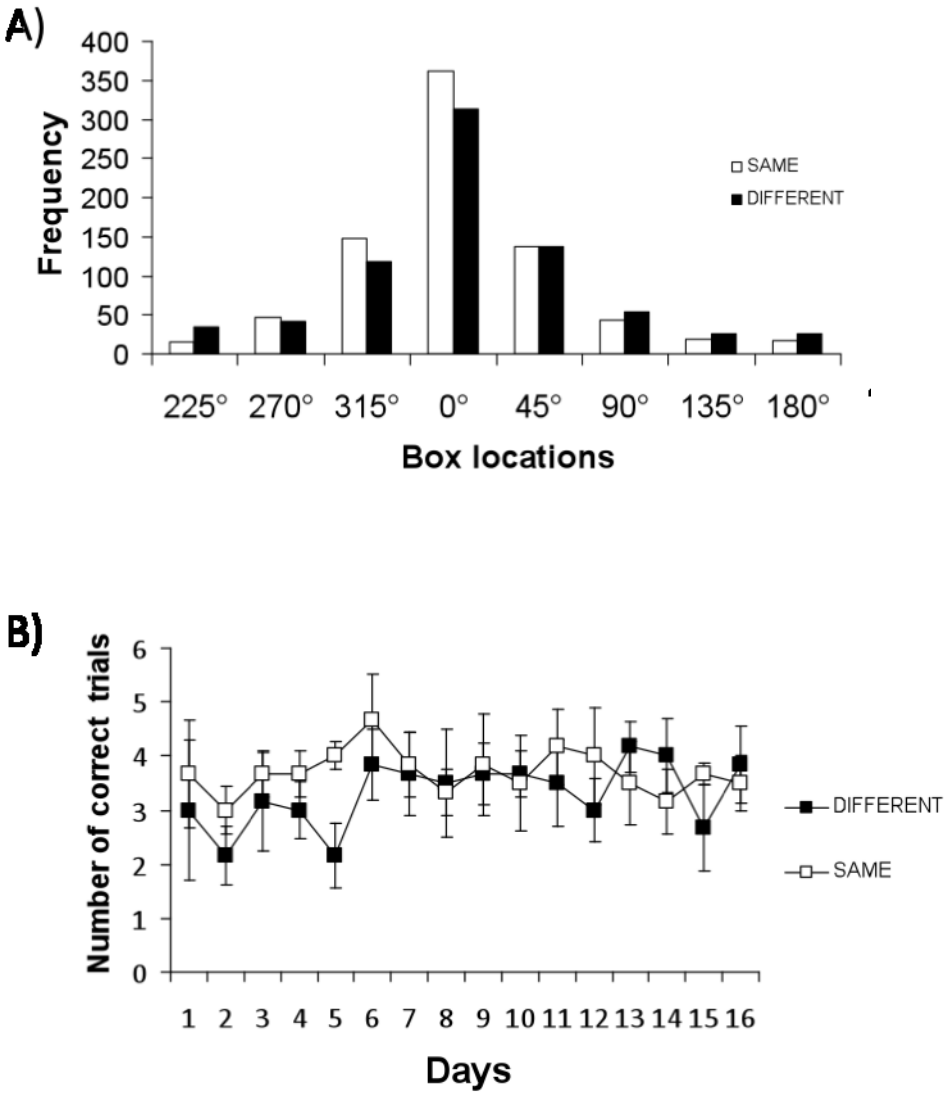

Figure 3.3 (A) Distribution of home box choices over 16 sessions of testing. The home box at 0° is the correct home box – the box from which the rat started its journey. Rats that started from the same home box repeatedly did not differ from those who started from different home boxes on different trials. (B) Across 16 sessions of testing, the number of correct trials – trials in which the rats' first choice was to the correct home box– did not differ for the rats starting from the same home box location compared to those starting each trial from a different home box location. Data is displayed as means ± SEM.

Memory for the home box location was delay-dependent Figure 3.4A shows the first box chosen by each animal on their return from the central platform following each delay. As is evident from the figure, the most frequently chosen location following no delay (0 s) was the home box of the animal. With delays of 30 and 60 s,

choices were concentrated on the home box and the adjacent locations. With delays of 120 and 240 s, home box choices were more variable.

For delays of 0 s, 30 s, and 60 s, the mean angles clustered significantly around 0° (u= 4.46, 3.34, and 3.59 respectively, n=12) with $p < 0.001$. However, for delay times above 60 s, the mean angles did not significantly cluster around 0° (120 s: $u = 0.47$, $p > 0.10$; 240 s: $u = 0.95$, $p > 0.10$).

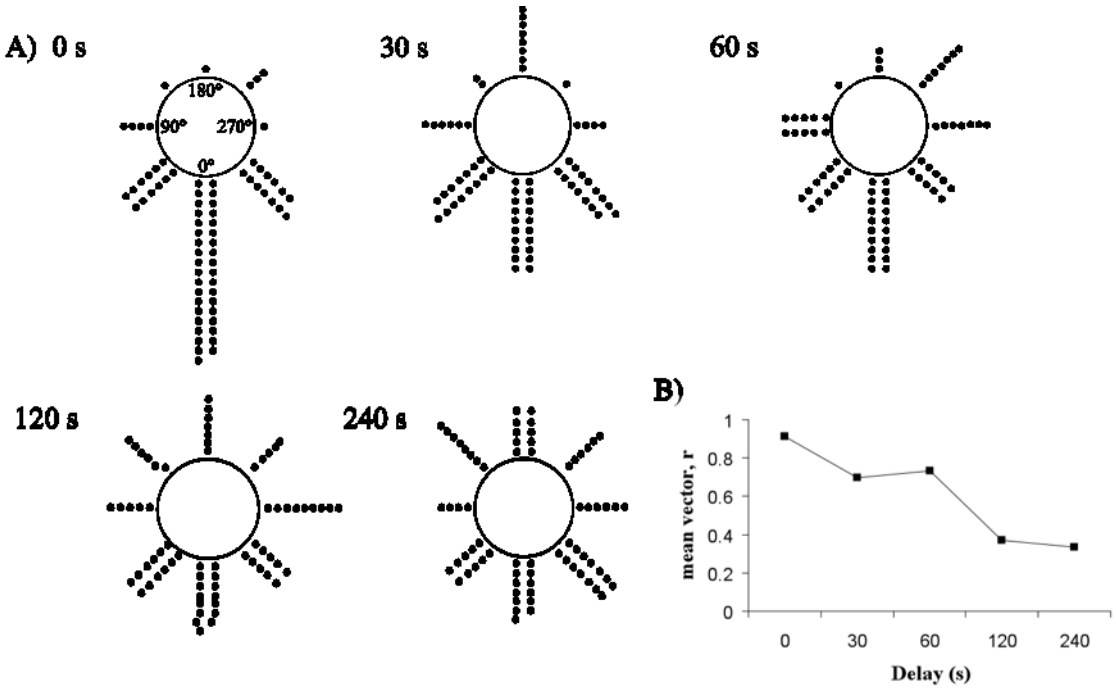

Figure 3.4 (A) Circular plots of home box choices with different delays before the home-bound journey. For delays of 0-60 s, the most frequently chosen home box was the correct box (at 0 degrees). For the 120 s and 240 s delays, home box choices were more varied. (B) The mean vector for these responses decreased across delays.

Rats made homing errors that corresponded to the direction of slow rotation. Seven animals were slowly rotated over 90° in both a clockwise and an anti-clockwise direction over 10 sessions. As is evident in top plots of Figure 3.5, although rats still chose the original home box (0^{o}) most frequently following rotation, they tended to make errors in the direction expected if the animals' sense of direction was fooled. Thus the mean angles for the choices following rotation are deflected in the direction

of the rotation [counter-clockwise (- 90^{o}) rotation average = 346.4 ^{o}; clockwise (+ 90^{o}) rotation average = 49.0^{o}; Figure 3.5, bottom plots].

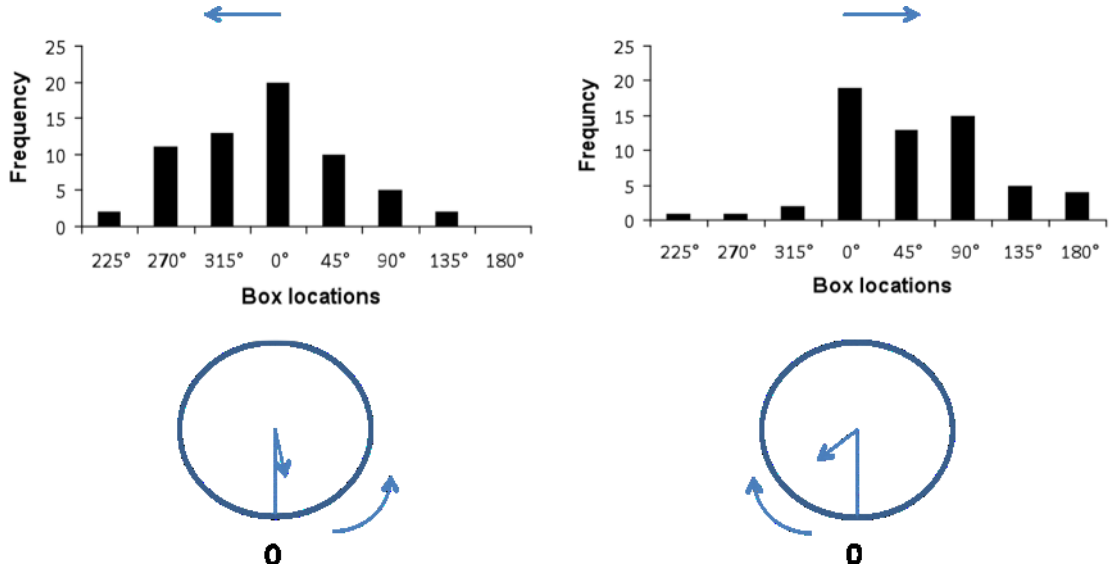

Figure 3.5 Home box choices when the rats were rotated by 90 degrees before their return journey. Right plots show that clock-wise rotations resulted in an apparent shift in home box choices (top) and mean vector (bottom) in the direction of the rotation. Left plots show a comparable finding from the counter-clockwise rotations of the rats.

Histology The infusions of ibotenic acid removed, on average, 66% of the postsubiculum (range 45 to 84%). Damage extended to other areas including the dentate gyrus (n=5), parasubiculum (n=2), subiculum (n=1) and retrosplenial cortex (n=4). Figure 3.6 shows a comparison of the largest lesion with the smallest lesion. Figure 3.7 shows a comparison of representative coronal sections of postsubiculum from control rats with lesioned rats.

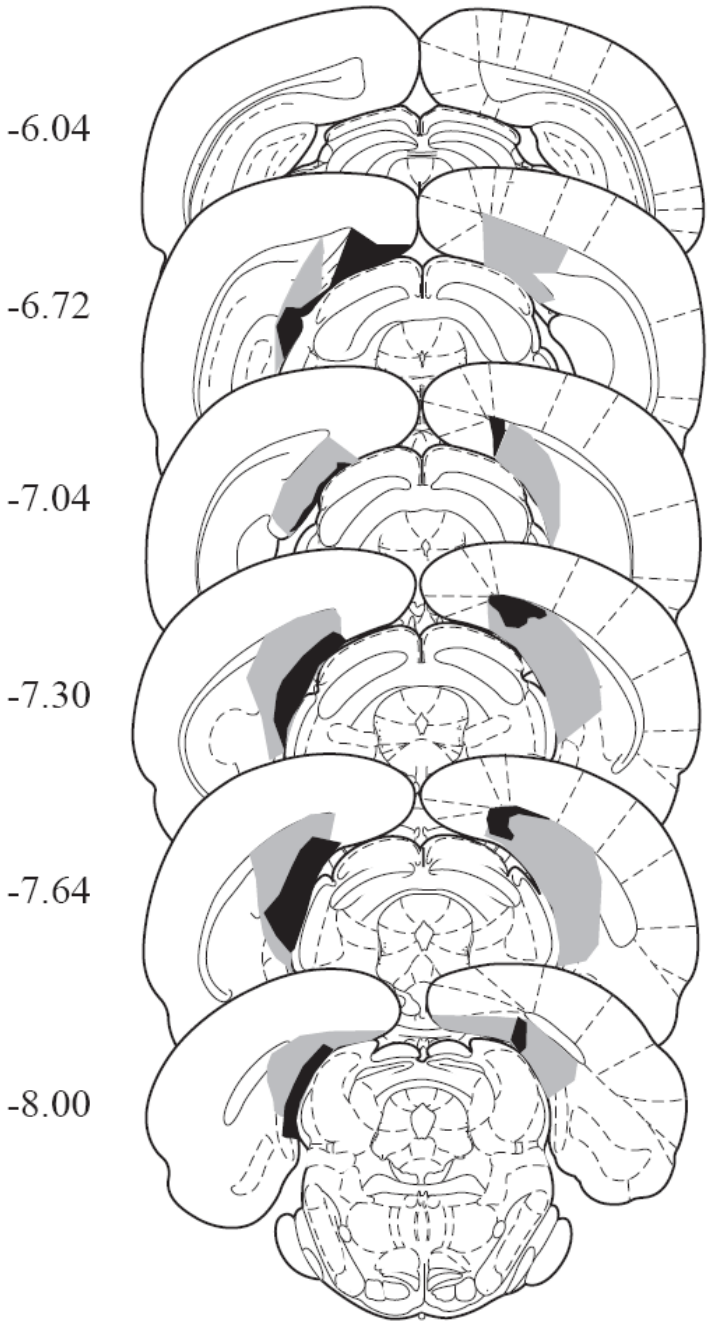

Figure 3.6 Illustration showing the extent of the lesions to the postsubiculum. The largest lesion (light grey) and smallest lesion (black) are plotted on brain sections (Paxinos and Watson, 1998). The numbers represent distance (mm) from bregma.

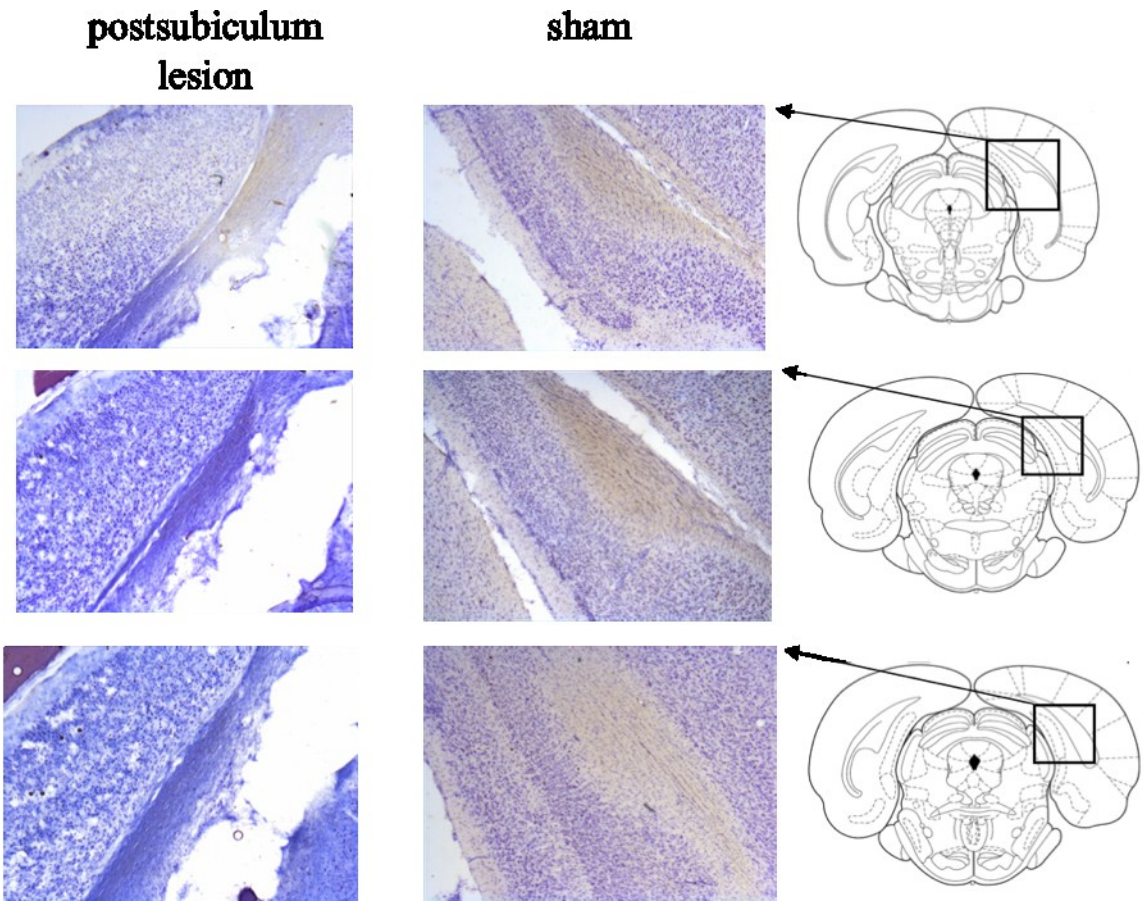

Figure 3.7 Photomicrograph of postsubiculum in a lesioned animal (left) and a sham animal (right). In this example, infusions of ibotenic acid produced a loss of tissue extending from the anterior postsubiculum (top) to more posterior sections (bottom). Atlas sections are from the Paxinos and Watson (1998).

Lesions of the postsubiculum fail to impair homing behaviour. Following recovery from surgery, rats were tested over 10 sessions on the type of trials in the pre-surgery baseline task. As is evident in Figure 3.8, both the postsubiculum-lesioned animals and the control animals readily returned to the home box (Box 0), and exhibited a nearly identical distribution of responses. A Chi-square test of independence confirmed that these distributions of home box choices did not differ [χ^2 (7) = 8.07, p = 0.32].

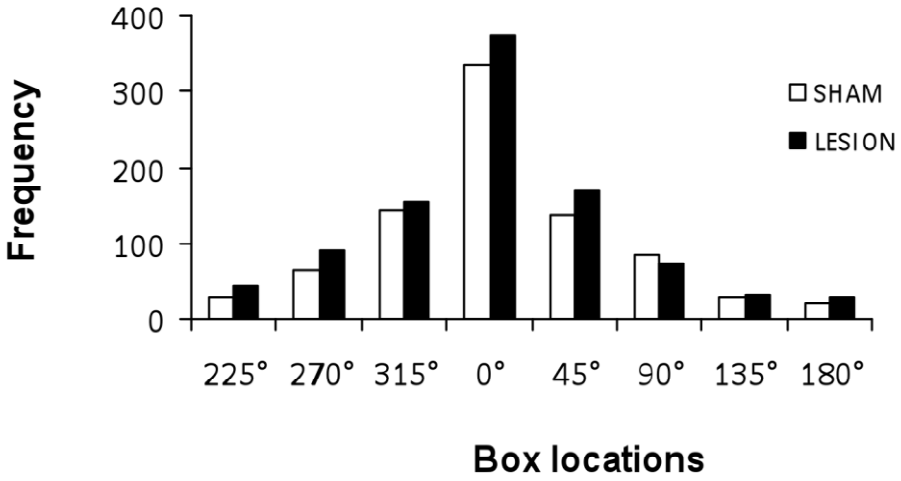

Figure 3.8 Home box choices for the animals with postsubiculum lesions (dark bars) and the sham-lesioned animals (white bars). Across 10 sessions following surgery, there were no differences in the accuracy of the lesioned and the control animals, and both groups selected the correct home box more than any other box.

Although both groups of animals displayed accurate homing in the standard task following surgery, their performance following the 60 s, 120 s, and 240 s delays in the centre was variable and not significantly oriented to the home box ($u = 0.74$, -0.47, and 0.89 respectively; all $p > 0.10$). The distribution of home box choices following the 60 s and 240 s delays did not differ between lesion and sham group [60 s: χ^2 (7) = 9.46, $p = 0.22$; 240 s: χ^2 (7) = 6.36, $p = 0.49$]. A difference was observed between the groups for the 120 s delay [χ^2 (7) = 20.7, $p < 0.005$], but neither group showed a significant homing to the home box at this delay [sham mean angle: 101.6 ^{o}, $u = -0.86$, $p > 0.1$; lesion mean angle: 319.5 ^{o}, $u = 0.20$, $p > 0.1$].

Both lesioned and control animals homed accurately in the dark. To control for unintentional visual cues in the environment, rats were tested in darkness with delays of 5 s, 10 s, or 20 s in the centre of the maze. In each of these delays, the lesioned and sham selected the home box more frequently than any other potential location (Figure 3.9A-C). Statistically, the distributions of home box choices by the lesion and sham groups did not differ [5 s: χ^2 (7) = 13.1, $p = 0.069$; 10 s: χ^2 (7) = 11.1,

p = 0.13; 20 s: χ^2 (7) = 7.53, p = 0.37]. For each delay, across groups, the choices were centred on the correct home box (5s: u = 10.62, p < 0.001; 10 s: u = 5.20, p < 0.001; 20 s: u = 8.88, p < 0.001).

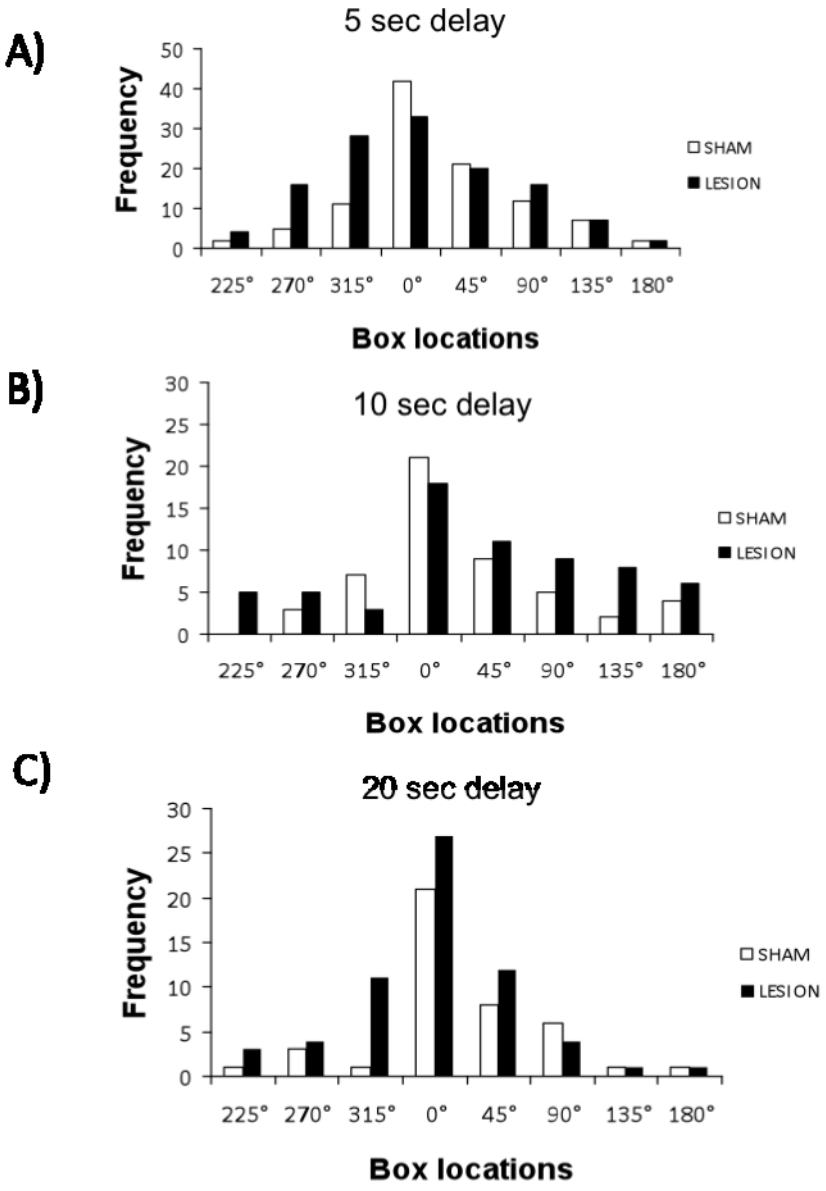

Figure 3.9 *(*A-C) Home box choices with 5-20 s delays before the return journey. The postsubiculum lesioned animals (black bars) and the sham-lesioned animals (white bars) performed accurately at these three delays, and their distributions did not differ.

Rats were also tested with rotation of the central platform by 45^o in the dark. Although we had observed some shift in home box choices in the direction of the earlier 90^o rotations, the 45^o rotation resulted in small shifts in the opposite direction

(clockwise (+45^{o}) rotation mean angle: 337^{o}; counter-clockwise (-45^{o}) mean angle: 29.5^{o}).

Control tests indicate that rats do not rely on odour cues to find the home box. When rats were started from a new home box, following repeated trials from a different home box, their responses were centred on the new location (sham: $u = 3.59$, $p < 0.001$; lesion: $u = 2.89$, $p < 0.005$). Thus, odours in the previous home box did not appear to control homing behaviour.

In a second probe session, when animals started each trial from a different home box, responses were again centred on the home box from which the trial began (home box = 0^{o}; sham: $u = 2.97$, $p < 0.005$; lesion: $u = 2.34$, $p < 0.01$). The distribution of responses for the lesion and sham groups did not differ [χ^2 (7) = 4.37, $p = 0.73$].

Further evidence that rats did not use an odour trail to find the home box was found in the third probe session. Rats were given repeated trials from the same home box, without cleaning the maze after each run, and both groups returned to the home box more than any other box (Figure 3.10A). The distribution of responses for the lesion and sham groups did not differ [χ^2 (7) = 6.71, $p = 0.46$].

On the following trial, the rats were then placed directly in the centre of the maze, and permitted to return home. In the absence of the outbound journey from the home box, neither the lesion nor the sham animals were able to return to its location [Figure 3.10B; sham: $u = 1.29$, $p > 0.10$; lesion: $u = 1.10$, $p > 0.10$].

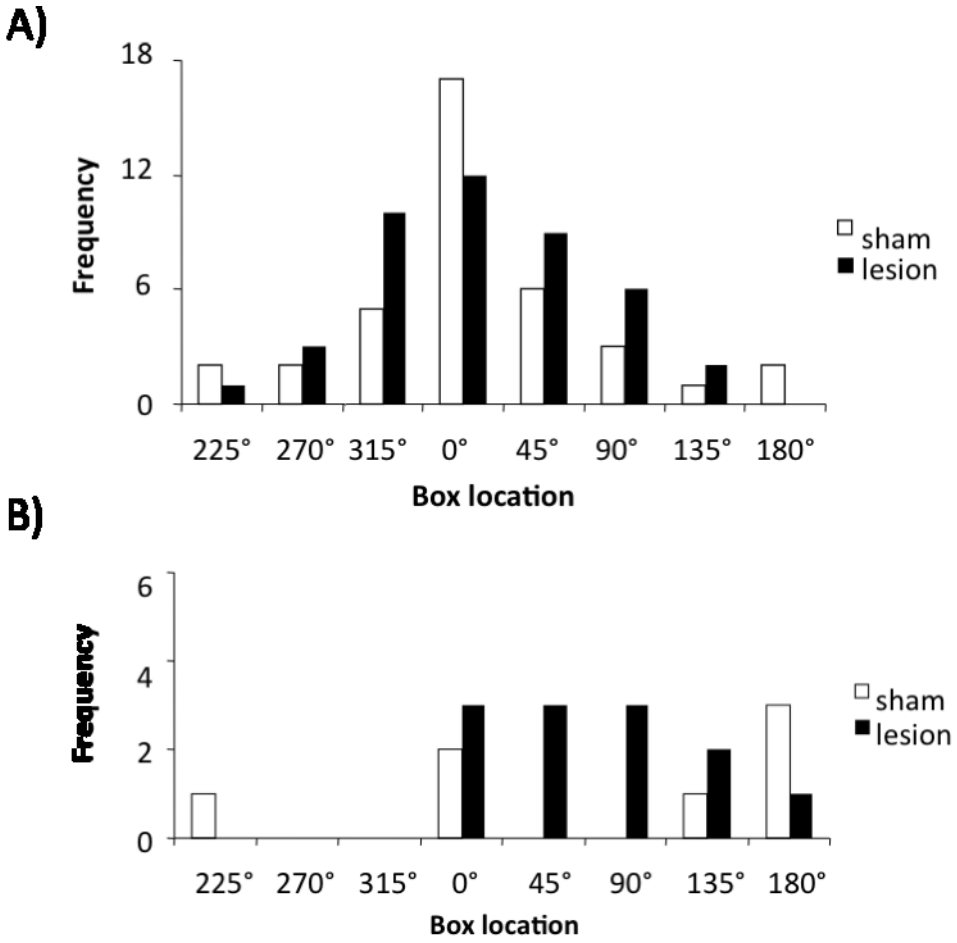

Figure 3.10 (A) Distribution of home box choices in darkness. Both the postsubiculum lesioned animals and the controls selected the correct home box more frequently than any other when tested in darkness. (B) Distribution of home box choices in the absence of path integration. When rats were simply placed in the centre of the maze, and did not experience the outbound journey, they no longer returned to the home box accurately.

In a final probe session we tested whether the confinement tube itself would disrupt homing accuracy. Following a 25 s delay in the confinement tube, both the lesioned and the sham animals returned to the correct home box [sham: $u = 2.57$, $p < 0.005$; lesion: $u = 5.16$, $p < 0.001$), and the distribution of home box choices did not differ [χ^2 (7) = 11.2, $p = 0.12$].

Postsubicular lesions impair spatial memory on a T-maze The lesion and sham groups were tested for 21 days on a delayed alternation T-maze task. Over this training, shown in 3-session blocks in Figure 3.11A, the number of correct responses

by the sham group improved, while those of the lesion group did so only to a limited extent. A repeated measures ANOVA on this performance revealed this interaction between lesion and sham group performance and session block approached, but did not reach, significance [$F(6,42) = 2.18$, $p = 0.065$]. There was no significant difference overall between the lesion and sham animals [$F(1,7) = 2.86$, $p = 0.135$], and overall performance improved across session blocks [$F(6,42) = 5.35$, $p < 0.001$].

As is evident in Figure 3.11B, animals with postsubiculum lesions were consistently worse than sham animals at all delays [$F(1,7) = 7.96$, $p = 0.026$]. This impairment did not differ across different memory delays [$F(2,14) = 0.54$, $p = 0.95$]. Overall, a slight decrease in performance is evident with longer delays (Figure 3.11B), but this did not reach significance [$F(2,14) = 1.36$, $p = 0.29$].

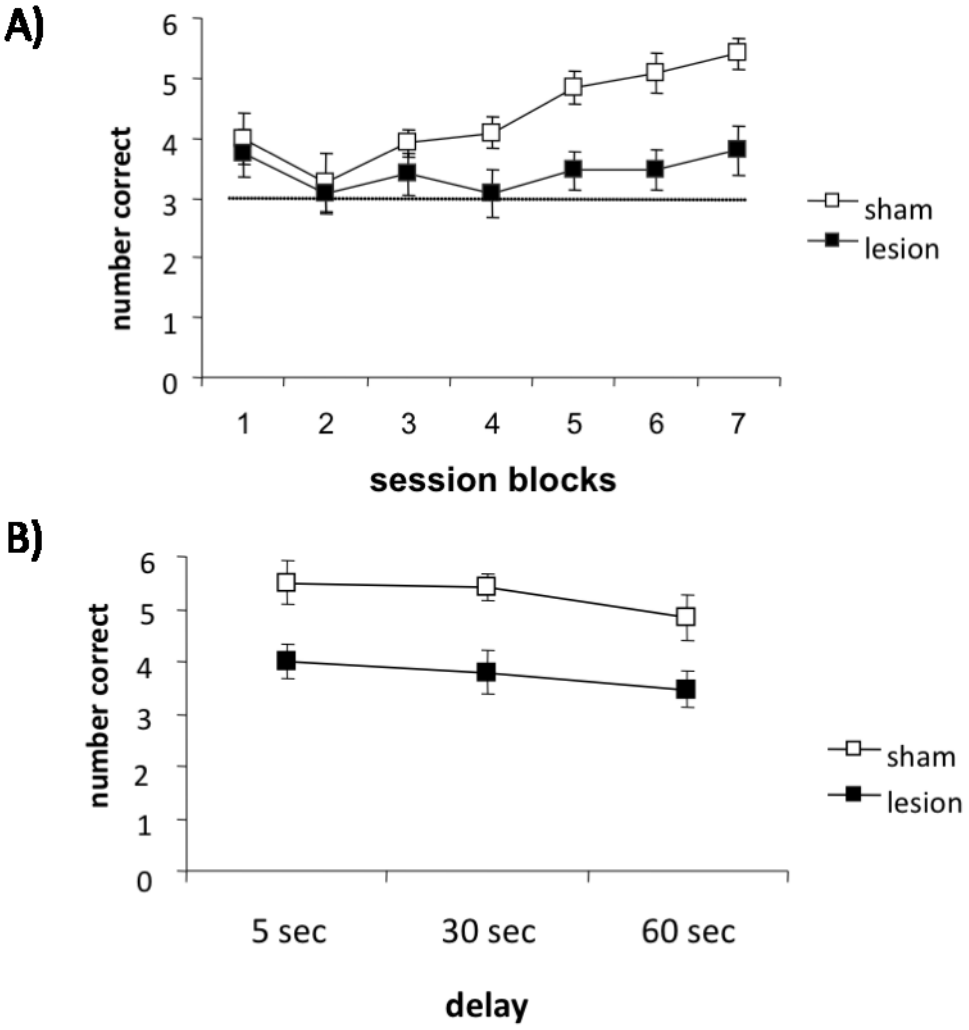

Figure 3.11 Performance of postsubicular- and sham-lesioned animals on a delayed alternation task in a T-maze. (A) Number of correct alternations, out of six, across session blocks. Each block is comprised of three test sessions. Horizontal line represents chance level. (B) Animals with postsubiculum lesions performed at a lower level than sham-lesioned animals at delays from 5-60 s. Data is displayed as means ± SEM.

3.4 Discussion

The aim of this study was to test whether the postsubiculum (dorsal presubiculum) is essential for path integration. To test this, rats with either lesions to the postsubiculum or sham-lesions were assessed on a homing task. A secondary aim of the current experiments was to further establish the validity of this task as a means of testing path integration behaviour, and to this end several manipulations were built in to this testing. It was found that animals appear to solve the task by path integration, and that damage to the postsubiculum had no effect on performance. However, it was found that postsubiculum is necessary for spatial memory in a T-maze task.

The postsubiculum is not necessary for homing Previous experiments with lesions of the postsubiculum, or combined lesions of the postsubiculum and parasubiculum, have assessed the effects of this damage on standard tests of spatial reference or working memory. Here, the effects of this damage on a different spatial ability were assessed, the capacity to return to a home site based on, presumably, an internal representation of the distance and direction travelled. As described below, manipulations implemented in the task during its acquisition and testing provide evidence that it tapped the animal's capacity for path integration.

Following removal of the postsubiculum, animals returned to the home box location as readily as intact animals. Their performance with a 5-20 s delay between the outbound and return (homing) journey was likewise unimpaired, suggesting that memory for the home box location does not critically depend on the postsubiculum. It is possible that deficits may have been more apparent at longer delays, but this appears unlikely for two reasons. First, at delays of 60 s or greater, even the sham-lesioned animals were not able to return to the home box accurately. Thus, the memory trace for the home box location, at least under the current testing conditions,

may be short-lived. Second, postsubiculum lesions produced a robust impairment on the T-maze task with delays of 5-60 s, suggesting that damage to this structure is sufficient to disrupt memory at these shorter delays.

The impairment in alternation on the T-maze is consistent with previous experiments in which the postsubiculum has been removed (Taube et al., 1992; Kesner and Giles, 1998; Liu et al., 2001). It is also consistent with findings that show that the postsubiculum is necessary for object-location memory (Liu et al., 2001, Bett et al., In preparation). Together with the lack of impairment in the path integration maze, these results suggest that the postsubiculum is necessary for some types of spatial memory, but not others. Specifically, the capacity to track self-motion, calculate a homeward vector, and retain this information for a short delay, appears to be independent of the postsubiculum.

Such a conclusion is surprising, given the types of spatial representations observed in this brain region. The postsubiculum was the first region in which head direction cells were observed, and also contains place-by-direction cells, border cells, and grid cells. Indeed, changes in the responses of head direction cells in this region have been shown to correlate with changes in the direction of homing (van der Meer et al., 2010).

There are several potential explanations for the observed results. First, it may be that the lesions spared portions of the postsubiculum and that these were sufficient to support path integration. While this possibility cannot be excluded, the lesions appeared to be comparable to those that produced instability in place cells (Calton et al., 2003). Second, it may be possible that grid cells and/or head direction cells in the parasubiculum, or other regions, are still present following the lesions, and are sufficient to enable path integration. In fact, it has been shown previously that the

retrosplenial cortex, which receives inputs from both the anterior and lateral dorsal thalamic nuclei, is essential for path integration under darkness (Cooper et al., 2001). It may be that the retrosplenial cortex is sufficient, without any postsubiculum, for path integration. Third, it may be that the type of information encoded by cells in the postsubiculum is not the only type of information that permits a representation of direction and distance travelled. Indeed, lesions of the postsubiculum produce only a minor impairment in the maintenance of preferred firing direction of head direction cells in the anterior dorsal thalamic nuclei when an animal walks from one environment to another (Goodridge & Taube, 1997), although only a limited number of cells were tested. Further, lesions of the lateral mammillary bodies, which presumably disrupt the head direction signal throughout the brain, fail to disrupt performance on several spatial tasks, including alternation of directions in the dark (Vann, 2011). It is also noteworthy that, whilst the postsubiculum lesions in the Calton et al. (2003) study led to hippocampal place fields being unstable *between* sessions, only small *within* session instability was found. It may be case that animals without postsubiculum are able to maintain their orientation within a given session and that this contributes towards successful path integration.

Alternatively, another potential explanation is that there were too few animals in the present study, resulting in insufficient statistical power which subsequently contributed to the lack of statistically significant differences between the lesions and sham groups. Due to this possibility, the results should be treated with caution.

Homing likely depends on path integration An additional contribution of the current study was to establish whether the current homing task truly requires path integration. The following findings support this is the case. First, animals that started each homing journey from a different location on the periphery of the maze were as

accurate as those starting from the same location. This suggests that the animals maintained their orientation relative to their departure point (the home box) and not its location relative to uncontrolled, fixed intra- or extra-maze cues.

Second, prior to surgery, a 90^{o} rotation of the animals below their vestibular threshold before their return journey caused a displacement in their home box choices in the direction of the rotation. This is consistent with previous work using this paradigm (van der Meer et al., 2010), and earlier work with desert mice (Mittelstaedt & Mittelstaedt, 1980). Less displacement was seen when this manipulation was tested with a smaller rotation (45^{o}), but these were slightly faster than the previous 90^{o} rotations (2.25^{o}/s vs. 1.5^{o}/s), and may have thus been more detectable to the animals.

Third, animals readily returned to the home box in darkness, even when the maze was cleaned before their return journey, or when the home boxed was switched to another location. Thus, the animals were returning to a specific direction or location, and not, as far as could be determined to a specific external cue. Taken together, these findings suggest that the rats used an internal sense of orientation to return to the home box.

Summary This study makes three contributions. First, it shows that the postsubiculum is not necessary for homing. Second, it confirms that the postsubiculum is essential for other forms of short term spatial memory, and specifically the memory of recently visited locations as demonstrated on the T-maze task. Third, it provides additional evidence that, in the absence of salient extra-maze landmarks, accurate homing is based on path integration.

Chapter 4: Place fields stability in novel environments

4.1 Introduction

Principal cells in the hippocampus, known as place cells, are known to have location specific firing (O'Keefe & Dostrovsky, 1971). One of the known properties of a place cell is that it can have a place field in a given environment and that this place field is likely to fire in the same location in space on the next exposure to the same environment. Place fields can be stable across multiple sessions, that is, they can fire in the same circumscribed portion of the recording environment even when there are days, weeks and even months between sessions (Muller et al., 1987; Thompson and Best, 1990). However, conflicts between a cylinder and a square shaped environment have been reported, that eventually resolve into distinct representations over time (Lever et al., 2002). In addition, if rats have sessions in multiple environments over days, place fields tend to remain in the same location in each environment so there is no interference between environments (Muller and Kubie, 1987; Thompson and Best, 1989). This means that there is some kind of memory for features of the environment that is represented by the place cell system.

This memory representation is constructed each time a rat enters a novel environment. Place fields are not present when a rat first enters a novel environment and take around five to six minutes before there is stability that lasts 24 hours in a maze consisting of narrow alleys (Frank et al., 2004). This timing is in line with a previous study by Wilson and McNaughton (1993), who reported that around eight to 10 minutes were necessary for place fields to fully develop in a box environment. This is unlike entorhinal cortex grid cells that appear to be hardwired and fire in a grid like formation very quickly upon entry to a novel environment (Hafting et al., 2005).

This time dependent formation of place fields, and the fact that place fields remap in different environments, suggests that synaptic plasticity is required for their formation. This was supported by a study by Xu et al. (1998) , in which LTP was induced in rats before being put into a novel environment or a familiar environment. They found that the novel environment blocked the early stage LTP in the stimulated pathway but didn't affect baseline levels in another pathway, suggesting that plasticity mechanisms employed within the hippocampus when the rats entered the novel environment were so strong that they outmatched the artificially potentiated pathway in competition for plastic resources. In a study by Kentros et al. (1998), an NMDAR antagonist was injected into rats prior to them entering a novel environment and the result was that place fields in the novel environment were stable when tested 1.5 hours later but had remapped and become unstable when tested at 24 hours. This indicated at least two separate synaptic learning mechanisms are involved: one for the short-term formation of place fields and another for their long-term consolidation.

However, most work on place cells focus on recording from familiar environments in which place fields are well established (Muller et al., 1996), including in the experiments cited above (Muller and Kubie, 1987; Muller et al., 1987; Thompson and Best, 1990). In these studies, the rats had become familiar with the environments over multiple sessions, and place fields tend to become established fairly quickly after first exposure to an environment. Muller et al. (1987) noted that after 32 minutes in one cylindrical environment, place fields contours were smoother than at four, eight or 16 minutes, suggesting that the fields were more fully formed. However, after four minutes, place fields were reasonably well established and improved over time. In this same study, the authors report the mean pixel-by-pixel correlation between firing rate maps of one cell in two sessions separated by one hour

was r = 0.7, and with six days between sessions, the correlation dropped to r = 0.4. However, it is not clear if the first of the two sessions separated by one hour was the *first* time that the cell had been in that environment; that is, if the first session was recorded in a completely novel environment.

In the Kentros et al. (1998) study, CA1 place fields were recorded in a novel environment for 16 minutes and then tested at 1.5 hours and 24 hours. The environment was a cylinder with a cue card. Place fields were stable after both time periods with correlation values of approximately r = 0.4-0.45.

One environmental feature that strongly influences the stability of place fields between sessions is stable visual landmarks (e.g. Muller and Kubie, 1987). It is known that 3D objects placed in the centre of a cylinder do not exert much influence but when the same objects are placed around the periphery they exert stimulus control over place fields (Cressant et al., 1999). In this study, place fields rotated along with the cues when they were rotated in the rats' absence.

The aim of this present study was to investigate place field stability in *novel* environments. This was achieved by manipulating the time between sampling the environment and a stability test. The underlying motivation for this experiment was to determine the optimal time delay between presentation of a novel environment to rats and a later test exposure to the same environment, such that place fields are reliably stable. When such a time delay was determined, it would then be used when designing the delay phase in the experiments reported in the following chapter (Chapter 5), where pharmacological manipulation of the postsubiculum during exposures to novel environments was conducted in conjunction with place cell recordings.

4.2 Methods

4.2.1 Overview

Prior to experiments, rats were chronically implanted with headcaps that had either 8 tetrodes (CA1) or four tetrodes (CA1) and bilateral cannulae (PoS). Rats with the cannulae were prepared for the experiments in Chapter 5 and no drug infusions took place in the experiments in this chapter. Rats with implanted microdrives containing either four or eight tetrodes aimed at CA1 were exposed to novel environments containing two 3D objects and a cue card. After three 10 minutesexposures to the environments, rats were then put back to their home cage for three, six or 24 hours. The environment was then rotated 90º clockwise or anti-clockwise and the floor and walls changed. Rats were once again introduced to the environment and then place fields were analysed for stability across the time delay. This was a within-subjects study, with all subjects experiencing all three delay times.

4.2.2 Subjects

Eight male adult Lister-Hooded rats (Charles River Laboratories, UK) were used in this study and were housed in individual cages and kept on a 12:12 light-dark cycle. All experiments took place during their light phase. The rats weighed between 350 – 420 g at the beginning of the experiment. During the experiment all rats had unrestricted access to water but were food restricted to around 85-90% of their *ad libitum* bodyweight. All procedures were compliant with the UK Animals Scientific Procedures Act, (1986) and with the European Communities Council Directive of 24 November 1986 (86/609/EEC) legislation governing the maintenance of laboratory animals and their use in scientific experiments.

4.2.3 Electrode preparation

The design for the recording microdrives used was a modified version of Kubie's (1984) tripod. Each microdrive had either 16 or 32 HML coated 17 µm (90% platinum; 10% iridium) wires (California Fine Wire, Grover Beach, CA) made into four or eight tetrodes. Tetrodes were made by twisting bundles of 4 wires together in a clockwise fashion and heat annealing using a heat gun (GHG660LCD, Bosch, Switzerland). Heat annealing was accomplished by directing the hot air flow from the gun at the bundled wires for a short time of around 5 s at close proximity. Tetrodes were then inserted carefully into a short length of 27-gauge (four tetrodes) or 23-guage (8 tetrodes) thin walled stainless steel cannula (27 Ga Hypo Tube/23 Ga Hypo Tube, Small Parts Inc, Mirimar, FL). Connection between the tetrodes and the recording system was achieved by way of a length of MillMax plug (MillMax, Oyster Bay, NY). For the eight-tetrode drives, two lengths of MillMax were used arranged in parallel (see figure 4.1). One end of each wire from all tetrodes was exposed at the tip carefully, using a flame from a gas lighter to burn the HML coating, and then each wire was connected to an individual MillMax pin on the MillMax plug. Some silver conductive paint ('Electrolube', RS Components, Derbyshire, UK) was applied to each of these connections to improve conductivity and to secure the connection. A 22 mm length of wire (Vishay) was soldered onto a spare pin on the MillMax plug to function as a ground wire (For eight-tetrode microdrives, two ground wires were attached, one to each of the two pieces of MillMax). The device was made drivable by using three anchors, or feet. Each foot consisted of a stainless steel screw (Small Parts Inc, Miramar, FL), a hexagonal nut (Small Parts Inc, Miramar, FL) and an Amphenol socket (Amphenol, Wallingford, CT). The socket was tapped and the screw then threaded through the nut and socket. Some cyanoacrylate superglue (Henkel Loctite

Ltd, Cheshire, UK) was applied carefully to fasten the nut (but not the screw) to the socket. The top end of the screws were fixed to the MillMax using dental cement (simplex Rapid Acrylic Denture Polymer, Kemdent). The tetrodes, which were left protruding from the thin walled cannula, were then cut to around 2 mm from the end of the feet and an "outer" cannula (18 Ga Hypo Tube, Small Parts Inc, Miramar, FL) was carefully placed over the narrower gauge cannula taking care to avoid contact with the tetrodes, and was held in place using some Vaseline (Lever Faberge Ltd., London, UK). Shortly before implantation surgery, the tip of each wire was gold plated to an impedance (tested in 0.9% saline) in the range of 200-300 kΩ at 1kHz to improve signal sensitivity.

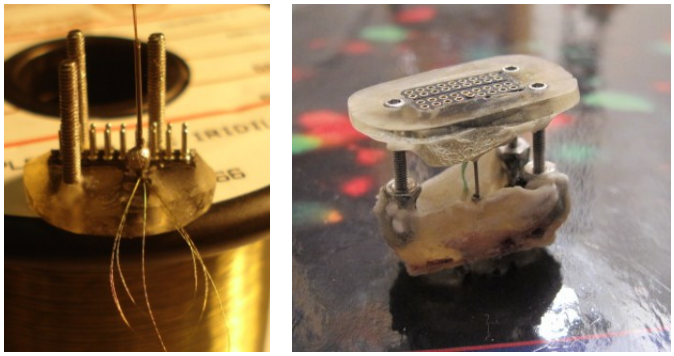

Figure 4.1 Left: Microdrive under construction showing the tetrodes inside the inner cannula adjoined to the MillMax. Right: Mircrodrive from rat's head.

4.2.4 Surgeries

Two types of surgeries were used in this experiment. Five rats were implanted with bilateral cannulae into the postsubiculum and also a four-tetrode microdrive. The rest of the rats, n = 3, were implanted with an eight-tetrode microdrive. Both surgeries were similar, but with the former type, the cannulae were positioned before the microdrive. The cannulae surgery is described in the next chapter (section 5.2.4).

Rats were anaesthetised using 5% isoflurane (Abbott Laboratories Ltd., UK) and their heads shaved then disinfected with clorohexidine scrub prior to surgery.

Rats were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and held in place using ear bars throughout the procedure. Isoflurane anesthesia was maintained throughout. A midline incision was made under sterile conditions and the skull exposed fully to the lateral ridges.

Next, a small hole was drilled through the skull over the left hippocampus for the electrodes. Five small screw holes were drilled as before, and screws inserted, for anchoring purposes. The dura over the electrode hole was punctured using a narrow gauge needle and the electrode drive was positioned and lowered into the brain, aimed just above the CA1 area. The outer cannula was then lowered to protect the exposed tetrodes. The ground wire on the drive was wrapped around a skull screw and the connection covered in silver paint. For eight-tetrode microdrives, two ground wires were used and attached to different screws. When this paint had dried, the drive was cemented in place, again using the skull screws as anchors. Cement was used to build "walls" around the exposed skull area so that the rat could not damage the drive post-surgery. Once the cement had fixed, the screws on the drive were turned ¼ to lower the tetrodes. A piece of electrical tape was attached around the cement base of the microdrive to protect the ground wires (and the electrodes) from the rat's claws. In some rats, a small piece of plastic was cemented around the back of the microdrive for this purpose,

Small Animal Rimadyl (0.08 ml/kg bodyweight) (Pfizer, UK) in 1 ml sterile saline was administered at the end of the procedure by subcutaneous injection as an analgesic. All rats were given a recovery period of at least 10 days before food restriction began and at least 14 days before screening for cells began.

4.2.5 Electrophysiology

4.2.5.1 Recording apparatus

Single units were recorded using an Axona 32-channel system (Axona, Hertz, UK). The recording microdrive on the rat's head was connected to a unitary gain operational amplifier via MillMax connectors for every screening or recording session. This amplifier was attached at the end of a long but light cable, which was connected to a commutator (Dragonfly Research and Development, Ridgeley, WV, USA) mounted onto the laboratory ceiling. The signal from the headstage amplifier then was passed to a pre-amplifier (Axona), which amplified the signal 1000 times before reaching the Axona recording system. Software (DacqUSB, Axona) was used to band-pass filter this signal between 600 and 6000 Hz. The software allowed further amplification of the signal, and allowed the gains of each individual channel (wire) to be set according to needs. The signal could be viewed on the oscilloscope option on the DacqUSB program, where 8 channels (2 tetrodes) could be viewed simultaneously on screen. The software also had the option to record the signals, and recorded 1 ms of activity across all wires over an adjustable pre-defined voltage threshold. When the signal from one wire in a tetrode exceeded this threshold, a 1 ms sample across all tetrodes was recorded, starting 0.2 ms before the threshold was triggered and ending 0.8 ms after, and time-stamped. The position of the rat was recorded by an overhead CCD camera, which was used to record (at 50 Hz sampling rate) infrared signals emitted by light-emitting diodes attached to the rat's headstage. These LED positions were also time-stamped by the DacqUSB software during recording.

4.2.5.2 Screening for cells

Rats were plugged in to the recording system up to twice daily to screen for cells after a recovery period of at least 14 days following surgery. The recording room was adjacent to the control room, which contained the recording system and computer. During screening, rats were put on a circular table top in the recording room and left to forage for small pellets (Dustless Precision Pellets, Bioserve, NJ, USA) thrown randomly by the experimenter. The signal on each wire in a tetrode was viewed as the difference between the signal and that of another wire from another tetrode, to reduce the signal-to-noise ratio. All recordings were also done in this manner. Principal cells were identified firstly by visual inspection of the signals on the oscilloscope and then, if deemed appropriate, by recording a short five to 10 minute session on the table top and analysing offline (see Data analysis). If no place cells were found, the electrodes were advanced by approximately 20 to 40 μm by turning the three screws on the rat's microdrive by between ⅛ and ¼ turn. Around six hours was allowed after advancing the tetrodes to allow them to settle before screening again. When suitable cells were found in a given rat, experiments then began with that rat.

4.2.5.3 Firing rate maps

Firing rate maps were generated using Matlab scripts written by Steven Huang. The script used an algorithm (Leutgeb et al., 2007), which involved dividing the experimental environment into bins of 5 cm x 5 cm. Then, the average firing rate for each single unit was calculated for each bin. This was done by dividing the total number of spikes in a given bin by the total time spent in that bin. A Gaussian smoothing function was then applied.

4.2.5.4 Place cell inclusion criteria

Single units were included for analysis if, after clustering, they fulfilled two criteria:

A) the average peak amplitude on one wire was >80 μV and the average width of largest waveform (peak to trough) was > 250 μs, and

B) the average firing rate for a session was between 0.1 Hz and 5 Hz and the Skaggs spatial information was > 0.5.

Since the analyses here involve the comparison of a single unit in two sessions, a cluster was accepted for analysis if the criteria in B) was fulfilled in at least one of the two sessions. This allows for the possibility that a place cell could remap between sessions.

Spatial information content was measured for each cell. This is a measure of how well the firing rate of the cell predicts the location of an animal within an environment (Skaggs et al., 1993). Skaggs spatial information = $\sum_i Pi \left(\frac{Ri}{R}\right) \ln\left(\frac{Ri}{R}\right)$, where i is the bin number in the spatial histogram, Pi is the probability that bin I is occupied, Ri is the mean firing rate of bin i and R is the overall mean firing rate.

4.2.5.5 Correlations

For each cell that satisfied the above criteria, correlations were carried out between place fields in one session and the corresponding place field in another session. This was to check the stability of the place field over time. To do this, the firing rate for each pixel was calculated and paired with the firing rate of the corresponding pixel from another session. Then a single correlation coefficient was calculated from these data.

4.2.6 Apparatus

Four very similar square-based boxes were used for this experiment. Each box was freshly painted black for the purpose of this study. The box walls were 51 cm high and each base was 50 x 50 cm. Each wall had a door insert in the centre, and the doors were kept in place throughout. There were a number of different cue sets, which could be put into any box. Cue sets consisted of a cue card, and two distinct 3-dimensional objects that were positioned around the periphery of the box in various locations. The objects in each cue set were kept in the same relative orientation to each other with respect to the boxes throughout the study. Example cue sets are shown in Figure 4.2.

Figure 4.2 Photographs of the black boxes used in the experiment with two examples of novel cue sets. Cue set were unique and differed from each other in the physical properties of objects themselves, as well as their relative positions within the box.

4.2.7 Procedure

Each rat was carried inside a high-walled bucket from the control room through to the recording room and inside the curtained enclosure, where the experimenter connected the recording cable to the rat's microdrive. The experimental box was covered over prior to this, so that the rat could not see the interior. The rat was then replaced into the bucket whilst gains and references were set for each recording channel. The rat was kept in the bucket for a five minute recording session, after which time the box was uncovered, before being placed into the box with a novel cue set configuration (Figure 4.3). The experimenter then left the curtained enclosure

and threw pellets intermittently so that the rat was motivated to explore the environment for the full length of the recording session. After 10 minutes, the rat was removed from the box and placed back in the bucket for five minutes. Two further box sessions were recorded in the same manner before the rat was unplugged and carried back inside the bucket to its home cage in the control room.

At this point, there was a delay of 3, 6 or 24 hours before the test phase. A different box was used for the test phase because this study was focused on place fields that were anchored to visual landmarks and not proximal cues on the box surfaces. The cue set was transferred to the new box (in the same configuration as the old box) with a rotation of 90° either clockwise or anti-clockwise. Note that, since most of the cue cards used were attached to a box door, when moving most cue cards from box to box the whole door was simply moved. The rat was then carried back through to the recording enclosure, connected to the recording apparatus and put in the bucket for a five minute recording session. Then, a 10 minute test session was recorded in the new box. The five minute bucket session was recorded as a check for activity of any cell "lost" during the test.

On those experiments when a rat did encounter a previously used box, the box was rotated in multiples of 90° relative to its orientation the last time the rat experienced it. Cue sets, boxes and order of delay times were varied between rats.

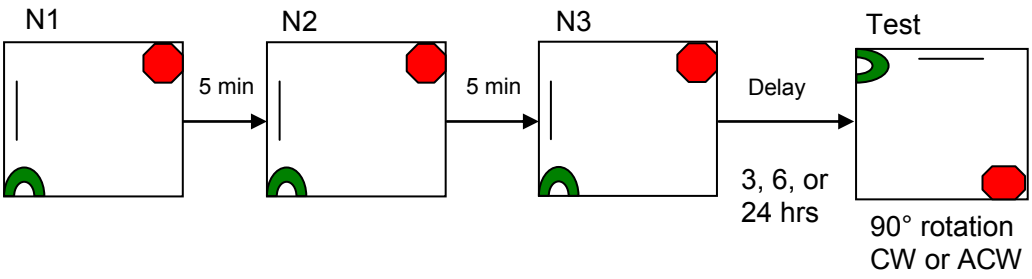

Figure 4.3 Schematic of experimental protocol. Rats were exposed to a novel environment three times at 10 min each whilst place cells were recorded. Following a delay of 3, 6, or 24 hours, the rats were returned to the environment with the cues rotated clockwise or anti-clockwise by 90º.

4.3 Results

4.3.1 Histology

Figure 4.4 shows a representative picture of an electrode tract over CA1. Electrode tips were difficult to determine; however, tracts were visible in all rats heading towards CA1.

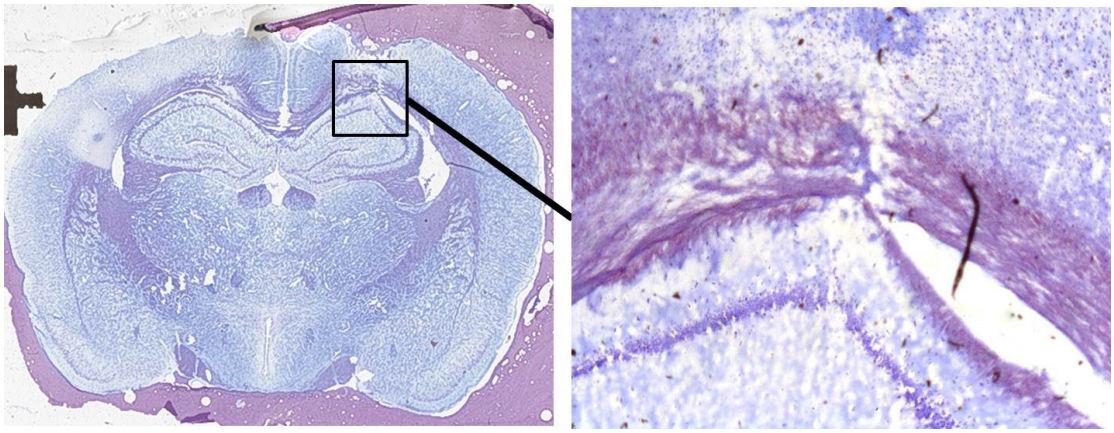

Figure 4.4 *Left* Cresyl-violet stained brain section showing electrode tract over CA1 in the right hemisphere. *Right* The same section but at higher resolution showing the right CA1. The exact electrode tip could not be found but the tract clearly shows the electrode heading directly towards CA1.

4.3.2 Stability of place fields across different time delays

Place fields appear relatively stable after a delay of 3 hours.

Representative place fields from cells recorded in each delay condition are shown in figures 4.5-4.7. Cells were found in each delay condition that appeared to have had stable place fields between sample and test. Visual inspection also revealed some remapping of place fields in each condition. However, performing pixel-by-pixel correlations between each place field between sample and test should give an indication of whether there was similar stability (or remapping of fields) between the different delay conditions.

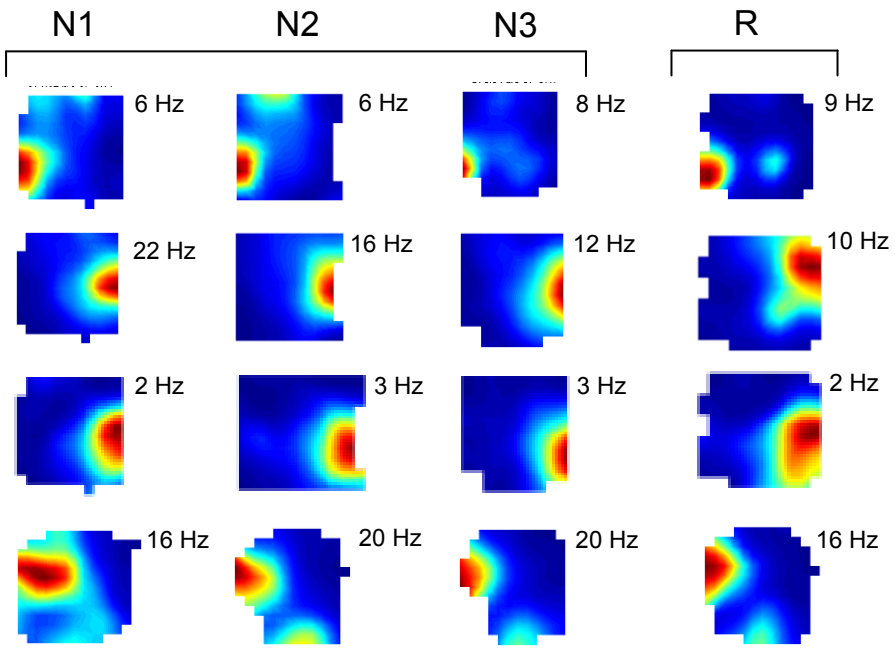

Figure 4.5 3 hour delay. Firing rate maps for the test sessions (R) are rotated such that the cues are aligned across all sessions. Peak firing rates are shown for cells in each session. Cells appeared to retain their previous firing location after the delay.

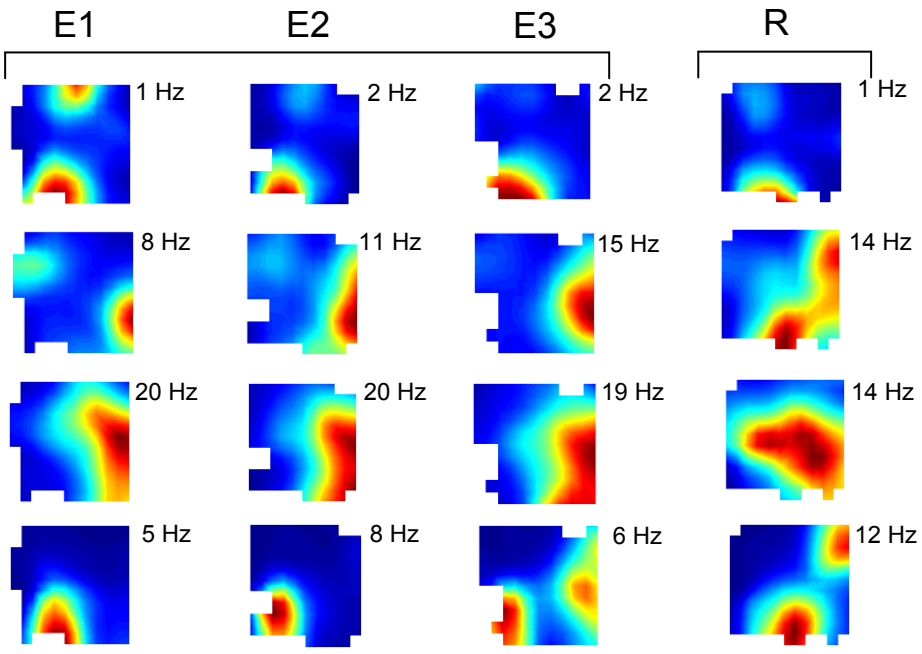

Figure 4.6 Six hour delay. Firing rate maps for the test sessions (R) are rotated such that the cues are aligned across all sessions. Peak firing rates are shown for cells in each session. Compared to 3 hour delay, cells appear to have lost some stability between initial exposures and test.

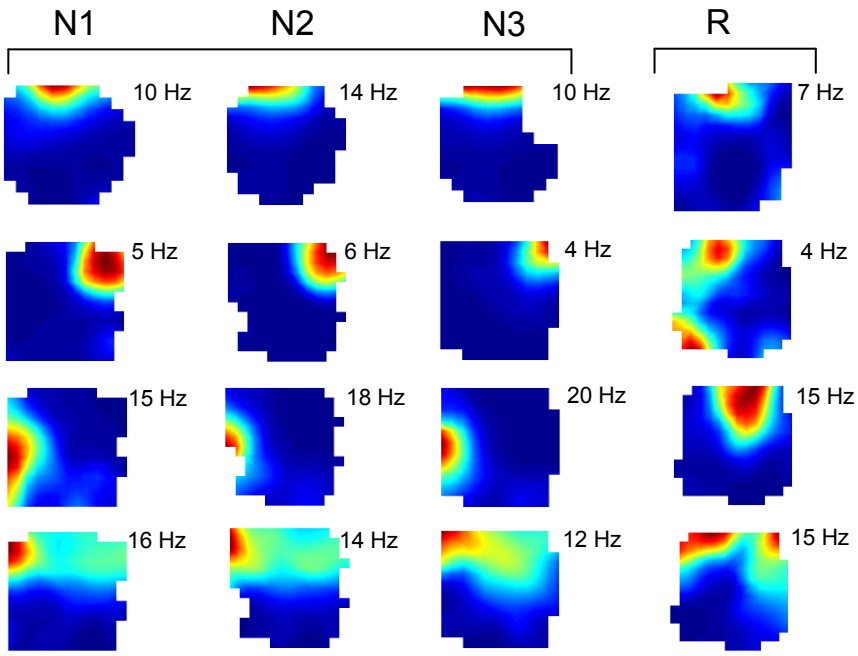

Figure 4.7 24 hour delay. Firing rate maps for the test sessions (R) are rotated such that the cues are aligned across all sessions. Peak firing rates are shown for cells in each session. Compared to the three hour and six hour delays, place fields lost some stability between initial exposures and test.

Comparisons of mean correlations between the three time delays are shown in Figure 4.8. One-way ANOVA revealed a significant main effect of time delay [$F(2,67) = 4.22$, $p = 0.02$]. Post hoc pairwise comparisons with Bonferroni corrections showed that correlations in the three hour delay were significantly ($p < 0.5$) larger than those in the 24 hour delay. There was no significant difference between six and 24 hour delay periods.

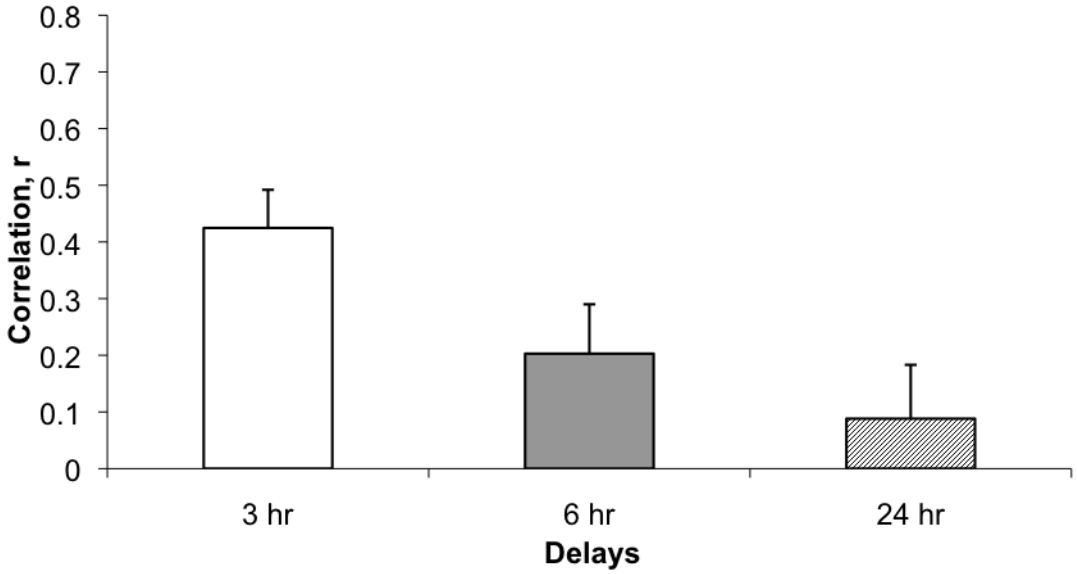

Figure 4.8 Correlations between N3 and R (rotation test) for all place cells recorded. Cells are from 8 rats: 3 hour, 38 cells; 6 hour, 20 cells; 24 hour, 12 cells.

Spatial information content was calculated for the last exposure sessions (N3) and the test (R) in each time delay. There was no difference in spatial information content between the sessions N3 and R in the three hour condition [$F(1,74) = 0.13$, $p = 0.72$], the six hour condition [$F(1, 28) = 0.87$, $p = 0.36$] or the 24 hour condition [$F(1, 28) = 0.56$, $p = 0.46$].

Difference in spatial information content between N3 and R was also examined (Figure 4.9). There were no differences in spatial information content between these session in any of the time delay conditions [$F(2,67) = 1.09$, $p = 0.34$].

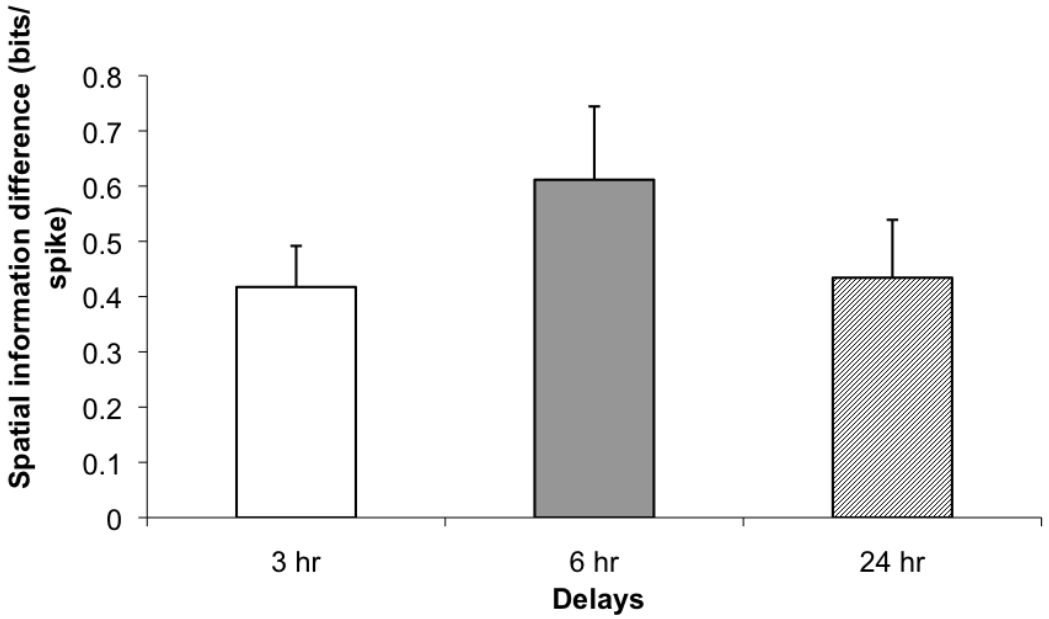

Figure 4.9 Spatial information content difference of place cells between the last exposure before the delay (N3) and the rotation session after the delay (R).

Correlations between N1-N2 (Figure 4.10) and N2-N3 (Figure 4.11) were calculated in all three time delay conditions as way of a control test. Since this was before the time delay and thus no experimental manipulation performed during these sessions in any, there should be no difference between the mean correlations between time delays. Indeed, this was the case for both N1-N2 [$F(2,44) = 0.76$, $p = 0.93$] and N2-N3 [$F(2,47) = 0.31$, $p = 0.74$].

Spatial information content was calculated for the initial exposures to the novel environments (N1, N2 and N3) for each time delay. The means for the 3 hour delay were: N1 = 0.82, N2 = 0.85, N3 = 0.94. The means for the 6 hour delay were: N1 = 0.84, N2 = 0.86, N3 = 0.80. The means for the 24 hour delay were: N1 = 1.03, N2 = 1.17, N3 = 0.80. There was no significant differences between the spatial information content of each session in any of the time delays [3 hr: $F(2,91) = 0.69$, $p = 0.5$; 6 hr: $F(2,41) = 0.15$, $p = 0.86$; 24 hr: $F(2,26) = 1.13$, $p = 0.32$]. Again, this

indicates that there was no difference in the intital exposures between any of the delay conditions.

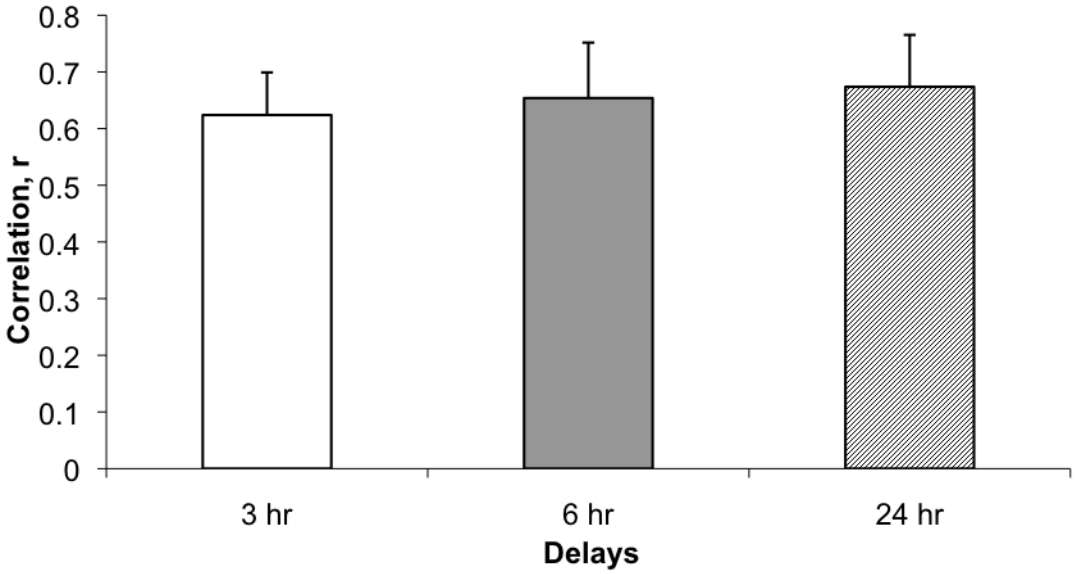

Figure 4.10 Correlations between sessions the first two exposures to the novel environment, N1 and N2, for all place cells recorded. Cells are from 8 rats: 3 hour, 26 cells; 6 hour, 12 cells; 24 hour, 9 cells.

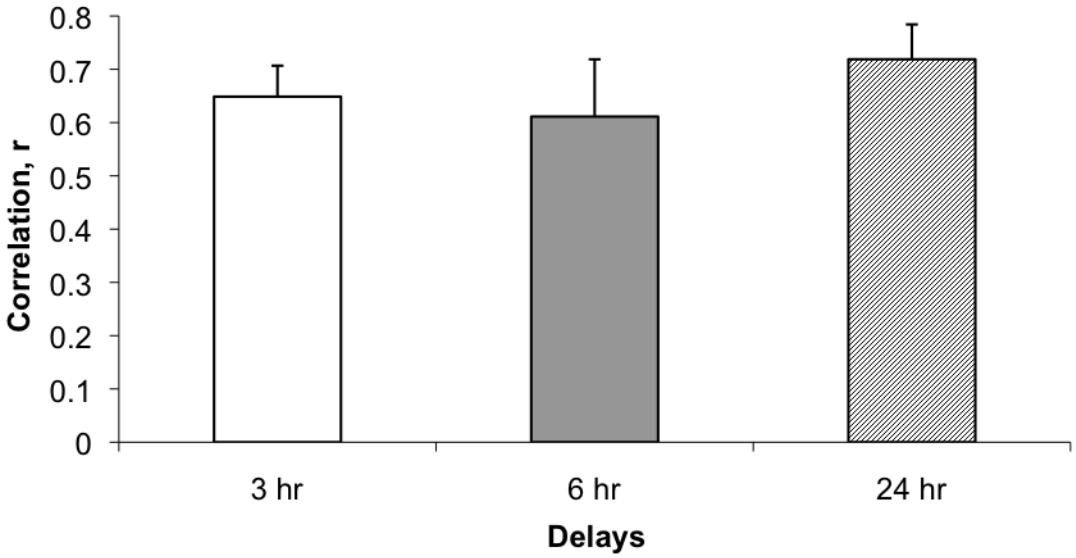

Figure 4.11 Correlations between sessions exposures two and exposure three to the novel environment, N2 and N3, for all place cells recorded. Cells are from 8 rats: 3 hour, 29 cells; 6 hour, 12 cells; 24 hour, 8 cells.

4.4 Discussion

This experiment tested place field stability between exposures to a novel cue-rich environment and a test exposure following a time delay of three, six or 24 hours. The test session involved a clockwise or anti-clockwise rotation of the cue constellation by 90°. The main results was that place fields from rats tested in the three hour delay condition were significantly more stable, as measured by pixel-by-pixel correlation firing rate maps, than the same rats in the 24 hour delay condition. Although the mean correlations were higher in the six hour condition compared to the 24 hour condition, this was not statistically significant.

These results indicate that there is a clear delay-dependent effect of place cell *memory* for novel environments. There were no differences between the place field correlations during the initial exposures to environments in any of the delay

conditions, suggesting that there was no initial bias before the test sessions. In addition, there were not any differences in the mean spatial information content between the place cells in N3 and R (Contestabile and Flumerfelt) at any time delay, or absolute spatial information difference, suggesting a uniformity of place cell quality.

The information is potentially valuable for experimental designs involving place field stability in novel environments. The experiments in the following chapter of this thesis, Chapter 5, rely on the results provided here. In the next chapter, a time delay is required between initial exposure to novel environments and a later test session. Although not statistically significant, the results here indicate that a six hour delay led to an increase in mean place field correlation, compared with a 24 hour delay. Figure 4.8 shows a monotonic trend in the mean correlation values from three hour through to 24 hour. One reason contributing to the fact that the result between these two time delays are not significant may be that there were fewer cells recorded in the 24 hour condition.

The time period of the initial exposures (3 x 10 min) was close to the 32 minutes that Muller and Kubie (1987) suggested as the optimal time for a place field to fully form. This suggests that more exposure time would not necessarily increase the place cell stability. However, perhaps stability over long delays could be improved upon by varying the geometry of the environment. Studies by Golob et al. (2001) and Breese et al. (1989) indicate that rats may not pay as much attention to visual cues in a square shaped environment, perhaps due to the four-fold symmetry. This explanation may help account for the relatively low 24 hour stability compared to that in Kentros et al. (1998). In the Kentros study, a cylinder with one polarising cue card was used

and mean between-session stability over 24 hours was r = 0.565 (Rats in saline condition; D1G1-D2G2 comparison).

Because the test sessions involved cue rotations, the results indicate that the cues were successful in exerting control over the place fields, and more so with shorter time delays. This is in line with previous studies that have demonstrated cue control in place cells with cue rotations (Muller and Kubie, 1987; Cressant et al., 1997; 1999).

In summary, in this chapter, delay time between exposure to a novel cue-rich environment and a later test exposure was systematically manipulated. Delays of three hours led to significantly greater place field correlations than delays of 24 hours.

Chapter 5: The role of the postsubiculum in novel environments: effect of inactivation on CA1 place fields

5.1 Introduction

When rats are put into an environment in which there are salient cues, head direction cells and place cells become *anchored* to the cues (Hetherington and Shapiro, 1997; Taube et al., 1990). This means that these cues have such control over the spatial firing of the cells that if the cues are moved, head direction and place cell firing will change in register with the cues. Specifically, place fields will rotate along with cue rotation, whereas the preferred firing direction of head direction cells will change by the same magnitude as the cue rotation. In addition to head direction and place cells, grid cells in the entorhinal cortex also shift their firing patterns with environmental landmark rotations (Hafting et al., 2005). Although rats can use different sensory modalities for this association of landmark with internal representation (e.g. Save et al., 1998; Hill and Best, 1981), visual landmarks in particular have strong control over spatially modulated cells (Muller and Kubie, 1987; Goodridge and Taube, 1995; Dudchenko and Zinyuk, 2005).

Although the head direction and place cell systems respond to the same stimuli, the relationship between them is not fully understood. Information from the head direction system is thought to project to, and be utilized by, the hippocampus. However, hippocampal place cells are still present after lesions of the mammillothalamic tract, which abolish the head direction signal upstream (Sharp and Koester, 2008). One possible role of the head direction system may be to provide

directional information to the hippocampus. Indeed, there is evidence that supports this idea.

Stackman and Taube (1997) showed that lesions to the vestibular system result in the loss of directional firing of head direction cells in the ADN. Similarly, inactivation of the vestibular system via tetrodotoxin injection led to CA1 place field degradation and loss of directional firing in postsubicular head direction cells (Stackman et al., 2002). This suggests that head direction information is required by the hippocampus for normal place cell function. In a study by Yoganarishima and Knierim (2005), head direction cells (ADN) and CA1 place cells were recorded simultaneously in a cylindrical environment containing a cue card. When the cue card was rotated between sessions, place fields tended to rotate and head direction cells tended to shift their preferred firing directions. Importantly, this phenomenon happened within the same rat, demonstrating a relationship between head direction cell system and the place cell system (Knierim et al., 1998, Yoganarasimha and Knierim, 2005). Although there is good evidence that the head direction and place cell systems can be strongly influenced by visual landmarks, it in not clear where this association of visual landmarks and internal representation occurs in the brain.

A study by Goodridge and Taube (1997) investigated the effects of ADN lesions on the firing of head direction cells in the postsubiculum, and conversely, the effects of postsubiculum lesions on head direction cells in the ADN. They recorded the lesioned rats in a cylindrical environment with a single polarising cue card and then rotated by 90º the cue card between sessions whilst the rat was removed from the recording arena and disoriented. No postsubiculur head direction cells were found in the rats with lesions to the ADN. Head direction cells were found in the ADN, although they were not as discriminative of the rats' head direction compared to

control animals; that is, the firing ranges of the preferred directions increased. In addition, the authors found that the preferred firing direction of the ADN head direction cells did not change in register with the cue card rotations, but shifted unpredictably. The preferred firing direction also shifted unpredictably between sessions in which the cue card remained in the same position. This is indicative that the postsubiculum plays some role in the forming associations of visual landmarks with the rat's internal representation of the environment.

The fact that no head direction cells were recorded in the postsubiculum is strongly suggestive that the head direction information normally arriving in the postsubiculum comes for the ADN and not the LDN, although both structures project to the postsubiculum. This premise was supported by work from the same laboratory that investigated postsubicular head direction cells following LDN lesions (Golob et al., 1998). They reported no effects of lesions on the properties of the postsubicular cells recorded.

Another study from the same laboratory (Calton et al., 2003) investigated effects of either ADN or postsubiculum lesions (as in Goodridge and Taube, 1997) but this time on CA1 place cells. After lesions (both ADN and PoS groups), place cells were present in a circular cylinder with one cue card. However, when the cue card was rotated in between sessions, place cells from the rats with postsubiculum lesions did not rotate with the cue card but rotated unpredictably. This further supports the role of the postsubiculum in the association of visual landmark with internal representation for environments. It is not clear how the head direction system contributes to place cell firing. One function that the signal from the postsubiculum may have is to associate visual landmarks with internal representation of environment.

The association of place cell firing with novel environments is disrupted by systemic injections of an NMDAR antagonist (Kentros et al., 1998). In this study, rats were injected with CPP before being introduced briefly (16 min) to a novel cylindrical environment containing a polarising cue card. Hippocampal (CA1) place cells were recorded after 1.5 hours and then again at 24 hours. Place fields were relatively stable between the first exposure and the exposure after 1.5 hours but not between either of these two sessions and the session at 24 hours. This strongly suggests that the long-term association of environmental features with the rat's internal representation takes place between 1.5 hours and 24 hours since there was no impairment up to around 1.5 hours. Additionally, the study suggests that long-term place cell *memory* for environments is NMDAR-dependent and that these receptors are required when the rat is in the environment (at the time of encoding).

Based on the evidence described above, the aims of the current study were to determine whether the postsubiculum is necessary for the association of visual landmarks with internal representation of location encoded by place cells. Specifically, one hypothesis of this study is that AMPAR-dependent neural transmission in the postsubiculum is necessary for the stability of place fields in novel environments. A second hypothesis is that NMDAR-dependent plasticity in the postsubiculum is necessary during exposure to novel environments for the long-term stability of the firing location of CA1 place fields. This is based on the presumption that NMDARs will facilitate the induction of LTP and that late-LTP is required for long-term place cell *memory*. Late-LTP occurs somewhere after two to three hours after induction (Sweatt, 1999) and involves gene transcription and plasticity proteins (Malenka and Bear, 2004).

In this current study, CA1 place cells were recorded in *novel* cylindrical environments with a single polarising cue card following bilateral infusion of the AMPAR antagonist CNQX, the NMDAR antagonist D-AP5, or ACSF. Rats were exposed to the same environment six hours later and place cells recorded for comparisons of place fields between sessions.

In this study a time period of six hours was used between the initial sampling of the novel environment and the stability test. This time period was chosen as it put the test of stability firmly in the time frame expected for late-LTP. A 24 hour time period as used by Kentros et al. (1998) was not used in this study due to preliminary results from the study described in Chapter 4 of this thesis, in which correlations over this time period were relatively very poor compared to shorter (3 and 6 hour) delays.

Inactivation of the postsubiculum at the time of encoding the novel environment should lead to reduced place field stability across the six hour delay. Specifically, if information from the head direction system is required to pass through the postsubiculum on route to the hippocampus, CNQX inactivation via AMPARs should result in reduced stability across the delay and between the sessions whilst under drug prior to the delay. If NMDAR-dependent plasticity is required in the postsubiculum to associate landmarks with internal representation of location by place cells, then AP5 inactivation should result in reduced place field stability across the delay only.

5.2 Methods

5.2.1 Overview

In this study, rats were implanted with cannulae in the postsubiculum for drug delivery. The same rats were also implanted with recording electrodes on a microdrive. CA1 place cells were recorded when the rats were exposed to novel environments whilst under the influence of AP5, CNQX, or ACSF. The same place cells were recorded six hours later in the same environment. If the postsubiculum is necessary for the association between landmarks and place field location, then inactivation should result in reduced stability between initial exposures and the post delay test sessions. Experiments were run in normal lighted conditions for all rats and also under darkness for two rats.

5.2.2 Subjects

Twelve male adult Lister-Hooded rats (Charles River Laboratories, UK) were implanted with electrodes and cannulae, however, data was only obtained from five of these. Rats were housed in individual cages and kept on a 12:12 light-dark cycle. All experiments took place during their light phase. The rats weighed between 350 and 400 g at the beginning of the experiment. During the experiment all rats had unrestricted access to water but were food restricted to around 85-90% of their *ad libitum* bodyweight. All procedures were compliant with the UK Animals Scientific Procedures Act, (1986) and with the [European Communities Council Directive of 24 November 1986 (86/609/EEC)] legislation governing the maintenance of laboratory animals and their use in scientific experiments.

5.2.3 Electrode preparation

The electrodes used were described in the previous chapter (section 4.2.3). Each microdrive contained four tetrodes.

Figure 5.1 Headcap from rat showing the 2 dummy cannulae and the recording microdrive.

5.2.4 Surgery

Rats were anaesthetised using 5% isoflurane (Abbott Laboratories Ltd., UK) and their heads shaved then disinfected with clorohexidine scrub prior to surgery. Rats were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and held in place using ear bars throughout the procedure. Isoflurane anesthesia was maintained throughout. A midline incision was made under sterile conditions and the skull exposed fully to the lateral ridges.

Twelve rats were implanted with bilateral cannulae into the postsubiculum and also a four-tetrode microdrive. Two small holes were drilled through the skull bilaterally over the postsubiculum for guide cannulae (Coordinates: AP 7.5 mm, ML 5 mm, DV 3 mm). Another 2 smaller holes were partially drilled through the skull in nearby locations for small stainless steel skull screws (Fine Science Tools GmbH, Heidelberg, Germany), which were screwed into the skull until stable. These screws were not intended to penetrate the brain. The dura was then punctured using a narrow gauge needle and a guide cannula (Plastics One, Bilaney, UK) was lowered through each of these holes into the brain, aimed at the postsubiculum. The guide cannulae

had dummy cannulae inserted at the time of implantation. The guide cannulae were cemented in place using dental cement (simplex Rapid Acrylic Denture Polymer) and the small screws acted as anchors for the cement. Vaseline was applied around the skull holes before cementing, to stop any cement getting into the brain.

Next, four-tetrode microdrives were fitted with tetrodes aimed at CA1, using the procedure detailed in section 4.2.4, and the rats given 14 days recovery period before screening for cells began.

5.2.5 Drugs and microinfusions

5.2.5.1 Drugs

The drugs used in this experiment were the competitive NMDA receptor antagonist AP5 [D-(-)-2-Amino-5-phosphonopentanoic acid, Tocris, UK] and the competitive AMPA/kianate receptor antagonist CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione, Tocris, UK). Phosphate-buffered artificial CSF was used as a vehicle and for as a solvent for the 2 aforementioned drugs. The concentrations of the AP5 and CNQX used were 30 mM (5.9 mg/ml) and 3 mM (0.89 mg/ml), respectively. Drug solutions were made in advance and divided into 50 μl aliquots which were frozen at -20°C; one aliquot of the drug(s) required was thawed and then spun in a vortex machine for up to 60 s on the day of the drug infusion sessions before use.

Drug concentrations and volumes used in this study were similar to those used by Bast et al. (2005), who infused the same drugs into hippocampus. The experimental protocol was also designed with the results from this study in mind. Specifically, Bast et al. found that, in anaesthetised rats intra-hippocampal infusions of CNQX led to impaired transmission at perforant path synapses onto dentate gyrus

granular cells for a period of around one hour. With this in mind, the experiments in this study were designed not to last more than 60 min after drug infusions.

5.2.5.2 Mock infusions

Each rat was subjected to a mock infusion prior to the drug infusion sessions. This was to habituate the rat to any stress involved in the infusion procedure. For all infusions, including mock infusions, a microinfusion pump was used. A motorised block on this pump drove two syringes containing either drug or control substance; however, for the mock infusions, the block was positioned such that the syringes were not driven. During mock infusions, rats were restrained by the experimenter and constrained a towel on the experimenter's lap. The experimenter removed both dummy cannulae and inserted an infusion cannula (33 gauge; C315; Plastics One, Bilaney, UK) into each guide cannula, which extended into the brain. Each injector was connected to the two syringes on the pump via a length of flexible plastic tubing. The pump was then turned on for five minutes (but nothing was actually pumped) and then a further two minutes were allowed to simulate the timings used in the actual drug infusions. Then, the injector cannulae were removed and the dummies replaced after being immersed in absolute alcohol and rinsed thoroughly with saline. The injector tips were similarly cleaned before and after each infusion.

5.2.5.3 Drug infusions

Drug infusions were carried out similarly to mock infusions. However, when the pump was turned on, the drug was pumped into the brain at a rate of 0.2 μL/min, such that, over five minutes, 1 μL was infused into each hemisphere of the brain. The injectors were left in place for a further two min to maximise the volume of drug

entering the brain by allowing time for all droplets of solution to enter the brain and limiting any back flow of solution through the guide cannulae.

5.2.6. Apparatus

Cylinders used for environments. In this experiment, it was necessary to have a number of different cylindrical environments that could be used on different sessions as novel environments for a given rat. Six such cylinders were used; three of these were large plastic plant pots (diameter 62 cm, height 45 cm) of varying colours and three were custom made using a wooden base with either bendy MDF (diameter 77 cm, height 59 cm) or cardboard for the walls (diameter 75 cm, height 38 cm; diameter 59 cm, height 59 cm). Each rat was assigned one cylinder as a familiar environment and the others used as novel environments**.** Cue cards were of varying colours and patterns. Within a given experiment, the cue cards in the familiar and novel cylinders had different orientations relative to the experiment room. Familiar cylinder cue cards remained in the same orientation throughout.

Cue cards and cue sets. Experiments were run either in normal lighted conditions (n=5) or under darkness (n=2). For the lighted experiments, a single cue card was used. During the dark experiments, no cue card was used, but three objects were attached to the bottom of the cylinder, around the periphery. A small red LED was used by the experimenter for illumination whilst putting the rat into the cylinder and removing him at the end of each session.

Rats were only exposed to each cylinder once (not including their familiar cylinder) during the experiments under normal light and the cylinders used for each drug condition were varied unsystematically across rats and drug condition. The same set of cylinders were used for the experiments under darkness, but with the cue cards removed and objects introduced. Distances between the individual objects in the cue

sets were also varied between the different cue sets to maximise the novelty of each set.

5.2.7 Electrophysiology

5.2.7.1 Recording apparatus

The recording apparatus was described in the previous chapter in section 4.2.5.1.

5.2.7.2 Screening for cells

Screening for cells typically took place twice daily, once in the morning and once in the late afternoon. This was described in the previous chapter in section 4.2.5.2. When a well-isolated cell, or cells, was found in a rat, the experiments began with the rat.

5.2.8 Behavioural training

Prior to surgery, rats were handled for five minutes daily for three or four days and were habituated to eating Weetos (Weetabix, UK) on the experimenter's lap. They were trained to eat facing away from the experimenter since this was the ideal position used post-surgery when plugging the recording cable into their microdrives. After surgery, the rats were trained to fetch food pellets (Bioserve, US) thrown by the experimenter on an open table-top maze whilst being screened for cells. During the recording experiments, the experimenters threw food pellets into various positions within the cylinders to encourage the rats to explore as much as possible whilst connected to the unit recording apparatus. This was so that there was adequate sampling of the environment such that recordings of place fields were reliable.

5.2.8.1 Procedure

Each rat was assigned one cylinder as a familiar environment. This was intended as a control environment for testing place field stability. The rat was exposed to its familiar cylinder prior to testing in novel environments. On the first day, each rat was exposed for the first time to its *familiar* environment for three 10 minute sessions whilst place cells were recorded, and then, re-introduced to the same environment after six hours. This was designed to give each rat time to become familiar with its environment, and also to determine that place fields were stable across the six hour gap between sessions.

The next step was the mock infusion session. Briefly, each rat was connected to the recording system and given three 10 minute sessions in its familiar environment before being disconnected and carried in a covered bucket to the infusion room. The mock infusion procedure is described above in section 5.2.5.2. Following this, place cells were recorded for another three 10 minute sessions. The infusion experimental sessions began on the next testing day for each rat.

Infusion experiment procedure

The experimental protocol is outlined in figure 5.2. The rat being tested was taken from its cage in the control room and placed into a high walled bucket with some animal bedding in the bottom. The bucket was covered with a white t-shirt and the rat was transported inside to the curtained environment in the adjacent recording room. Here, the rat was connected to the recording system and given five minutes inside the transport bucket. This time allowed the experimenter to set references and gains on each recording channel. Then, the rat was given three 10 minute sessions of pellet chasing within its familiar cylinder, each separated with a five minute period inside the bucket. Then, the rat was unplugged from the recording system and

transported inside the covered bucket to another room for the drug microinfusion (described above). The rat was then taken back to the recording room inside the bucket and plugged in, followed by one session in its familiar environment. The experimenter then swapped the familiar cylinder for a novel cylinder and the rat was given three 10 minute pellet chasing sessions, interspersed with one three minute, and two one-minute bucket sessions, respectively. The rat was then returned to its home cage for six hours before being plugged in once again and given two sessions in the novel cylinder, followed by one in the familiar, each interspersed with five minute bucket sessions.

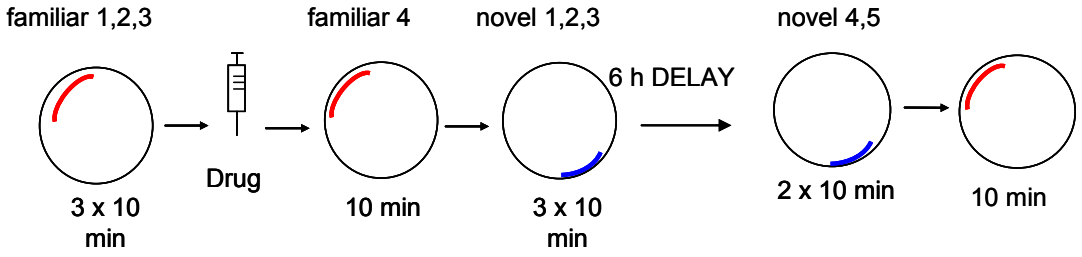

Figure 5.2. Experimental design. Rats received infusions of 0.2µL of the NMDA receptor antagonist AP5 (30mM), the AMPA receptor antagonist CNQX (4mM), or ACSF in the postsubiculum prior to entering the novel environment for the first time. Drugs and environments were counterbalanced across rats.

5.2.9 Perfusion and histology

At the end of the experiment, rats were terminally anaesthetised with an overdose of sodium pentobarbital (Euthatal, Merial Animal Health, UK) and perfused transcardially with 0.9% saline and then 4% formalin. The brains were removed and stored in 4% formalin for at least 24 hours before being egg embedded (see section 3.2.6 for details). They were then cut into 30 µm coronal sections with a cryostat. Approximately one in 3 sections were mounted on to gelatine coated slides and stained in cresyl violet solution.

5.2.10 Data analysis

5.2.10.1 Single unit islolation

Unit recording data was stored on a computer hard disk and analysed after the experiment or screening session was complete. Position and time information for recorded spikes were processed using a combination of Matlab scripts written by Dr. Steven Huang (with modifications by Michael T. Smith, University of Edinburgh) and free open source programs available online. Multiple session data was merged together using a Matlab script for the purposes of clustering waveforms of single units. This meant that clustering was uniform across all sessions. Two features only were used, energy and first principle component, so that data dimensions were minimised. Clustering followed two steps, (i) automatic clustering using Klustakwik 1.5 (http://klustakwik.sourceforge.net/, written by Harris. K) and then (ii) manual clustering using Klusters (L. Hazan, Buzsaki lab, Rutgers, Newark NJ, klusters.sourceforge.net). Manual clustering involved inspecting the automatically clustered waveforms and the auto- and cross-correlograms of these clusters. Other parameters were also looked at, including amplitude of waveforms, spike width, and time.

5.2.10.2 Firing rate maps

Firing rates were generated as detailed in Section 4.2.5.3.

5.2.10.3 Place cell inclusion criteria

The criteria for place cell inclusion were identical to that described in Section 4.2.5.4.

5.2.10.4 Correlations

For each cell that satisfied the above criteria, correlations were carried out between place fields in one session and the corresponding place field in another session. This was to check the stability of the place field over time. To do this, the firing rate for each pixel was calculated and paired with the firing rate of the corresponding pixel from another session. Then a single correlation coefficient was calculated from these data.

5.3 Results

5.3.1 Histology

Data was collected from five rats (two of the five were tested under normal illumination and dark conditions). Cannulae positions were verified as being in the postsubiculum in all rats. Figure 5.3 shows a representative section demonstrating this.

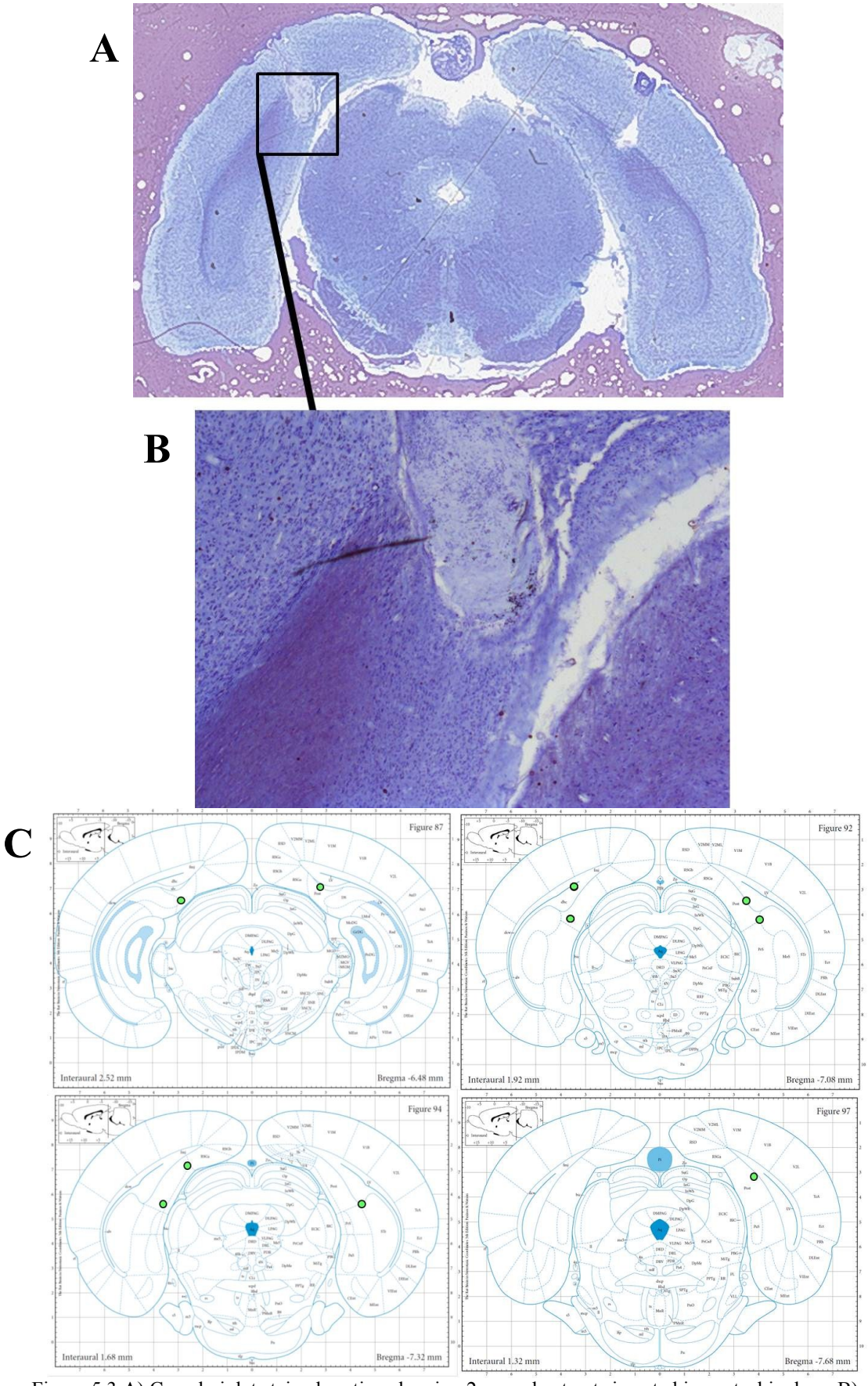

Figure 5.3 A) Cresyl violet stained section showing 2 cannulae tracts inserted in postsubiculum. B) A higher resolution image of a cannula tract entering the postsubiculum. C) Schematic of the cannulae placement in all of the five rats used for data, showing that each enters the postsubiculum.

5.3.2 Experiments in the light

Place fields from cells in five rats were used for comparisons. All rats had one experiment using ACSF and two experiments with either drug, D-AP5 or CNQX, except for one rat that had only one experiment in each drug condition. No attempt was made to identify the same cells over multiple days. The main comparison used was between sessions N3 and N5. N3 was the last session whilst the rats were under drug and N5 was the second novel session after the six hour delay.

5.3.2.1 Comparisons of place fields over the six hour delay

Place fields were relatively stable in all three drug conditions from N3-N5. Figure 5.4 shows representative place field firing maps from cells in N3 (left) and N5 (right). In each drug condition, there were cells that look stable across the delay and there were cells that remap.

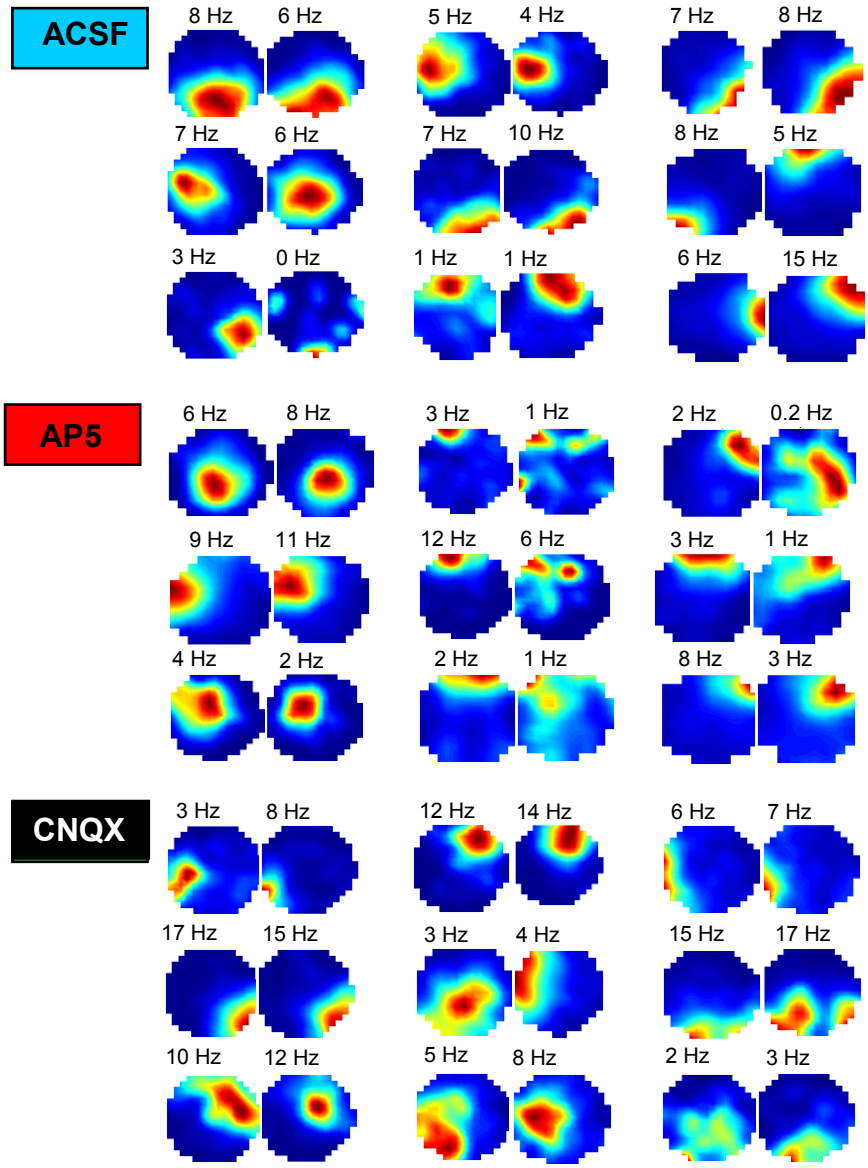

Figure 5.4 Representative pairs of place cell firing maps for each drug condition across the six hour delay period. Maps on the left of each pair represent the session N3 (under drug) and the maps on the right of each pair represent session N5 (post delay).

Correlations of firing rate maps between sessions N3 and N5 were similar for each drug condition (Figure 5.5). Place fields were as stable for CNQX and AP5 conditions as they were for the ACSF condition [$F(2, 61) = 0.064$, $p = 0.938$].

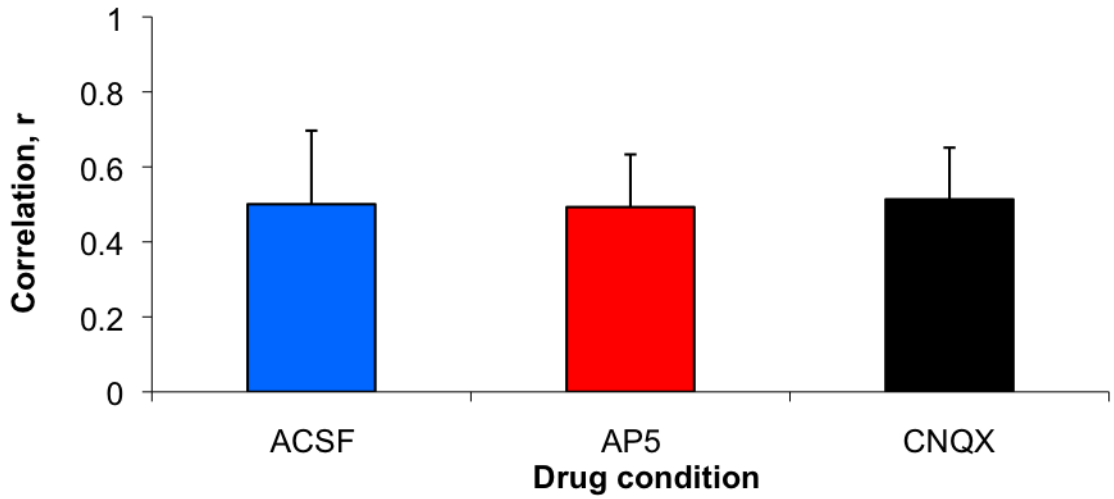

Figure 5.5 Correlation coefficients for place fields in sessions N3 and N5 across the six hr delay for ACSF, AP5 and CNQX conditions. Cells included in comparisons from 5 rats: ACSF 12, AP5 25, CNQX 27.

Spatial information content for the last novel session (N3) under drug and the two novel sessions after the six hour delay (N4 and N5) was calculated. There was a significant main effect of session for spatial information in the ACSF drug condition [$F(2,31) = 3.362$, $p = 0.048$]; however, pairwise comparisons with Bonferroni corrections for multiple comparisons were not significant. In the CNQX condition, there was a significant main effect of session [$F(2,65) = 4.033$, $p = 0.02$]. Pairwise comparisons using Bonferroni correction for multiple comparisons revealed that spatial information content was higher in N5 compared to N4 ($p = 0.02$). In the AP5 condition, there were no significant differences between spatial information content means over these three sessions [$F(2,68) = 0.176$, $p = 0.839$].

Figure 5.6 shows the mean difference in spatial information for all cells included in the correlation analysis above between the last novel session N3 under drug and the second novel session after the six hour delay (N5). There was a significant main effect of drug condition [$F(2,55) = 4.357$, $p = 0.018$]. Pairwise comparisons with Games-Howell correction for unequal variances revealed that spatial information content difference was significantly larger during the CNQX

condition compared to the AP5 condition ($p = 0.005$) but not compared to the ACSF condition ($p = 0.329$).

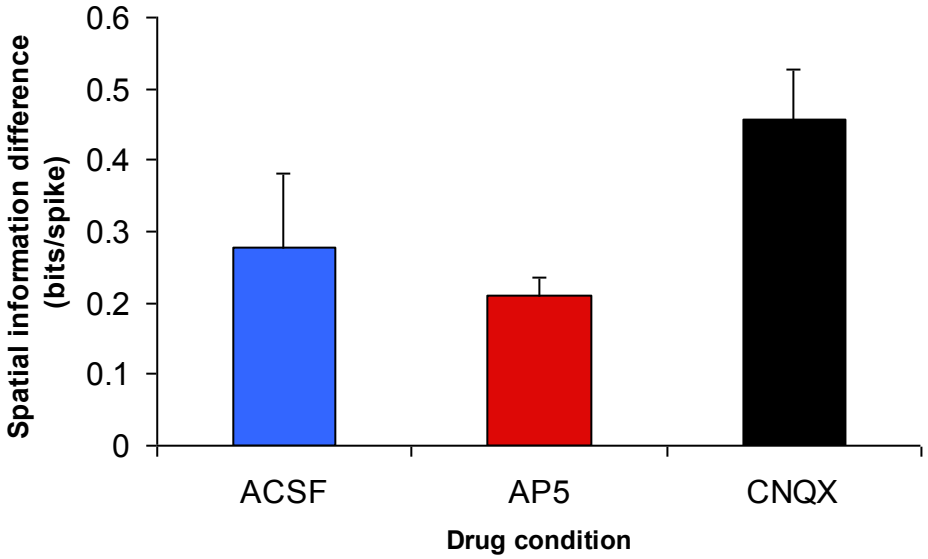

Figure 5.6 Mean difference in spatial information (bits/spike) between place fields in sessions N3 and N5 in all three drug conditions.

Familiar environment stability was assessed across the six hour delay. Figure 5.7 shows the correlations of place fields between the last familiar session before the infusion and the second familiar session after the six hour delay. There is a trend for decreasing correlation coefficients from ACSF to AP5 to CNQX, although any differences did not reach statistical significance [$(F(2,66) = 1.103, p = 0.338$].

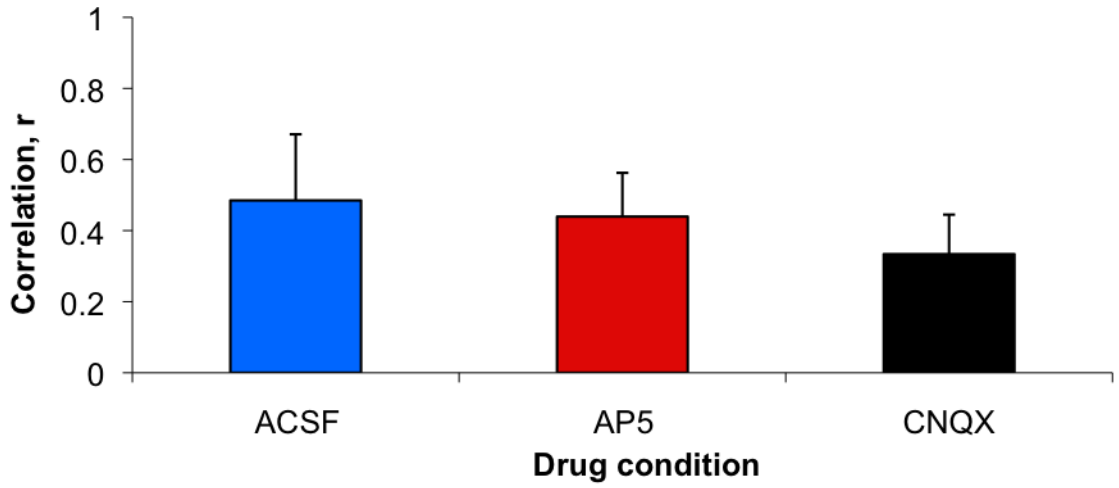

Figure 5.7 Correlation coefficients for place fields in sessions F3 and F5 across the six hour delay for ACSF, AP5 and CNQX conditions. Cells included in comparisons from 5 rats: ACSF 14, AP5 28, CNQX 27.

Spatial information content was calculated for place fields in the familiar environment sessions over the drug infusions (F3, F4) and over the six hour delay (F5) separately for each drug condition. In the ACSF drug condition, the means are similar for all sessions [($F(2,42) = 0.769$, $p = 0.47$]. In the CNQX drug condition, there was a significant main effect of session [($F(2,83) = 6.097$, $p = 0.003$]. Pairwise comparisons using Bonferroni correction for multiple comparisons revealed that the only significant difference in spatial information content was between sessions F4 and F5, and that F5 was higher ($p = 0.003$). In the AP5 drug condition, there were no differences in spatial information content means [($F(2,75) = 0.337$, $p = 0.715$].

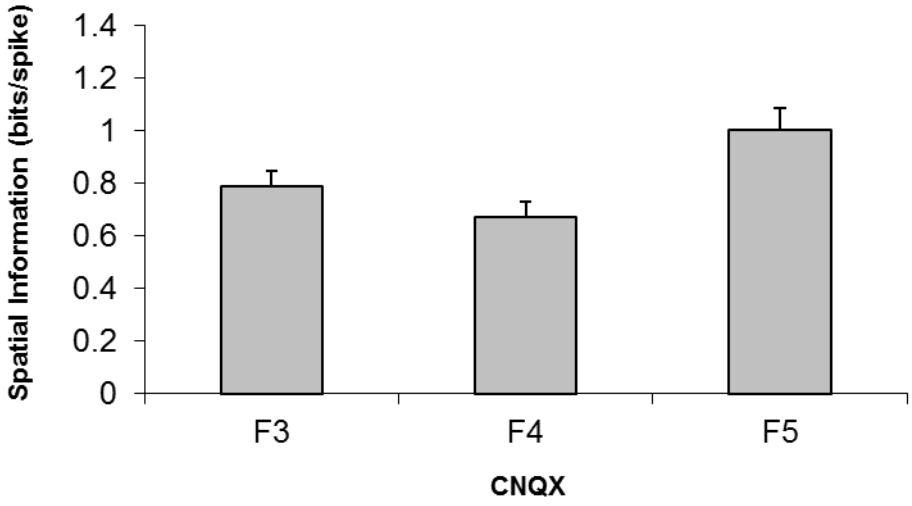

Figure 5.8 Mean spatial information (bits/spike) for place fields in sessions F3, F4 and F5 in the CNQX drug condition.

5.3.2.2 Comparisons of place fields in the three novel sessions under drug

Place fields were relatively stable in all three drug conditions from N1-N4. Figure 5.9 shows the correlation between place fields in sessions N1 and N2. Place field stability was similar in all drug conditions [$F(2,41) = 2.313$, $p = 0.112$]. Figure 5.10 shows the correlation between place fields in sessions N2 and N3. Again place field stability was similar in all drug conditions [$F(2,52) = 1.513$, $p = 0.23$].

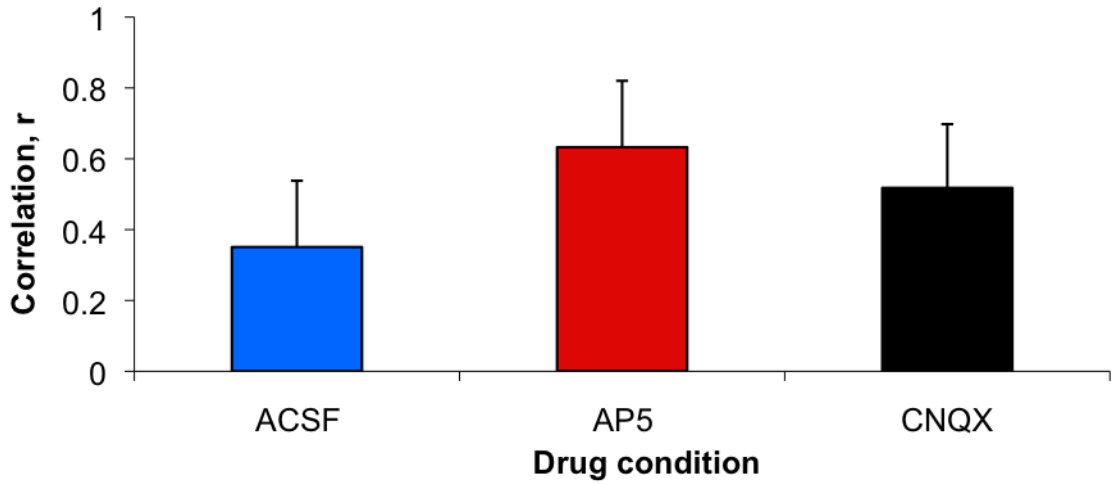

Figure 5.9 Correlation coefficients for place fields in sessions N1 and N2 immediately following drug infusion for ACSF, AP5 and CNQX conditions. Cells included in comparisons from 5 rats: ACSF 10, AP5 18, CNQX 16.

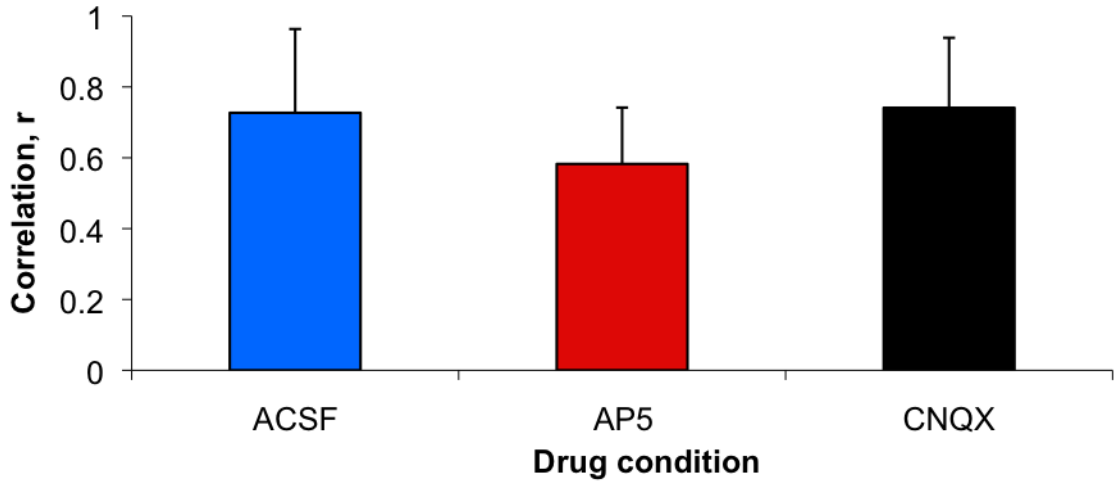

Figure 5.10 Correlation coefficients for place fields in sessions N2 and N3 following drug infusion for ACSF, AP5 and CNQX conditions. Cells included in comparisons from 5 rats: ACSF 13, AP5 23, CNQX 19.

Spatial information under drug was calculated for the novel sessions under drug (N1, N2 and N3). In all three drug conditions, spatial information content was similar [ACSF: $F(2,30) = 1.921$, $p = 0.164$; CNQX: $F(2,55) = 0.429$, $p = 0.653$; AP5: $F(2,64) = 0.342$, $p = 0.712$]. There were no differences between the same session under the different drug conditions either.

Spatial information difference between drug conditions and N1-N2 and N2-N3 is shown in figure 5.11. There were no differences between groups in the either N1-N2 [$F(2,40) = 0.724$, $p = 0.491$] or N2-N3 [$F(2,51) = 0.043$, $p = 0.958$].

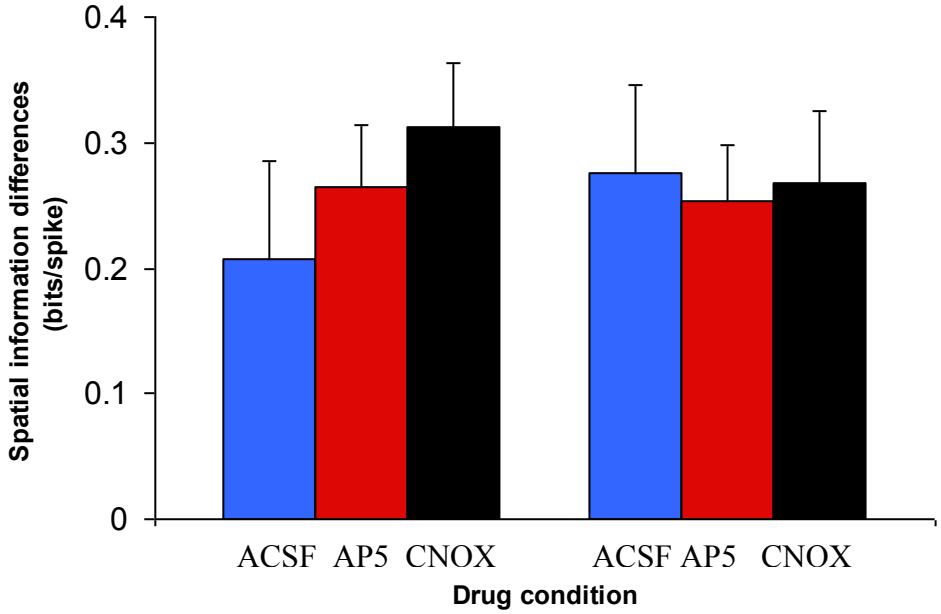

Figure 5.11 Mean difference in spatial information (bits/spike) between place fields from the same cell in sessions N1 and N2 (left), and also N2 and N3 (right), in all three drug conditions.

5.3.2.3 Comparisons of familiar environment place fields across the injection

Comparisons were made of the place fields recorded in the familiar session immediately before (F3) and the session immediately after (F4) the injection. Figure 5.12 shows the mean correlations between the place fields recorded in each drug condition.

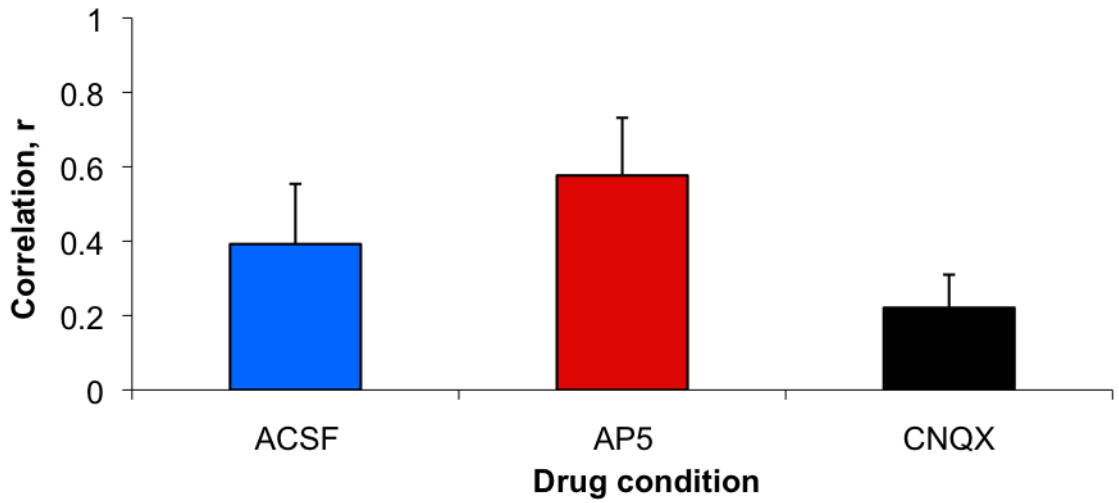

Figure 5.12 Correlation coefficients for place fields in familiar environments in the session prior to (F3) and immediately following (F4) infusion, between ACSF, AP5 and CNQX conditions. Cells included in comparisons from 5 rats: ACSF 15, AP5 23, CNQX 28.

There was a significant main effect of drug condition [($F(2,63) = 5.313$, $p = 0.007$)]. Pairwise comparisons with Bonferroni correction revealed that correlations in the CNQX condition were significantly lower than those in the AP5 condition ($p = 0.005$), but not different from ACSF.

5.3.3 Experiments in the dark

5.3.3.1 Comparisons of place fields over the six hour delay

Place cells were recorded in two rats in the darkness in all drug conditions. Correlations between place fields over the six hour delay are shown for all drug conditions in figure 5.13. There was no differences in mean correlation between the different drug groups [$F(2,27) = 1.12$, $p = 0.34$)].

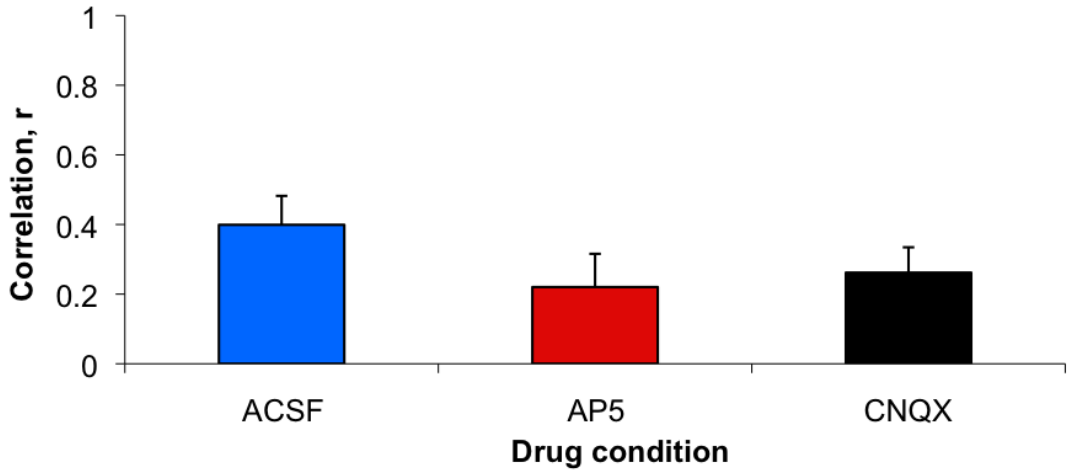

Figure 5.13 Correlation coefficients for place fields in sessions N3 and N5 across the six hr delay between ACSF, AP5 and CNQX conditions. Cells included in comparisons from 2 rats: ACSF 9, AP5 12, CNQX 9.

Spatial information content for the cells under drug were calculated in novel sessions N1, N2 and N3. In the ACSF condition there were no differences between spatial information between any of the sessions [$F(2,28 = 0.1$, $p = 0.91$], nor in the AP5 condition [$F(2,29) = 0.85$, $p = 0.44$]. In the CNQX condition, there was a trend for cells to increase over session from N3 to N5. This, however, failed to reach significance [$F(2,22) = 1.55$, $p = 0.24$].

Spatial information content differences between sessions N3 and N5 are shown in figure 5.14. The was no main effect of drug group [$F(2,26) = 1.14$, $p = 0.34$].

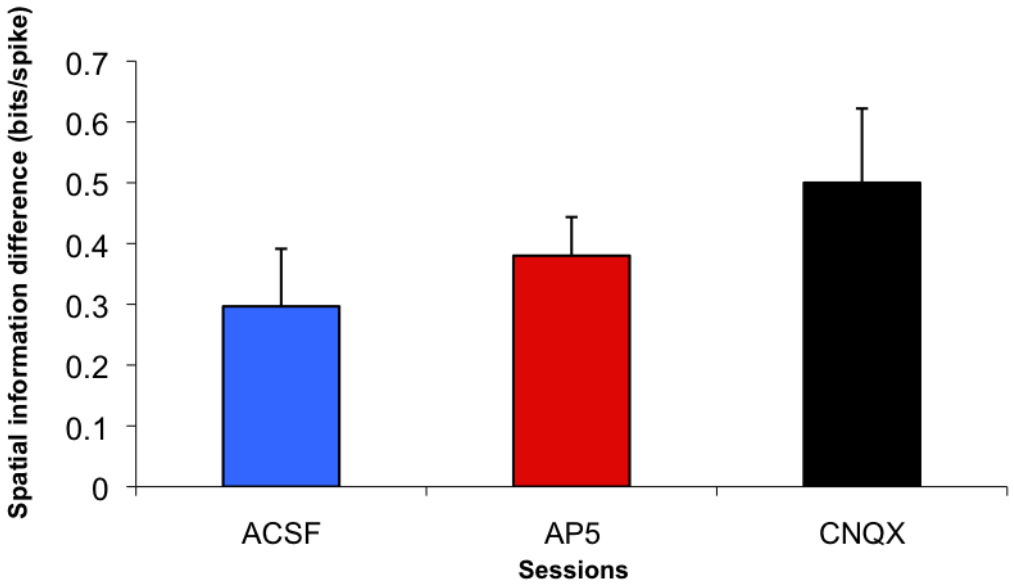

Figure 5.14 Difference in spatial information content difference of cells in the correlation of novel sessions N3 and N5 across the six hour delay in all drug conditions.

Correlations between place fields in the familiar sessions over the delay are shown on figure 5.15. There was no main effect of drug condition [$F(2, 26) = 1.14$, $p = 0.37$].

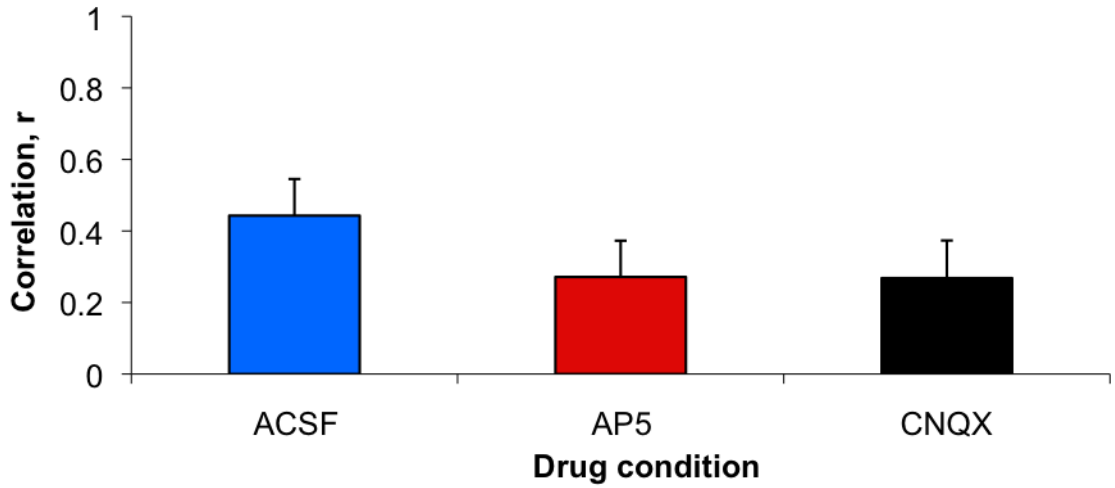

Figure 5.15 Correlation coefficients for place fields in sessions familiar environments in the session prior to infusion (F3) and after the six hr delay, between ACSF, AP5 and CNQX conditions. Cells included in comparisons from 2 rats: ACSF 12, AP5 11, CNQX 11.

Spatial information content from place cells in the familiar sessions F3, F4 and F5 was calculated for the three drug conditions. In the ACSF and AP5 conditions, there means were similar [ACSF: $F(2,33) = 0.74$, $p = 0.49$; AP5: $F(2,32) = 0.56$, $p = 0.58$]. However, in the CNQX condition, there was a significant main effect of session [$F(2, 18.14) = 4.121$, $p = 0.03$]. Pairwise comparisons with Games-Howell corrections for multiple comparisons reveal that session F3 has significantly higher spatial information content than F4 ($p = 0.03$).

Place field correlations in darkness between the first two novel sessions after infusion are shown in figure 5.16. There was no main effect of drug group [$F(2,12) = 3.31$, $p = 0.07$], perhaps failing to reach statistical significance because there were only 2 cells in the CNQX drug condition. Correlations of place fields in the next two sessions after the infusions, N2 and N3, are shown in figure 5.16. There was no main effect of drug condition between sessions N2-N3 [$F(2,15) = 3.48$, $p = 0.06$]. Once again, perhaps the reason that statistical significance was approached, but not reached was due to the small sample size in the CNQX group ($n = 3$).

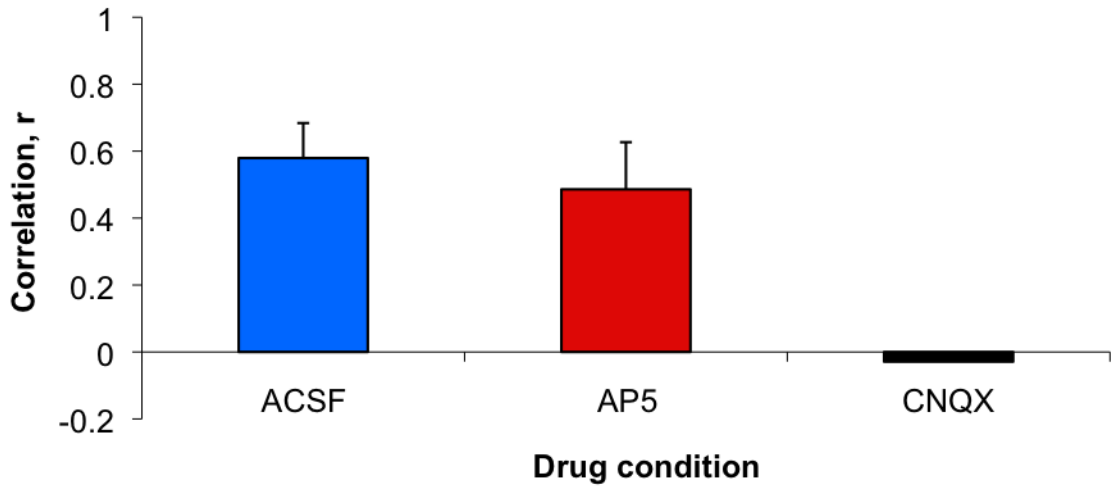

Figure 5.16 Correlation coefficients for place fields in sessions N1 and N2 immediately following drug infusion for ACSF, AP5 and CNQX conditions. Cells included in comparisons from 2 rats: ACSF 7, AP5 6, CNQX 2.

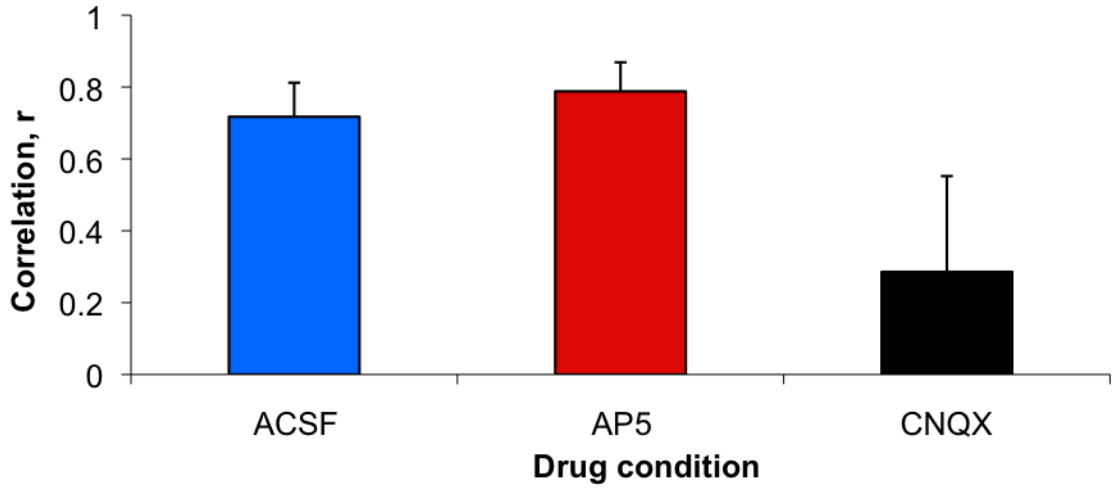

Figure 5.17 Correlation coefficients for place fields in sessions N2 and N3 immediately following drug infusion for ACSF, AP5 and CNQX conditions. Cells included in comparisons from 2 rats: ACSF 8, AP5 7, CNQX 3.

Spatial information content of place cells in the novel sessions (N1, N2 and N3) following infusions was calculated for each drug condition. There were no differences in the means in either condition [ACSF: $F(2, 23) = 1.55$, $p = 0.23$; CNQX: $F(2,11) = 0.46$, $p = 0.65$; AP5: $F(2, 20) = 0.50$, $p = 0.62$]

Spatial information difference was also calculated between N1 and N2 over the three drug conditions and for N2-N3 (Figure 5.18). There were no statistically significant differences in either case [N1-N2: $F(2,12) = 0.34$, $p = 0.72$; N2-N3: $F(2,15) = 0.73$, $p = 0.5$].

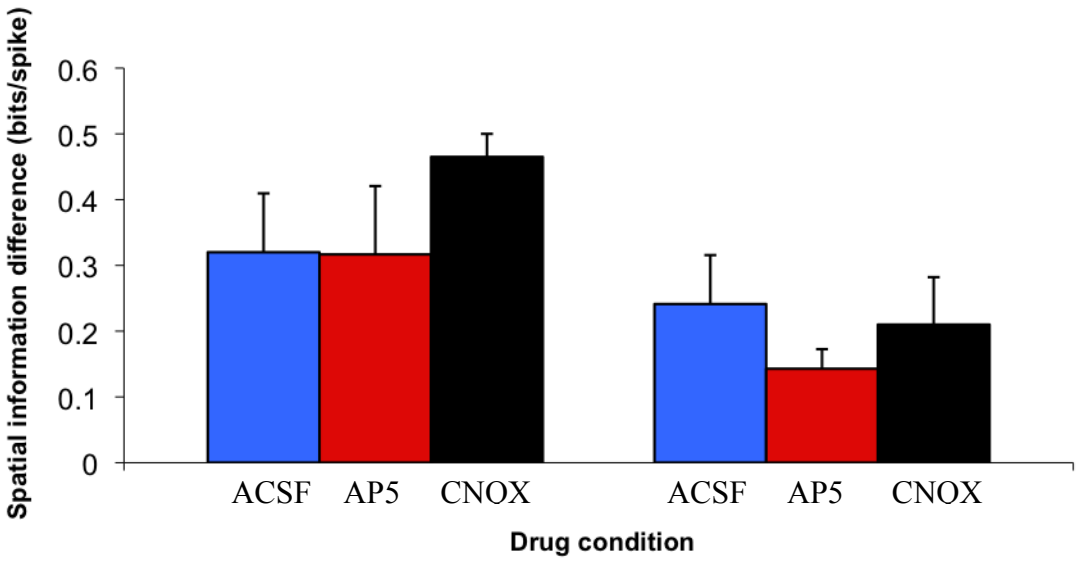

Figure 5.18 Mean difference in spatial information (bits/spike) between place fields from the same cell in sessions N1 and N2 (left), and also N2 and N3 (right), in all three drug conditions.

5.4 Discussion

Novel environments

In this study, the stability of CA1 place fields over the six hours following exposure to a novel environment under normal illumination was investigated. Stability was tested under three drug conditions: following intrapostsubicular infusions of D-AP5, CNQX or ACSF vehicle. Correlations of place fields in each of the three conditions showed no significant differences. This does not support the hypothesis that (i) AMPAR-dependent fast neuronal transmission is necessary in the postsubiculum for the association of visual landmarks with place cell representation or that (ii) NMDAR-mediated plasticity in the postsubiculum is necessary for the long-term stability of place fields in newly learned environments.

Histology showed that in all rats, the cannulae were inserted in the postsubiculum. In light of the negative result of the correlations over the six hour delay, it is worthwhile noting that results from in vivo injections of CNQX and AP5 into the postsubiculum suggest that the drugs do have a biological effect in this brain region (Shires et al., 2008, Bett et al., In preparation). In these experiments, fEPSPs were measured in the postsubiculum during and after infusion of ACSF, CNQX or AP5 into the same region. Figure 5.19 A and B show that administration of CNQX into the postsubiculum successfully blocked baseline transmission in the postsubiculum, but neither AP5 nor ACSF did.

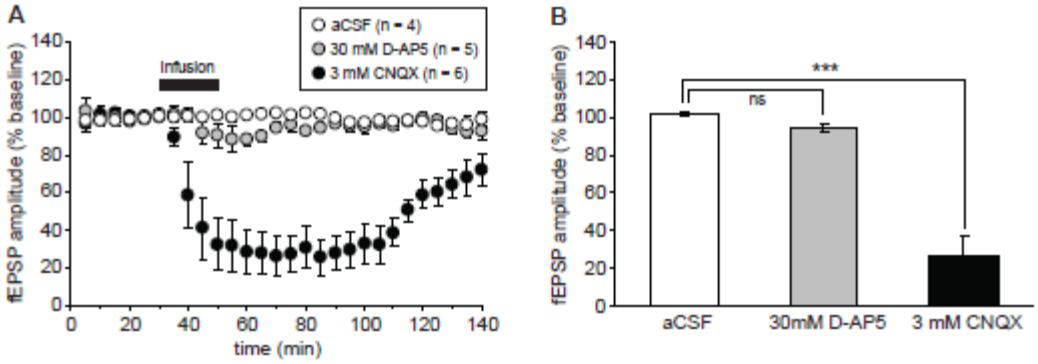

Figure 5.19 Infusions of CNQX into the postsubiculum showed a significant decrease in fEPSP compared to infusions of ACSF.

In addition, results from an object-place recognition study in which the present author performed drug infusions into the postsubiculum (Stevenson, 2010, Bett et al., In preparation) suggest that CNQX infusions have a behavioural effect. In these experiments, object-recognition was compared with object-place recognition. Postsubicular dug infusions of ASCF, AP5 or CNQX were administered prior to sampling of novel objects and tested 24 hours later (Figure 5.20). Infusions of both CNQX and AP5 resulted in rats having no preference for the displaced object, indicating that AMPAR-dependent transmission and NMDAR-dependent plasticity in the postsubiculum is required for the encoding of object-place associations, but not for the recognition of objects themselves.

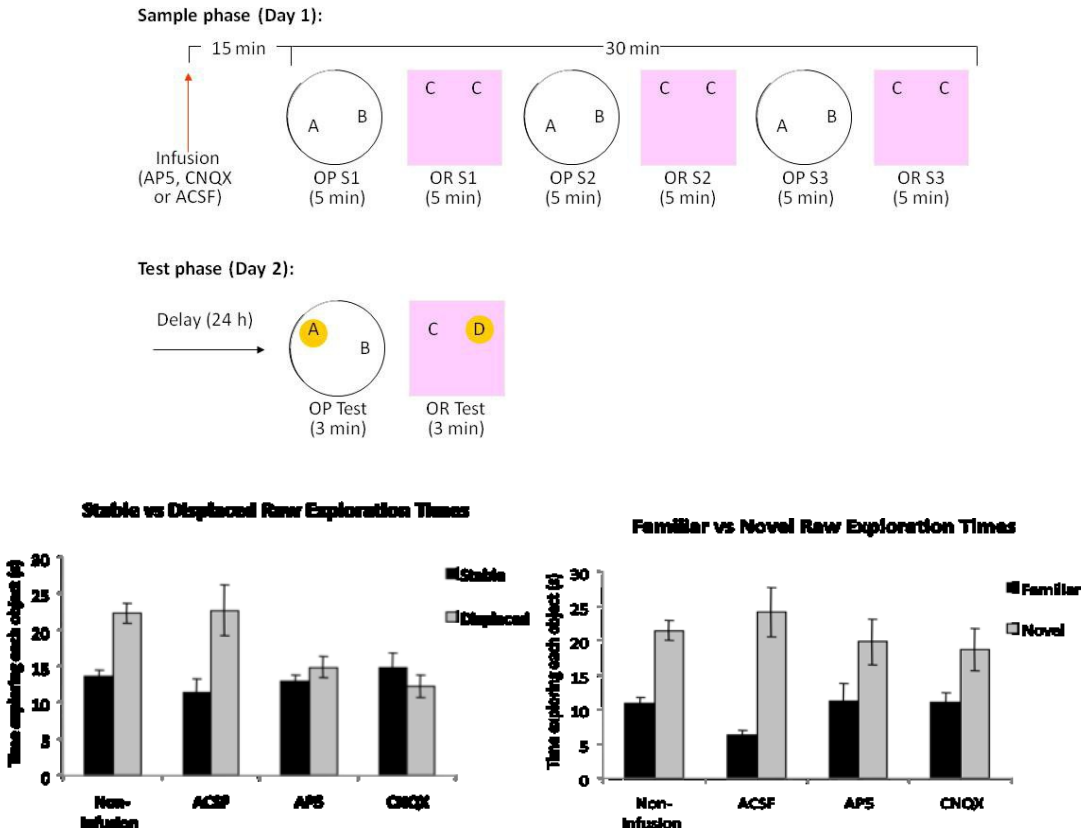

Figure 5.20. Top: Experimental design. Rats received infusions of 1µl of the NMDA receptor antagonist AP5 (30 mM), the AMPA receptor antagonist CNQX (4 mM), or ACSF in the postsubiculum prior to object-place learning (OP; cylinders) or object-recognition learning (OR; squares). The next day, their object-place memory was tested by displacing one of the previously seen object, and their object-recognition memory was tested by replacing one of the previously seen objects with a novel object. Bottom: Results. Infusions of AP5 and CNQX resulted in no preference for exploring the displaced objects. Object-place Object recognition was not affected by any drug condition.

Why then was an effect on place field stability not found in the current study using the same drugs at the same concentration injected into the same brain area? One possibility is that the postsubiculum is not essential for the association of internal representation of orientation with external representation of visual landmarks under the conditions in which this experiment was conducted.

The postsubiculum receives head direction information from the ADN, and then this information is presumably passed to the medial entorhinal cortex by the postsubiculum and eventually to the hippocampus. However, the ADN also projects to the retrosplenial cortex, which contains cells that respond to head direction. Both the postsubiculum and the retrosplenial cortex receive information from visual areas

17 and 18b (Vogt and Miller, 1983). Thus, both structures are in a position to process allocentric head direction information and visual information. Bearing in mind that both structures can perform this function, there are potential anatomical projections that could carry information about visual landmarks to the CA1 if the postsubiculum is inactivated or lesioned. The retrosplenial cortex projects to the entorhinal cortex directly (van Groen and Wyss, 1992), and also to the parasubiculum that in turn projects to the dentate gyrus. In addition, the retrosplenial cortex projects to the CA3 directly.

The results from this chapter are in contrast to the Calton et al. (2003) study that reported less cue card influence on place fields in rats with postsubiculum lesions compared to control rats (and ADN lesioned rats). One difference in methodology in these two studies is that Calton et al. compared place field stability in sessions in which the rats were always disoriented first, before being placed into the recording cylinder. In this case, any potential head direction information bypassing the postsubiculum, whether to the retrosplenial cortex first, where the association of external representations of visual landmarks and internal representation of orientation may occur, or more directly towards CA1, would likely be unstable between sessions due to the disorientation. Bear in mind that in the Goodridge and Taube (1997) study, after postsubiculum lesions, ADN head direction cells were less influenced by visual landmarks and thus unstable between cue-card rotation sessions. So it is entirely possibly that in the Calton et al. study, place cell instability between sessions may have resulted from the combination of unstable head direction information from the ADN *in addition to* the lack of postsubiculum (which presumably has the potential to anchor internal head direction to external visual landmarks). In contrast, the current study did not purposefully disorient rats, leaving the possibility that path integration

could have been used between the novel sessions N1, N2 and N3 to keep track of direction. Bear in mind that the experiments in Chapter one of this thesis suggest that the postsubiculum is not necessary for path integration. However, the retrosplenial cortex has been implicated in path integration (Cooper et al., 2001, but see Clark et al., 2010). Interestingly, lesions of the retrosplenial cortex have been reported to cause instability *within*-session in ADN head direction cells under normal illumination (Taube, 2007, unpublished data from Wang and Taube). This points to a role for this structure in processing visual information. Clark and colleagues (2010) also report that retrosplenial cortex lesions disrupt ADN head direction cells' preferred firing direction, in light and dark conditions.

This difference in methodologies may also account for the difference in the object-place task described above (Stevenson, 2010). In this task, following the infusions, rats were kept inside a lidded bucket (with air holes) for around five minutes before being taken out through the infusion room door that was kept closed (and thus required the experimenter to open whilst carrying the rat), across a corridor and through two doorways (one which was again closed) to the experiment room where they were placed directly into the sample environment. It is not unreasonable to suggest that, under these conditions, the rat was disoriented, although not purposefully. This is in contrast to the present study in which rats were taken from their home cage in a room adjoining the experiment room and transported inside a covered bucket to the experiment room and immediately taken out of the bucket and connected to the recording apparatus (approx. 10-15 seconds inside bucket). Although they were then kept in a covered bucket for five minutes, the recording cable ensured that the coverage was not complete and the recording cable itself may have provided an uncontrolled polarising cue.

If the association of visual landmarks and internal representation encoded by place cells does not occur in the postsubiculum or the retrosplenial cortex, there are other anatomical pathways that can potentially carry head direction information from the thalamic nuclei that bypass the postsubiculum. The nucleus reuniens projects to CA1 directly and also to the subiculum, which in turn projects to the entorhinal cortex and from there, the entorhinal cortex projects to the hippocampus. Another point worth mentioning is that in the Calton et al. (2003), study, following lesions to the ADN, the CA1 was still able to anchor place fields to visual landmarks successfully, despite the fact that there *should* have been no head direction cells in the postsubiculum at the time, as found in the Goodridge and Taube (1997) study with ADN lesioned rats. This suggests that any function attributed to the postsubiculum in associating landmarks with CA1 place cells is not due to its head direction cells that arise from ADN inputs. The evidence seems to suggest that head direction information can be used by the hippocampus in the absence of head direction cells in the postsubiculum.

Another possibility for the current results is that the effects of the drugs in the postsubiculum on hippocampal place fields are small and may not be detected with correlations. The effects of postsubiculum lesions on CA1 place cells found by Calton et al. (2003) were relatively small. They did not do pixel-by-pixel correlations of place fields between sessions, but reported reduced spatial information content compared to controls. Spatial information content is a measure of how well the firing rate of the cell predicts the location of an animal within an environment (Skaggs et al., 1993). The current study did not find reduced spatial information content scores of drug compared with ASCF infusions. This possibility of the correlations not detecting a putative drug effect seems unlikely though, because in the Calton et al. study, the

authors could clearly see place cell remapping between sessions from looking at the firing rate maps. In the current study, the between-session *differences* in spatial information content of place cells in sessions N3 and N5, however, were largest in the CNQX condition. This is a measure of the mean difference per cell between N3 and N5 and takes into account that spatial information may increase for some cells and decrease for others between the two sessions and thus may reflect extent of place field remapping between these sessions.

Familiar environments

Place field correlations in the familiar sessions across the delay (F3-F5) were similar between all drug groups. This was expected as F3 was prior to infusion and F5 was seven hours after infusions. This was intended as a control environment in which place cells would be stable over time. This was confirmed by the results. However, in the CNQX drug condition, there was a difference on spatial information between familiar sessions F4 and F5. Spatial information content on F4, the first session after infusion, was significantly lower than seven hours later in F5. This suggests that CNQX may have temporarily affected the spatial firing of cells in the familiar environments immediately following the infusion. Another interesting finding in the familiar environments was that the correlation between F3-F4, immediately prior to and immediately following the infusion was significantly lower in the CNQX condition compared to the AP5 condition (but not compared to ACSF). If more cells had been recorded in the ACSF condition then the mean correlation increase between place fields in this condition and those in the CNQX condition may also have reached statistical significance. Since this mean correlation difference only occurred between the CNQX and AP5 drug conditions, stress caused by infusions can be ruled out as an

explanation. There was no CNQX effect on place cell stability from session F3 to F5, suggesting that the decrease from F3-F4 was a drug effect.

It is not clear why there was an effect of CNQX in familiar environments but not in the novel environments. There was no difference in correlations between N1-N2 or between N2-N3. One possible reason is that the drug effect wore off quicker than expected, bearing in mind that in the object-place study (Stevenson, 2010) the sample phases lasted only around 30 minutes. This is unlikely, however, as there was an effect of CNQX in the postsubiculum in anaesthetised rats past 60 minutes (Shires et al., 2008) which was the maximum length of time used in following infusion the current experiments.

Experiments in darkness

In darkness, the only significant difference found was between F3 and F4, showing that F3 had higher spatial information content than F4. This indicates a possible drug effect. There were too few cells in the CNQX condition to make strong conclusions about this, however. Differences in place field correlations between the drug conditions in sessions N1-N2 and N2-N3 approached statistical significance. It may be that the postsubiculum becomes important in darkness for the association of landmarks with internal representation and warrants further investigation.

This leaves open the question of the postsubiculum's involvement in the association of external representation of visual landmarks with internal representation as encoded by place cells. A limitation of the current study is that no rotation sessions were conducted. Rotation of the familiar environment could have given information on whether the cue card influence was strong enough to exert control over cell firing. Future experiments could explore this possibility by repeating the experiment but

including an experimental group that receives a disorientation procedure before each novel environment session and between the last two sessions (N5-F5).

In conclusion, CNQX infusions directly into the postsubiculum prior to exposures to novel environments had minimal effect of place cell stability across a six hour period, and infusions of AP5 had no effect. One possible reason for this is that rats were not disoriented prior to entering the novel environments. It would be beneficial if future studies investigated the effects of disorientation compared with no disorientation between recording sessions, on place field stability, whilst the postsubiculum is inactivated pharmacologically separately with AMPAR and NMDAR antagonists.

Chapter 6: General Discussion

The experiments reported in this thesis investigated the postsubiculum's role in spatial cognition; specifically, its role in path integration in a homing task and its role in associating external representations of visual landmarks with internal representation of orientation as encoded by hippocampal place cells. In addition, its role in associating environmental cues with CA1 representation was looked at under darkness.

6.1. The postsubiculum and path integration

The postsubiculum has received relatively little attention in experimental studies in spatial cognition, despite the fact that (i) it was the first region known to have head direction cells, (ii) it receives inputs from ADN, LDN, and retrosplenial cortex, and (iii) projects strongly to the hippocampus.

In experiments chapter 3, rats were trained to use path integration in a food-collecting homing task. The task was designed to have diffuse distal stimuli by having black floor-to-wall curtains surrounding the maze and eight virtually identical equidistant home box locations of equal size and shape around the circular platform's periphery in an effort to make visual cue use extremely difficult. In addition, auditory cues were masked with radio noise and potential olfactory cues reduced/masked by wiping the maze with a detergent soaked cloth between trials. Rats with lesions to the postsubiculum managed to home as accurately as sham operated controls, even when the room lights were switched off. This strongly suggests that the postsubiculum is not necessary for path integration.

This is interesting because head direction cells are thought to underlie sense of direction, which seems essential for path integration. Besides, there are published reports of spatial memory impairments following postsubiculum lesions, including impairments on a hidden-platform water maze task, a radial arm maze task (Taube et al., 1992), and a T-maze alternation task (lesion of pre- and parasubiculum, Liu et al., 2001). However, none of these tasks explicitly tested path integration abilities. The water maze task is likely solved using locale navigation, involving room cues and an allocentric reference frame that requires a cognitive map. Thus, the water maze task does not require path integration. The 8-arm radial arm maze used by Taube et al. (1992) had a working memory component, as did the delayed T-maze alternation task in chapter 3. Despite the fact that the radial arm maze can potentially be solved using an egocentric strategy, for example, always turning left from the central platform after exiting an arm, rats are likely to use an allocentric reference frame, which has the consequence of employing working memory. Both tasks involved memory of previously visited location and rats with postsubiculum lesions were impaired in both. Path integration involves continually computing direction and distance travelled and is known to depend upon structures containing head direction cells (Frohardt et al., 2006). Interestingly, in the Frohardt et al. study, lesions to the DTN led to larger path integration impairments than lesions to the ADN. Most researchers believe (although unconfirmed experimentally) that the origin of the head direction signal outside the classic Papez circuit arises from the pathway DTN > LMN > ADN > Postsubiculum (Taube, 2007). And if you take into account the results from chapter 3 and Frohardt et al., it definitely appears that the more downstream in the circuit the lesion is, the less the effects on path integration are pronounced behaviourally. A likely explanation of this is that, the more the signal is processed and transmitted through this pathway, the

more the signal is dispersed to other brain regions, making it likely that a lesion will not block the signal to the entorhinal cortex, which is one region that has recently been implicated in path integration (McNaughton et al., 2006) and/or hippocampus. For example, DTN projects to LMN such that lesion to DTN should, and do, block the signal (Bassett et al., 2007), whereas LMN projects to both ADN and LDN and lesions to LMN block head direction in the ADN (Blair et al., 1998). Further downstream, ADN projects to postsubiculum, retrosplenial cortex, parasubiculum and CA1 (via nucleus reuniens). This means that, by the time the head direction signal reaches the postsubiculum, it has also reached other areas that could potentially compensate.

With lesions to the postsubiculum, rats may be able to track their direction through head direction cells in the retrosplenial cortex. This region is known to be essential for navigation in darkness on an 8-arm radial arm maze (e.g. Cooper et al., 2001) and lesions to it result in preferred head direction drift in the ADN under darkness (Clark et al, 2010), indicating impaired use of idiothetic cues. In addition, it has been reported that the retrosplenial cortex is important for T-maze alternation under darkness but not under normal illumination (Pothuizen et al., 2008), thereby suggesting a possible role in path integration.

6.2 The postsubiculum, visual landmarks and CA1 place cell stability

In the experiments in chapter 5, rats were given temporary pharmacological inactivations of the postsubiculum via infusion of AMPAR antagonist CNQX followed by exposure to novel environments with polarising cue cards. Place cell stability in the CA1 was used to assess the memory the place cells had for the

environment six hours later. The result of this was that there was no difference in place field stability compared to a control infusion condition. As mentioned in the discussion section in Chapter 5, this is likely because the rats did go through a disorientation procedure first.

If both the postsubiculum and the retrosplenial cortex function to associate head direction information with visual landmarks (Goodridge and Taube, 1997, Calton et al., 2003, Clark et al., 2010), then it follows that loss of one region may be compensated by the function of the other. This is because both structures receive head direction input from ADN (and LDN) and inputs from visual areas 17 and 18b. Compensation did not occur in Calton et al., but this can perhaps be explained by the disorientation procedure used before each testing session. Disorientation has the effect of causing ADN cells (and presumably also head direction cells afferent to it) to shift their preferred firing directions randomly, and this information will in turn be sent to the retrosplenial cortex. However, when the ADN is lesioned, there is relative stability and landmark cue-control of CA1 place cells (Calton et al. 2003), despite the fact that neither the postsubiculum nor the retrosplenial cortex should have any head direction information (Goodridge and Taube, 1997). This is according to the model proposed by Clark et al. (2010). But this model does not account for all for the evidence. It seems that there is a missing piece of the puzzle if we accept that PoS head direction is needed to associate visual landmarks with internal representation as assessed by CA1 place cells. If head direction information is necessary to associate visual landmarks with CA1 representations, then how does it occur when ADN is removed? One possibility is that there is more than one source of head direction information in the brain and that the head direction information flowing from the DTN to the postsubiculum is complemented by another source coming through the visual cortices

towards the retrosplenial cortex and to the postsubiculum. This may explain why removal of the postsubiculum, in addition to disorientation, results in instability of CA1 place fields between sessions. If the ADN is present but simply under the effects of disorientation, there is still a signal arriving in the retrosplenial cortex, which may be used to associate landmarks and CA1 representation. However, since the preferred firing direction of ADN head direction cells is unstable when there is no postsubiculum, this would lead to unstable CA1 representation between sessions. This can account for the results of Calton et al. (2003). However, if there is no ADN signal (due to lesion), head direction information from the retrosplenial cortex may be used to influence the spatial firing of CA1 place cells. This accounts for both the Goodridge and Taube (1997) report of no head direction cells in the postsubiculum following ADN lesions and the Calton et al. result of cue-control of CA1 place cells following ADN lesion. A function of the postsubiculum, then, may be to relay head direction information back to the ADN (also to LMN) with information from the retrosplenial cortex, thereby integrating the two sources of head direction information. This could be used to update the preferred firing direction of ADN (and LMN) head direction cells in cases where the animal is disoriented. Thus, when the postsubiculum is lesioned and the rat is disoriented, CA1 stability is impaired between sessions.

However, it should be noted that unilateral lesions (Jenkins et al., 2002) and bilateral lesions (Jenkins et al., 2004) to the ATN (including the ADN) result in reduced expression of the immediate early gene c-Fos in the retrosplenial cortex following either a working memory 8-arm radial arm maze or a simple foraging task, respectively. In addition, a recent lesion study reported impaired synaptic plasticity in the form of long-term depression (LTD) in brain slices six months after lesions of the

anterior thalamus (Garden et al., 2009). This implies that, in fact, the retropslenial cortex may be impaired, such that in cannot influence CA1 place cells.

The above account could be verified experimentally in the novel environments used in Chapter 6 using rats implanted with drug infusion cannulae in the ADN, retrosplenial cortex, or postsubiculum and with CA1 recording electrodes. A design incorporating inactivation via CNQX infusion of each structure in separate experiments could be used. In addition to the three structures, a condition of disorientation or no disorientation could be used. Figure 6.1 has a table describing expected outcomes based on the model proposed.

Inactivate	**Disorientation?**	**Prediction of CA1 stability between sessions**	**Reason**
RSPL	Yes	Impaired	Unstable ADN head direction cells (Clark et al., 2010)
RSPL	No	Unimpaired	PoS intact
ADN	Yes	Unimpaired	RSPL head direction cells use (Calton et al., 2003)
ADN	No	Unimpaired	RSPL intact (Calton et al., 2003)
PoS	Yes	Impaired	ADN unstable (Calton et al., 2003)
PoS	No	Unimpaired	RSPL + stable ADN (present thesis Ch5 result)

Figure 6.1 Table demonstrating prediction of model along side explanation. ADN, anterior dorsal thalamus; PoS, postsubiculum; RSPL. Retrosplenial cortex.

Conclusion

The experiments contained in this thesis contribute (i) that the postsubiculum is not necessary for path integration in a homing task and that (ii) the postsubiculum is not necessary for the association of visual landmarks in a novel environment with the internal representation of the environment as assessed by CA1 place fields. In light of

this and other published studies cited above it is very probable that, (i) path integration is not dependent on the postsubiculum, (ii) visual landmark and internal representation associations are dependent upon both postsubiculum and retrosplenial cortex together in the condition where the rat is disoriented and the signal from the ADN is variable between session, but when the rat is not disoriented either structure is sufficient.

Appendix I

In the experiments in Chapter 3, an attempt was made to train the rats to use cues for guiding homing. The methods and results are described below.

Cue training. This stage involved training the animals to use intra-maze cues to navigate back to their home box. For this, 3 cues were selected: 1 tall red cue with 4 legs attached to a base (height 29.5 cm; width 15 cm; length 15 cm), 1 tall white/red circular tube attached to a base (height 35 cm; width 18.5 cm; length 18.5 cm) and 1 white tub with a blue cap (height 14.5 cm; width 8 cm; length 8 cm). The cues had a fixed configuration across sessions relative to each other (see Figure 1) and each animal was assigned a home box location relative to the cues, which was maintained across all cue sessions.

Habituation to cues. The animals were given 3 x 15 minute sessions to habituate to the cues. During these sessions, an abundance of food was placed on the central platform and the animals were uninterrupted for the session (unless eating on the central platform occurred in which case the rat was picked up and returned home). The rats' choices made whilst homing were recorded.

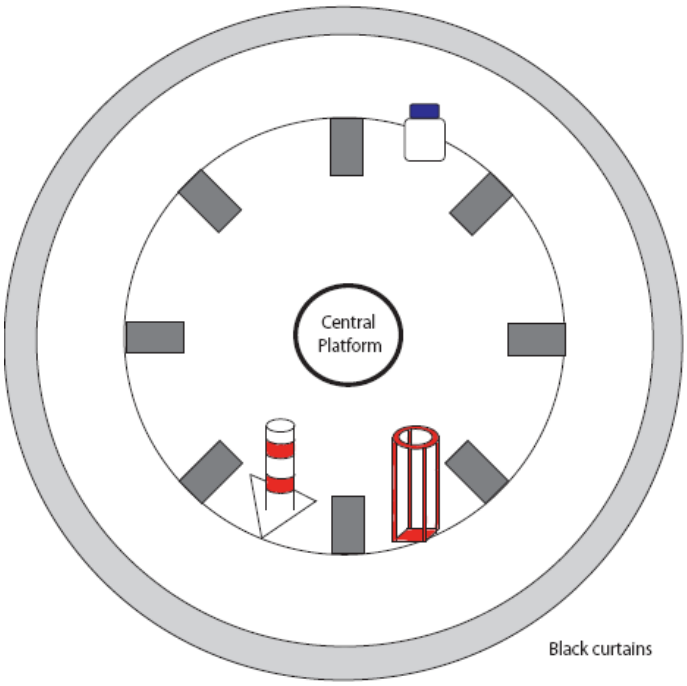

Figure 1. Schematic of configuration of intra-maze cues. Each animal was designated one HB location relative to these cues, which stayed constant across all trials.

Intra-maze cue training. The rats were then given 7 sessions which tested their ability to use the cues. In these sessions, the rats were allowed 6 uninterrupted fetches (6 halved Wotsits were placed on the central platform before the rats were released from the TB). On the 7th fetch, the animals were trapped inside the tube and removed from the maze to a holding bucket containing bedding and 3 Wotsits and then moved to another part of the room for 3 minutes. During this delay, the maze was wiped down with a detergent soaked cloth and the cues were then either rotated 90° clockwise, 90° anti-clockwise, 180°, or not rotated. The home box location was also moved so that it stayed in its original configuration relative to the cues. 2 whole Wotsits were put inside the raised tube onto the central platform. Following this, the animals were returned to the raised tube and the experimenter withdrew from the curtains and lowered the tube. The rats' choices were recorded when returning home.

Based on poor performance from all animals on these rotation sessions, it was then decided to run sessions with no rotations. Seven sessions were conducted, this

time without rotation of cues/ home box and without removing the animal from the maze. This time, on the 7th fetch, the rats were contained inside the raised tube for 2m and subsequently released after 2 Wotsits were thrown in. Again, the experimenter was outside the curtained maze area.

Cues probe session. A probe session was conducted with 5 rats to determine if the cues were aiding homing. In these trials, the animals were started off as usual from their home box /cue configuration and allowed 3 uninterrupted fetches. Then the animals were placed back in the transport box and the maze wiped down. The animals were then placed back into the same position and allowed 3 fetches. Then the animals were placed again inside the transport box and the maze wiped down. This time, the animals were returned to the location 180° from the original home box location. In addition, the lid covering the box now in the original home box location was opened. If the animals were using the intra-maze cues, we would expect them to return to this newly opened box.

Results

Rats did not use intra-maze cues to aid homing.

To determine whether or not the postsubiculum is required for navigation using landmarks, 3 intra-maze cues were introduced in a set configuration. In the first attempt, the animals were removed from the maze for a 3 minute delay before their homeward journey. It was found that out of 68 trials across all 11 animals there were (i) 7 instances where an animal reached the new home box location on the 1st choice (10.3% of trials;), (ii) 17 instances where an animal found the new home box location within the first 2 choices (25% of trials), and (iii) 20 instances where an animal

returned to the old home box within the first 2 choices (29.4% of trials). The probability of selecting any particular box by chance is 12.5%. This suggested that the animals were not using the cues to navigate home.

This was investigated further by using the same cues/configuration again, but without doing rotations. This time, the animals were delayed on the central platform and kept there on the maze for a shorter delay of 2 minutes. It was expected that this task should yield greater homing performances since no rotations were occurring and there was no disruption to the animal caused by removal from the maze. In addition, the delay time was 1 minute shorter. Performance was better this time: the animals found the home box on the first choice 28.5% of the time and found the home box by the second choice 46.4% of the time. Following this, a probe session was conducted in which animals were allowed to fetch from their normal home box location relative to the cues, and then put in transport box, and then returned to the same location after the maze was cleaned. Then, after fetching, the animals were again put in the transport box and the maze cleaned. This time, the animals were returned to the location 180° from their original location, thus disrupting the cues/home box configuration. Five animals were tested and on the probe trials and 2 animals found the new location, whilst the other 3 did not find either the old location or the new location after 2 choices. This suggests that the cues were not being used for navigation and further attempts to train the animals in this way were suspended.

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