Behavioural and electrophysiological investigations of three-dimensional navigation.

Jonathan James Wilson

Thesis submitted to University College London for the Degree of Doctor of Philosophy in Neuroscience

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

Acknowledgements

I would firstly like to thank my supervisors Hugo Spiers and Kate Jeffery for their guidance and support throughout my PhD. In particular, I would like to thank Kate for her enthusiastic support when I most needed encouragement and her seemingly endless patience, not only throughout my PhD, but also in previous years during my BSc and MSc studies.

My PhD was funded by a BBSRC CASE studentship with support from Axona Ltd. I would like to thank both organisations, and in particular Jim Donnett for his assistance in developing recording protocols using Axona hardware. I would also like to thank all of my colleagues at the UCL Institute of Behavioural Neuroscience, whose support and friendship have made long days in the lab fly by (in particular Giulio, Liz, Josh, Pierre and Laurenz). I would especially like to thank Robin Hayman for teaching me many of the techniques I have used over the past few years. I am also grateful to all of the students who have assisted me in the development and running of experiments, including: Mathilde, Elizabeth, Alasdair, Megan, Ningyu, Alice and Benjamin.

This work could not have been completed without the support and words of encouragement from my family, in particular my Dad, Cheryl and Anna. I would also like to thank all of my housemates past and present, but in particular Joe, George, Isabel and Andy. I would especially like to thank Joe for his friendship and late night neuroscience discussions. Finally, I would like to thank Amber and Chris and all those at The Charlotte Despard for helping me wind down over a beer and a game of pool after a long week's work.

Abstract

The world through which animals navigate is complex and three-dimensional, yet the majority of studies of navigation have been conducted in two-dimensional planar environments. The aim of this thesis, therefore, was to test whether animals are able to represent three-dimensional space, and if so, to determine the manner in which this space is represented in the brain. Behavioural and electrophysiological techniques were employed to answer these questions. In the first set of experiments, the ability of mice to complete working and reference memory tasks on a three-dimensional radial arm maze was compared with their ability on a two-dimensional analogue of the three-dimensional maze. The findings showed an equally good level of working memory in two and three-dimensional mazes, but reduced reference memory in the three-dimensional maze. These results suggest an intact representation of three-dimensional space over short time scales, but impairment in the formation, retention and/or recall of these representations over longer timescales. The second study, using electrophysiological techniques, tested the manner in which the brain represents orientation in three-dimensional environments. A three-dimensional apparatus was developed to test whether head direction (HD) cells encode orientation in a planar, multi-planar or volumetric manner. Head direction cells, which are known to be responsive to spatial orientation in 2D environments, were recorded as rats climbed between different vertical walls on cuboidal climbing apparatus. The findings showed that the HD cell system represents orientation in a multi-planar manner, in which the animal's plane of locomotion and the position of that plane relative to the azimuth inform the firing direction of HD cells. It will be argued in this thesis that the HD system is optimised to allow animals to translate their representation of orientation from vertical planes back to the horizontal plane without the accumulation of heading errors. Together, the findings presented in this thesis suggest that rodent use a multi-planar reference frame to aid navigation in complex three-dimensional environments.

Publications arising from this work

Wilson, J. J., Harding, E., Fortier, M., James, B., Donnett, M., Kerslake, A., O'Leary, A., Zhang, N. & Jeffery, K. (2015) Spatial learning by mice in three dimensions. Behavioural Brain Research, 289, 125-132. doi:10.1016/j.bbr.2015.04.035

Hayman, R. M. A., Casali, G., Wilson, J. J., & Jeffery, K. J. (2015). Grid cells on steeply sloping terrain: evidence for planar rather than volumetric encoding. *Frontiers in Psychology*, 925. http://doi.org/10.3389/fpsyg.2015.00925

Jeffery, K. J., Wilson, J. J., Casali, G., & Hayman, R. M. (2015). Neural encoding of large-scale three-dimensional space—properties and constraints. *Frontiers in Psychology*, 927. http://doi.org/10.3389/fpsyg.2015.00927

List of abbreviations

2D: Two-dimensional

3D: Three-dimensional

ADN: Anterodorsal nucleus of the thalamus

AHV: Angular head velocity

AMN: Anteromedial nucleus of the thalamus

ATI: Anticipatory time interval

ATN: Antherior thalamic nuclei

AVN: Anteroventral nucleus of the thalamus

BVC: Boundary vector cell

CA: Cornus ammonis

CCW: Counter-clockwise

CW: Clockwise

DG: Dentate gyrus

DTN :Dorsal tegmental nucleus of Gudden

FCC: Face centered cubic

HCP: Hexagonal close-packed

HD: Head direction

IP: Interpeduncular nucleus

LFP: Local field potential

LMN: Lateral mammillary nucleus

MEC: Medial entorhinal cortex

MVN: Median vestibular nucleus

NpH: Nucleus prepositus hyperglossi

PFD: Preferred firing direction

PoS: Postsubiculum

Rd: Retrosplenial dysgranular cortex

Rg: Retrosplenial granular cortex

RSC: Retrosplenial cortex

Sub: Subiculum

V1: Primary visual cortex

Contents

Index of figures

Overview

The ability to accurately navigate throughout our surroundings is a vital survival tool for all mobile animals. Whether searching for food or potential nesting locations, or avoiding predators, it is necessary to be able to represent the world around us and to recall the position of specific salient locations – for example, the location of a water source. This ability has been studied extensively in a wide range of species, from the remarkable dead-reckoning ability of desert ants (*Cataglyphis fortis*) over relatively featureless terrains (Wehner, 2003), to the large-scale navigation of flying Egyptian fruit bats (*Rousettus aegyptiacus*; Tsoar et al., 2011).

Over centuries of research, scientists have developed behavioural, and more recently, electrophysiological techniques to understand how animals navigate, and how the brain represents space. This work has primarily focused on spatial cognition in simple two-dimensional environments – however, the world in which we live, and through which we navigate, is highly complex and often involves movement through three-dimensional space. For example, many animals navigate in the Earth-vertical plane as well as the horizontal plane: squirrels (*Sciuridae*) can be seen climbing and rotating around tree trunks towards their nests; the Norwegian burrowing rat (*Rattus norvegicus*) will navigate through tunnels without the use of external visual cues; arboreal primates are known for their movement through forest canopies and humans regularly move through three-dimensional space when navigating multi-level buildings and sloped terrains.

The overarching question addressed throughout this thesis therefore concerns how three-dimensional space is represented in the brain, and whether this representation differs from that of two-dimensional space. Two different experimental approaches were taken to answering these questions. First, the behaviour of mice was tested in a three-dimensional environment to ascertain whether they could hold short- and long-term representations of three-dimensional space, and whether their ability to do this differs from two-dimensional space.

Second, the neural representation of orientation in three-dimensional environments was tested using electrophysiological recordings of a subset of spatially modulated neurons known to be sensitive to the animal's heading direction. The present work therefore tested the ability of animals to represent three-dimensional space, and further explored the functioning of a neural system underpinning one important aspect of navigation (judging one's orientation) during movement on a three-dimensional structure.

The findings presented in this thesis reveal the ability of rodents to recall locations distributed in three-dimensional space, albeit less well than in two-dimensional space. Further investigation of the neural systems for this three-dimensional representation showed that during locomotion on three-dimensional structures the neural system for determining orientation is updated in a manner that allows animals to maintain a surface-anchored representation of orientation that can always be reliably translated back to the horizontal plane without the accumulation of heading errors. These findings not only display the ability of animals to navigate complex three-dimensional environments, but they reveal a mechanism by which the neural representation of orientation is maintained during navigation of three-dimensional structures, thus providing one of the components required for successful navigation of complex three-dimensional spaces.

Prior to the presentation of the experimental findings of this thesis (Chapters 4 and 5), a detailed review of the behavioural and electrophysiological studies of spatial cognition in two-dimensional environments (Chapter 1) and three-dimensional environments (Chapter 2) is presented, providing a broader context to the questions addressed in this thesis. Following this, an in-depth review of a subtype of neurons known to be modulated by an animal's heading orientation is given (Chapter 3), providing the background for the electrophysiological experiments presented in this thesis. Finally, a discussion of the experimental findings and their implications for understanding the neural basis for navigation in three-dimensional environments is presented in Chapter 6.

Chapter 1 Navigation in two-dimensional environments

Until recently, the study of navigation and spatial cognition has been carried out in relatively simple flat environments. These studies have provided a great deal of insight into the behavioural processes involved in navigation, as well as the underlying neural systems that aid the representation of space. This chapter will first provide a brief overview of the history of navigation studies, before detailing some of the findings of behavioural studies of navigation in two-dimensional space, followed by an introduction to the neural substrates of spatial cognition. Together, the studies presented in this chapter reveal that animals are able to hold and subsequently use an internal representation of space, and that several subsets of neurons found within the brain provide the information required for such a representation. These neural systems ultimately allow for the implementation of complex navigational behaviours in two-dimensional environments. The findings reviewed in this chapter will serve to provide the context for the discussion of three-dimensional navigation throughout the rest of this thesis.

1.1 History of the study of navigation

At the beginning of the twentieth century our understanding of navigation was underpinned by the main tenets of behaviourism, in which accurate navigation was considered to be a result of learnt stimulus-response associations, known as Thorndike's Law of Effect (Thorndike, 1911). These associations were thought to be inflexible, and by extension, navigation was also thought to be a rigid process. This theory of navigation led to the conclusion that animals are only able to use learnt associations to guide themselves to goal locations, and would thus be unable to navigate around obstacles on their learnt route, or take shortcuts.

Edward Tolman challenged this theory in the 1930's and 40's with a series of behavioural experiments. These experiments revealed that rather than being inflexible, navigation was a fluid process in which behaviour was guided by an internal representation of space. Tolman coined this representation the "cognitive map" (Tolman, 1948). This idea that animals possess a cognitive map has since become the focal theory of the study of navigation.

Among the first experiments to reveal the presence of a cognitive map was Tolman's sunburst maze experiment (Tolman & Kalish, 1946). In this study rats were first trained to run from a platform along a circuitous path towards a goal location to receive a food reward (Fig 1.1). After training, rats were then reintroduced to the platform, but rather than having the one path towards the goal, there were a number of different paths radiating out from the platform (Fig 1.1). When introduced to this new platform, named the sunburst maze, rats would preferentially choose to run down the path offering the shortest possible route to the goal – even though the rat had never previously been introduced to this path. This revealed that navigation is not an inflexible process, as rats chose to take a shortcut to their goal location, indicating the presence of an internal representation of space. However, it must be noted that a light over the goal location may have provided an external cue, and it has since been argued (O'Keefe & Nadel, 1978) that the stimulus-response association between the light and food reward guided the animals' behaviour.

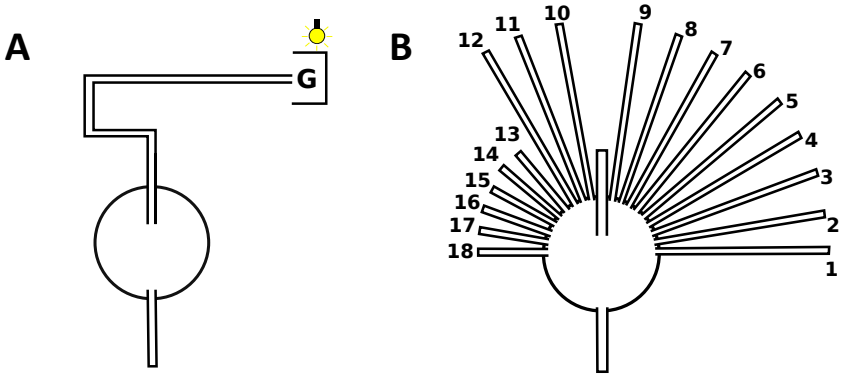


Figure 1.1 Tolman sunburst maze. A) Training maze B) Sunburst maze. Upon realisation that their learnt path was blocked, animal's preferentially chose to follow path 6 towards the goal location.

Despite this potential confound of Tolman's sunburst maze experiment, much of his research from the 1930's onwards provided strong support for the presence of a cognitive map. One of these experiments studied the latent learning of rats in a complicated T-maze (Tolman & Honzik, 1930; Fig 1.2A). Rats were required to reach a goal location in this maze, and could only do this following a correct sequence of left or right turns. Two cohorts of rats were studied; one group received a food reward upon reaching the goal location from the first trial, while the other did not receive any reward, or other positive reinforcement such as being removed from the maze at the goal location, until the eleventh trial. Unsurprisingly, the time taken (task latency) for the rats in the rewarded condition to receive the goal location decreased steadily from the first day. In the no-reward condition, there was no decrease in task latency over the first ten trials. However, once this group of animals was given a food reward at the goal location on the eleventh trial, there was a rapid decrease in task latency. The rate of reduction in this group was much greater than that of the rewarded group, indicating that even when there is no reward to motivate the rats, they appear to be developing an internal representation of the maze (or a "cognitive map"), and that once the goal location is rewarded they are able to rapidly put this representation to use.

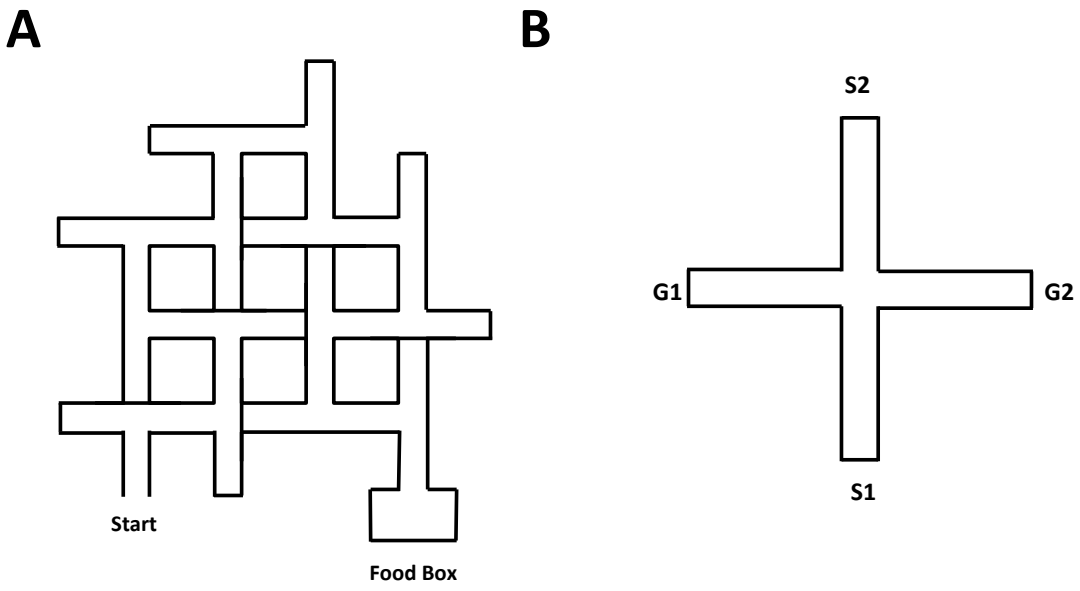


Figure 1.2 A) Tolman's complicated T-maze B) Tolman's plus maze

As well as the sunburst maze and latent learning experiments, Tolman, Ritchie & Kalish (1946) compared place and response learning on a simple plus maze (Fig 1.2B), in which there were two starting locations and two possible goal locations. Rats were either trained in a response group, in which they were required to always turn right to the goal location, or they were trained in a place group in which they were required to always move to the same goal location relative to the external cues, such that the motor commands required to reach the goal differed depending on the animal's starting location. The authors found that the place group animals learnt the task more readily than the response group, supporting the idea that animal's preferentially use place learning strategies – a result consistent with the hypotheses of the cognitive map.

Since Tolman first introduced the idea of the cognitive map, there have been a great number of more recently introduced behavioural approaches that have advanced our understanding of how space is represented and recalled. These approaches will be discussed in the next section.

One of the next great steps in understanding navigation was the introduction of a neurological approach to memory and spatial cognition. Research by Scoville & Milner (1957) on a group of patients with severe psychiatric and neurological conditions elucidated the hippocampus as a vital brain structure for the formation of memories. The study of one patient in particular, patient H.M. (Henry Molaison), provided evidence for the relationship between the hippocampus and memory. H.M. had been diagnosed with severe epilepsy with drug-resistant symptoms. In order to alleviate his symptoms, surgeons carried out a bilateral temporal lobe resection of H.M.'s brain. While this surgery was successful with regards to his epilepsy, it resulted in a complete loss in the ability to form new memories (anterograde amnesia) and an amnesia for memories formed prior to his surgery (retrograde amnesia). These retrograde memory effects were initially thought to affect patient H.M.'s memories from two years prior to surgery (Scoville & Milner, 1957), however it has since been argued that he exhibited retrograde amnesia for

events in the eleven years prior to his surgery (Sagar et al., 1985) but with a memory for more distant memories relatively well preserved. A more recent study of a patient with bilateral hippocampal damage (patient TT) indicated that the hippocampus is particularly important in facilitating navigation when detailed spatial representations need to be accessed (Maguire, Nannery & Spiers, 2006).

Tolman's hypothesis that animals hold a cognitive map for space was developed upon in the 1970's with the introduction of electrophysiological recordings of the hippocampus in rodents. Most notably, O'Keefe & Dostrovsky (1971) implanted electrodes in the hippocampus of rats, and recorded single-neuron activity of these animals while they were awake and freely moving. These recordings led to the discovery of neurons in the hippocampus that would fire action potentials when the animal was in specific locations within the recording environment. These neurons, now known as place cells, led to O'Keefe and Nadel to suggest that the hippocampus is the neural basis of Tolman's cognitive map (O'Keefe & Nadel, 1978).

Since the 1970's, electrophysiology in awake behaving animals has become one of the most commonly used techniques for understanding how the brain represents space, and has provided some important insights into the neural systems for encoding space. These include the discovery of a subset of neurons which are directionally modulated, known as head direction cells (Ranck, 1986); neurons which fire in multiple equally spaced locations within an environment, known as grid cells (Hafting, Fyhn, Molden, Moser, & Moser, 2005); and neurons which respond specifically to borders in an environment, known as border cells or boundary vector cells (Solstad, Boccara, Kropff, Moser, & Moser, 2008; Lever, Burton, Jeewajee, O'Keefe, & Burgess, 2009). Together these cell types are thought to provide the positional, directional and odometry information required to form a cognitive map. These cell types and their characteristics will be described in further detail later in this chapter.

1.2 Behavioural studies of navigation in two-dimensional environments

Successful navigation is thought to rely upon the processing of two types of information, external cues and internally generated cues derived from self-motion. External cues, otherwise known as allothetic cues, include visual, olfactory and auditory landmarks, while internally generated cues (idiothetic cues) are provided by the vestibular, optic and motor systems. One particular feature of successful navigation is the ability to use path integration, in which idiothetic cues alone are used to define the distance and direction of an animal's movement (Barlow, 1964; Mittelstaedt & Mittelstaedt, 1982; Mittelstaedt & Glasauer, 1991). The process of path integration requires the processing of direction and distance vectors that are subsequently used to determine current position. The ability to use path integration has been demonstrated in a wide range of animals including desert ants. Muller & Wehner (1988) demonstrated that desert ants could use path integration to return to their nest with a high degree of accuracy even after circuitous routes taking the animal over 100m from their nest location. These animals could even find their way to their nest location after a large barrier obstructed their initial route (Schmidt, Collett, Dillier, & Wehner, 1992). Both of these studies reveal that in the absence of external cues ants could estimate both the distance and direction of their previous movements to determine the locations of their nest.

Idiothetic cues alone are not always sufficient to guide behaviour, as evidenced by the widely reported phenomenon of humans walking in circles when lost in deserts or thick forest terrains (Souman et al., 2009). Extended reliance on path integration alone tends to lead to an accumulation of error, in which consistent under- or over-estimations of turn angles result in errant directional heading away from an intended goal location (Etienne, Maurer, & Séguinot, 1996). External landmark cues are thought to provide the stable information that allows for consistent correction of any errors provided by idiothetic signals. In order for these external landmarks to be useful, a successful navigator will also need to know how a constellation of

external landmarks are related to each other (in terms of distance and direction) and how these are related to the intended goal location.

Accurate navigation therefore requires the integration of idiothetic and allothetic cues, as well as a memory for the relation of external landmarks to goal locations. This section will describe some of the spatial navigation tasks that have been used to test the ability of animals to represent the position of goal locations, and the role of allothetic cues in guiding behaviour.

1.2.1 Memory for locations in two-dimensional environments

Laboratory studies of navigation have allowed researchers to understand how animals integrate idiothetic and allothetic cues during navigation. In particular, the radial arm maze, developed by David Olton in the 1970's, and the Morris maze developed by Richard Morris in the 1980's have been invaluable in elucidating the neural underpinning of navigation in two-dimensional environments. As versions of the radial arm maze were used in the behavioural experimental studies presented in this thesis (Chapter 4), an extensive review of the behavioural tasks using this apparatus will be presented first.

1.2.1.1 Radial arm maze

The radial arm maze, first introduced by Olton & Samuelson (1976), was designed to test the ability of rats to recall the position of food rewards, and their ability to use working memory to avoid revisiting previously rewarded locations. Working memory is defined as a process by which newly acquired information can be integrated with short-term memory (Miller, Galanter & Pribram, 1960). Such a system is consistently updated to guide an animal's behaviour based on recent past experience and novel incoming information.

The radial arm maze classically consists of a central platform from which eight arms project outwards (Fig 1.3A). In Olton & Samuleson's (1976) initial report, rats were required to retrieve food rewards from the ends of each of the arms. These rewards were not replaced over the course of the training sessions, meaning the rewards at the end of each arm became depleted as each trial progressed. In the first five days of trials, animals visited an average of 5.7 different arms within their first eight choices. This number increased to 7.6 in the final five days of trials, indicating that the animals had learnt that arms became depleted and that they could remember which arms they had previously visited in any given trial. This result provided evidence that rats are able to hold a working memory for locations on the maze.

To ensure that this result was not an effect of stereotypic behaviour the authors also analysed the sequences of arm visits, reporting that rats tended not to use a neighbouring arm strategy of persistently visiting the nearest arm in either a clockwise or counter-clockwise direction. Further to this finding, Olton & Samuelson (1976) also tested for the effects of intramaze cues on performance by randomly shuffling the position of the arms between trials, thus creating a discrepancy between intramaze and extramaze cues. They found that the accuracy of the first eight arm visits did not decrease as a result of interchanging the arms. These results support Tolman's notion of the cognitive map, as the rats were neither using stereotyped behaviours following a simple heuristic (such as always turning right), nor were they following intramaze cues (such as odour) to guide their behaviour in a stimulus-response manner. Instead, rats appeared to be able to recall the positions of previously visited arms, and to inhibit any subsequent visits to these arms.

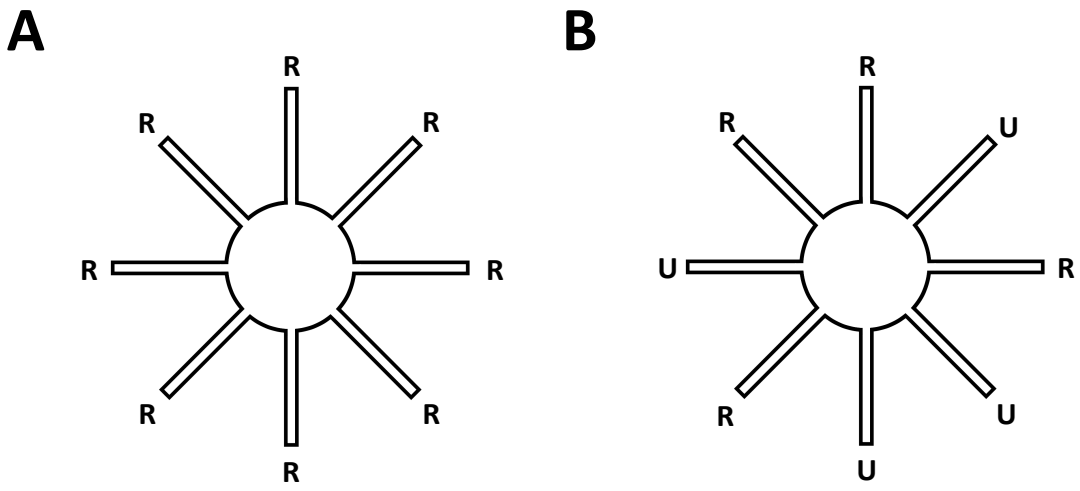


Figure 1.3 The radial arm maze tasks. A) The working memory task in which all arms of the maze are initially rewarded (R) B) The reference memory task in which only a subset of arms are initially rewarded (R), while the remaining arms remain unrewarded (U) throughout the experiment.

In an extension of their initial study, Olton, Collison & Werz (1977) developed a radial arm maze with 17 arms instead of eight. Similar to results found by Olton & Samuelson (1976), the authors found that, on average, 14 of the first 17 arm visits were to different arms, and that over the course of each trial there was a decrease in response accuracy, with more visits to previously visited arms occurring as the arms became depleted. Further to this, they reported that animals exhibited a preference for visiting arms closer to their previously visited arm, indicating that rats tended to prefer to visit neighbouring arms. The authors also reported that confinement in the central platform after each arm visit worked to reduce the strategy for visiting nearby arms. This technique of confinement has since been regularly used in experiments using the radial arm maze task, thus reducing the likelihood of animals to follow simple behavioural heuristics.

Another task that is commonly used on the Olton radial arm maze tests reference memory. The reference memory task, designed by Olton and Papas (1979), was used to determine whether rats can hold a long-term fixed representation of the positions of food rewards on the radial arm maze (Fig 1.3B). Classically, reference memory is considered to be the knowledge of aspects of a task that remain

constant over several trials of that same task. Reference memory, unlike working memory, requires consolidation between trials and can be thought of as a form of long-term semantic memory (Nadel & Hardt, 2010). In order to test reference memory in the radial arm maze, only a subset of arms (8 out of 17 arms) was ever baited. Rats were required to learn the positions of arms that were baited, and only to visit these arms. They found that over the course of the experiment rats reached a criterion level of performance in which 7 arms out of the first 8 visits were distinct baited arms. This result shows that, with experience, rats could learn the specific positions of baited arms relative to the external environment, and use these cues to direct their behaviour so as to only ever visit the baited arms. In order to complete this task, rats needed to hold a long-term representation of the positions of baited arms (reference memory), together with a short-term representation of the arms they had previously visited within a trial (working memory).

The key information used to drive response behaviours during radial arm maze tasks is provided by extramaze cues. Suzuki, Augerinos & Black (1980) carried out a set of experiments to determine how rats use visual extramaze cues to guide their performance on the working memory task. First, they compared two groups of rats trained with or without access to salient visual extramaze stimuli (stimulus-rich vs. stimulus-poor). In the stimulus rich group extramaze cues were placed at the ends of each arm, while there were no cues in the stimulus-poor group. The authors found that rats in the stimulus-rich group showed more correct choices over the course of the experiment, but that both groups performed above chance levels. This was explained by the strong tendency of the stimulus-poor group to carry out response-chaining behaviours, in which they carried out more stereotyped movements (e.g. always visiting the adjacent arm to the right of the current arm) than the stimulus-rich group.

In a second experiment (Fig 1.4), the authors either transposed or rotated the extramaze cues in a forced-choice paradigm (see Zoladek & Roberts, 1978). In this task, rats were forced to choose three specific arms on the maze before being

constrained on the central platform for 2.5 minutes while the extramaze cues were moved. The rats were then required, in a free-choice paradigm, to collect rewards from the remaining five baited arms. In the rotation condition, all extramaze cues were rotated so that the relationship between the previously visited arms and cues in the free-choice segment of the task differed to those in the forced-choice segment. The arms chosen in the subsequent free-choice task were rotated by an amount commensurate with amount of rotation of the cues. In the transposition task the position of three of the cues was changed in order to scramble the configuration of the cues. This manipulation resulted in a strong disruption of correct choices by the animals, with only 2.9 of the first five visits deemed to be correct choices. Together, these results indicate that rats use extramaze cues to guide their choices in the working-memory task on the radial arm maze, with the disruption of behaviour in the transposition task suggesting that the rats were using a configuration of external cues as opposed to individual external cues to guide their behaviour.

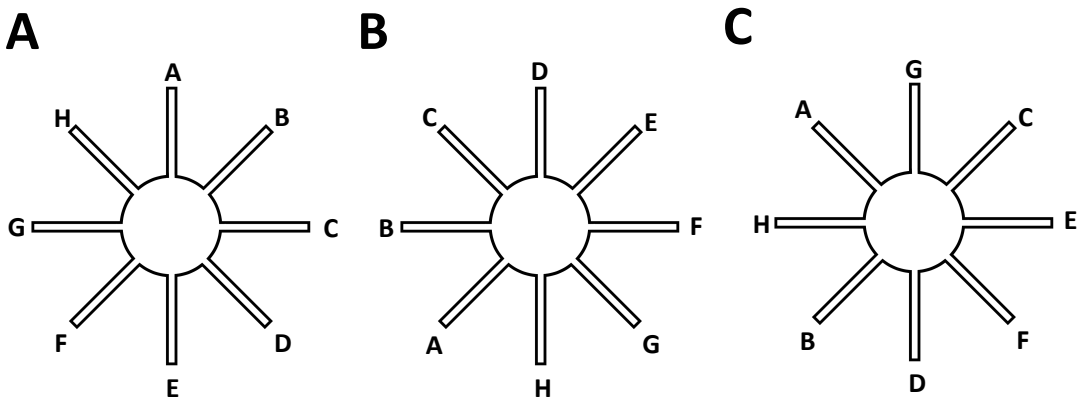


Figure 1.4 A) Training condition B) Rotation condition C) Transposition condition. Letters represent the location of each extramaze cue for each condition. Figures adapted from Suzuki, Augineros & Black (1980).

While rats have been shown to perform well on the radial arm maze, readily learning both the working memory and reference memory tasks, studies of mice on the radial arm maze have provided mixed results, with the first report of mice on the radial arm maze suggesting that mice did not improve over the course of the

working memory task (Mizumori, Rosenzweig, & Kermisch, 1982). While both mice and rats exhibited above-chance performance for visiting as-yet-unvisited arms on the first day of trials, rats were shown to improve over the course of the experiment, with all rats making more than 7 correct choices out of the first 8 choices from day four of trials. In mice, this number never exceeded 6.33, even after 20 days of trials.

Contrary to the findings of Mizumori et al., (1982), another experiment using a reference memory task on a six-arm radial arm maze described the ability of mice to reduce both working memory errors (revisits to previously visited arms) and reference memory errors (visits to never-baited arms) over the course of 20 trials (Levy, Kluge, & Elsmore, 1983).

Lesion studies of learning on the radial arm maze have also revealed the importance of the septo-hippocampal system in spatial memory. Olton (1977) studied the learning of animals during the working memory task on the radial arm maze, after they had undergone lesions of various regions of the neocortex and septo-hippocampal network. Lesions of cortical regions had no effect on the probability of animals visiting baited arms, while lesions of the entorhinal cortex and the fornix resulted in a rapid decrease in the probability of animals visiting baited arms. For example, after only two arm visits, the percentage of control animals visiting a baited arm was 90-100%, while only ~30% of fornix and entorhinal lesioned visited a baited arm on their third arm visit. These studies reveal the ability of animals to hold a working memory for previously visited locations, and the importance of the hippocampal formation in spatial memory.

While the radial arm maze is more traditionally used to assess working and reference memory in rodents, it has also been used to test spatial memory in humans and to compare the spatial working memory capacity between species. Aadland, Beatty & Maki (1984) assessed the performance of children aged 18 to 71 months and college students on free-choice and forced choice working memory

tasks on an eight arm radial maze. The authors found that in a free-choice paradigm, in which the participants were permitted to access the arms of the maze in any order they wished, the accuracy of arm selection in the fifth to eighth choices increased with age, with children over 50 months exhibiting a choice accuracy of over 90% compared to a choice accuracy of ~50% for children aged under 30 months. The authors also reported striking similarities to rodents with regards to behavioural strategies used to complete the free-choice task. Children over 40 months of age regularly used a neighbouring arm strategy, in which their first four arm choices were to adjacent arms.

In another study of working memory on the radial arm maze O'Connor & Glassman (1992) reported that in a 17 arm radial maze that humans exhibited very similar spatial working memory to rats. The authors used a paper analogue of the radial maze, which consisted of seventeen cardboard flaps arrange radially around a centre point. The participants were required to lift as many cardboard flaps as possible in a random order without repetition. Participants lifted an average of 15.4 different flaps in their first 17 attempts compared to an average of 14 arm visits by rats on a 17 arm radial maze (Olton, Collison & Werz,1977).

Together, both the working memory tasks and reference memory tasks developed on the radial arm maze have shown that rodents can hold flexible short-term memories of locations, and that they can hold long-term representations of fixed locations over the course of several days. These representations are further aided by the presence of extramaze cues. The use of this apparatus with humans also indicates a similarity in the behavioural strategies and spatial working memory capacities of humans and rodents.

1.2.1.2 Morris watermaze

Another apparatus that has been useful in the study of navigation is the Morris watermaze (Morris, 1981). The purpose of the watermaze was to demonstrate that rats could learn the position of an object that they could not see or smell, and

therefore use only external landmarks (extramaze cues) and idiothetic information to guide their behaviour (Fig 1.5). In this maze, rats are required to escape from a pool of opaque water onto a hidden platform that is located just below the surface of the water. Morris reported that when the platform was located in a fixed position across trials, that rats readily learnt the location of the platform within the watermaze, resulting in a reduction in the path length and task latency of the animal's search for the platform (i.e. rats recalled the location of the platform, even without the presence of intramaze cues to guide their behaviour).

In a second experiment, transfer tests were carried out, in which the starting location of the rats was changed between trials, thus changing the direction of the shortest possible route to the platform. During this task, rather than using a previously learnt path direction, rats were shown to quickly reorient their path to swim directly towards the goal location. This further indicates that rats were able to use extramaze cues to determine their starting position, and adapt their path appropriately to minimize the length of time in the water. This adaptation of behaviour further refutes the notion that animals use S-R associations to guide their behaviour, and supports the idea that animals can hold and utilise an internal representation of space, allowing them to determine their current and goal locations in relation to distal cues.

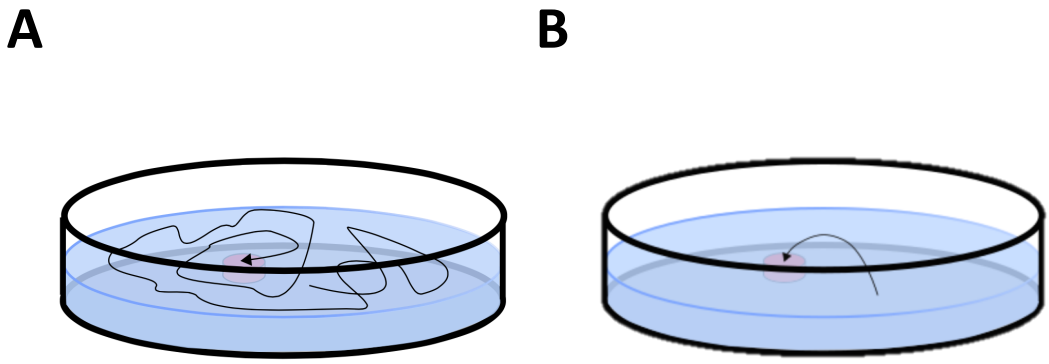


Figure 1.5 Morris watermaze. Schematic of the animal's path (black arrow) in the Morris watermaze in A) early trials and B) late trials. Red cylinder shows the position of the hidden platform.

The watermaze apparatus has also been used to reveal the importance of the hippocampus in navigation. Morris et al. (1982) studied the behaviour of hippocampal or cortically lesioned rats in the watermaze, and found that hippocampal lesions impaired the ability of rats to learn the position of the platform, demonstrated by a significantly slower reduction in path length of hippocampus lesioned rats than controls and cortically lesioned rats. In a further control experiment, the three groups of rats were also studied when the platform was placed above the water, and therefore providing a visual cue as to its location. In this experiment, rats with hippocampal lesions performed at the same level as controls and cortically lesioned animals – indicating that the hippocampus lesioned animals were equally able to complete the basic task of swimming directly to the platform. This study, alongside the discovery of place cells in the hippocampus (discussed in the next section), was among the first to reveal the importance of the hippocampus in navigation.

The water maze and virtual reality analogues of the water maze have also been succesfully used to test the spatial memory of other animal models, such as mice and humans. Mice were similarly able to learn the non-cued watermaze task (Florian & Roullet, 2004; Stackman, Lora, & Williams, 2012) as rats. Stackman and colleagues (2012) revealed that mice rely upon extramaze cues to guide their behaviour. A rotation of the extramaze cues by 90 degrees resulted in a corresponding shift of initial path direction by the mice. These authors also reported that lesions of the anterior thalamic nuclei (ATN) – a region known to contain directionally modulated cells (discussed in the next section) – reverse the search strategies of mice, causing them to use intramaze cues rather than extramaze cues to guide their search for the platform.

Jacobs, Laurance & Thomas (1997) developed a virtual reality watermaze that could be used with humans. The task they developed was an analogue of the water maze task developed by Morris (1981). The authors found that humans, like rats in the non-cued watermaze task, were able to learn the position of goal locations within a

virtual environment even when the goal was not visible to the viewer. Like rats, over the course of training the amount of time taken to find the invisible target and the length of path to that target decreased.

1.2.2 Summary

Behavioural investigations of navigation in two-dimensional environments have shown that a wide variety of species are able to integrate internally generated (idiothetic) cues with external landmark-based (allothetic) cues while navigating to form an internal representation of space (the "cognitive map"). Apparati such as the radial arm maze and the Morris water maze have revealed that rodents and humans are able to retain this internal representation of space, and to use the information contained within to flexibly direct behaviour during navigation. The next section will introduce the neural systems thought to contribute to the cognitive map.

1.3 Neural correlates of two-dimensional spatial cognition

Since the discovery of place cells by O'Keefe & Dostrovsky (1971) there has been a great deal of research into the hippocampal formation and its involvement in spatial cognition. This research led to the discovery of head-direction cells, grid cells and border cells, all of which are thought to contribute to the neural basis of the "cognitive map". This section will provide an overview of the firing characteristics of these cell types in two-dimensional environments, giving a broader framework for the subsequent discussions of the neural basis of three-dimensional navigation, presented throughout the remainder of this thesis.

1.3.1 Place cells

Place cells are neurons that exhibit complex-spiking properties when an animal is in a specific location within its environment, known as the cell's place field (Fig 1.6).

Remarkably, a given place cell will exhibit little to no firing activity when an animal is outside of its place field. O'Keefe & Dostrovsky initially recorded these cells from the CA1 (cornus ammonis 1) region of the hippocampus, and these cells have since been recorded throughout the hippocampal formation, including the CA2 and CA3 regions and the dentate gyrus (Fig 1.6 C-D).

Quantitative analysis of place cell firing has allowed researchers to probe the types of information – both idiothetic (internally generated) and allothetic (externally generated) – that place cells use to determine the location of their firing. The firing features of place cells, such as their firing rates, place field size and place field location are some of the most-studied aspects of place cell activity.

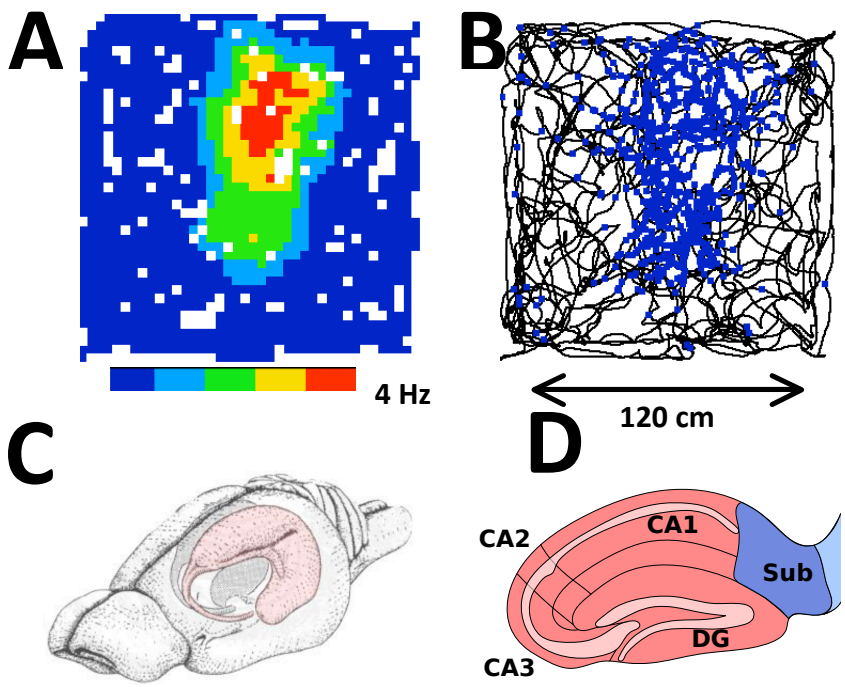


Figure 1.6. A place cell recorded from the CA1 region of the hippocampus in a 1.2m x 1.2m square recording arena. A) Firing rate map of the place cell, with hot colours representing a high firing rate and cool colours representing a low firing rate. B) Spike plot of the same cell, showing the path of the animal (black lines) and the above threshold spiking of the place cell (blue dots). C) Location of hippocampus within the rodent brain, with the left hippocampus highlighted in pink (Image from Amaral & Witter (1989). D) Representation of the cross-sectional anatomy of the hippocampus, detailing the cornus ammonis regions (CA1-CA3), dentate gyrus (DG) and subiculum (Sub). Firing rate map and spike plots supplied by Giulio Casali.

One example of a study investigating place cell firing characteristics revealed place cells sometimes behave differently in different environments. Kubie and Muller (1991) reported that place cells recorded in one environment may not be active in a second environment, and that those cells that are active in both environments tend to fire in different locations relative to a cue card, or with differing firing rates. These changes in place cell firing, known as remapping events, can be divided into several subtypes. Firstly, a place cell that is active in one environment but not another is said to have undergone complete remapping (Bostock, Muller, & Kubie, 1991). A population of place cells that are active in two different environments but with different firing locations in the two environments are said to have undergone global remapping, whereas a population of place cells that are active in similar locations in both environments but with different firing rates are said to have undergone rate remapping. These different types of remapping events can occur concurrently.

Together, these remapping events indicate that place cells are able to function as a population to represent multiple differing environments, while still maintaining some relative firing for environments with similar features. Further studies of place cell remapping have shown that context can also drive remapping events. Anderson & Jeffery (2003) recorded place cells in a variety of environments that either differed in visual (black or white box) or olfactory (lemon or vanilla) contexts. Some place cells were shown to remap as a result of colour or odour changes alone, while many other cells also required a change in both visual and olfactory context for remapping. This implies that representing context requires a population level encoding of different aspects of context by the place-cell system.

The effects of environmental manipulation on place field size have also been studied. O'Keefe and Burgess (1996) found that morphing a small square recording arena into larger rectangles or a larger square resulted an increase in place field size. For example, stretching the environment into a rectangle along the x-axis of the environment resulted in an elongation of the place field along the same axis.

This led the authors to conclude that the borders of an environment important in driving the location and size of place field firing.

The presence of place cells in non-rodent species has also been investigated. In primates, there is as yet no evidence for the presence of neurons that exhibit specific place-modulated activity. However, cells recorded from the hippocampus of primates have been shown to exhibit selectivity for the spatial view of animals (Rolls & O'Mara, 1995; Rolls, 1999; for review, see Rolls & Xiang, 2006). Rolls (1999) reported that hippocampal cells recorded from primates were selectively active for the allocentric direction in which the primates were looking and fired irrespective of the animal's location in the room. For example, a spatial view cell would be active when the animal looked towards the centre of a specific wall independently of where the animal was standing in the recording room.

More recently, Ekstrom et al. (2003) carried out multi-unit recordings of hippocampal and parahippocampal cells in the human brain. The authors implanted microelectrodes into the brains of patients with pharmacologically intractable epilepsy who had undergone intracranial electrode implants to identify the seizure focus for surgical treatments. While patients were implanted with these electrodes, Ekstrom et al. (2003) recorded from neurons as the patients were tested on a virtual-reality way-finding task. The authors found that of 317 cells, 12% responded to the spatial-view of the participants, while 11% responded specifically to place. The majority of place cells were recorded from the hippocampus, while spatial view cells were recorded from the parahippocampal region.

Together, electrophysiological studies of the hippocampus during navigation have shown the presence of a well-conserved mechanism for representing allocentric space, with rodent, primate and human hippocampi all shown to be involved in the representation of allocentric position or spatial view. Place cells are likely to be an important component of the cognitive map, as they provide the information required to determine one's position. However, a map requires other features if we

are to be able to navigate accurately through the world. One of these features is the ability to determine direction. Neurons that are believed to encode for heading direction are discussed next.

1.3.2 Head direction cells

In 1984, while the majority of spatial cognition researchers were occupied with the role of the hippocampus in encoding, James B. Ranck Jr. started to implant microelectrodes in the rat subiculum – a region of the hippocampal formation that receives a strong input from CA1 neurons. In what turned out to be a serendipitous series of events, Ranck had actually consistently implanted the microelectrodes in the postsubiculum (PoS) – a region which projects to the hippocampus via the entorhinal cortex.

Ranck noted that the cells he had been recording in the PoS were strongly modulated by the animal's heading direction. Initially these cells were not analysed quantitatively, but a video he recorded showed that these head direction (HD) cells exhibited firing in an absolute horizontal direction within the environment, and that the firing appeared to be independent of other behaviours such as rearing or the animal's position in the environment (Ranck, 1986). Over the next six years Taube & Muller, together with Ranck, developed techniques to quantitatively analyse the relation between heading direction and activity of neurons in the PoS.

Taube and Muller recorded the heading direction of animals using a pair of different coloured LEDs attached above the rat's head. The angular displacement (angle between the two LEDs) of these LEDs in the environment could then be computed to determine the animal's heading direction in the horizontal plane.

The first quantitative analysis of HD cells showed that of 239 cells recorded from the PoS, 26% were directionally modulated (Taube, Muller, & Ranck, 1990). These cells were shown to have directionally modulated tuning curves, such that the firing rate of a given cell exhibited a single peak in its tuning curve when plotted against

heading direction, with the angle at the peak of this curve being used to define the cell's preferred firing direction (PFD). The mean peak firing rate of these HD cells was 35Hz. The firing range of cells is defined as two standard deviations of the Gaussian tuning function. HD cells on average had a mean firing range of 83.4°, with low levels of spiking activity (usually <1Hz) outside of the Gaussian curve. There was also an even distribution of the HD cells' PFDs when analysed at a population level, indicating that there was not a preference for a population of HD cells to all fire towards a particular direction in the environment. Alongside this, HD cells did not exhibit spatially localised firing as seen in place cells; instead, they would fire in all locations of an environment whenever the animals faced in a given cell's PFD. An example of an HD cell's directional tuning can be seen in Figure 1.7.

HD cells have since been recorded in several other brain regions throughout the limbic system. These include the cortical regions such as the retrosplenial (RSC) and entorhinal cortex (EC), and thalamic regions including the anterodorsal nucleus of the thalamus (ADN), lateral mammillary nuclei (LMN) and the dorsal tegmental nuclei (DTN).

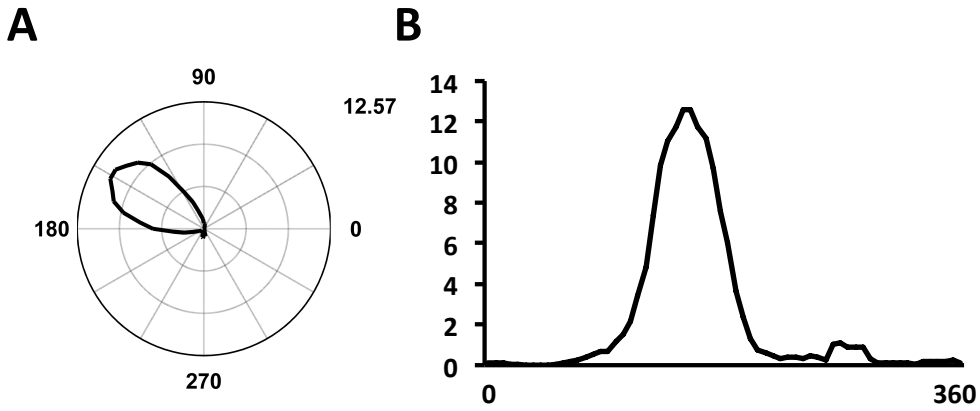


Figure 1.7 Firing activity of one HD cell shown in two formats. A) Polar plot showing the firing rate of the head direction as a function of animal's heading direction B) Line chart of the same cell showing the Gaussian tuning of HD cells. Note that the firing direction of the cell is ~150 degrees, with a peak firing rate of 12.57 Hz. Figures created using data collected in Chapter 5.

As with place cells, researchers have also investigated the presence of head direction cells in primates and humans. Robertson et al. (1999) recorded neurons from the presubiculum of primates, as well as from other hippocampus related structures such as the subiculum and parahippocampus. The authors found that HD cells exist within the presubiculum of the macaque, and that their firing characteristics are highly similar to those of HD cells recorded from the rat postsubiculum. First, HD cells recorded from the macaque presubiculum fired in the same allocentric direction independently of where the animal was stood within the room. Second, primate HD cells had an average tuning width of 76 degrees. Finally, the activity of HD cells outside of the cells' PFDs was under one spike per second.

Directionally modulated cells have also been recorded in the entorhinal cortices of human patients. Jacobs et al. (2010) recorded cells from the entorhinal cortex of patients as they played a virtual reality taxi driving game. Participants were required to drive a virtual taxi around a ring road of a simple town. During completion of the task, participants could choose to drive to the goal locations following on clockwise or counter clockwise route. The authors found that 6% of cells were modulated by the direction of movement around the virtual town, with these cells exhibiting spiking activity for either clockwise or counter-clockwise paths. The authors did not report the presence of cells that could be described as HD cells, however, the finding that directionally sensitive cells exist in the human entorhinal cortex further indicate that the systems representing allocentric space are highly conserved across mammalian species.

The characteristics of the HD cell system will be discussed in greater detail in Chapter 3, as the electrophysiological experimental chapter of this thesis centres on the activity of head direction cells in three-dimensional environments. This includes the differential firing properties between different HD-containing brain regions, the idiothetic (internal) and allothetic (external) determinants of HD cell firing, the anatomy of the HD cell circuit, and theoretical models of HD cell firing.

1.3.3 Grid cells

Place cells and head direction cells are believed to encode for location and heading direction, respectively. However, one more required feature of a cognitive map, as supposed by O'Keefe & Nadel (1978), is an ability to encode for distance.

One potential neural correlate for distance encoding is a subset of neurons found in the medial entorhinal cortex (MEC). Fyhn et al. (2004) first reported a strong spatial modulation of MEC neurons, showing that these cells, unlike place cells, fired in multiple positions in an environment, and that the distance between these firing fields increased at more ventral regions of the MEC. Shortly after this report, Hafting et al. (2005) found that the firing pattern of these multi-peaked MEC cells was not just random, but instead they conformed to a structure that can be described as a regular grid of tessellating equilateral triangles. An example of a grid cell's firing properties can be seen in Figure 1.8.

As with place cells, these 'grid cells' can be described by their firing rates and field sizes. As well as this, they can be described by the orientation of the grid pattern and grid spacing (the characteristic distance between two neighbouring fields), and are commonly defined as grid cells based on their conformity to their hexagonal patterning, known as the cell's 'gridness'.

Importantly, grid cells have been shown to maintain their firing patterns as rats move in darkness (Hafting et al., 2005) suggesting that they may be an ideal mechanism for path integration, and distance encoding. Moreover, the spacing between grid fields is greater in more ventral regions of the MEC than the dorsal regions (Hafting et al., 2005; Brun et al., 2008) with some cells exhibiting field spacing of over 70cm. This indicated that grid cells are able to encode for a wide range of distances. As well as encoding for distance, some grid cells exhibit higher firing rates at higher running speeds and show modulation by direction as well as location (Sargolini et al., 2006).

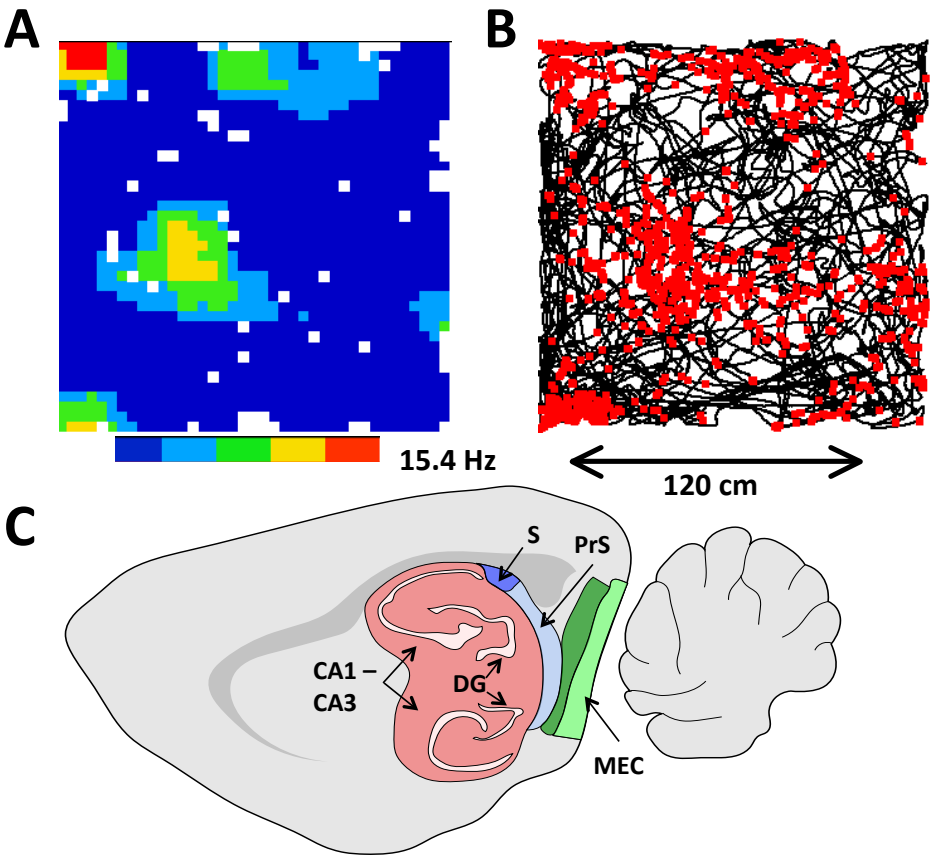


Figure 1.8 A grid cell from the MEC recorded in a 1.2m x 1.2m square environment. A) Firing rate map of the grid cell, with hot colours representing high firing and cool colours representing low firing. B) Spike plot of the cell, with the cell's above-threshold spiking (red dots) overlaying the animal's path (black lines). The rate map and spike plot were provided by Giulio Casali. C) Illustration of the location of the MEC in relation to the hippocampus, subiculum (S) and presubiculum (PrS). Image adapted from Paxinos & Watson, 2006.

More recently evidence of grid-like neural activity has been reported in humans. Doeller, Barry & Burgess (2010) used function magnetic resonance imaging to look for evidence of spatially modulated activity of the entorhinal cortex. Specifically, the authors used the understanding of grid cell activity in rats to predict a modulation of the fMRI signal by running direction. As shown by Sargolini et al., (2006) some rodent grid cells are modulated by direction. The preferred firing direction of these conjunctive grid x direction cells tend to be aligned with one of the three axes of the grid cell firing pattern. Doeller et al., (2010) predicted that if grid cells are present in the human entorhinal cortex, that the fMRI signal would be modulated by running direction on a virtual maze, such that the signal would show a six-fold rotation symmetry in its modulation by direction. The authors confirmed this prediction in their study, as well as showing that, like rodent grid cells, the fMRI signal of the

entorhinal cortex was also modulated by running speed. While this study does not show direct evidence for the presence of grid cells in the human brain, it does at least indicate that activity in the human entorhinal cortex is modulated by similar components of navigation (direction and speed) as has been shown in the rodent entorhinal cortex.

These initial results all indicate that grid cells might be highly informative for path integration, as they are modulated by distance, speed and directional information. More recently they have been shown to be affected by external landmark information. For example, Barry et al. (2007) reported that when introduced to a novel environment grid cells increase in their scale, and decrease in their firing regularity. Over time, with increasing exposure, the grid cell firing will return to firing similar to that of a more familiar environment. Changes in environmental geometry have also been shown to affect the scaling and spatial symmetry of grid cell firing Krupic et al. (2015).

Together, these results indicate that grid cells may be important for encoding distance and path integration, but that they are unable to provide a universal spatial metric as they are easily distorted by changes in geometry and novelty of environments.

1.3.4 Boundary cells

Based upon the findings of O'Keefe & Burgess (1996), which showed the elongation of place fields upon the extension of environmental walls, a subset of boundary sensitive neurons were predicted (O'Keefe & Burgess, 1996, Burgess, Jackson, Hartley, & O'Keefe, 2000; Hartley, Burgess, Lever, Cacucci, & O'Keefe, 2000). These hypothesised neurons, known as boundary vector cells (BVC), were predicted to be active whenever the animal was oriented at a specific direction and from a specific distance. The firing location of place cells is thought to be informed by the summation of BVC's synapsing onto a given place cell.

Boundary-sensitive neurons have since been found in both the subiculum (Lever et al., 2009) and MEC (Solstad et al., 2008). Solstad et al. (2008) reported that entorhinal 'border' cells exhibited firing along at least one, but sometimes more, environmental borders. For cells to be defined as border cells, the length of their firing fields was required to run tightly alongside at least one border (Fig 1.9). This is somewhat different from the predicted firing behaviour of BVC's, in which the length of a firing field of a BVC was predicted to run parallel to at least one border, but could be located at some distance from the borders of the environment. However, some of the cells recorded from the MEC, but that were not classified as border cells, exhibited BVC-like firing away from the edges of the environment. To date, the presence of border-like cells ahs not been reported in non-rodent species.

Lever et al. (2009) reported the presence of BVC's in the subiculum. Unlike MEC border cells, none of the recorded subiculum BVC's were responsive to all borders of the environment. Moreover, BVC's maintained boundary-sensitive firing after the removal of environmental walls, while MEC border cells did not.

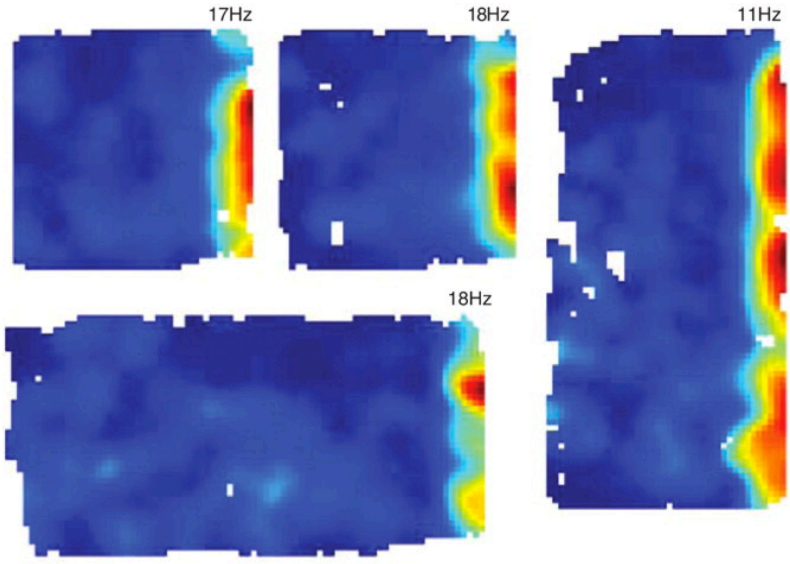


Figure 1.9 Border cell recorded from layer two of MEC. Firing rate maps of the same cell are shown in four different environments recorded within the same experimental room. Note that the cell fires along the East wall of each environment. Image taken from Solstad et al. (2009)

Despite the apparent differences between border cells and boundary vector cells, the discovery of boundary sensitive cells in two anatomically distinct regions of the hippocampal formation suggests that the encoding of boundaries is important for the function of the cognitive map. More specifically, they provide a boundary-determined reference frame to anchor the firing of place and grid cells within a local environment.

1.4 Summary

Several decades of research have revealed that animals hold an internal representation of space, which they use to aid their navigation throughout the world around them. The ability of animals to form short and long-term memories in two-dimensional planar spaces has been well established, and advances in electrophysiological techniques have revealed several subsets of neurons that are thought to encode for position, direction and distance. These are the three major components required for successful and flexible navigation of space.

The majority of studies of navigation, both behavioural and electrophysiological, have been limited to relatively simple, two-dimensional, horizontally oriented environments. This, of course, is not representative of the world through which we and other animals navigate on a daily basis. The real world is vastly more complex, and many animals navigate regularly through three-dimensional space. Successful navigation in three-dimensional space might require a representation of height as well as horizontal displacement, and the representation of orientation might also need a component measuring elevation angle of the head as well as its azimuthal angle.

The next chapter will provide an overview of the research literature that has investigated navigation in three-dimensional environments; first detailing the additional computational issues that arise during movement in three-dimensional

space, followed by a review of behavioural and electrophysiological studies of navigation in three-dimensional environments.

Chapter 2 Navigation in three-dimensional environments

As discussed in the previous chapter, the study of navigation has provided considerable insight into how animals navigate in horizontal planar environments. In particular, this work has shown that animals can hold long-term representations of location and how animals use cues to guide these representations. However, the real world is three-dimensional and animals are often required to move in the vertical as well as the horizontal plane. Movement in the vertical plane not only requires additional energy costs to move against gravity, but it also adds a greater deal of complexity to the computational issue of representing space. This chapter will first detail the potential issues that arise when navigating in a three-dimensional environment, followed by a review of the behavioural studies of three-dimensional navigation. The subsequent sections will first introduce several predictions of place, grid and head direction cell firing in three-dimensional environments, followed by a review of the few investigations that have studied these cells in such environments.

2.1 Moving in three-dimensional space

The computational problem of navigating accurately gains considerable complexity when we consider navigation through three-dimensional as opposed to two-dimensional environments. While the position of an animal in a two-dimensional environment can be described in Cartesian co-ordinates by its location relative to two perpendicular axes (usually x and y axes), its position in three-dimensional space is described by its location relative to three perpendicular axes; x and y to describe the animal's horizontal displacement, and the z axis to describe the animal's vertical position. Even in species whose movement is primarily anchored to surfaces, much of their navigable space can be considered to be in three dimensions. Consider a common squirrel climbing up a tree trunk to its nest; it

needs to know the height as well as the horizontal displacement of its goal location. Or, consider a surface-travelling animal navigating over an undulating terrain – the absolute distance travelled by the animal relative to the surface is different from the distance travelled as the crow flies. This discrepancy, introduced due to the additional height component of an undulating terrain, could lead to navigational errors if left unaccounted for.

Another vital metric for navigation is the understanding of one's heading direction. Again, this can be described simply during navigation of horizontally aligned environments (the animal is facing East, or 90 degrees relative to a pre-defined direction). The picture becomes more complex when we consider heading direction in three-dimensional space, wherein the orientation of an animal must be described by a displacement vector relative to the horizontal (azimuth angle) and the vertical (elevation angle).

One way to describe the rotations carried out by an animal, or indeed any rigid body, uses egocentric terms – that is, a description of rotations relative to the body axes of the animal (Fig 2.1). These terms, known as Bryan angles (Fraiture, 2013), are yaw, pitch and roll rotations. With respect to heading direction, yaw rotations are described as rotations of the head around the dorso-ventral axis of the head, pitch rotations are rotations around the inter-aural (transverse) axis of the head, and roll rotations are around the antero-posterior axis of the head. Herein, these terms will be used only to describe the egocentric rotations of the head.

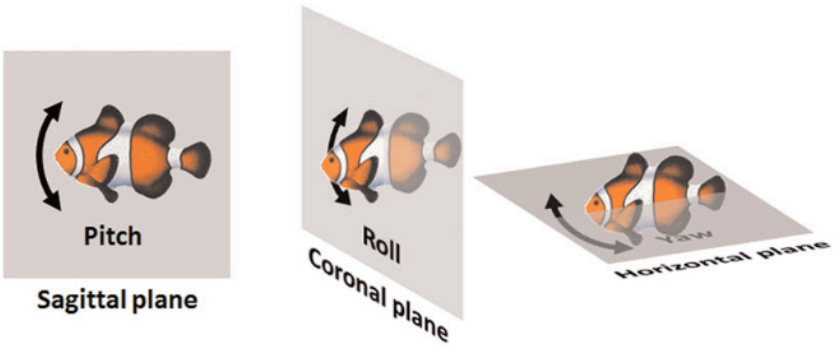


Figure 2.1 Terminology for the three Bryan rotations. Left – pitch rotations are carried out in the sagittal plane around the medio-lateral axis of the head. Centre – roll rotation are carried out in the coronal plane around the antero-posterior axis of the head. Right – yaw rotations are carried out in the horizontal plane around the dorso-ventral axis of the head. Image from Jeffery et al. (2013).

One important characteristic of these three types of rotations is that they are order-dependent (non-commutative) – this occurs because any given rotation redefines the allocentric axes from which any subsequent egocentric rotation occurs. For example, if an animal, whose starting position is with an East-facing heading direction with 0 degrees of roll or pitch, carries out a 90 degree roll rotation to the left followed by a 90 degree upwards pitch rotation and a 90 degree clockwise yaw rotation, the animal's head will be pointed straight upwards, with the underside of the head pointing to the East. Now, if we change this sequence, the animal (from the same starting position) carries out a 90 degree upwards pitch rotation followed by a 90-degree clockwise yaw rotation and then a roll to the left. The final heading direction of the animal is South, with 0 degrees of pitch and 0 degrees roll. Despite the fact that the animal carried out the same rotations, the different order of these rotations results in a different final heading direction (Fig 2.2). This has very important implications when we consider navigation through three-dimensional space, and any neural system that may encode for heading direction may need to account for such issues in order to maintain a stable representation of three-dimensional space.

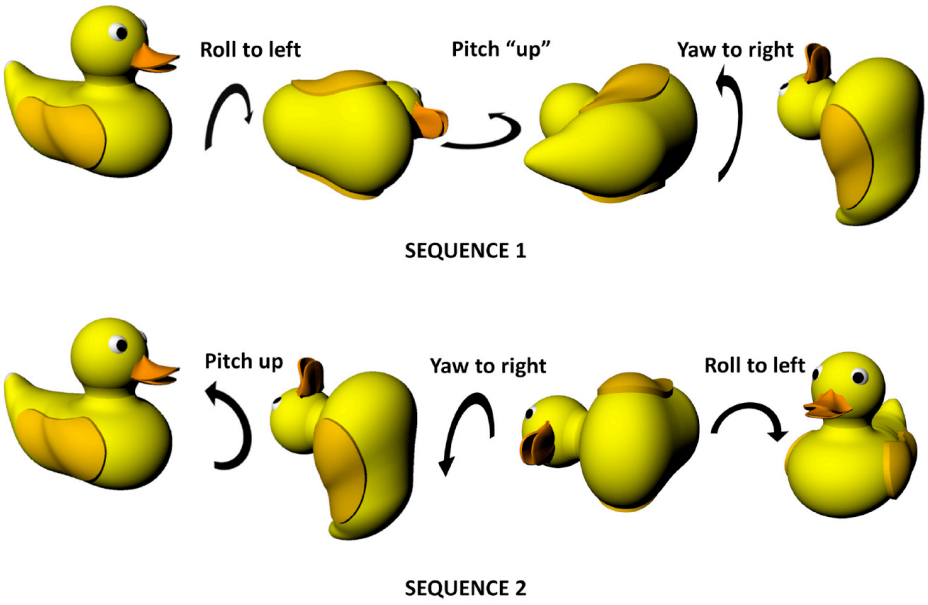


Figure 2.2 Non-commutativity of egocentric rotations. The two sequences displayed show a rubber duck carrying out the same egocentric rotations (90° roll to the left, 90° upwards pitch and a 90° yaw to the right) in two different orders. Note that even though the rubber duck has the same starting orientation and carries out the same rotations, the different order in which those rotations are carried out results in a different final orientation. Image taken from Jeffery et al. (2015).

2.2 Behavioural studies of three-dimensional navigation

The ability to process and represent three-dimensional space has been studied in a range of animal species, from surface bound animals such as rodents and humans to animals that can freely move through volumetric space, such as birds, bats and fish. This section will describe the behavioural studies of navigation in three-dimensional environments across different species, with a focus on differing navigational strategies between species and task types, and the ability to assess and use information about the vertical axis during navigation. The studies presented below will provide a context to the questions addressed in the behavioural experiments presented in Chapter 4.

2.2.1 Rodents

Grobety & Schenk (1992a) were the first to study navigation in non-horizontal environments. Initially, rats were trained on a place-learning task on either a horizontal, 45° tilted or vertically oriented plane, with the rats required to navigate from a starting location to a single rewarded goal location. The task latency and path lengths of animals were compared between the three mazes across the eight days of trials, and also for transfer tests in which the food reward was removed from the maze. In the transfer tests, the proximity of their searching locations to the original goal location was compared between the three different maze orientations. Rats successfully learnt the place task on all three mazes, albeit with a greater task latency on the vertical maze than on the tilted or horizontal mazes. The path lengths of animals were also greater on the vertical maze than on the other two mazes. Analysis of the two transfer tests, in which the reward was removed from the goal location, somewhat surprisingly showed that a greater percentage of movements in the vertical and tilted mazes occurred in close proximity to the goal location than in the horizontal maze. It was only in a second transfer test that rats in the horizontal maze were able to perform at the same level as rats trained on the other two apparati.

In a second experiment, Grobety & Schenk (1992a) studied the place learning of rats in a lattice maze. This maze consisted of 512 elementary cubes, which were arranged in an 8x8x8 structure. As in the previous mazes, rats were able to locate the position of the goal reward, suggesting an ability to encode position in the vertical as well as the horizontal plane. In the first transfer test rats spent the majority of their time in the correct horizontal plane of the goal location (i.e. at the correct height), but did not locate the correct horizontal position of the goal location. On the second transfer test rats showed an improved ability to locate the correct horizontal position of the goal, and continued to spend the majority of their search time in the correct horizontal plane, or the plane directly adjacent to the correct horizontal plane. The authors suggested that the results of these

experiments show first, that rats can recall positions in three dimensional space, and second, that they are likely to do this in a two-step process, with the vertical location encoded/recalled before the horizontal location.

Foraging and detour experiments using rats have found contradictory results to those reported by Grobety & Schenk (1992a). Jovalekic et al. (2011) studied the behaviour of animals as they searched for food rewards on two mazes, a 4x4x4 version of the lattice maze and a vertically oriented pegboard maze (Fig 2.3). During foraging experiments rats were required to retrieve pseudo-randomly positioned food rewards (equal numbers of rewards for each horizontal layer of the mazes) on the lattice and pegboard mazes. These experiments found that rats exhibited a strong layer-by-layer preference, in which they carried out fewer movements between different layers than they did within the same layer, indicating an aversion to vertical movements.

In the detour experiment rats were first trained to move towards a goal location before returning to their starting location. Once training was complete, a barrier was positioned to block the most direct route between the goals, forcing the animals to carry out a detour to move between the start and goal locations. Rats exhibited a strong preference for moving to the correct horizontal location before moving vertically to the goal location. This was true of both the outbound path to the goal location, and the inbound path back to the starting location.

Together with the results of Grobety & Schenk (1992a), these studies show that rats have the ability to recall the locations of single rewards in three-dimensional environments, but that any preference for movement in one dimension over the other appears to be task-dependent. During foraging, where reward locations are not known, rats prefer to search for food on a layer-by-layer basis. It is likely that this is a combined effect of uncertainty of reward locations alongside the added cost of moving against gravity.

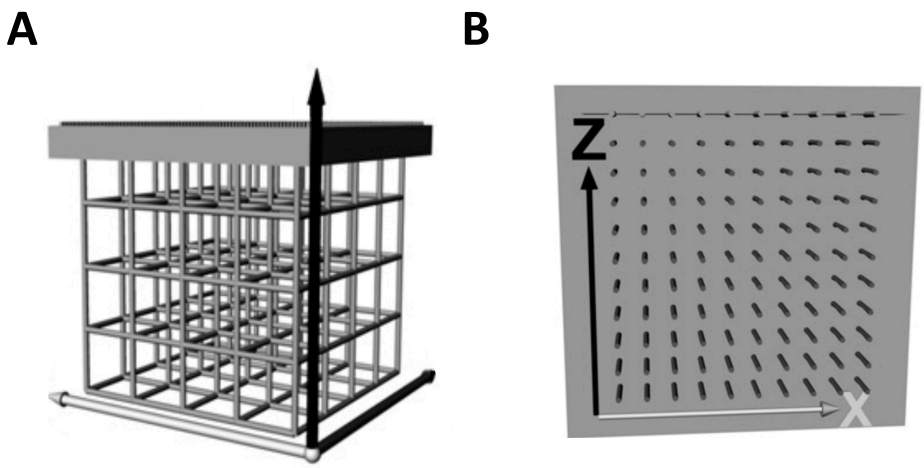


Figure 2.3 Apparatus used in Jovalekic et al. (2011). A) Lattice maze B) Pegboard maze. (Images from Jovalekic et al., 2011).

Rats have also been shown to use information about the slope of environments to cue their behavioural responses. Grobety & Schenk (Grobéty & Schenk, 1992b) studied the responses of rats in three different versions of the radial arm maze. In this study, rats were either trained on a classic eight-arm version of the radial arm maze, or on two irregularly structured eight-arm mazes: either a maze with arms tilted at different angles ("varying-tilt maze"; Fig 2.4), or one with differing angular distances between each arm ("varying-angle maze"). It was hypothesised that the irregularities of each of these mazes could provide cues to increase working memory accuracy. Animals were either trained in a free-choice paradigm, or in a forced-choice paradigm. In the forced-choice paradigm, rats were initially only given access to four of the eight arms before being removed from the maze for one minute, during which doors were removed from the other four arms. Upon return to the maze, rats were required to visit the four previously unvisited arms. In the variable-angle maze there were significantly more errors (visits to previously visited arms) than in the classic maze or variable-tilt maze in both the free-choice and forced-choice paradigms. In fact, only half of the animals on the varying-angle maze reached performance criterion within 18 days of training on the forced-choice trials. Comparison of the forced-choice trials between the other two mazes revealed that animals reached criterion performance levels significantly sooner on the variable-tilt

maze than the classic maze, indicating that the differential tilt of arms may act as a cue for encoding and recalling information about previously visited arms.

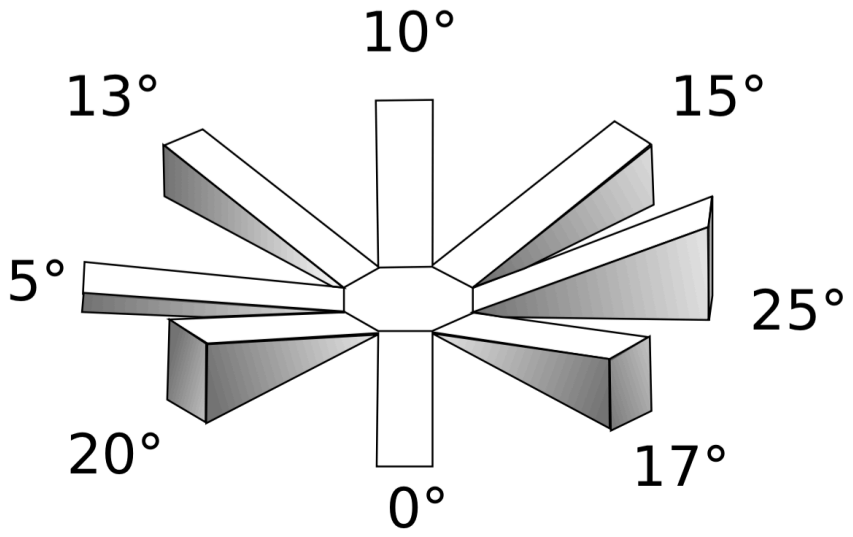


Figure 2.4 Varying tilt radial arm maze used in Grobety & Schenk (1992b). Image adapted from Grobety & Schenk (1992b).

Further evidence supporting the use of terrain slope in navigation was shown by Moghaddam et al. (1996). The researchers tested the ability of rats to reach a feeder placed at the apex of a cone in dark conditions. The apex of the cone was set at different heights (0, 1, 2 and 4cm) therefore providing different increment angles for each condition. The authors found that the rats displayed lower task latencies and shorter path lengths to the feeder at higher cone increment angles (the 2 and 4cm conditions) than during the flat or 1cm condition. Rats were therefore considered to have used the vestibular and proprioceptive information provided by the cone slope to guide their behaviour to the goal location at the apex of the cone.

2.2.2 Fish

Place learning studies in fish have also revealed differences in how fish use the vertical and horizontal planes. Holbrook & Burt de Perera (2009) studied the place learning of sighted banded tetra (*Astyanax* fasciatus) on a Y-maze (Fig. 2.5). The Y-maze could either be aligned with the two potential goal locations in the same vertical plane or in the same horizontal plane. The fish were required to move to a goal arm to retrieve a food reward, with a choice of moving upwards or downwards in the vertical condition, or left or right in the horizontal condition. The accuracy of responses was comparable between the vertical and horizontal conditions.

In a second experiment, the authors oriented the Y-maze at 45 degrees, with the rewarded location either upwards and left or downwards and right. Fish were shown to be equally able to learn the positions of rewards as in the first experiment. Following training, fish were tested in probe trials in which the Y-maze was reoriented either in the horizontal position or the vertical position. In the horizontal probe, fish accurately selected the horizontal direction in which they had previously been rewarded, while the fish could not accurately determine the previously rewarded vertical component in the vertical probe. In a third experiment the maze was at first aligned at 45 degrees (for example with the arms facing upwards and left, and downwards and right) before being rotated by 90 degrees, with the arms now in conflict with the previously learnt arrangement (the two arms facing down and left, and up and right). During these conflict trials fish preferred to move to the vertically consistent arm, rather than the horizontally consistent arm. In a follow-up study, Holbrook & Burt de Perera (2011) used the conflict experiment in an environment with several salient horizontal and vertical cues. Even with the presence of salient visual landmarks in the horizontal plane, fish still preferred to use the previously learnt vertical component over the horizontal component. Another interesting finding of this follow-up study was that the presence of visual landmarks improved the speed of response by fish, indicating that fish, like rats, are able to integrate visual landmarks to aid their navigation.

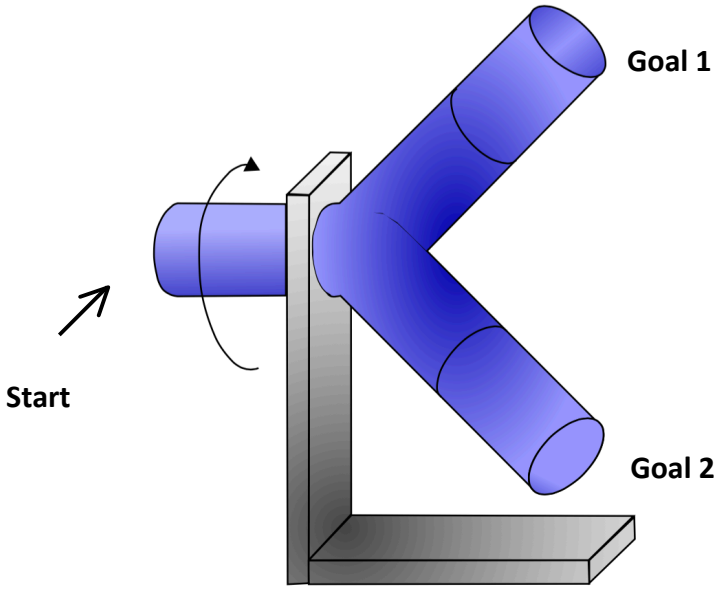


Figure 2.5 Y-maze used in Holbrook & Burt de Perera (2009; 2011). The horizontal and vertical position of goals could be altered through rotation of the Y-apparatus. Image adapted from Holbrook & Burt de Perera (2009).

Similar to the findings of Grobety & Schenk (1992a), these studies indicate that fish may separately encode vertical and horizontal components of space, with a preference for movement in the vertical plane. In fish, this may be due to the additional cues provided by hydrostatic pressure or even through a pressure sensing mechanism provided by the swim bladder (Taylor, Holbrook, & Perera, 2010)

2.2.3 Birds

Birds, like fish, are able to move freely in volumetric space. With this ability comes the potential that they may be equally able to represent the vertical and horizontal planes. Healy & Hurly (1995) initially reported that hummingbirds are able to hold a working memory representation of rewarded locations in a circular array. This study holds many similarities with rodent studies using the radial arm maze. Eight flowers were arranged in a circular array, each of which contained a vial of sucrose. Birds were either given a free choice or forced choice to visit four of the eight flowers. They were then removed from the apparatus for up to an hour. On their return the

birds exhibited an above-chance preference for visiting the four flowers they had not previously visited, indicating a memory for the locations of the previously rewarded flowers.

Following this study, Henderson, Hurly & Healy (2001) carried out a similar experiment with flowers arranged at two different heights. The birds exhibited a strong preference for visiting higher flowers. On the return trials, after the initial free or forced choice trials, the birds were able to identify and visit the flowers they had not previously visited. Their performance on the 3D apparatus was better than on the 2D arrangement described above, indicating that the birds were able to use the height information of the flowers to improve their differentiation of different flowers.

In another study, Flores-Abreu, Hurly & Healy (2013) compared the performance of hummingbirds on three different linear arrays of flowers. These arrays were oriented horizontally, vertically or diagonally (containing both horizontal and vertical components). The authors reported that hummingbirds were able to locate the position of a rewarded flower in the horizontal but not vertical array. Birds trained on the diagonal array were tested with the array rearranged into a conflicting diagonal orientation, in which birds could visit the flowers at either the correct horizontal or correct vertical position in which they were trained. Unlike fish, hummingbirds exhibited a preference for visiting the correct horizontal position over the correct vertical position of flower.

More recently, Flores-Abreu, Hurly, Ainge & Healy (2014) compared the place learning of hummingbirds in a lattice maze with that of rats. As reported by Grobety & Schenk (1992a), rats were found to move more in the z-axis than they did in the x or y axes. Hummingbirds, however, exhibited no preference for movement in any direction. In probe trials, in which a previously rewarded location was found empty, hummingbirds were found to move horizontally in search of a reward, while rats were found to move vertically. Again, this indicates that hummingbirds exhibit a

preference for moving horizontally, perhaps due to a greater ability for encoding position in the horizontal than the vertical plane.

2.2.4 Humans

Studies of human navigation have also revealed that the addition of verticality information to an environment changes the navigational strategies that people use. Similar to the studies discussed in previous sections, studies of humans have tested how terrain slope affects navigation and whether people exhibit preferences in movement in the vertical or horizontal planes during tasks set in multi-layered environments. This section will first discuss the way humans perceive and use geographical slant information to guide navigation, followed by a discussion of the navigation strategies used by humans in multi-layer environments.

Humans have been shown to overestimate the geographical slant angle of terrain slopes (Kammann, 1967; Proffitt, Bhalla, Gossweiler, & Midgett, 1995; Creem & Proffitt, 1998). Geographical slant is defined as the angle of a slope relative to a fixed environmental frame – most usually the earth horizontal plane (Proffitt et al., 1995). Kamman (1967) found that when asked to judge the geographical slant angle from the top of a 34° hill, males estimated the hill to be 48° and females estimated the hill to be 55°.

In a further series of experiments, Proffitt et al. (1995) showed that the overestimation of hill slope occurred whether participants stood at the top of a hill looking down the slope, or at the base of the hill looking towards the slope. In their experiments, verbal and visual estimations of a 15° hill was reported to be on average 30°, and a 30° hill slope was reported by participants to be 60°. Interestingly, the authors also carried out a haptic measure for hill slope judgement, in which participants, without looking at their hand, were asked to tilt a board to their estimated geographical slant. These measures were shown to be highly accurate, with the estimated geographical slant to be consistent with the actual slant angle of the hill, irrespective of whether the participant stood at the top or the

base of hill. Creem & Proffitt (1998) extended these studies by testing participants' memory for geographical slant after the initial perception tests. After only one day, verbal reports of geographical slant showed an increase in estimated slant angle, while there was no change in the haptic estimation for geographical slant.

Despite these overestimations of geographical slant, the studies discussed above also show that humans are at least able to perceive the difference in slopes. These findings led researchers (Restat, Steck, Mochnatzki, & Mallot, 2004; Steck, Mochnatzki, & Mallot, 2003) to investigate the practical implications of slope judgement during navigation. In these studies participants navigated a virtual reality town called Hexatown (Fig. 2.6) in either a flat condition, or one of two sloped conditions in which the angle of the town was tilted at 4° or 7°. Several navigational tasks, including place-finding and place-pointing tasks, showed that participants exhibited fewer errors in the sloped conditions than the flat condition. For example, in a task in which participants had to return from a novel location to a previously learnt goal condition, participants on the sloped tasks made fewer moves away from the intended goal location than on the flat task (Restat, Steck, Mochnatzki, & Mallot, 2004).

These results, like the studies of rodents on the variable-tilt maze (Grobety & Schenk 1992b) and the cone maze (Moghaddam et al., 1996) show that terrain slope can be important in guiding navigation. However, studies of slant orientation and the importance of geometric cues indicate that while geographical slant can provide guiding information, when the slant angle is put into conflict with external geometric cues participants tend to rely more on geometric properties of an environment than the terrain slope of an environment (Kelly, 2011).

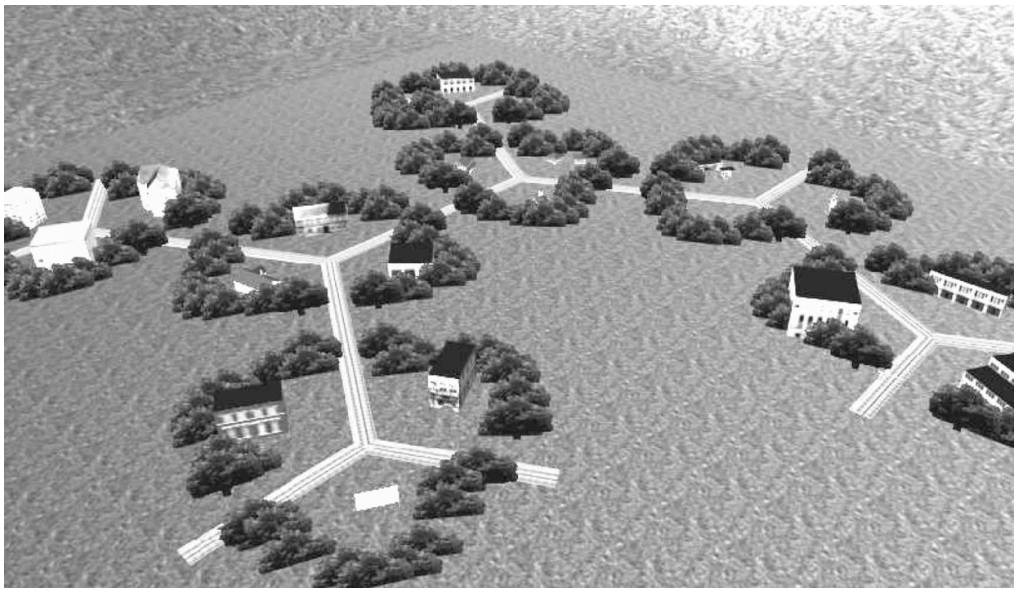


Figure 2.6 Hexatown. An overhead view showing the layout and junctions of hexatown used in Steck et al. (2003) and Restat et al. (2004). Image from Restat et al. (2004).

As well as the studies of the effects of slope orientation on navigation, researchers have also tested humans' ability to navigate multi-layered environments. These studies, like those of Grobety & Schenk (1992a) and Jovalekic et al. (2011) examined whether participants exhibited preferences for movements in either the vertical plane.

Buechner (2007) investigated the path-choice heuristics of participants asked to navigate to goal locations in a 3x3 grid. There were three goal locations on each of three floors, with each of the floors accessible by three equidistant staircases. Participants spontaneously divided the building into regions inherent in its structure, with some participants grouping the position of landmarks into three columns representing the staircase of the building or into three rows representing the three floors of the building. In goal-search tasks, in which participants navigated to the position of one of the nine goal locations, those participants that represented the building on a floor-by-floor basis chose to move to the correct vertical position of the goal prior to movement to the correct horizontal locations. Those

participants that represented the building on a column-by-column basis did the opposite, preferring horizontal movements to vertical movements.

Hölscher et al. (2006) also tested participants' navigational strategies during a place-finding task in a multi-level building. In this experiment, the building use was more complex than that used by Buechner (2007), with some goal locations only accessible by specific staircases, and with different floors having a different layout of rooms and corridors. The authors found that participants familiar with the building more commonly used a floor-by-floor strategy to reach goals while participants less familiar with the building tended to use well-known parts of the buildings such as main connecting corridors to find their way, even if this resulted in considerable detours from the optimum path. Together, these findings show that humans regionalise multi-level buildings based on salient aspects of the building such as the position of staircases into specific floors, and use these representations to determine whether to move in a floor-by-floor or column-by-column manner. They also show that the complexity of the building can lead to other navigational strategies such as using familiar locations as guiding points to subsequent movements. Similar to the reports of rodent navigation in the lattice maze (Grobety & Schenk 1992a; Jovalekic et al., 2011), the strategies humans use to navigate multi-level environments can differ depending on the demands of the task at hand.

2.2.5 Summary

Although the behavioural research of navigation in three-dimensional environments is relatively scarce, it has revealed two key themes in the way that animals use and represent 3D space. First, several different species have been shown to have preferences for movement in one plane over the other, whether they are able to move freely in volumetric space or not. Interestingly, the particular direction of this preference differs between species (e.g. fish preferring vertical movement and hummingbirds showing a preference for encoding horizontal space) and also differs within species depending on task demands (e.g. foraging vs place learning in rats).

The second, and perhaps most important, theme of these investigations is that animals are able use information about the vertical position of rewards or the elevation angle of an environment to guide their behaviour.

One aspect of three-dimensional navigation that has not been thoroughly investigated is the ability of animals to hold working or reference memories of locations distributed throughout three-dimensional space, and whether this differs to memory of two-dimensional space. Studies using the radial arm maze (discussed in the first chapter) reveal that rodents are able to encode and utilise such memories in two-dimensional environments, but it is an open question whether they are able to do this in a three-dimensional environment.

On the radial arm maze, the working memory task tests the ability to integrate incoming information (such as current position) with the short-term memory of previous events (which locations have already been visited), and then to utilise this information to reduce the number of visits to previously visited (and now unrewarded) arms. The reference memory task tests the ability to hold a long-term memory for the positions of rewarded arms with relation to extramaze cues. Successful completion of either of these tasks requires the animal to know both its own position (present and past) on the maze and the position of rewarded arms relative to the position of external cues. They next need to implement this knowledge to guide behaviour towards the rewarded arm – constantly updating their position and orientation. Using these tasks on a three-dimensional radial arm maze can therefore provide insight into the ability of animals to represent three-dimensional space.

An inability to perform either of these tasks successfully on a three-dimensional radial maze would indicate an inability of animals to accurately encode or recall locations distributed throughout three-dimensional space, while accurate performance on these tasks would indicate that animals possess the cognitive machinery to navigate effectively in a three-dimensional world. The experiments

presented in Chapter 4 therefore ask whether mice can learn both the working and reference memory tasks on a three-dimensional radial arm maze, and whether learning on this maze differs from learning on two two-dimensional radial arm mazes.

2.3 Neural correlates of three-dimensional spatial cognition

When considering the potential for encoding of three-dimensional space by place, grid and head direction cells, we must first take into account how animals move through such space. While the movement of many animals is constrained to movement on planar surfaces (e.g. humans and rodents etc.), there is also a wide range of species able to move freely throughout volumetric space (e.g. fish, birds, flies, bats etc.). This section first introduces the potential mechanisms of encoding three-dimensional space, and will discuss a variety of hypothetical outcomes for: (1) horizontal-only encoding (2) surface-defined planar mapping in both horizontal and vertical planes and (3) systems encoding volumetric space. Following this, the recent electrophysiological studies of three-dimensional spatial encoding will be discussed.

2.3.1 Theoretical considerations

This section will provide an overview of potential types of neural encoding of three-dimensional space by place, grid and head direction cells. More specifically, the firing characteristics of each cell type are discussed for instances in which the spatial map is a purely two-dimensional (horizontal-planar) map, a surface mapping (multi-planar) map, or a volumetric representation. As well as these predictions, potential computational issues with encoding direction in 3D space are introduced. Many of the predictions presented below have previously been suggested by a range of authors (Jeffery, Wilson, Casali, & Hayman, 2015; Jeffery, Jovalekic, Verriotis, & Hayman, 2013; Taube & Shinder, 2013; Ulanovsky, 2011).

2.3.1.1 Place cells

Place cell activity, as mentioned earlier, is defined by a localised peak of firing activity in a specific location within an environment (Fig. 2.7). In horizontal environments the location of this peak firing is known as a place field.

If the cognitive map is purely two-dimensional and only encodes for position on horizontal planar surfaces, there are two ways in which place cells might fire during locomotion in volumetric space or on a vertically oriented planar surface (Jeffery et al., 2015). Either place fields will be constrained to firing only on the floor of an environment (Fig 2.7A), or they will continue firing along the vertical axis, but with no modulation of cell firing rate by height – in this case, a place field would appear to be columnar (Fig 2.7B). For each of these examples, place cells would not encode for position along the vertical axis, and would thus provide no information about height.

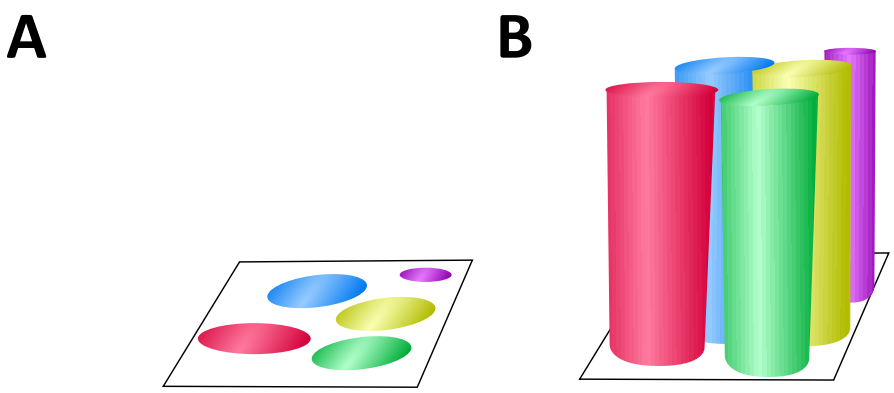


Figure 2.7 Horizontal encoding of position. (A) Place cells fields may be defined purely by the horizontal surface, and not extend their firing in the z-axis. (B) Place cells may extend their firing in the z-axis but without height modulation. Neither of these outcomes would allow for the encoding of height.

Another alternative is that place cells are modulated by height, but that they still require a planar surface to define the position of firing (Taube & Shinder, 2013; Ulanvosky; 2011). This can be thought of as surface encoding, in which there is

localised place cell firing in the animal's plane of locomotion (Fig. 2.8). In this case, an animal climbing on a vertical plane would exhibit a place field at a specific height and horizontal position (e.g. a position defined by x-z or y-z co-ordinates). It should also be noted that a surface encoding system might not necessarily be able to encode for height at the same resolution as horizontal axes. It is possible that height is more weakly modulated than horizontal position, which would result in an elongation of a place field in the z-axis compared to the x or y axes. Within such a system, place cells might repeat their relative position on both horizontal and vertical planes (i.e. a similar distance from the left boundary of the planes), essentially treating the horizontal and vertical surfaces as repeating entities; or the firing of a given place cell could be in different relative locations for each plane.

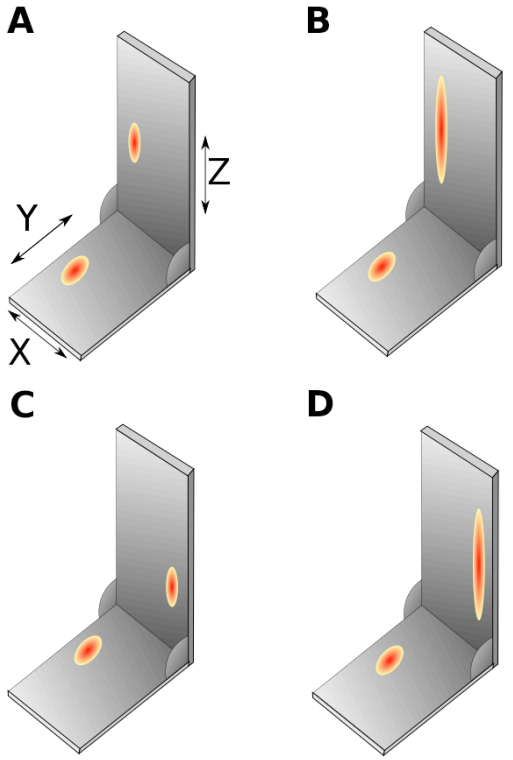


Figure 2.8 Surface-mapping of place fields. Expected firing pattern of place cells encoding for position relative to the plane of locomotion with repeating locations of place fields (A-B) or distinct firing locations between planes (C-D). Fields in A and C have the same resolution in the vertical plane as the horizontal plane. Fields in B and D are more strongly modulated by horizontal position (narrower field) than height (broader field).

A second potential effect of a surface-encoding system is that not all place cells necessarily fire in both vertical and horizontal planes. Within a population of place cells there may be some cells that are active only in the horizontal plane, some active in only the vertical plane, and some active in both planes. Again, those cells that are active in the vertical plane might not necessarily represent height with the same resolution as they represent position in the horizontal plane (Fig 2.9).

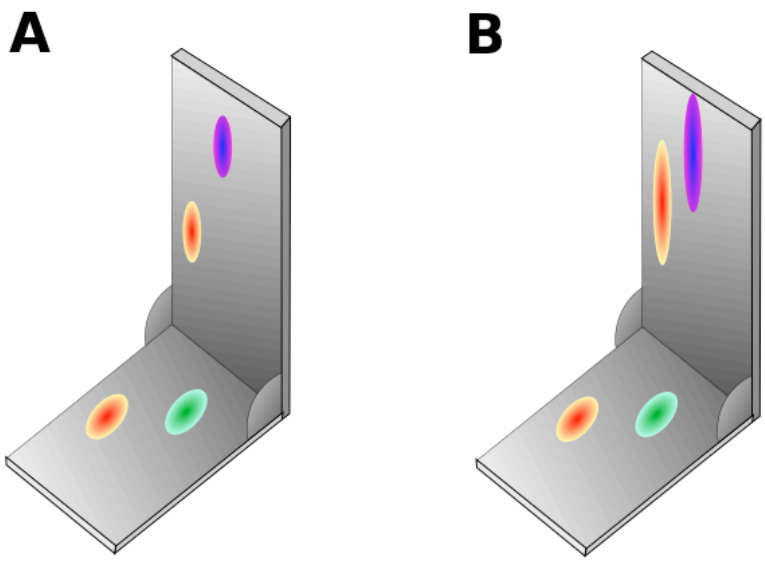


Figure 2.9 Population encoding of surface mapping place cells. A given place cell might fire on both planes (red), just the horizontal plane (green) or just the vertical plane (purple). A) Place cells show same resolution of encoding in both planes B) Place cells that do fire on the vertical plane show weaker encoding of height than horizontal position.

Finally, place cells may be able to encode for position in volumetric space (Jeffery et al., 2015; Yartsev et al., 2013). While this is particularly difficult to test in surface travelling animals, there have been recent advances in recording place cells in bats (discussed in the next section). If volumetric position can be encoded by place cells, one would expect their place fields to be three-dimensional, with modulation of firing in the x, y and z axes. In a system that encodes height at the same resolution as horizontal position, one would expect place fields to be spherical. Alternatively, it is possible for place cells to be modulated more weakly in one axis (e.g. height) than the other axes. For such a system, one would expect

place fields to have an anisotropy, in which they are elongated in the vertical plane compared to the horizontal plane (Fig 2.10).

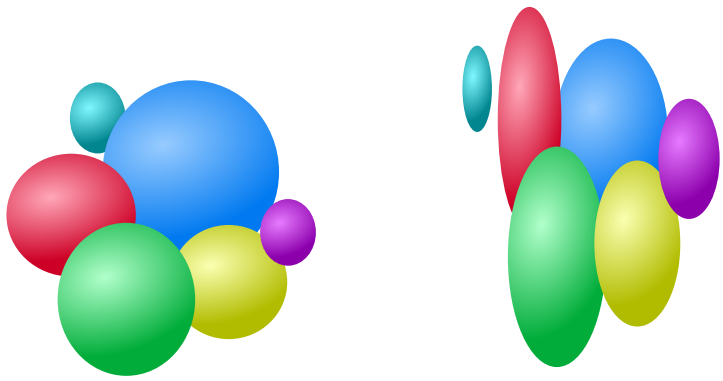


Figure 2.10 Volumetric encoding of position by place cells. A) Population of place cells encoding for volumetric position with same resolution in vertical and horizontal planes. B) Population of volumetric place cells encoding the vertical plane at a lower resolution than the horizontal plane.

2.3.1.2 Grid cells

While place cells are characterised by a single localised firing field, grid cells are defined by the hexagonal pattern of multiple firing fields in the horizontal plane. The potential firing characteristics of grid cells in three-dimensional space can be thought of along the same lines as place cells.

If the cognitive map is purely two-dimensional, we expect grid cells to fire in their characteristic grid like pattern on a horizontal plane, with either no firing in the vertical plane (Fig 2.11A) or an extension of firing into the vertical plane that is not modulated by height (Fig 2.11B) – respectively, the firing of grid cells would appear to be either flat or columnar (Jeffery et al., 2015).

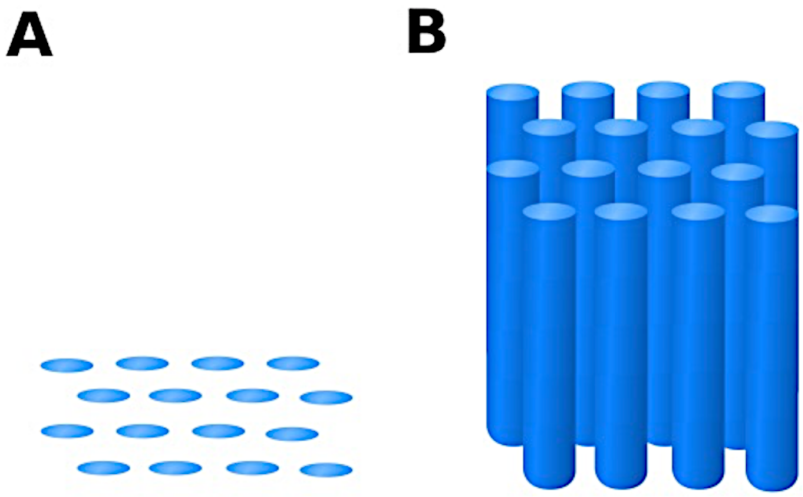


Figure 2.11 Horizontal encoding by grid cells. A) Firing pattern of a grid cell with no firing in the vertical plane. B) Firing pattern of a grid cell with firing extended into the vertical plane, but without modulation by height. Neither of these patterns are able to provide metric information for height.

According to a surface-encoding system, if animals move on a vertical plane, one might expect grid cells to maintain strong grid-like firing in that plane. With such a system, two-dimensional co-ordinates would still define grid cell firing, but the axes of those co-ordinates would lie in the animal's plane of locomotion. As with place cells, height may be encoded by grid cells at a lower resolution than their encoding of horizontal position. Such a system might therefore result in an extension of individual grid fields in the vertical plane, or a distortion of the grid structure in which field size and/or field spacing is different in the vertical plane than in the horizontal plane.

Finally, grid cells might represent volumetric space (Jeffery et al., 2015). In this case, grid cells might have multiple grid fields organised in a close-packed array throughout three-dimensional space – either isotropically (spherical fields) or anisotropically (ovoid fields; Fig. 2.12).

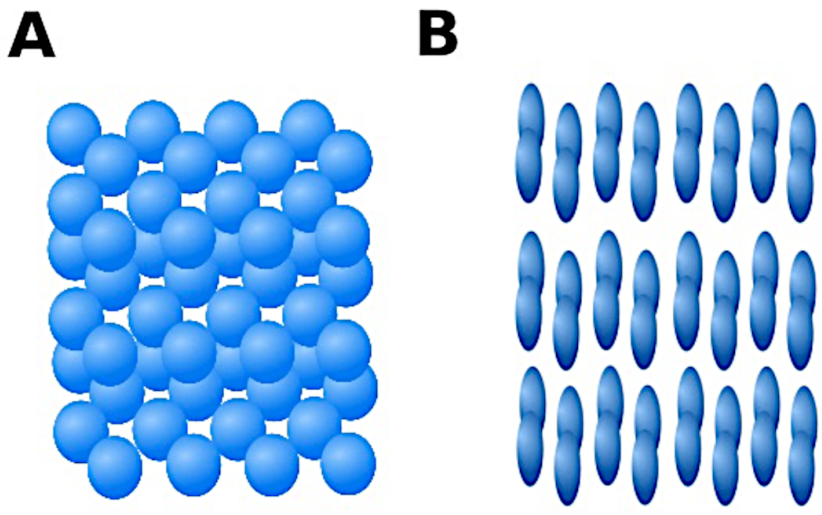


Figure 2.12 Volumetric encoding by grid cells. Expected firing pattern of grid cells firing in a A) close-packed array of sphere or B) close-packed array with anisotropic encoding of height and horizontal position.

2.3.1.3 Head direction cells

As discussed in the first chapter, defining one's orientation in three-dimensional space presents a number of unique computational problems. First, there are three types of egocentric head rotations to which HD cells may be sensitive. These are yaw rotations (rotations around the dorso-ventral axis of the head), pitch rotations (rotations around the inter-aural axis) and roll rotations (rotations around the antero-posterior axis). Second, a vector with x, y and z components is needed to describe allocentric orientation in three-dimensional space, with orientation in the x-y plane providing the animal's azimuth angle and the angle between the z axis and horizontal plane providing the elevation angle. Mapping orientation in volumetric space therefore requires two-components – the azimuth angle and elevation angle. A robust system for encoding three-dimensional orientation must therefore be able to integrate the three types of egocentric head rotation with allocentric head direction (defined as the pointing direction of the nose).

If we first consider the three types of egocentric rotations relative to allocentric heading as an animal moves on a horizontally oriented surface, we can see that a yaw rotation of the head changes heading direction in azimuth but not in elevation. An animal could carry out a full 360° yaw rotation without any change in the

elevation angle of it's heading direction. A pitch rotation results in a change in elevation angle, but does not affect azimuth heading direction until the head has rotated ± 90° from the starting horizontal position. Beyond ± 90° the animal becomes inverted, and the heading direction in the azimuth is rotated by 180 degrees. A roll rotation on the other hand does not result in a change in heading direction relative to the azimuth or elevation angle. This issue becomes more complex when we consider a series of different head-rotation types. As explained earlier in this chapter, the order of egocentric rotations defines the final allocentric heading direction, and the sequence of the same rotations, but in a different order, will result in different final allocentric headings.

If we now consider these rotations as an animal moves on a vertically oriented plane, the three different egocentric rotations have different effects on allocentric heading, and these effects are dependent upon the starting direction of the animal. For example, if the starting position of an animal on a vertical plane is with the animal's nose pointing upwards, a downwards pitch rotation would have no effect on the animal's heading in the azimuth until the animal rotates past 180°. A yaw rotation, however, would change the elevation angle and the azimuth angle of the head. A roll rotation would not change the azimuth or elevation angle of the head. On the other hand, if the starting position of the animal on the vertical plane is with the animal's nose pointing to the right of the plane; a yaw rotation would change the animal's allocentric heading direction relative to both azimuth and elevation angle, a pitch rotation would change the animal's heading direction relative to azimuth but not elevation angle, and a roll rotation would not change heading direction relative to either azimuth or elevation angle. These examples show that the degrees of freedom for rotation types, alongside the range of possibilities of rotation sequences, make determination of heading-direction in three-dimensional co-ordinates computationally difficult.

One potential mechanism for avoiding these difficulties is to maintain a purely horizontal two-dimensional cognitive map. In this case, head direction cells would

be defined purely by heading direction in the azimuth, and would only respond to yaw-rotations of the head (Fig 2.13). For this to be the case, HD cells would only be modulated by yaw-rotations during movement on horizontally oriented surfaces. Of course, while computationally simple, this would mean that heading direction could not be determined as animals move on sloped, or vertical surfaces, never mind through volumetric space.

A potential extension of a purely horizontal two-dimensional map would be a planar map that is defined by the animal's plane of locomotion, rather than by the earth-horizontal plane (as noted by Stackman et al., 2000). In this case, HD cells are expected to be informed purely by yaw rotations, but to anchor their reference frame to the plane of locomotion. For example, if an animal is moving on a vertical plane, HD cells would still be modulated by yaw rotations of the head, with the direction of firing being relative to the vertical plane, and not to the azimuth heading of the external environment (Fig 2.13). This would allow animals to maintain their orientation relative to a local reference frame, but would not allow animals to maintain a globally defined orientation.

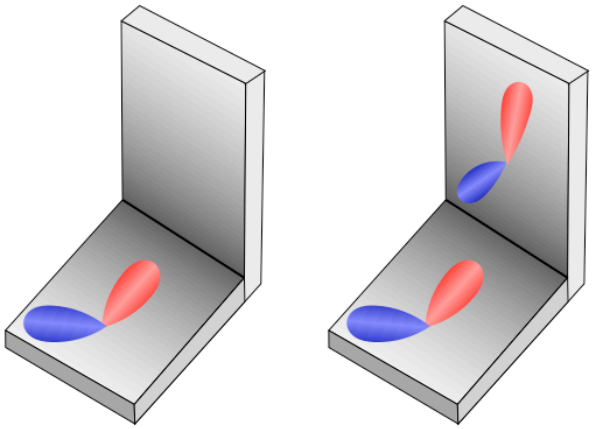


Figure 2.13 Planar representation of orientation. Expected firing of two HD cells (red and blue teardrops represent the polar tuning curves of the two cells) for instances in which the HD system used a purely horizontal reference frame (left) and when the HD cells defines its firing direction by the plane of locomotion (right). Note that for this example HD cells maintain the same plane-relative orientation after a transition from horizontal to the vertical plane.

If we add further complexity to the potential for a multi-planar map, we might expect HD cells to be anchored to an animal's plane of locomotion, but to also be modulated by the position of this plane in the azimuth (Fig 2.14). In this case, HD cells would still be modulated by yaw rotations on horizontal, sloped and vertical planes, but the direction of their firing relative to these planes would be further informed by the position of these planes relative to the external world. For example, if we imagine an HD cell firing upwards on one vertical plane (say, an East facing wall), it may fire in a different direction relative to a differently oriented vertical plane (i.e. downwards on a West-facing wall). Such a system would still essentially be two-dimensional, and anchored to a plane of locomotion, but it would further inform the animal of the position of any given plane relative to the external environment. The potential computational issue here, is that such a system would also require updating of HD cell activity by pitch and roll rotations.

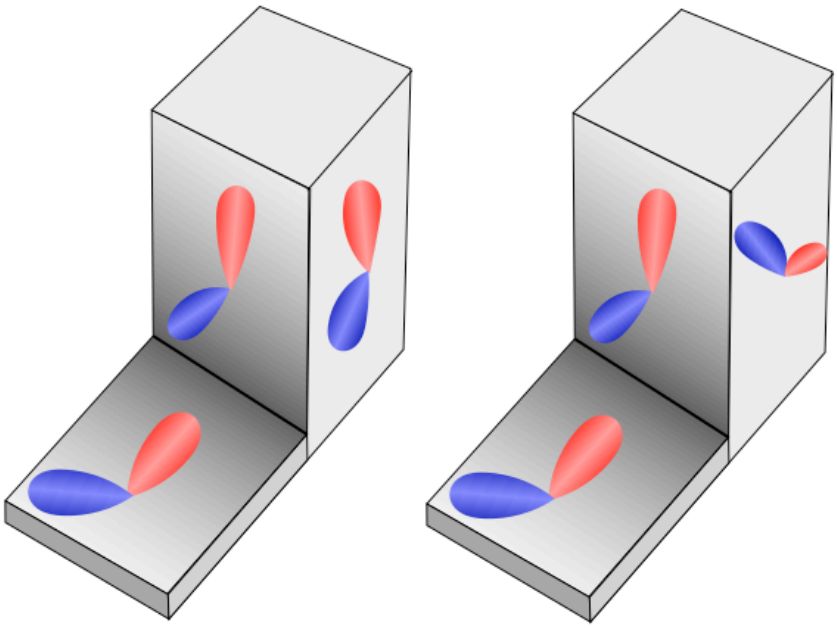


Figure 2.14 Representation of multi-planar spaces. In multi-planar space HD cells may use the local features of the plane of locomotion to orient themselves, and maintain the same direction of firing relative to each plane (left). On the other hand, they may have a multi-planar representation in which the relative position of each plane further informs the HD system. In this case, the firing of HD cells would be expected to rotate relative to the plane of locomotion between different vertically oriented planes (right). In each case, the coloured teardrops represent the polar tuning curves of two cells (red and blue) at three different planes of a three-dimensional structure – the horizontal plane, and two orthogonal vertical planes.

As well as these potential planar and multi-planar representations of orientation, there of course remains the possibility that HD cells are modulated by allocentric direction in all three-dimensions. In this case, the HD system would at the very least be required to contain cells modulated by pitch as well as yaw (Ulanovsky, 2011; Finkelstein et al., 2015; Geva-Sagiv, Las, Yovel, & Ulanovsky, 2015). Further to this, it is likely that such a system would need cells that are conjunctively modulated by both pitch and yaw (e.g. a cell firing when the animal's head faces upwards by 45° and 90° in the azimuth). Again, because of the non-commutative nature of head rotations, such a system would likely require roll-modulated cells, not to define volumetric allocentric heading, but to be integrated with the other rotation sensitive cells to prevent error accumulation after complex rotation sequences.

2.3.1.4 Summary

As is evident from the hypotheses laid out above, there is a wide range of predictions that can be made for the firing activity of place, grid and head direction cells in three-dimensional spaces, ranging from the computationally simple mechanisms in which the position and orientation are only encoded in horizontal two-dimensional environments, to more complex volumetrically-encoding systems that require the integration of a wider-range of inputs. The few studies that have investigated the activity of spatially correlated neurons in three-dimensional environments are presented in the next section.

2.3.2 Experimental findings

2.3.2.1 Place cells

Among the first electrophysiology studies of three-dimensional spatial cognition was Knierim & McNaughton's (2001) comparison of place cell firing between a horizontally oriented rectangular track and a track tilted at 45°. They first recorded place cells on the horizontally oriented track, before tilting the maze for a second session of recordings, which was then followed by a second flat session. Perhaps

unsurprisingly there was a high correlation of place field firing location between the two flat session recordings. The correlation of place field location was, however, significantly less between the tilted session and the flat sessions, indicating that rats were encoding the maze differently when it was tilted.

Of the place cells recorded, one third were able to maintain highly correlated firing between the tilted and flat sessions, indicating that at least a subset of the population of place cells recorded maintained the same firing irrespective of whether the maze was tilted or not. Perhaps, rather than indicating a difference in encoding the z-axis by place cells, these results describe a partial remapping of place cell firing which was driven by the modification of external cues available to the animals, or the introduction of the tilted session induced a contextual change the environment, in which the rectangular track once tilted was considered to be a new environment altogether.

Another experiment testing the effect of slope on place cell firing revealed that in the absence of all other cues the slope of a terrain could be used as a polarising cue to inform the location of place cell firing (Jeffery, Anand, & Anderson, 2006). Moreover, correlations of place field maps after reorientation of the maps relative to the room, intramaze cues or slope direction showed the highest correlation between training and probe test maps when the slope was oriented in the same direction for the correlation analysis. This indicates that the terrain slope provided the most powerful orienting cues for place cell firing.

Place cells have also been recorded in micro-gravity conditions. Knierim, McNaughton & Poe (2000) recorded place cells on a three-dimensional climbing apparatus during the Neurolab Space Shuttle mission of 1998. The climbing apparatus was a three-dimensional track that required animals to make three clockwise 90° yaw turns, interleaved with three 90° upwards pitch rotations. The structure of the apparatus, named the ‘Escher staircase track’, and the non-commutative nature of egocentric rotations (discussed earlier in this chapter)

meant that one full lap around the track returned the animals to their starting location and direction, even though the animals only carried out 270° of yaw rotations.

Place cells were shown to have the same spatial information content and mean firing rates in micro-gravity on the Escher staircase track as on the pre-flight recordings of the cells in a horizontal two-dimensional environment. Recordings of place cells in the Escher staircase track showed that place fields remained stable over time, firing in the same position on the track in different recording sessions. This suggests that place cells remain spatially modulated without the input from the gravity-sensing otolith organs and despite the non-commutative nature of the rotations carried out by the animal. It is possible that the stability of these cells could be explained by the presence of salient visual extramaze cues providing an animal an allothetic reference to their position on the track.

More recently, Hayman et al. (2011) returned to the question of how place cells encode vertical space. They created two apparatuses that had vertical as well as horizontal components, and compared the firing on these apparatuses to recordings of cells in a purely horizontal environment.

One piece of apparatus Hayman et al (2011) used was the pegboard maze (see Jovalekic et al., 2011). This maze was oriented vertically against a wall, and had pegs on which rats could climb. While not truly three-dimensional, this maze had a single horizontal component (the x-axis) and one vertical component (the z axis). During recording, rats were required to climb on pegs inserted into the maze to forage for food. They found that compared with the horizontal recordings, place cells exhibited elongation of the place fields in the z-axis, while their firing in the x-axis was comparable to that of the horizontal recordings.

Place cells were also recorded as animals ran up and down a helical track. This track had both x and y components, as well as a vertical component. Again, Hayman et al. (2011) found that place cells exhibited an elongation of their firing fields in the

vertical axis, without an expansion of their firing fields in the x-y plane. Together with the results of the pegboard maze they concluded that there is an anisotropy in place cell firing during movement in three-dimensional environments – that is, place cells are less strongly modulated by movement in the vertical plane than they are in the horizontal (Fig 2.15).

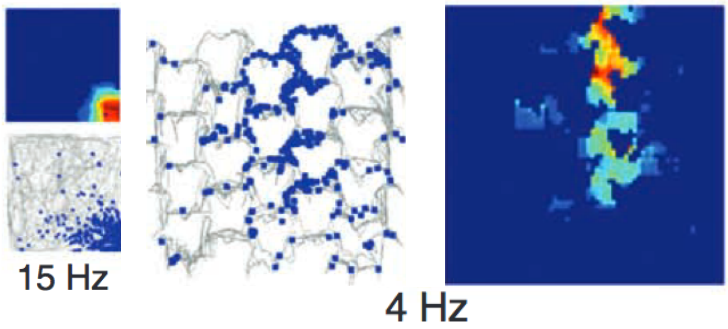


Figure 2.15 Place cells recorded on the pegboard. Left – firing rate map (top) and spike plot (bottom) showing the firing field of a place cell in a horizontal environment. Centre – Spike plot of the same cell on the pegboard. Right- Firing rate map of the cell on the pegboard. Note the elongation of the cell's firing activity in the z-axis. Image taken from Hayman et al. (2011)

The Ulanovsky lab has driven a recent progression in the electrophysiological study of three-dimensional spatial cognition. Rather than studying the firing properties of rodent place cells in 3D space, they instead record from bats as they fly, or crawl on tilted surfaces. These studies are particularly informative as, unlike rats, bats can freely move through volumetric space, and are therefore not constrained to travelling on surfaces. Contrary to the findings in rats, place cells in bats exhibit volumetric firing fields, in which the place cells are as equally modulated by the z axis as they are by the x and y axes (Yartsev & Ulanovsky, 2013; Fig 2.16).

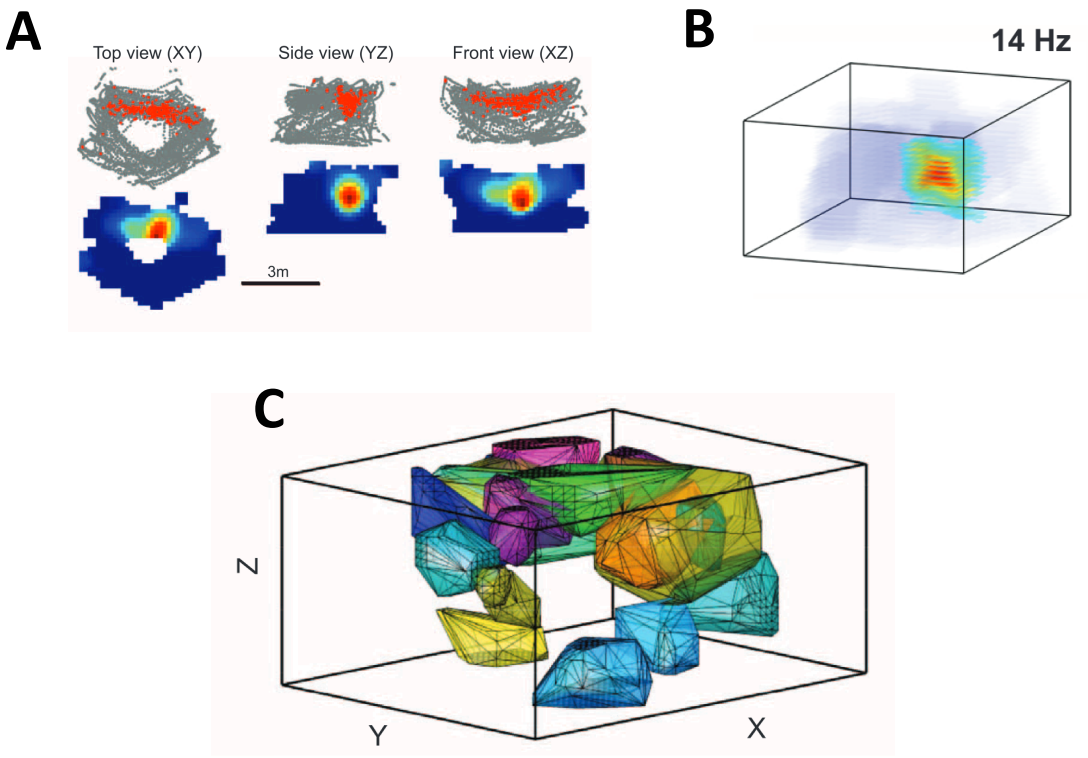


Figure 2.16 Firing activity of place cells in the bat hippocampus. A) Spike plots (top) and rate maps (bottom) for a place cell recorded during flight. The cell is shown from three viewpoints. B) Three-dimensional rate map for the same cell. C) Polygons displaying the three-dimensional encoding by a population of place cells. Images from Yartsev & Ulanovsky (2011).

2.3.2.2 Grid cells

Alongside their report of place cell properties in 3D environments, Hayman et al. (2011) also reported grid cell properties on the pegboard maze and the helical track. On the pegboard maze, grid cells fired multiple firing fields along the horizontal axes but were only very weakly modulated by position in the z-axis of the maze. This resulted in the appearance of multiple columnar firing fields on the pegboard maze (Fig 2.17A), indicating that, like place cells, grid cells represent the vertical plane with lower resolution than they do the horizontal plane. Corroborating this evidence, results from the helical track showed multiple firing fields in the x-y axes of the helical track, with repeating firing at each vertical level of the helical track (Fig 2.17B). Together, these results suggest that grid cells in rats are not modulated by height.

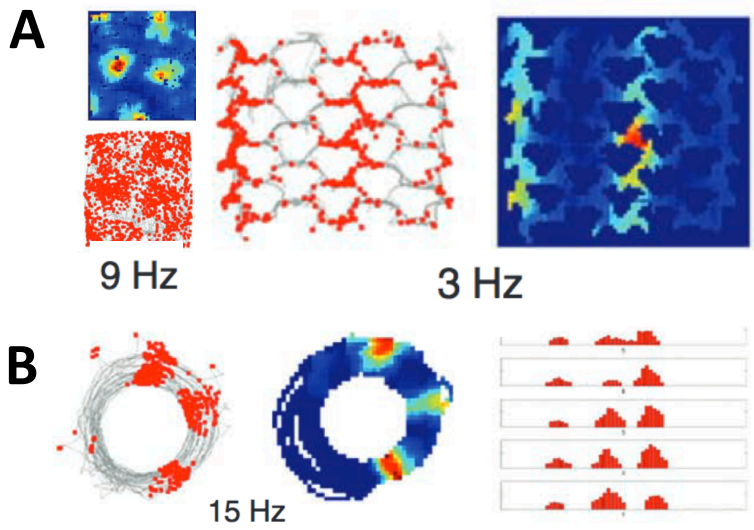


Figure 2.17 Grid cells in the vertical plane. A) Left – firing rate map (top) and spike plot (bottom) for a grid cell recorded in a horizontal environment. Centre – Spike plot of the same cell on the pegboard. Right. Firing rate map of the grid cell on the pegboard. Note the elongation of grid fields in the z-axis. B) Spike plot (left) and rate map (centre) of a grid cell on the helical maze. Right – histograms for the spike position at each level of the helical track. Note the repetition of activity across levels. Images from Hayman et al. (2011).

More recently, Hayman et al. (2015) compared grid cell properties between a flat surface and a surface tilted at 40° (Fig 2.18A). The experiment was designed to determine whether grid fields conform to a volumetric close-packed array or whether they are constrained to the surface of locomotion i.e. does the tilted surface of the apparatus transect through a volumetric array or not? (Fig 2.18B)

During this experiment rats were able to traverse freely between the flat and tilted surface as they foraged for food rewards. The firing of grid cells on this surface was compared to the expected firing behaviour based on models of hexagonal close-packed arrays (HCP) or face-centered cubic (FCC) close-packed arrays. For both the HCP and FCC models, field size, number of fields, surface coverage of fields, and their hexagonal symmetry was predicted to be lower if the lattices were transected at 40° (Fig 2.18C). However, results from the electrophysiological recordings revealed that grid cells exhibited the same number of fields on the tilted surface as on the horizontal surface, and an increased number of fields and increased surface

coverage by the fields. However, grid field symmetry was degraded slightly, albeit less than predicted for either the HCP or FCC models.

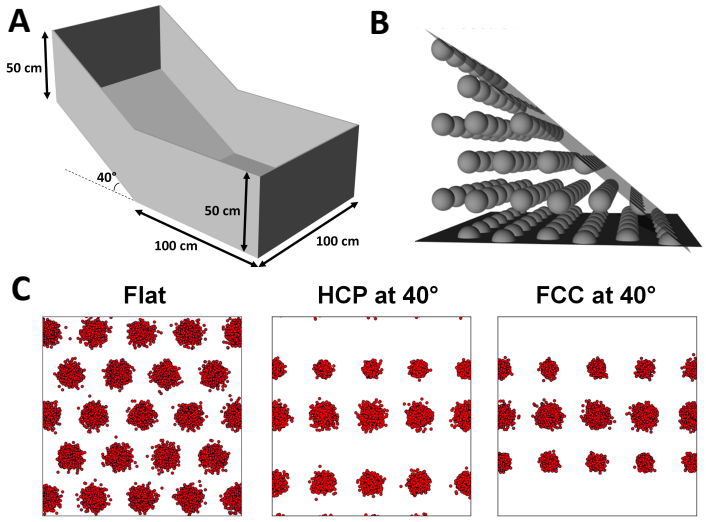


Figure 2.18 Grid cells on a sloping environment. A) Apparatus used in Hayman et al. (2015) B) Schematic of a 40° slope transecting a spherical lattice. C) Computationally generated spike plots for grid cells on a flat surface (left) and for 40° transections through a hypothesised HCP lattice (centre) and FCC lattice (right). Images taken from Hayman et al. (2015).

Hayman et al. (2015) noted that the spatially periodic firing of grid cells found on the flat surface deteriorated during movement on the sloped surface. Despite this, grid cell firing did maintain some level of spatial periodicity on the slope, with the same number of firing fields on the slope as the flat surface, and the same occupancy of the surfaces by grid cell firing fields.

It appears from the recordings of grid cells that the neural map of space is not volumetric, and instead appears to be constrained to the plane of the animal's locomotion. However, it must be noted that the studies of grid cell activity have themselves been constrained to surface-dwelling animals moving on planar surfaces rather than through volumes. It is entirely possible that free movement through volumetric space may encourage the development of a close-packed volumetric representation of space. Recordings of freely flying animals, such as bats, will further elucidate this issue.

2.3.2.3 Head direction cells

One of the first indications that head direction cells may be able to encode directional information in the vertical plane came from Stackman and Taube's (1998) study of neurons in the lateral mammillary nucleus (LMN). This study revealed that the LMN contained neurons that encode for horizontal head direction, horizontal angular head velocity, and, most pertinently to this chapter of the thesis, neurons which responded to the pitch angle of the animal's head. Unlike head direction cells, these head pitch cells did not display a Gaussian tuning curve, nor did the population of cells recorded equally represent all head pitch angles. While the recording setup was limited to estimating head pitch from ~ -45° (head pitched 45° downwards) to +90° (head pitched straight upwards), 13 of the 14 cells recorded displayed peak firing rates when the head pitched upwards by 60 degrees or more. Of these 13 cells, 9 displayed a peak firing rate when the head was pitched upwards by over 80 degrees. Importantly, the presence of head pitch cells in rats indicates that a brain region known to contain HD cells also contains neurons that encode heading elevation – albeit at a lower resolution than its encoding of horizontal direction.

Following on from this discovery, Stackman et al. (2000) recorded from head direction cells as animals climbed on a vertical plane. In this study, HD cells were recorded as animals climbed between the floor of cylindrical apparatus and a ring-shaped platform at the top of the cylinder, using a wire mesh ladder. This ladder was either oriented in line with the PFD of the HD cell on the floor (0° condition), ±90 degrees away from the cell's PFD or 180 degrees away from its PFD. They found that HD cells maintained their firing activity as rats climbed from the floor of the apparatus up the climbing wall during the 0° condition. When the rats climbed back down from the annulus during the 0° condition the firing rate of HD cells degraded. During the 180° condition, the results were inverted, such that HD cell firing was maintained as the animals climbed down the wire mesh ladder, but not when they climbed up the ladder from the floor. For the ±90 degree conditions, there was a

degradation of HD cell firing during both upwards and downwards climbs. These results suggest that the HD signal is maintained during movement in the vertical plane, and that the reference frame to which HD cells are anchored is defined by the animal's plane of locomotion (Fig 2.19).

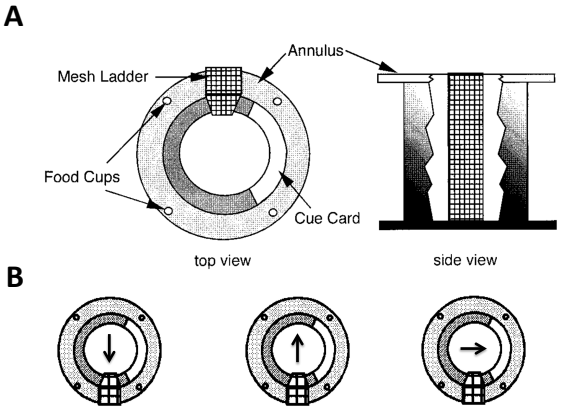


Figure 2.19 A) Climbing apparatus used in Stackman et al. (2000) B) Direction of HD cell firing on the floor for cells that only fire when the rat climbs upwards (left) or downwards (middle), and when the cell firing degrades during both upwards and downwards climbing. Images taken from Stackman et al. (2000).

One issue with this study was that yaw rotations of the head while the animal climbed in the vertical plane could not be recorded, so it was uncertain whether or not HD cells maintain a Gaussian yaw-modulated tuning curve during locomotion in the vertical plane. To investigate this issue, Taube et al. (2013) recorded HD cells on a new vertical apparatus that was developed to allow rats to carry out yaw rotations while climbing. HD cells were recorded as rats climbed around a spiral track on a vertically oriented wall. This spiral track allowed rats to sample all 360° of potential yaw heading directions while climbing. First and foremost, this study showed that HD cells maintained a modulation by yaw rotations while animals climbed on a vertical surface, showing Gaussian tuning curves similar to those of floor sessions.

In a second manipulation in this experiment, HD cells were recorded on the apparatus at the 4 cardinal locations of the experimental room (North, South, East and West) as animals either climbed actively from the floor to the spiral track via a ramp, or were passively placed at the starting point of the track. When rats actively climbed onto the spiral track, the direction of HD cell firing relative to the track

changed depending on the orientation of the apparatus within the room. For example, a cell firing towards the North of the experimental room on the floor would fire upward as the animal climbed the South-facing apparatus, but it would fire to the right of the apparatus when the animal climbed the West-facing apparatus. While at first, this may seem counterintuitive; this rotation of the cell PFD relative to the animal's plane of locomotion can also be viewed as maintenance of a room-based reference frame in which the PFD of the cell relative to the horizontal plane remains consistent (Fig 2.20B). Interestingly, during the passive placement condition, the firing direction of rat HD cells was defined by the local reference frame of the animals – that is, HD cells firing upwards on the spiral track when the apparatus was South-facing would also fire upwards on the spiral track at the other 3 cardinal orientations. This indicates that active locomotion, and the idiothetic cues associated with it, is required to update the functioning of HD cells as they move between different planes.

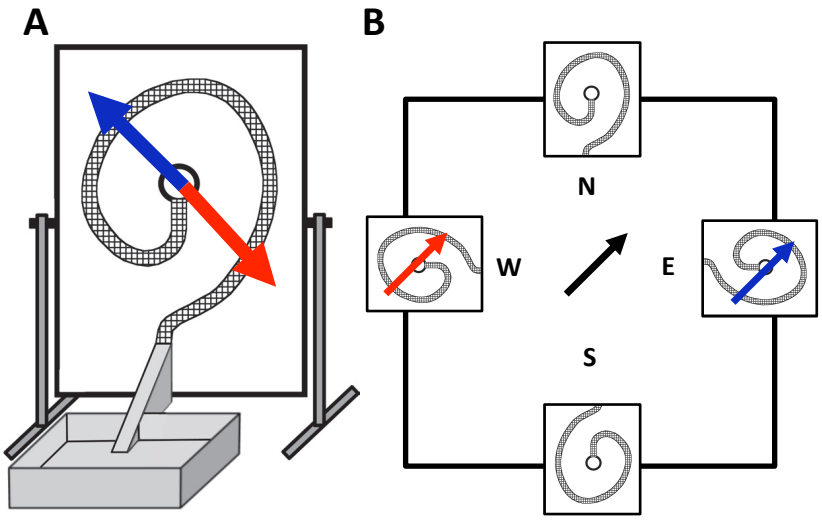


Figure 2.20 HD cells on a vertical plane. A) Spiral apparatus used in Taube et al. (2013). Rats climbed from a starting box, up a ramp, and around a spiral mesh track to a central goal location. B) Flattened schematic of the apparatus positions in the experimental room. Arrows represent the firing of a schematic HD cell based upon the results of Taube et al. The PFD of the cell in the starting box is shown in black, while red and blue arrows represent the PFD of the same cell on the West and East walls, respectively. Note in (A) that the firing directions of the cells relative to the vertical plane differs by 180°, while the firing directions relative to the horizontal plane (shown in B) remains consistent. (Images adapted from Taube et al., 2013).

Another topic of interest in the study of HD cells during locomotion in 3D space is whether these cells are able to maintain directional modulation during inverted locomotion. Calton & Taube (2005) recorded HD cells as rats climbed around a ring apparatus in which HD cells were recorded as the rats move from the floor of the apparatus up one wall, and then moved inverted across the ceiling of the apparatus before descending down another wall back to the floor. While climbing on the walls of the apparatus, HD cells maintained their PFD relative to their locomotor plane, as shown in Stackman et al. (2000). However, when animals climbed inverted on the ceiling, 47% of HD cells lost all directional modulation, with the 53% of cells maintaining directionality exhibiting attenuation of their directional tuning with an increase in background firing rate and a less distinct PFD. Moreover, the PFD of those cells that were directionally selective on the ceiling shifted randomly when compared to the floor trials. This suggests first, that HD cells are less able to main directional tuning during inverted movement, and second, that their PFD relative to a room-based reference frame becomes unpredictable, and detached from the reference frame held during vertical or horizontal locomotion. Calton &Taube suggested that this result might have been an effect of distortion of vestibular functioning during inversion. In particular, the functioning of the otolith organs has previously been shown to become distorted during non-upright orientation (Walsh, 1960; Plotnik, Freeman, Sohmer, & Elidan, 1999)

Yoder & Taube (2009) subsequently recorded from mice with otolith organ dysfunction (otoconia-deficient). While the peak firing rates, background rates, and tuning width of HD cells in the otoconia-deficient mice were comparable with wild type (C57BL/J6) mice; they did show a distinct lack of stability in PFD between recording sessions. This lack of stability had two key characteristics: first, over the course of five recording sessions there was a large decrease in the directional tuning of cells from the first to the final recording session; second, those cells that did remain directionally sensitive between the first and fifth trials showed a greater deviation in PFD than in the wild type mice. Together these data show the

importance of the otolith signal for robust HD cell activity, and provide a possible explanation for the variability of HD activity during inverted locomotion.

More recently, HD cells have been reported in bats (Finkelstein et al., 2015). As bats are able to move throughout volumetric space, one might expect their representation of orientation to be three-dimensional and described by a 3D vector. Indeed, Finkelstein et al. (2015) found that HD cells in the presubiculum of bats were not just modulated by yaw rotations of the head, but that some cells were modulated in a Gaussian fashion by pitch rotations of the head, and were also modulated to a lesser extent by roll rotations of the head. This is quite unlike what has been reported in rats, where HD cells are thought to be purely modulated by yaw rotations of the head.

While the majority of HD cells in bats were modulated by yaw, 21% of cells were pitch modulated and 12% were modulated by roll. The authors also reported the presence of cells which were conjunctively tuned to 3D head direction, with the majority of these cells being responsive to yaw and pitch together (27%) and a smaller percentage (11%) conjunctively tuned to yaw and roll, and an even smaller number (2.5%) modulated by all three rotation types (Fig. 2.21).

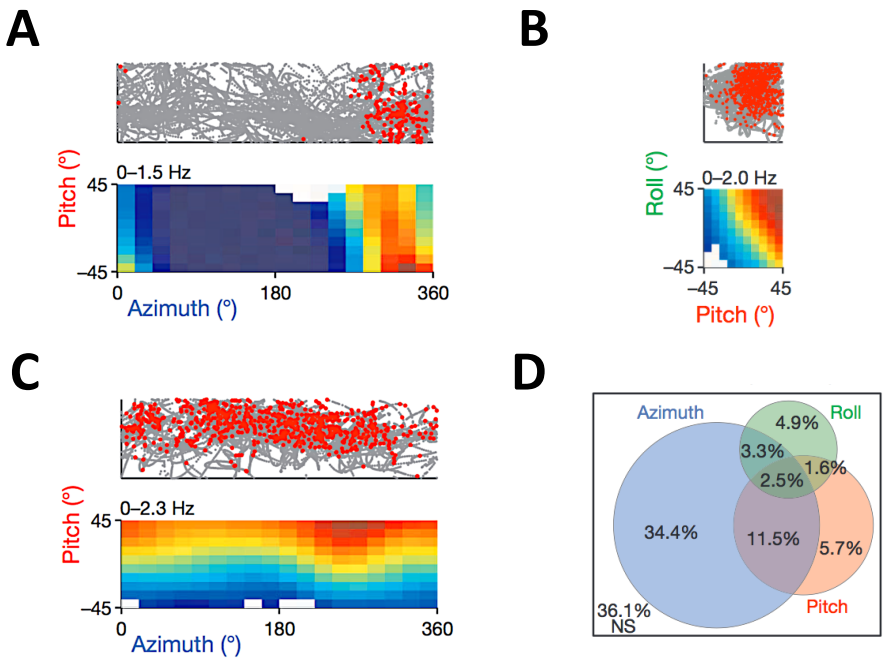


Figure 2.21 HD cell firing in bats. A) Spike plot (top) and rate map (bottom) showing the firing activity of a yaw cell. Hot colours represent higher spiking activity. In this example, the cell is active at ~340 degrees in azimuth, but is not modulated by pitch. B) Spike plot and rate map of an HD cell conjunctively modulated by roll and pitch. This cell is most active at a 45° upwards pitch angle, and a 45° clockwise roll angle. C) Spike plots and rate map of an HD cell which is only responsive to pitch. This cell is active at all azimuth angles, but only when the pitch is ~45°. D) Venn diagram of the distribution of cells encoding for azimuth, pitch and roll. Images taken from Finkelstein et al. (2015).

Perhaps the most surprising of the results reported by Finkelstein et al. (2015) was that the 38% of yaw-modulated cells which remained directionally tuned during inverted locomotion shifted their PFD 180° when inverted. For example, an HD cell which fired when the animal's noise pointed West during upright movement would fire when the animal's nose pointed East during inverted movement. This is certainly surprising if our concept of an animal's heading direction is defined by the direction the nose points (i.e. along the rostro-caudal axis), as has been done in the study of rodent HD cells. However, if instead of defining heading direction by the rostro-caudal axis we define it with relation to the inter-aural axis, we can see that if a cell fires when the left ear of the bat faces South (and the nose points West) during upright locomotion, when the animal is inverted and the HD cell continues

firing the left ear still points to the South, even though the pointing direction of the nose is now towards the East.

Considering the effects of inversion on HD cell activity in bats and rats together, it is clear that while at first there appears to be some divergence in HD functionality between species, there are also some rather striking similarities. First, both bats (Finkelstein et al., 2015) and rats (Calton and Taube, 2005) exhibit a strong attenuation of their firing during inversion, with only 38% of bat HD cells and 53% of rat HD cells maintaining directionality during inversion. This attenuation of directional firing indicates that the underlying systems driving the HD signal are highly conserved, irrespective of the different behavioural affordances of the two species. However, the key difference between bats and rats is that of those cells that did remain directionally modulated during inversion, the preferred direction of HD cells in bats during inversion could be consistently shifted by 180 degrees between upright and inverted positions, whereas HD cells in rats showed weaker directionality, and inconsistent preferred firing directions during inversion. While it is possible that these differences are driven by an evolutionary divergence between species, it remains possible that the lack of consistent directionality of HD cells in rats during inversion is due to a lack of experience in being inverted.

2.3.3 Imaging studies of three-dimensional navigation

While the majority of studies of the neural basis of three-dimensional navigation have used single-unit electrophysiology of rodents, there have been more recent attempts to understand how the human brain represents vertical space. Studying the human brain during complex three-dimensional navigation is particularly difficult because brain imaging techniques such as fMRI (functional magnetic resonance imaging) often require subjects to remain completely still while navigating a virtual environment. This constraint means that the idiothetic processes such as motor-efference, proprioception and vestibular signalling do not contribute to the navigation tasks that are used. As will be discussed in later

chapters, these internal inputs are thought to be very important in the generation of signals that aid navigation in rodents. Perhaps because of this, there are a limited number of studies investigating the neural basis for three-dimensional navigation in humans.

To date, the only study that has attempted to understand the neural basis for vertical navigation in humans used PET (positron emission topography) to compare brain metabolism between horizontal and vertical navigation (Zwergal et al., 2015). Subjects were either required to complete navigation tasks in either a single floor of a building, or between multiple floors. The authors found that, as with other animals (Jovalekic et al., 2011; Flores-Abreu et al., 2014; Holbrook & Burt de Perera, 2009), humans exhibit a behavioural anisotropy in which they performed better in the horizontal navigation task than they did on the vertical navigation task. As well as this, they authors reported differences in their measures of brain activity between the two tasks, with vertical navigation activating hippocampus bilaterally and horizontal navigation primarily activating the right hippocampus.

As there is so little data describing human brain activity during three-dimensional navigation, it is difficult to draw conclusions on whether the human brain may represent 3D space in a similar way to the rodent brain. The results of Zwergal et al. (2015) do at least indicate that there is an anisotropy in both the behaviour and neural correlates of navigation between vertical and horizontal spaces. This work could perhaps be further expanded on with human single-unit recordings similar to those by Ekstrom et al., (2003) and Jacobs et al., (2010) or with the use of mobile EEG (electroencephalogram) techniques.

2.4 Summary of navigation in three-dimensional environments

While the literature covering the neural representation of 3D space is relatively sparse, it has started to reveal a distinction in the neural representation of space by

different species. Studies of rats indicate that the firing of place, grid and head direction cells is planar, and defined by the surface on which the animals is moving, whereas studies of bats indicate that place cell firing is volumetric, and that head direction cells are responsive to pitch and roll rotations, as well as yaw rotations.

It must, however, be noted that it is not yet possible to confirm whether the apparent distinctions between place and head direction cells in bats and rats are a result of an evolutionary divergence or because of differences in experimental methodology. With regards to place cells, researchers have been unable to record rodent place cells in volumetric environments, while the recording of bats during flight has allowed for the study of bat place cells in volumetric space. It is possible that if rodents could be recorded in an environment similar to the lattice maze used by Jovalekic et al., (2011) that rodent place cells would also exhibit volumetric firing.

With regards to head direction cells, the use of a four LED tracking setup by Finkelstein et al., (2015) allowed for the recording of roll and pitch rotations of the head, whereas studies of rat HD cells have only used a two LED tracking setup meaning that only a single egocentric rotation could be studied at any given time. The discovery of pitch-modulated cells in the rat LMN by Stackman & Taube (1998) indicate that there may indeed be cells modulated by non-yaw rotations within the rodent HD network. As such, further studies of both rat and bat HD systems using similar experimental designs are required in order for a consensus to be reached on whether or not there is a distinction in the neural networks involved in navigation.

The electrophysiological study presented in chapter five of this thesis focuses on the functioning of head direction cells in three-dimensional environments. This reason for this focus on HD cells is because normal HD cell functioning is needed for the accurate representation of space by place and grid cells. For example, lesions of the postsubiculum result in a loss of spatial-coherence in place cells (Calton et al., 2003) and lesions of the anterior thalamic nucleus (ATN) severely distort the hexagonally patterned firing of grid cells (Winter, Clark & Taube, 2015). Understanding the

activity of the HD system during locomotion over three-dimensional structures can therefore aid the prediction of specific hypotheses for the functioning of other spatially modulated neurons in complex three-dimensional environments.

In rats, head direction cells are modulated by yaw rotations during locomotion on vertical surfaces, just as they are on horizontal surfaces. The results of Taube et al., (2013) revealed that active locomotion between horizontal and vertical planes allows animals to maintain a representation of orientation that always remained related to a room-based horizontal reference frame. However, it is unclear, whether this result was due to an active updating of the animal's orientation between planes, or whether the HD system was merely unresponsive to the rotation between planes.

If we imagine, for example, an animal moving between two perpendicular walls, a maintenance of heading direction relative to a room-based horizontal reference frame would require a rotation of the preferred firing direction of the HD cell by 90° (as shown in Fig 2.12 *right*). This would require an active updating of head direction cells between the two planes, possibly requiring information from neural systems sensitive to non-yaw rotations. On the other hand, if the HD system is unresponsive to rotations between planes, one might expect HD cells to fire in the same direction relative to each plane (as shown in Fig 2.12 *left*). Finally, as rodent HD cells have not yet been recorded in environments with multiple vertically oriented planes, it remains possible that HD cells might fire in the same direction relative to a global volumetric position.

The electrophysiological experiment presented in Chapter 5 was therefore designed to test each of these hypotheses, to determine whether the rodent HD system is planar, multi-planar, or volumetric. As the recording of HD cells forms the basis for the electrophysiological experiments in this thesis, the next chapter will provide a detailed overview of the anatomy of the HD cell circuit, the differences in firing characteristics between different HD-cell-containing regions, and allothetic and

idiothetic determinant of HD cell firings. This will provide a basis for the later discussion of the experimental results of the present work.

Chapter 3 Head direction cells

As discussed in Chapter 1, HD cells are characterised by a modulation of their firing rate by orientation in the animal's plane of locomotion. This can be shown as a Gaussian peak in directional tuning, in which the peak firing rate defines the cell's PFD, and the angular width of the curve above the background firing rate defines the cell's tuning width. HD cells have been discovered in various regions within the limbic system. This chapter outlines the anatomy of the HD cell circuit, and compares the firing characteristics of HD cells found throughout the HD system. Following this, the allothetic (external) and idiothetic (internally generated) determinants of HD cell firing will be described, followed by a discussion of computational models that have been developed to describe the functioning of the HD cell circuit.

3.1 The head direction cell circuit

3.1.1 Subicular complex

As outlined in Chapter 1, head direction cells were first discovered by Ranck (1986) in the postsubiculum (PoS). The PoS is one of several regions that make up the subicular complex, which itself is part of the hippocampal formation (Amaral and Witter, 1995). The subicular complex can be divided into four subregions: the subiculum (Sub), parasubiculum (PaS), presubiculum (PrS) and the postsubiculum (PoS). While initially described as the dorsal presubiculum, the PoS has been shown to have a neurochemical organisation that is distinct from the presubiculum (van Groen & Wyss, 1990a) and as such is considered as distinct from the PrS. Furthermore, van Groen & Wyss (1990b) reported that the connectivity of the PoS was distinct from the PrS – for example, the presubiculum has dense projections to the antero-ventral thalamic nucleus, while the PoS does not. A figure illustrating the relation of the PoS to the hippocampal formation can be seen in figure 3.1.

The PoS itself can be grouped into two sets of laminae each containing three layers. Layers I to III form the superficial (external) laminae, while layers IV to VI form the deep (internal) laminae. The neuroanatomy of the PoS was further shown to be distinct from PrS and retrosplenial cortex (RSc) by van Groen and Wyss (1990b) using Nissl and acetylcholinesterase staining. The distinct staining of the deep layers for acetylcholinesterase distinguish the PoS and the RSc, and the clustering of cells in layer II and parallel rows of cells in layer III further distinguish the superficial layers of PoS from PrS.

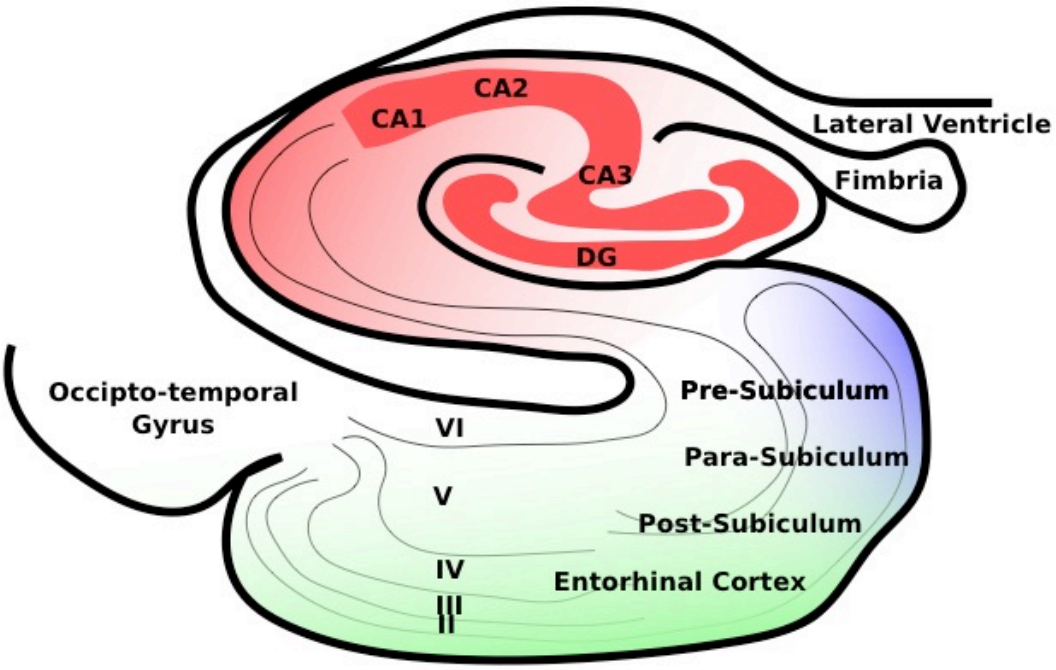


Figure 3.1 Illustration of a horizontal slice through the subicular complex. The position of the subicular complex (blue) is shown in relation to the hippocampus (red) and the entorhinal cortex (green). HD cells have been recorded in the post-subiculum and entorhinal cortex. Image adapted from Strafstrom (2005).

In the first quantitative analysis of HD cells Taube et al. (1990a) found that ~25% of all cells recorded from the PoS were HD cells, and that the majority of HD cells (35/38) were recorded from the deep layers of the PoS (Layers IV to VI). These deep layers (internal laminae) were later shown by van Groen & Wyss (1992a) to contain small pyramidal cells.

HD cells recorded from the PoS have very similar firing characteristics to HD cells recorded elsewhere in the limbic system, with their firing rates and tuning width no different from those of HD cells recorded in the ADN and RSC (Sharp, 2005). One difference in behaviour of the PoS HD cells to other regions is that they appear to encode for the exact current heading direction of the animal, whereas HD cells in the ADN exhibit anticipatory firing before the animal faces a given cell's PFD. This firing characteristic is discussed in more detail in the next section.

3.1.2 Thalamic nuclei

The thalamic nuclei consist of three main sets of nuclei: the anterior, lateral and reticular nuclei. The anterior nuclei themselves can be split into three distinct sets of nuclei: the antero-dorsal (ADN), antero-medial (AMN) and antero-ventral (AVN) thalamic nuclei; while the lateral nuclei can be split into two distinct nuclei: the ventro-lateral (VLN) and latero-dorsal (LDN) nuclei. An illustration of the location of the ADN and AVN can be seen in figure 3.2.

The anterior thalamic nuclei can be distinguished from each other on the basis of cell morphology and neurochemistry. In particular the ADN contains medium-sized cells (over 15µm) that show dense Nissl staining, while the AVN contains smaller cells with very sparse Nissl staining (Dekker & Kuypers, 1976; Oda, Kuroda, Kakuta, & Kishi, 2001) The AMN, on the other hand, is harder to distinguish and in most cases holds more similarity with the AVN than the ADN.

Within the thalamic nuclei, HD cells have primarily been recorded in the ADN; however they have also been reported in the LDN (Mizumori & Williams, 1993). The first report of HD cells in the ADN (Taube, 1995) revealed that ~60% of cells in the ADN could be described as HD cells – a much greater yield than seen in the PoS. The HD cells recorded by Taube (1995) were qualitatively indistinguishable from those recorded in the PoS: the PFDs of the population of ADN cells together represented all horizontal directions within the environment, ADN HD cells were similarly

responsive to cue rotations as shown in Taube (1990b), and the firing rates of HD cells in the ADN and PoS are similar.

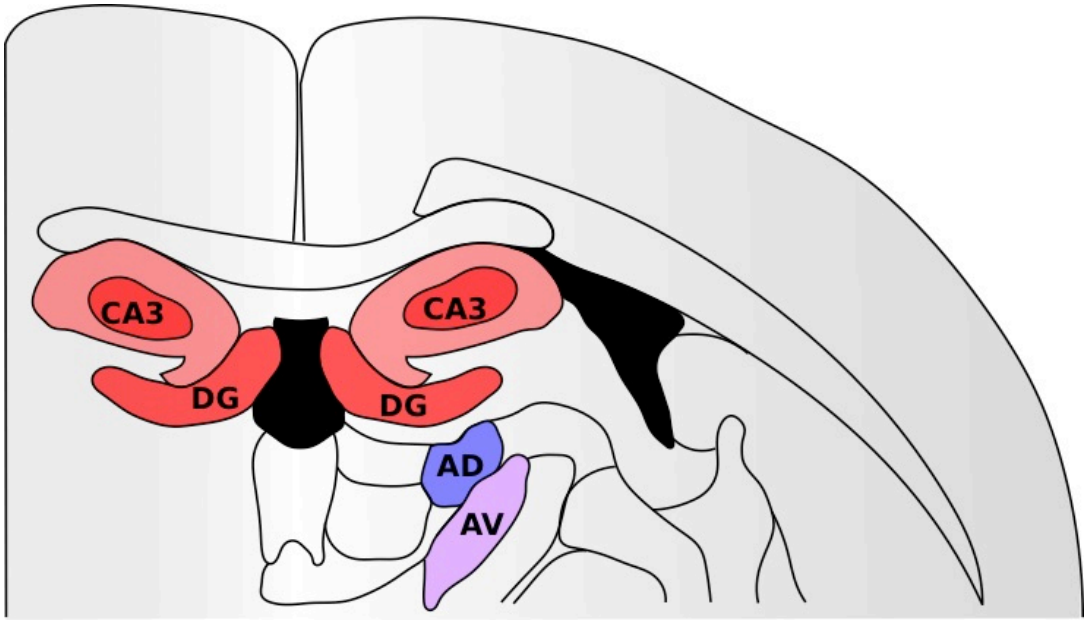


Figure 3.2 Illustration of a coronal slice through the ADN. The location of the ADN (AD) and AVN (AV) are shown in relation to the hippocampus (CA3) and dentate gyrus (DG). Image adapted from Taube (1995).

Blair & Sharp (1996) also recorded HD cells from the ADN and compared their firing characteristics to those of PoS. While the firing characteristics of HD cells in the two regions were mostly similar, the authors found a striking effect of angular head velocity in the ADN. Having recorded HD cells over 30 minute foraging trials, Blair & Sharp (1996) divided head movements into clockwise (CW) and counter-clockwise (CCW) movements. They found that ADN HD cell firing anticipated heading direction, such that cells would start firing CCW of the preferred firing direction during CW rotations of the head, and CW of the PFD during CCW rotations of the head. Furthermore, the authors reported that the velocity of the rotation affected the amount of shift between CW and CCW rotations, with higher velocity rotations causing an increased shift between CW and CCW rotations. This anticipatory firing, seen in the ADN and not the PoS, indicates that the HD system uses angular head velocity information to determine firing activity. This evidence of angular head

velocity by head direction cells (AHV x HD cells) has been widely used in the computational models of the HD system (discussed at the end of this chapter).

The discovery of AHV x HD cells by Blair & Sharp (1996) was further supported by Taube & Muller's (1998) investigation of ADN and PoS cells. However, Taube & Muller found that not all ADN HD cells exhibited anticipatory firing, and that some PoS cells did exhibit this anticipatory firing characteristic. One of the prime candidates for the production of angular head velocity signals was the vestibular system (discussed later in this chapter). Brain regions heavily linked to the vestibular system include the mammillary bodies, which are connected to the vestibular apparatus via the dorsal tegmental nucleus of Gudden (DTN).

3.1.3 Mammillary bodies

The mammillary bodies consist of the lateral mammillary nucleus (LMN) and three subdivisions of the medial mammillary nuclei: pars lateralis (MML), pars medialis (MMM) and pars medianus (MME). To date, HD cells have only been recorded in the LMN (Blair, Cho, & Sharp, 1998; Stackman & Taube, 1998)

Stackman & Taube (1998) reported that the LMN contained three key types of directionally sensitive cells: HD cells, AHV cells and head pitch cells. Of the cells recorded, they reported that ~ 14% of cells were sensitive to head pitch, as opposed to classic HD cells which are responsive purely to yaw rotations. HD cells constituted 23% of all recorded cells – a yield similar to reported values of PoS cells (Taube et al., 1990a). The firing characteristics of LMN HD cells reported by Stackman & Taube (1998) were markedly different from those recorded in the PoS, with LMN HD cells exhibiting: 1) higher peak firing rates; 2) broader directional tuning; 3) higher background firing rate; and 4) larger anticipatory time intervals (ATI). In particular, the presence of a strong ATI indicates that LMN HD cells are heavily driven by angular head velocity.

As with ADN and PoS HD cells, LMN HD cells were shown to rotate accordingly with the rotation of visual cues. Blair et al., (1998) also reported the presence of HD x AHV cells in the LMN. Unlike the study by Stackman & Taube (1998), Blair et al., (1998) recorded these cells from the LMN in both hemispheres. Interestingly, they found a laterality in the HD cells responses to CW and CCW rotations, with HD cells recorded form the left hemisphere exhibiting tighter tuning during CW rotations than CCW rotations, and the inverse being true of HD cells recorded from the right hemisphere. This laterality is not seen in the ADN. The presence of such laterality in the LMN suggests a lateralised input from angular velocity signals produced by the vestibular system.

As well as HD x AHV cells, Stackman & Taube (1998) also reported the presence of pure angular head velocity cells (AHV) in the LMN. These cells either showed a positive correlation of firing rate and angular head velocity (fast AHV cells), in which an increase in angular head velocity increase the cell firing rate, or the cells showed a negative correlation of firing rate and AHV (slow AHV cells). Heading direction did not modulate these cells.

3.1.4 Dorsal tegmental nuclei

HD, head pitch and AHV cells have also been reported in the dorsal tegmental nuclei of Gudden (DTN) (Bassett & Taube, 2001), a region that contains reciprocal connections with the LMN (Takeuchi, Allen, & Hopkins, 1985; Allen & Hopkins, 1989).

Bassett & Taube (2001) reported that ~11% of all recorded cells in the DTN were HD cells, while 75% of recorded cells were modulated by AHV. These AHV cells could be split into two groups, based on their firing characteristics. They either responded symmetrically (48% of cells) to CW or CCW rotations of the head, or asymmetrically (27% of cells) with, for example, an increase in firing rate with increasing angular velocity during CW rotations, and a decrease in firing rate with increasing angular velocity during CCW rotations. Of the AHV cells recorded, ~40% were also

modulated by linear head velocity, although more weakly than they were by AHV. There was also a small percentage of AHV cells that were modulated by head pitch (16%), similar to the reports of head pitch cells in LMN (Stackman & Taube, 1998). All of these head pitch cells were conjunctively modulated by pitch and horizontal AHV.

3.1.5 Posterior cortex

The rat posterior cortex has also been shown to contain HD cells. In particular the medial prestriate (area Oc2M) and the retrosplenial cortex (RSC) contain directionally modulated neurons, some of which are further modulated by specific behavioural modes (Chen, Lin, Green, Barnes, & McNaughton, 1994). An illustration of the position of the RSC in relation to the PoS, subiculum and hippocampus can be seen in figure 3.3.

The retrosplenial cortex can be divided into two sections, the retrosplenial dysgranular cortex (Rd) and the retrosplenial granular cortex (Rg), which itself can be subdivided into two parts, RgA and RgB (van Groen & Wyss, 1990c, 1992a). HD cells have been reported in both the Rd and Rg (Chen et al., 1994; Cho & Sharp, 2001). HD cells found in the Rg have similar firing properties to those found in the PoS, with no modulation by motion (such as turn direction or linear velocity). By contrast, HD cells found in the Oc2M (6% of all cells recorded from Oc2M) were predominantly modulated by motion (~ 57% of the recorded HD cells). The firing characteristics of cells recorded from the Rd fell somewhere in between, with ~10% of cells modulated by direction, of which 19% were behaviour-selective. The yield of HD cells in the posterior cortex appears to be much lower than that of the PoS (~25%) and ADN (~60%).

Cho & Sharp (2001) further demonstrated that HD cells in the RSC have narrower tuning curves than those found in other regions such as the PoS, LMN and ADN, and that RSC HD cells are influenced by angular head velocity, with a broader tuning function of cells during ipsiversive turns. This suggests that the RSC receives

lateralised AHV input from the vestibular system, potentially via other HD cell containing regions.

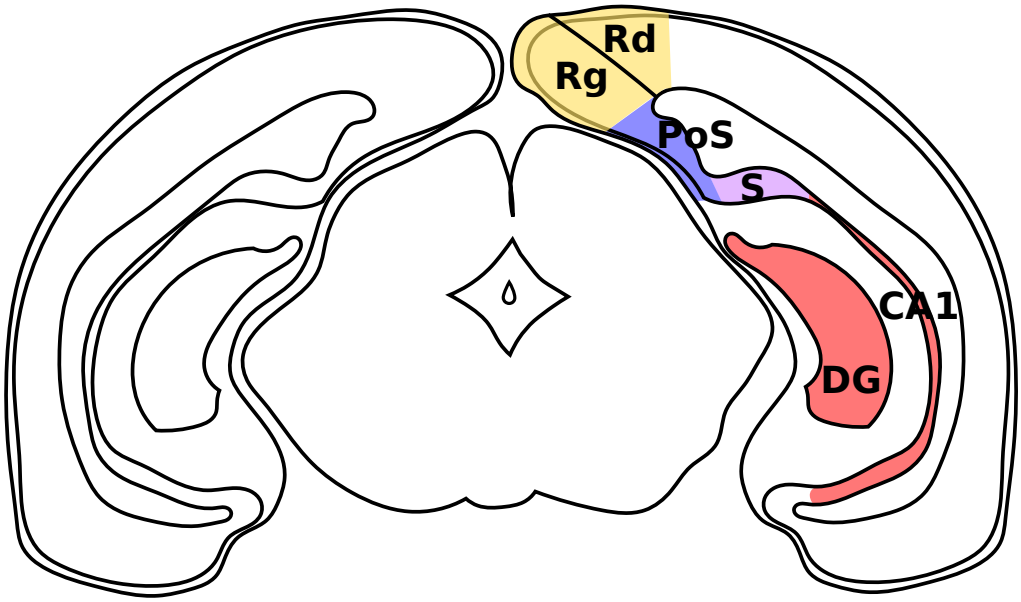


Figure 3.3 Illustration of a coronal slice through the RSC. The locations of the granular (Rg) and dysgranular (Rd) regions of the RSC are shown in relation to the locations of the postsubiculum (PoS), subiculum (S) and hippocampus. Image adapted from Taube et al. (1990a).

3.1.6 Entorhinal cortex

The medial entorhinal cortex (MEC) has also been shown to contain HD cells (Sargolini et al., 2006). As discussed in previous chapters, this region had previously been shown to contain grid cells (Hafting et al., 2005), which are thought to be important in providing odometry information for the spatial cognition circuit. Sargolini et al. (2006) found that ~73% of cells recorded from layers III-VI of the MEC were significantly directionally modulated, while there were no HD cells recorded from layer II. The directionally modulated cells included those that did not exhibit grid-like firing, and cells that had both grid-like and directional modulation. In layer III 66% of grid cells were conjunctively modulated by direction, and in layer V 90% of grid cells were conjunctively modulated. These conjunctive cells were also shown to rotate with landmarks during a cue rotation experiment, showing that these cells are similar to ADN, PoS and RSc HD cells in their use of visual landmarks to anchor their firing.

Further studies of HD cells in the MEC of mice revealed differences in the tuning properties of HD cells recorded from different layers of MEC (Giocomo et al., 2014). HD cells recorded from layer II, but not layers V-VI, of MEC were shown to have a dorso-ventral gradient in their tuning widths, with cells at more dorsal regions exhibiting tighter tuning curves than those in more ventral regions.

3.1.7 Summary

HD cells are present throughout the Papez circuit, which is described as the pathway between the hippocampal formation, mammillary bodies, anterior thalamic nucleus, cingulum and entorhinal cortex (Papez,1937) and in brainstem structures such as the DTN. Their firing characteristics are remarkably similar in each of these brain structures, with only small variations in tuning width between some regions (e.g. broader tuning curves in the LMN and DTN). There is vast interconnectivity between the HD containing brain regions (Taube, 2007), with some of these regions receiving direct input from visual cortices and motion-processing regions. The next two sections will therefore describe how external (allothetic) sensory information and internally generated (idiothetic) signals inform the firing of HD cells.

3.2 Allothetic inputs to the HD system

One potential determinant of HD cell firing comes from the information provided to the HD cell system from the processing of sensory information available in the external world. This sensory information can be derived from any of the sensory modalities, and includes visual, olfactory, auditory and tactile information. It stands to reason that the HD system may use such information to anchor the animal's orientation with respect to the external world. This section will detail the investigations of the allothetic inputs to the HD system, and contrast the importance of cues from each of the modalities on the determination of HD cell activity.

3.2.1 Visual inputs

Having characterised the basic firing properties of HD cells in the postsubiculum, Taube et al. (1990b) investigated the effects of environmental manipulations on the firing properties of HD cells. First, Taube et al. (1990b) studied the importance of visual cues on the HD system with the use of a cue-rotation paradigm. In this study, a cue card covering 100° of arc within a cylindrical environment was moved to different positions between recording sessions. The position of this card was rotated by either 90, 180 or 270 degrees between sessions, and the animal was also mildly disoriented within an opaque box between sessions. After each rotation of the cue card, HD cells were shown to rotate their PFD accordingly – for example, after a 90° degree cue card rotation HD cells also rotated ~90°. This result shows that visual cues are used by the HD system to help anchor their PFD to the external environment. Interestingly, these results are in agreement with cue card rotation experiments during the recording of place cells, in which the position of the cell's place field rotates accordingly with the cue card rotation (Muller & Kubie, 1987). It must also be noted that HD cells tended to exhibit an under-rotation of their firing, which has since been shown to be a common effect in cue-card rotation experiments (Taube et al., 1990b; Taube, 1995; Knight et al., 2013). It is possible that other environmental cues such as olfactory and tactile cues may account for this under-rotation. This control of HD cell PFDs by visual landmarks has been shown throughout the HD cell circuit (ADN: Taube; 1995, RSc: Chen et al., 1994; LMN: Stackman & Taube, 1998, MEC: Sargolini et al., 2006).

Taube et al (1990b) also showed that, while visual landmarks can drive the PFD of HD cells, they are not necessary for the maintenance of a stable directional signal. In a cue card removal paradigm, the authors showed that HD cells maintained directional modulation, exhibiting comparable peak firing rates and cell tuning widths to sessions with the cue card present. They did observe a shift in the PFD of HD cells between the cue card and the cue-removal sessions, which were likely driven by error accumulation from idiotohetic signals (e.g. under or over estimation

of the degree of rotations). Goodridge et al. (1998) obtained similar results when they recorded HD cells from the ADN and PoS while rats were blindfolded. These HD cells maintained strong directional modulation without the presence of visual cues, with comparable peak firing rates and tuning widths for standard and blindfolded sessions. One interesting characteristic of these results was an average PFD shift of ~23° degrees over the course of the blindfolded session – a shift which is considerably larger than that of the standard sessions (~6°). This indicates that while HD cells do not require visual information for directional modulation, they do require landmark information to anchor their preferred firing direction.

The experiments discussed above established the importance of visual landmarks for maintaining stable PFDs; however, during navigation in more naturalistic settings, we are often presented with a range of different visual landmarks. These landmarks tend to have differing characteristics – some may be more stable and therefore more predictable than others, some may be more familiar, and one constellation of external landmarks may be more proximal than others.

To assess the characteristics of visual cues that anchor HD cells, Zugaro et al. (2001) recorded HD cells from the ADN while rats either had access to local cues only, or to both local and distal cues. In this study, three objects were placed at equal distances around the periphery of a circular platform. This platform was either enclosed within a cylinder (proximal background condition), or left open to allow animals to view a distal curtained backdrop (distal background condition). The PFDs of HD cells were compared between a baseline session and a rotation session, in which the three objects were rotated by 120 degrees. In the proximal background condition HD cell PFDs rotated accordingly with the rotation of the objects, while there was no evidence of PFD rotation in the distal background condition. Following this finding, Zugaro et al. (2004) recorded HD cells in which a foreground and a background cue card were presented to the animal simultaneously. Following a baseline recording session, the two cue cards were rotated by 90° in opposite directions. The authors found that on the majority of occasions (30/53) the PFD of

HD cells rotated in the direction of the distal cue card, while cells rotated in the direction of the proximal cue card on 5/53 occasions. On the other occasions, cells did not shift their PFD (5/53) or showed inconclusive preference for proximal and distal cue cards (13/53). Together, these experiments indicate that background visual landmarks are favoured by the HD system as anchoring points over foreground visual landmarks.

3.2.2 Olfactory and auditory inputs

As well as studying the role of visual landmarks on HD cell firing, Goodridge et al. (1998) also carried out manipulations of olfactory and auditory cues. In their study of olfactory cues, four cotton buds were taped at equal distances around the edge of a cylindrical apparatus. Of the four cotton buds, three were left odourless, while one was dipped in peppermint extract. The firing characteristics of HD cells were then compared between a baseline session, and a rotation session, in which the cotton buds were rotated by 90 degrees. Similar to the visual landmark rotation experiments, the majority of HD cells rotated in the same direction as the rotation of the olfactory cue. However, the amount of under-rotation in the olfactory cue rotation experiment was ~55 degrees, compared to ~16 degrees in the visual landmark rotation experiment. Moreover, during return rotation trials, in which the olfactory cue was placed back in the same position as baseline trials, there was an average deviation of PFD of ~89 degrees. These results show that, while HD cells do appear to use olfactory cues to anchor their directional firing, they rely less on these cues than they do on visual landmark information.

Goodridge et al. (1998) used a similar approach to determine the importance of auditory cues in anchoring HD cell firing. Auditory clicks, or in one case noise bursts, were presented to animals from one particular direction during baseline sessions, and from a direction 90 degrees clockwise to this in the rotation condition. HD cells showed an unexpected mean deviation from their expected firing direction of 112 degrees after the auditory cue rotation, without any tendency for cells to shift

their PFD in the direction of the auditory click rotation. This is greatly different from the results shown with visual landmark and olfactory rotation, indicating that auditory information does not exert control over the direction of HD cell firing.

3.2.3 Summary

Studies of the driving allothetic inputs for the HD system reveal a dominance of visual cues in the anchoring of HD cell directionality, with the position of olfactory cues playing a minor role in directing HD cell firing. Auditory cues appear to be even less important, as reorientation of auditory cues does not result in a concordant rotation of HD cell PFD. While visual and olfactory cues appear to help anchor the directionality of the HD system, removal of these cues does not result in a degradation of directionally modulated firing. This indicates that allothetic cues are not necessary for the generation of the HD signal. The next section, therefore, will discuss the importance of idiothetic cues for the HD system.

3.3 Idiothetic inputs to the HD system

Accurate navigation has been shown to be possible without the presence of external landmark information. This type of navigation, known as path integration, is thought to rely on the presence of internally generated signals, known as *idiothetic* signals (Mittelstaedt & Mittelstaedt, 1980). Some evidence of this was shown in the cue removal (Taube et al., 1990b) and blindfolding (Goodridge et al., 1998) studies presented in the previous section which highlight the ability of the HD signal to maintain directionally modulated firing using idiothetic signals alone. These may be in the form of vestibular information, motor efference copies, proprioception, or optic and auditory flow. This section will outline the evidence for the integration of idiothetic cues in the generation and maintenance of the HD cell signal.

3.3.1 Vestibular input

The primary sources of information used to generate the HD cell signal are the vestibular end organs (Stackman & Taube, 1997; Stackman et al., 2002; Yoder et al., 2011). These organs consist of three differently oriented semicircular canals and two otolith organs (Fig 3.4). The otolith organs consist of the saccule, which is responsive to linear head acceleration in the vertical plane, and the utricle, which is responsive to linear head acceleration in the horizontal plane. The semicircular canals are sensitive to rotations of the head. The horizontal semicircular canals are responsive to yaw rotations of the head, while anterior and posterior semicircular canals are responsive to pitch and roll rotations of the head respectively. The horizontal canal neurons on the left side of the head increase their firing rate with a leftwards yaw rotation of the head, and suppress their firing rate with a rightwards yaw rotation of the head. The inverse is true of the right horizontal canal. It is this modulation of the horizontal canal neurons by yaw rotations that makes them the ideal candidate for the generation of the HD signal.

The vestibular system is remarkably well conserved across mammalian species (Brown, 1874) with all mammalian vestibular systems thought to contain all three semicircular canals and otolith organs. The three semicircular canal planes are thought to be orthogonal within 4-8 degrees for rats, humans and other species (Daunicht & Pellionisz, 1987, Blanks & Torigoe, 1989, Glasauer, 2005). Davies et al., (2013) also reported that the bat vestibular system is highly similar to that of rats, with the biggest differences between bat species associated with echolocating bats possessing relatively large cochlears compared to non echo-locating bats.

The role of the vestibular system in perceptual and motor tasks has been studied in a wide range of species. Some of the most commonly studied mechanisms that have been studied relate to the role of the vestibular system in maintaining balance and posture (Inglis et al., 1995), spatial navigation (Stackman & Taube, 1997), gaze stabilization (Angelaki, 2004) and the vestibulo-ocular reflex (VOR; Lorente de No,

1933; Angelaki & Hess, 1994; Huterer & Cullen, 2002). The range of mechanisms that have been studied in the vestibular system display its importance in the functioning of some of the most basic of sensory-motor reflexes (the VOR) as well as high level perceptual and spatial processing (for review, see Angelaki & Cullen, 2008).

Perhaps the most studied aspect of the vestibular system is the vestibulo-occular reflex because of its conservation across species, including afoveate animals such as rats (Artal et al., 1998) and foveate animals such as higher primates and humans (Angelaki & Hess, 1994). This reflex causes an equal and opposite rotation of the eye to the rotation of the head to allow for gaze-fixation of the eye on a visual target even during head movement. The rotation of the eye in response to head rotation work within three degrees of freedom (Glasauer, 2005), meaning that tilt and roll rotations of the head elicit a VOR response as well as yaw rotations of the head. For example, in rats, a pitch rotation of the head elicits an otolith-driven VOR response (Brettler et al., 2000).

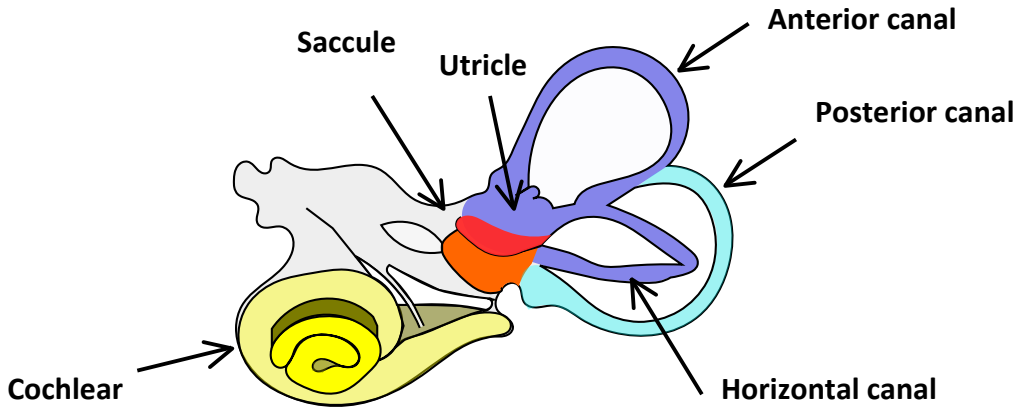


Figure 3.4 The vestibular apparatus. The anterior, posterior and horizontal canals are sensitive to angular rotations of the head. The utricle and saccule are sensitive to linear acceleration. This figure was adapted from Saylor Academy (2012) under a Creative Commons BY CA license.

The wealth of research showing the role of the otolith and semicircular canals in driving the vestibulo-occular response provided researchers with an ideal candidate to start determining the source of the HD signal. Some of the strongest evidence for the necessity of the vestibular signal in HD activity generation came from investigations of the effects of neurotoxic lesioning of the vestibular end organs on HD cell activity. Stackman & Taube (1997) lesioned the vestibular apparatus using sodium arsanilate to cause vestibular hair cell death. Screening for HD cells in the ADN did not yield any HD cells in these vestibular lesioned rats, despite histological analysis confirming that microelectrodes had passed through the ADN. Following this, the authors implanted a new batch of rats in the ADN prior to lesioning. Having identified HD cells, they then carried out the vestibular lesions, again using sodium arsanilate. They found that these lesions eradicated the directional tuning of HD cells over the course of 96 hours. Following these studies, Stackman et al. (2002) carried out temporary lesions of the vestibular end organs using tetrodotoxin. The authors found a temporary loss of directional tuning in PoS HD cells, and a loss of place cell location-specific firing. Together, these experiments reveal that the vestibular end organs are necessary for the generation of the HD signal in both the ADN and PoS, and that the loss of directional modulation in these areas is correlated with place cell localisation.

Other evidence for the role of the vestibular end organs in maintaining the HD signal comes from passive rotation experiments, in which rats are passively rotated at differing angular velocities. Blair & Sharp (1996) recorded HD cells as animals foraged in a cylindrical environment that was rotated 90 degrees at either high or low velocities. The low velocity rotations were below the vestibular threshold, and recordings were carried out in either light or dark conditions. In light conditions, the authors found that high-velocity rotations did not result in a rotation of HD firing directions, while low velocity sub-threshold rotations resulted in a ~90 degree rotation of HD cell firing direction. They concluded that the vestibular system could not update the HD system during low velocity rotations, as the vestibular system could not detect the rotations. During high velocity rotations, the vestibular system

detected the rotations of the apparatus, and could therefore maintain correct firing directions of HD cells.

In the dark condition the results were more mixed, with an equal likelihood of HD cells rotating by 90 degrees, or not rotating at all, in both high and low velocity signals. Whilst at first surprising, this effect may be explained by a re-weighting of the importance of olfactory cues in darkness. Studies of place cells during sub-threshold rotations have revealed similar results, with place cells rotating their place-field location accordingly with the degree to which animals had been passively rotated on a rotating platform (Sharp, Blair, Etkin, & Tzanetos, 1995; Jeffery, Donnett, Burgess, & O'Keefe, 1997)

In another study, Zugaro et al. (2002) recorded HD cells during fast and slow passive rotations while animals were immobile. Rather than restraining the animals, which was previously shown by Taube (1995) to reduce HD cell firing rats, Zugaro et al. (2002) trained their rats to stay immobile to receive a water reward while an experimenter manually rotated the platform at either high or low angular velocities. The authors found that HD cell firing rates were significantly higher during fast rotations of the platform than they were during slow rotations. Because of the design of this experiment, it could be concluded that vestibular information, and not motor signals, was driving the firing rate of HD cells.

As discussed in the previous chapter, linear velocity signals are also thought to be important for the maintenance of directional firing. Yoder et al., (2011) recorded from otocania-deficient "*tilted"* mice, and compared the firing characteristics of these mice to those of wild type mice. They found that *tilted* mice, which have a deficiency in the function of their otolith organs, exhibited degradation in directional firing of HD cells over the course of five recordings sessions – an effect that was not seen in wild type mice.

3.3.2 Motor cues

Motor cues may be provided to the animal, and the HD system, in the form of motor efference copy, proprioceptive (the ability to sense joint movement and position) and motor command signals.

Early evidence for the role of motor cues in generating the HD signal came from passive rotation experiments in which rats were either restrained or immobile. Initially, it appeared that passive rotation while the rat was restrained in a towel caused a significant reduction in peak firing rate of HD cells recorded from the PoS (Taube et al., 1990b) and ADN (Taube, 1995) but not the LDN (Mizumori and Williams, 1993). These results, while somewhat inconsistent between brain regions, indicate that motor signals may be important in influencing the firing rate, but not directional modulation or tuning, of HD cells.

Following these studies, Stackman et al. (2003) adapted a previous study by Taube & Burton (1995). In the original experiment, HD cells were recorded as rats moved along a U-shaped passageway from a familiar cylindrical environment into a novel rectangular environment. They found that the PFDs of HD cells were stable between the two environments, showing no effect of novelty on HD cell firing. Stackman et al., (2003) added further manipulations to this study. Rats either actively moved between environments during light or dark conditions, or were passively transported between the two environments during light or dark conditions. The authors found that passive transport of rats between the two arenas – even during light conditions – resulted in a robust shift in the PFD's of HD cells compared to the active movement condition. This indicates that the motor cues provided by active transport are important in maintaining a stable directional signal between two environments.

More recently, Shinder & Taube (2011) recorded HD cells from the ADN while rats were restrained and head-fixed to a rotating apparatus. The firing characteristics were compared with those of active transport conditions and hand-held conditions.

In contrast to the previous findings discussed above, the authors found that the firing rates and PFD's of HD cells during passive rotation (in both the hand-held and head-fixed) conditions were comparable to those of active transport. The authors also reported that head-fixed passive rotation in darkness resulted in similar firing rates and firing directions as in light conditions, with the only consistent difference being an increased tuning width of HD cells. This study suggests that in contrast with previous studies, the vestibular system is sufficient for an accurate directional representation of HD cells, and that motor cues have minimal importance for the maintenance of stable directional modulation of HD cells.

The effects of passive transport have also been studied in three-dimensional environments. As described in Chapter 2, Taube et al. (2013) recorded HD cells as animals climbed on a vertically oriented spiral track after animals had either climbed up a ramp to the track (active transport condition) or been placed on the track by the experimenter (passive transport condition). The authors found that HD cells used a local reference frame in the passive transport condition, and a room-based reference frame in the active transport condition. It is therefore possible that while motor cues may have minimal importance in the modulation of HD cells in the horizontal plane, they may be needed to update the HD system when animals move between the horizontal and vertical planes.

3.3.3 Optic flow

Optic flow comes about as a result of the relative motion of an observer, and the world around them. Movement of the head, or the eye, results in a rotation of the visual field on the retinae, which can in turn provide information about head motion. This information is therefore a candidate for influencing the head direction signal.

The experiment carried out by Stackman et al., (2003) described above, also investigated the importance of optic flow in determining heading direction. HD cells were recorded from the ADN as rats either actively or passively moved between the

cylindrical and rectangular arenas in darkness. During active transport in darkness there was an observed moderate shift in HD cell PFD between the two arenas, which was not statistically significant. This indicates that the optic flow does not strongly affect the HD system when other idiothetic cues, such as vestibular and motor cues, are present. During the passive transport trials in the dark condition – in which motor and optic flow cues were removed – the shift of HD cell PFDs was no different from the passive transport in light condition. Again, this indicates that optic flow is not as necessary for maintenance of HD cell firing as motor efference or vestibular cues.

Further to this study, Shinder & Taube (2011) also observed the responses of HD cells as animals were passively rotated in darkness. The authors observed no differences in the directionality or peak firing rates of HD cells in these trials. This indicates that removal of motor and optic flow cues was not sufficient to disrupt the modulation of HD cells by horizontal rotation, further supporting the notion that HD cells only require vestibular input for the generation and maintenance of the HD signal, and that motor and optic cues are not vital for the maintenance of HD cell firing.

3.3.4 Summary

Taken together with the previous section (3.2), it is evident that while allothetic cues are required to anchor HD cell firing to external landmarks, idiothetic signals generated by the vestibular system are required for the generation of the HD signal (Stackman & Taube, 1997; Stackman et al., 2002). In order to maintain stable directional firing relative to the external world, both allothetic and idiothetic cues are therefore required. The ways in which these cues are integrated are presented in the next two sections – first from an anatomical viewpoint, followed by a discussion of computational models describing the HD system.

3.4 Connectivity of the HD system

As discussed in the previous three sections, there is considerable evidence that the HD signal is generated by the vestibular apparatus through responses to angular head velocity in the horizontal plane, and that the stability of the preferred firing direction of HD cells is aided by allothetic cues, in particular visual landmarks. When addressing the integration of these idiothetic and allothetic cues, we can consider two separate streams of information that converge within the head direction circuit. The ascending stream is thought to relay idiothetic information from the vestibular apparatus, while the descending stream is thought to relay allothetic information such as highly processed visual information from the visual cortex. These streams, proposed by Bassett & Taube (2005), were based on a growing wealth of recording and lesion studies of the HD system. This section will first describe the proposed separate streams, before discussing lesion studies examining the interconnectivity of the various structures containing HD cells, and the evidence for the existence of these streams. An overview of the HD cell network can be seen in figure 3.5.

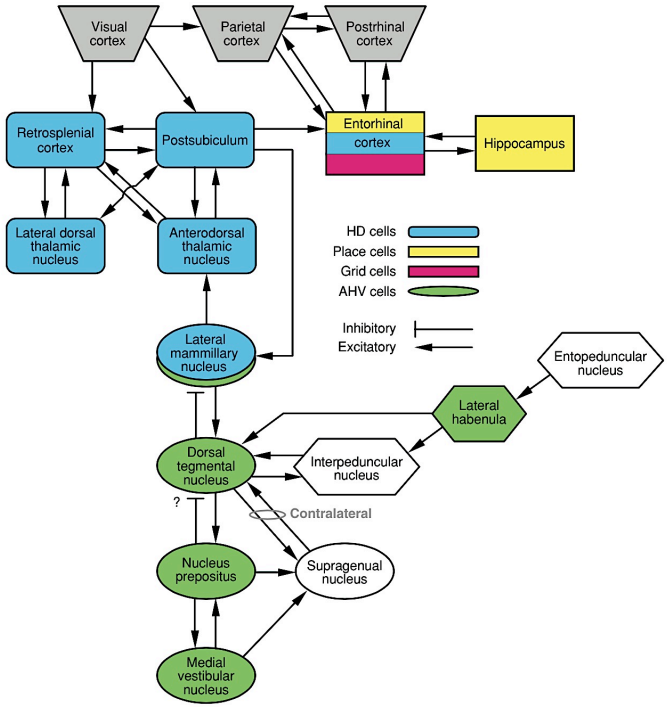


Figure 3.5 Summary of the HD cell network. Flow chart showing the interconnectivity of the HD cell network, and its relationship with the hippocampus and entorhinal cortex. Image taken from Taube (2007).

3.4.1 Ascending pathway

As the directional modulation of HD cells is severely disrupted by the lesions of the vestibular apparatus, while relatively unaffected by the removal of allothetic cues, it is apparent that the HD signal is generated by the vestibular end organs, in particular the horizontal semicircular canals and the medial vestibular nucleus (MVN). Velocity information generated by the vestibular end organs flows from the MVN via the nucleus prepositus hyperglossi (nPH) to the DTN, which is the first region along the ascending pathway containing cells that exhibit directionally modulated firing (Fig 3.6). The DTN itself contains reciprocal connections with the LMN, with the afferents to the LMN being largely inhibitory. The stream then flows from the LMN towards the ADN, which in turns sends afferents to the granular layers of the RSc and to the PoS. The ascending pathway then flows towards the superficial layers of the entorhinal cortex, and then on towards the hippocampus via the perforant pathway.

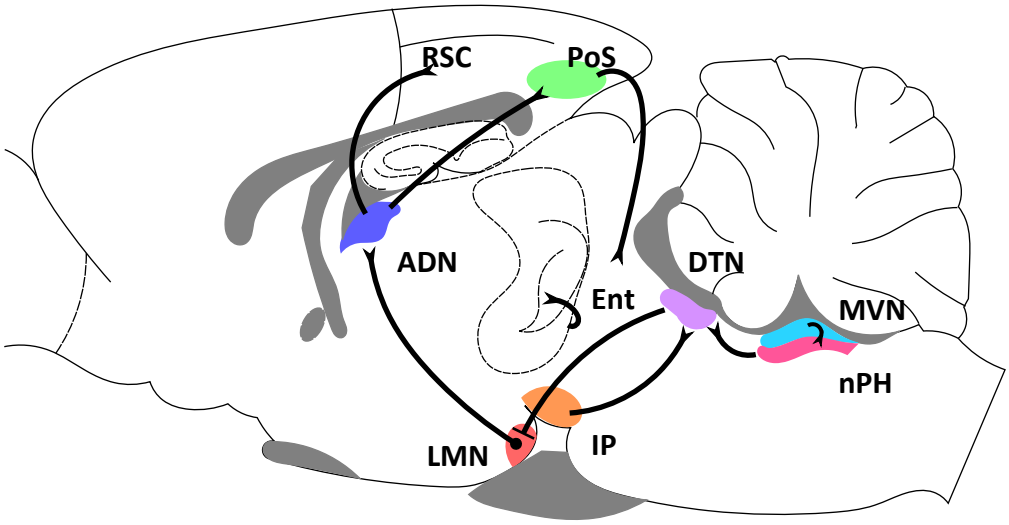


Figure 3.6 Connectivity of the ascending stream. Information from the vestibular apparatus enters the median vestibular nucleus (MVN) and moves onto the nucleus prepositus hyperglossi (nPH). The nPH projects to the dorsal tegmental nucleus of Gudden (DTN), which also receives input from the interpeduncular nucleus (IP). The DTN projects to the lateral mammillary nucleus (LMN) that in turn projects to the anterodorsal nucleus of the thalamus (ADN). The ADN then relays information to the retrosplenial cortex (RSC) and postsubiculum (PoS), which in turn relay information to the entorhinal cortex (Ent). Image adapted from Bassett & Taube (2005).

3.4.2 Descending pathway

The descending pathway (Fig 3.7) is thought to provide information from allothetic cues to the HD circuit. Cortical inputs from the visual cortex and other sensory regions provide input to the PoS and the RSC – two regions that are heavily interconnected through reciprocal connections between the dysgranular RSC (Rd) and the PoS, and between the granular B section of the RSC (RgB) and the PoS. As well as this, the RgA section of the RSC sends afferent connections towards the PoS. Information then flows from the RSC and PoS towards the ADN – which itself sends afferents back to each of the structures along the ascending pathway. As well as this projection to the ADN, the PoS relays information ventrally to the LMN, which in turn projects back towards the DTN.

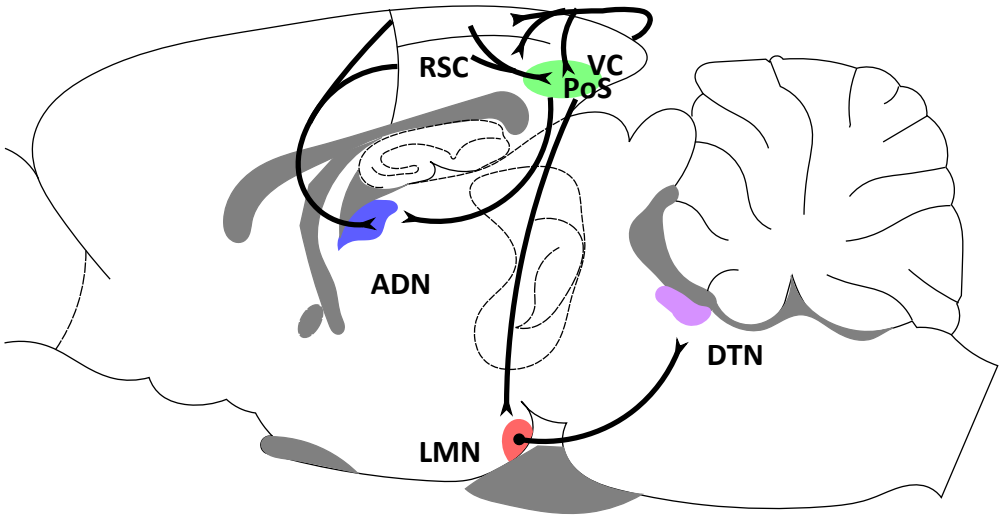


Figure 3.7 Connectivity of the descending stream. Inputs enter the PoS from the visual (VC) and retrosplenial cortices (RSC). The ADN receives direct input from the RSC and from the PoS. The PoS also sends direct input to the LMN, which in turns projects to the DTN. Image adapted from Bassett & Taube (2005).

3.4.3 Lesion studies of connectivity

As can be gathered from the above descriptions of the ascending and descending pathways, the HD system contains a complex set of interconnected brain regions, with reciprocal connections between many of the structures, and with upstream

regions of the ascending pathway (LMN and DTN) receiving direct input from the most upstream regions of the descending pathway. In order to disentangle the integration of information between the various brain regions of the HD cell circuit, researchers have carried out complementary lesion and recording studies between differing brain regions. The effects of HD circuit lesions on HD cell, place cell and grid cell activity will be discussed in the order of the ascending pathway from lesions of the NpH and DTN to lesions of the retrosplenial cortex.

Lesions studies of the most upstream regions of the ascending pathway have revealed the strength of the input from the vestibular system. Butler & Taube (2015) recorded HD cells from the ADN after lesions of the NpH. Complete bilateral lesions led to a degradation of directionally modulated HD cell activity in the ADN – none of the recorded cells maintained a consistent within-trial PFD. However, these cells did exhibit some directional modulation, displaying epochs of directionally modulated bursting of firing. Each epoch of bursting was in a different direction, highlighting the importance of the NpH in maintaining consistent directional modulation of HD cells. In animals with incomplete lesions HD cells maintained stable directionally modulated firing in standard sessions but lost PFD stability during dark sessions.

Complete bilateral lesions of the DTN yielded similar results, with none of the cells recorded in the ADN exhibiting directional modulation (Bassett, Tullman, & Taube, 2007). Even incomplete lesions of the DTN resulted in a severe disruption of HD cell activity, with only 5.6% of all ADN cells classifiable as HD cells, compared to 46.6% of cells in control animals. Further along the ascending pathway from DTN is the LMN. Again, complete bilateral electrolytic lesions of the LMN resulted in a complete degradation of HD cell modulation by direction. These cells, recorded from the ADN, maintained a similar background firing rate to the pre-lesion recordings, but did not show any tuning of firing rate by direction (Blair, Cho, & Sharp, 1999). Incomplete lesions of the LMN had a different effect. HD cells in the ADN showed a decrease in firing rate after lesions, and some exhibited a shift in

PFD. These cells maintained a consistent firing direction and firing rate over several sessions after the incomplete electrolytic lesions.

Together these results indicate the importance of the upstream regions of the ascending pathway in determining directional modulation of cells recorded from the ADN.

Moving further along the ascending pathway from the LMN, lesions of the ADN have also been shown to have an effect on HD modulation in the PoS and other downstream structures. Goodridge & Taube (1997) carried out complete lesions of the ADN and found that none of the 384 cells recorded from the PoS could be classified as HD cells. In the one instance of incomplete lesions, the authors found four HD cells in the PoS, only one of which was sufficiently distinguishable from background firing rates to determine its directional tuning range. The range of this cell was ~240° - a value much greater than those reported from intact animals (Taube et al, 1990b).

In the same report, Goodridge & Taube (1997) carried out lesions of the PoS while concurrently recording from the ADN. Of the cells recorded, 26.1% could be classified as HD cells – a somewhat smaller yield than had previously been reported in intact animals (Taube, 1995). These HD cells had similar peak and background firing rates to control animals, but did exhibit an increased directional firing range. As well as their increased directional firing range, HD cells recorded from PoS-lesioned animals were not controlled by the rotation of landmarks. In intact animals, the rotation of a cue card by 90 degrees resulted in a shift in the PFD of HD cells by a similar amount. In the PoS-lesioned animals, ADN cells did not exhibit any consistency in the extent of rotation, nor the direction of the rotation. This indicates that while the ADN is vital for directional modulation of cells in the PoS, the PoS is required for the integration of visual landmarks by the ADN, albeit not for the generation of directional modulation of firing. This again highlights the importance of the ascending stream in the provision of the HD signal, alongside the importance

of the upstream regions of the descending stream in anchoring HD cell firing to external landmarks.

Lesions of the HD network also affect the firing of other spatially modulated cells. Calton et al. (2003) recorded place cells from CA1 in the hippocampus after lesions of either the PoS or ADN. After lesions of either region, place cells exhibited a decrease in their spatial coherence and their spatial information content. PoS lesions had the strongest effect, with place cells in these animals showing more inter-session instability with random rotations of the place fields, while ADN-lesioned animals had maintained fairly consistent place field localisation as well as appropriate rotations of the place field in accordance with cue rotations. In particular, these results show the HD signal is not necessary for the localised firing of place cells, but that it does help to drive the position of place fields relative to external landmarks.

More recently, Winter, Clark & Taube (2015) carried out temporary and permanent lesions of the anterior thalamic nucleus (ATN) while recording from the MEC. Temporary lesions of the ATN using lidocaine caused a disruption in the regularity of grid cell firing, with high doses of lidocaine resulting in a greater decrease in grid scores of grid cells than low doses. Permanent lesions of the ADN had a permanent effect on the grid scores of grid cells. As well as the effect on grid cells, HD cells recorded in the MEC showed degraded directional modulation by high-dose lidocaine temporary lesions, and by permanent lesions of the ATN. The studies of Calton et al. (2003) and Winter, Clark & Taube (2015) highlight the importance of the HD system for the wider range of structures supporting the neural representation of space.

In summary, the lesion studies performed on the HD system reveals the great importance of vestibular input to the ascending stream in generating the HD signal, as well as displaying the role of the sensory input to the descending stream in refining the directional sensitivity of HD cells elsewhere in the HD circuit. In a wider

context, lesion studies have also shown that the HD signal is vital for the stability of place cell firing and the generation of grid cell firing, showing its importance in the wider functioning of the neural representation of space.

3.5 Models of head direction cell activity

Since the first reports of HD cells (Taube et al., 1990a; 1990b) it has become clear that there is a strong network of interconnected brain regions which contain these directionally modulated cells, and that there are small but not-insignificant differences in the firing properties of cells between these regions. It has also been shown within a single brain region that when multiple HD cells are recorded simultaneously during cue rotation experiments, then the cells all rotate their PFDs by the same amount and in the same direction. Such results indicate that HD cells work in conjunction with one another, and suggest that there is likely to be a great deal of interconnectivity between different HD cells. The way in which this interconnectivity drives and maintains a consistent HD signal has been evaluated using various computational models.

The first computational hypothesis of HD cell activity came from McNaughton, Chen & Markus (1991), only one year after the initial report of HD cells by Taube et al. (1990a). The model hypothesised that HD cell tuning is maintained through the connections between four major cell types: HD cells, AHV cells, HD x AHV cells and "local view" cells. This model assumed that HD cells, and AHV and HD x AHV cells, could be treated as linear vectors that were interconnected. HD cells would report on the current heading direction, while AHV cells provide a linear input to predict final heading direction after a given rotation of the head. They also predicted the presence of HD x AHV cells, which act as an intermediary between the AHV and HD connectivity and respond to heading direction only at a given angular head velocity.

Indeed, subsequent to this prediction, AHV cells and HD x AHV cells were discovered in many parts of the HD cell circuit, including the DTN (Bassett & Taube,

2001) and the LMN (Stackman & Taube, 1997). Beyond the presence of these directional and angular velocity sensitive cells, McNaughton, Chen & Markus (1991) suggested that local view cells could work to anchor the signals to the external landmark information. This type of anchoring is required for the rotation of HD cell PFD during cue rotation experiments. While this first model made great steps in its prediction of AHV and HD x AHV cells, it was constrained by its layout as a linear associative mapping system, meaning that it could not explain the Gaussian feature of HD cell tuning curves, nor could it explain the differences in tuning curves between different brain regions.

Rather than using linear associators, the majority of subsequent models of HD cells activity have been based on the attractor hypothesis (Skaggs et al., 1995; Zhang et al., 1996; Goodridge & Touretzky, 2000). Attractor models are used to describe a highly interconnected system whose state evolves over time, known as a dynamic system. For such systems to be useful, they require stable states to which the system can return even after being disturbed. The most simple of these attractors is known as a point attractor, in which a set of states is drawn towards a given stable point. This can be thought of a landscape with a single valley (or a point of lowest altitude). Now, if we are to imagine water running on this landscape, it will eventually be drawn to and collect at this point of lowest altitude, known as the attractor basin. If there are any disturbances to the system due to noise or another source of error the attractor basin should always attract the state of the system back to its stable point.

A point attractor, or even a set of point attractors, is insufficient for the modelling of HD cell activity, because the head can be oriented in any direction at any one time. This means that the attractor needs to have a continuous set of local minima, and in the case of head direction, is most often thought of as a one-dimensional circle with continuum of stable states, or a closely related set of stable states. This type of attractor model, known as a continuous ring attractor, is the most

commonly used system for modelling HD activity. An example of the energy landscape of point and ring attractors can be seen in Figure 3.8.

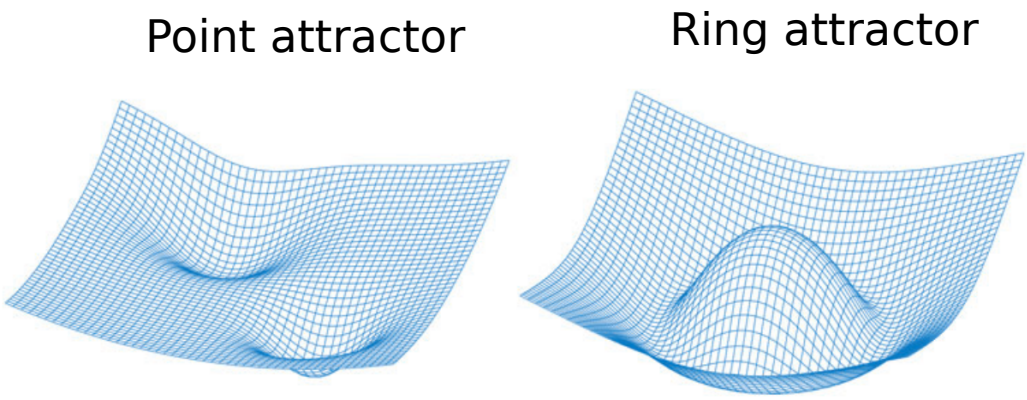


Figure 3.8 Energy landscape of point and ring attractors. Note that on the point attractor example there are two local minima in which a stable state could form. In the ring attractor there is one continuous circular local minimum, in which a stable state can form at any point of the ring. Image taken from Knierim & Zhang (2012).

Skaggs et al. (1995) were the first to conceptualise the HD system within a ring attractor. Expanding on the work of McNaughton, Chen & Markus (1991), their proposed model was developed to explain the Gaussian tuning curves of HD cells. Within the model there are four key cell types arranged in circular layers (for ease of explanation, rather than to reflect anatomical organisation). These cell types are HD cells, rotation cells, vestibular cells and visual input cells. Within the model, the HD cells have strong intrinsic connections, with the strongest excitatory connections between cells encoding for neighbouring directions, and the strongest inhibitory connections between HD cells encoding for opposing directions. There are two external driving mechanisms on the HD ring attractor, in the form of vestibular and visual information. Vestibular cells, which are sensitive to left or right rotations of the head, influence the rotation cells which in turn influence the HD cells. For example, a rotation of the head to the right results in an activation of “vestibular right” cells, which in turn send excitatory input to the “rotation right” cells. These rotation right cells, which receive excitatory input from the currently active HD cell,

also send excitatory input to the HD cells to the right of the currently active HD cell. This causes a shift in excitation of the HD cells to the right, as well as a shift in the excitation of the rotation cells to the right. As long as there is continuous input from the vestibular system, the bump of activity will continue shifting in the same direction around the ring attractor. Once excitation from the vestibular system stops, the rotation of the activity bump will also stop, allowing for a maintained firing of HD cells encoding for the final direction of the head.

The ring attractor model (Fig 3.9) also uses visual landmark information to anchor HD activity to specific directions. Visual cells are thought to provide weak input to all HD cells, and allow for the stability of HD activity to account for error within the vestibular system.

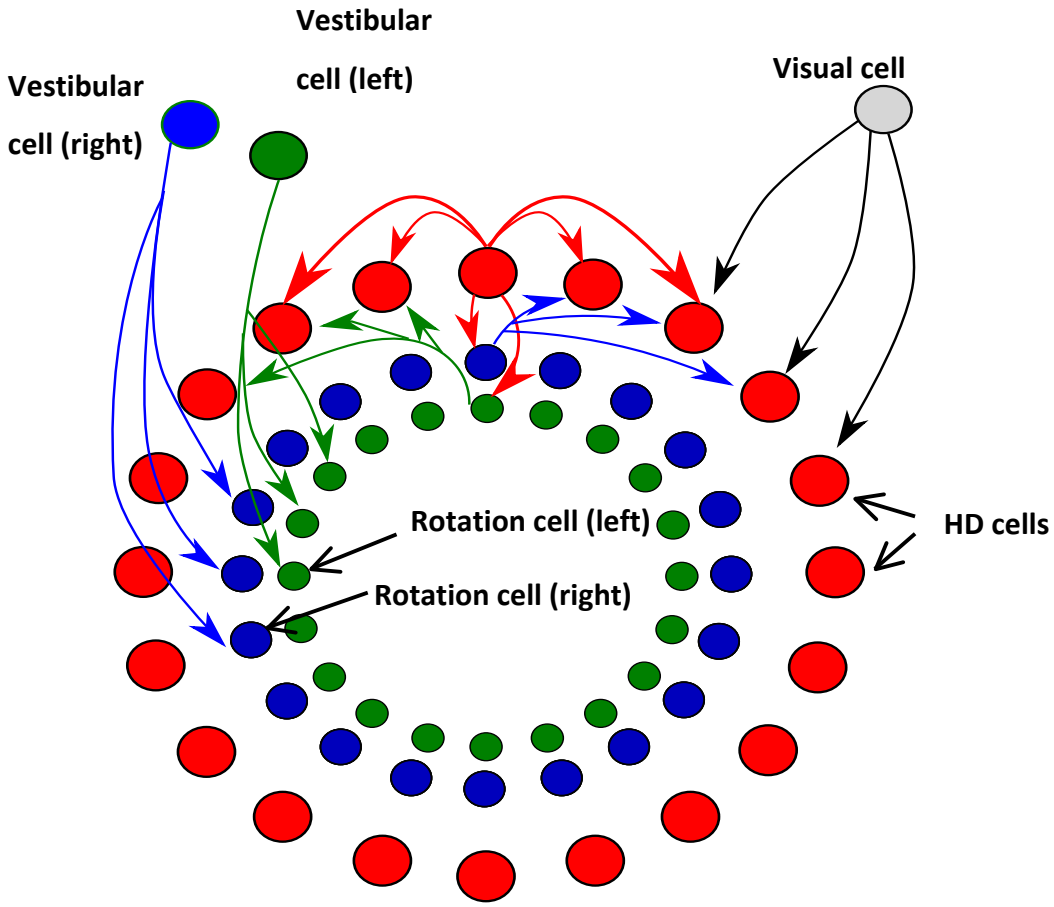


Figure 3.9 Ring attractor model. Architecture of the HD cell ring-attractor model proposed by Skaggs (1995). Image adapted from Skaggs (1995).

The concept that HD cells hold reciprocal weighted connections put forward by Skaggs et al. (1995) was subsequently mathematically formalised by Zhang (1996). A set of simulations in which the weight of connections could be modified was used to describe the shift in the activity bump around a ring attractor. During stable states, the influence of neighbouring HD cells remains equal from all sides of the attractor. This allows the currently firing HD cell to maintain the same level of excitation and continue to fire. However, when the head rotates, a vestibular signal is sent to the HD system and causes an asymmetric reweighting of inputs to HD cells, resulting in a shift of the activity bump in the direction of the head turn. The simulations carried out by Zhang (1996) showed that such asymmetrical weightings between HD cells can result in an active shift of tuning curves.

Some support for the presence of such ring attractors has come from ensemble recordings of HD cells, in which a population of co-recorded cells appear to rotate together after manipulation of visual cues. However, some of the differences in HD firing characteristics, such as the lower ATI's in the PoS and the larger tuning widths found in LMN HD cells, indicates that the whole HD system may not work within a single ring attractor.

More recently, the presence of a directionally sensitive ring attractor system has been reported in *Drosophila* (Seelig & Jayaraman, 2015). Remarkably, cells in the ellipsoid body of the fly central brain are sensitive to orientation in the azimuth, with their activity organised around a ring of neurons. The firing characteristics of these cells, recorded with 2-photon calcium imaging, have many of the hallmarks of a ring attractor. The activity of cells is organised into a localised bump. This bump of activity moves around the ring of neurons during self-motion and was even shown to drift in the absence of visual cues. Moreover, the reintroduction of strong visual input resulted in abrupt jumps in activity, which could be seen as a strong reorientation upon access to external landmark cues.

Of course, the rodent brain has multiple HD containing regions, and processes allothetic cues and idiothetic cues in a different way to *Drosophila*. For example, the vestibular system in *Drosophila* is integrated with its hearing system in a region known as the Johnston's organ, with a relatively simple set of mechanosensory neurons encoding for both (Kamikouchi et al., 2009). Nevertheless, the discovery of a ring-attractor-like system in such a simple organism provides strong physiological evidence for the potential of such systems in the rodent brain.

3.6 Summary

The findings outlined in this chapter show that the HD system consists of a wide range of interconnected brain regions. The directionally modulated firing that is characteristic of HD cells was shown to be dependent on input from the semicircular canals of the vestibular system, and further anchored to the external world through the processing of sensory information, in particular visual cues. Lesion studies have been particularly important in determining how different HD cell containing regions interact, and have also shown that the HD system is important for the functioning of place cells and grid cells – two other vital components of the cognitive map. The study of HD cells in three-dimensional environments therefore not only tests how HD cells represent orientation in three-dimensional space, but also provides a gateway to understanding and predicting how the cognitive map as a whole represents three-dimensional space.

The two experimental chapters presented next tested how three-dimensional space is represented. First, a behavioural study tested whether mice are able to represent three-dimensional space; and second, the manner in which HD cells represent three-dimensional space was tested as rats moved on a three-dimensional structure. Together, the studies presented suggest that the rodent cognitive map represents three-dimensional space in a multi-planar manner.

Chapter 4 Behavioural investigation of place learning in a three-dimensional radial arm maze

The findings presented in this chapter have been published.

Article reference:

Wilson, J. J., Harding, E., Fortier, M., James, B., Donnett, M., Kerslake, A., O'Leary, A., Zhang, N. & Jeffery, K. (2015) Spatial learning by mice in three dimensions. Behavioural Brain Research, 289, 125-132. doi:10.1016/j.bbr.2015.04.035

4.1 Introduction

As has been discussed in previous chapters, the majority of research on spatial memory has focussed on navigation in horizontal two-dimensional environments, with focus only recently turning to navigation in more dimensionally complex spaces. One aspect of navigation in three-dimensional environments that has not yet been tested is whether animals can hold both working and reference memories for locations distributed in three-dimensional space.

As described in chapter one, the radial arm maze apparatus has been used successfully to concurrently test both long-term (reference) and short-term (working) memory (Olton & Samuelson, 1976; Olton & Papas, 1979). Successful completion of the working and reference memory tasks require animals to know both their own position (present and past) on the maze as well as the position of rewarded arms relative to the position of external cues. They also need to continuously update this information to guide their behaviour towards rewarded arms.

While the ability of animals to hold such representations of two-dimensional space is well established, it remains an open question as to whether they can do this in three-dimensional environments. As such, the experiments described in this chapter tested whether mice could form working (WM) and reference memory (RM) representations of a three-dimensional radial arm maze. An inability to perform the WM and RM tasks on the three-dimensional radial arm maze would indicate that mice are unable to represent locations distributed in three-dimensional space, while the ability to successfully complete these tasks on the three-dimensional maze would indicate that mice possess the cognitive mechanisms needed to represent three-dimensional space. Specifically, variations of the radial arm maze were used in this set of experiments because they can concurrently test for spatial working and reference memory and because the general design of the radial arm maze can be easily adapted into a three-dimensional structure without having to significantly alter experimental design. Other tests of spatial memory, such as the water maze (Morris, 1981) can not be so easily adapted for the purpose of investigating three-dimensional spatial memory. This set of experiments therefore used a well-established behavioural paradigm to test whether mice are able to form, recall and use a representation of three-dimensional space to guide their behaviour to reward locations.

The novel three-dimensional radial maze developed for this set of experiments, named the radiolarian maze[1] because of its spherical symmetry, consisted of a spherical centre from which reward-baited arms projected outwards. For each horizontal arm co-ordinate there were two vertical arm co-ordinates (Fig 4.1A). Animals were therefore required to encode horizontal and vertical information simultaneously in order to remember and dynamically update their representation of the distribution of food rewards. Mice were used for this set of experiments instead of rats as they have a naturally 3D ecology and are skilled climbers. Secondary to this, mice weigh about ten to fifteen times less than rats (~25 grams

[1] Radiolaria are zooplankton having radial symmetry: see https://en.wikipedia.org/wiki/Radiolaria

for an adult mouse compared to ~300 grams for adult rats). This allowed for the production of a 3D apparatus that could be suspended within the experimental room. While mice have been shown to perform less well on the radial arm maze than rats (Mizumori et al., 1982) there is evidence that over the course of a 20 trial experiment mice reduce both working and reference memory errors on a radial maze (Levy, Kluge & Elsmore, 1983). This reduction in error-rate over the course of the experiment can still therefore be used to compare learning between different types of radial maze.

Mice were trained on two versions of the radial maze task: the standard working memory version in which all arms were initially baited and became depleted without replacement over the course of the trial, and the reference memory task in which only a subset of arms was ever baited. In the reference memory task, mice were required to learn the position of these baited arms with relation to extramaze cues distributed throughout the experimental room. If mice are unable to encode height, we expected they would have difficulty learning both versions of the task, due to confusion between upper and lower layers of arms.

Learning on the radiolarian maze was compared with a version of the original radial arm maze (the classic maze), and with a two-dimensional analogue of the 3D maze (the hexagon maze). The hexagon maze was developed to produce a two-dimensional radial arm maze that held similar geometric properties to the radiolarian maze. The results show that mice were equally able to learn the working memory task on the radiolarian maze as in the two two-dimensional mazes. In the reference memory task, there was a reduction in learning in the radiolarian maze when compared to its two-dimensional analogue. These results indicate that mice are able to simultaneously represent vertical and horizontal components of a spatial memory task, but that doing so results in processing difficulties that diminish their learning. These findings indicate that mice possess the cognitive mechanisms to represent three-dimensional space.

4.2 Methods

4.2.1 General methods

4.2.1.1 Subjects

Subjects were 40 male C57BL/6J mice obtained at 8-10 weeks of age from Charles River Laboratories, individually housed and mildly food restricted to maintain body weight at 90% of free feeding weight. A 12 hour reversed light/dark cycle was used with 30 min simulated dawn at 23:30 and simulated dusk at 11:30; all mice were trained during their dark cycle between 12:30 and 15:00. Experiment 1 used three cohorts of mice (each n = 8) while Experiment 2 used two (each n = 8). All mice were naïve to the experimental apparatus prior to habituation. All procedures carried out during these experiments were licensed by the UK Home Office, subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act of 1986. Data were collected with the assistance of Elizabeth Harding, Mathilde Fortier, Benjamin James, Megan Donnett, Alasdair Kerslake, Alice O'Leary and Ningyu Zhang.

4.2.1.2 Apparatus

The apparatus comprised three versions of the classic radial arm maze: a 3D version named the *radiolarian maze* (Fig. 4.1A), a 13-arm version referred to throughout as the *classic maze* (Fig. 4.1B), and a two-dimensional analogue of the radiolarian maze, named the *hexagon maze* (Fig. 4.1C). Arms were baited with condensed milk applied to dressmakers' pins inserted at the end of each arm. All three mazes were used for Experiment 1, and just the radiolarian and hexagon mazes for Experiment 2. All experiments were carried out in the same well-lit room, with consistent visual extramaze cues available to mice throughout data collection.

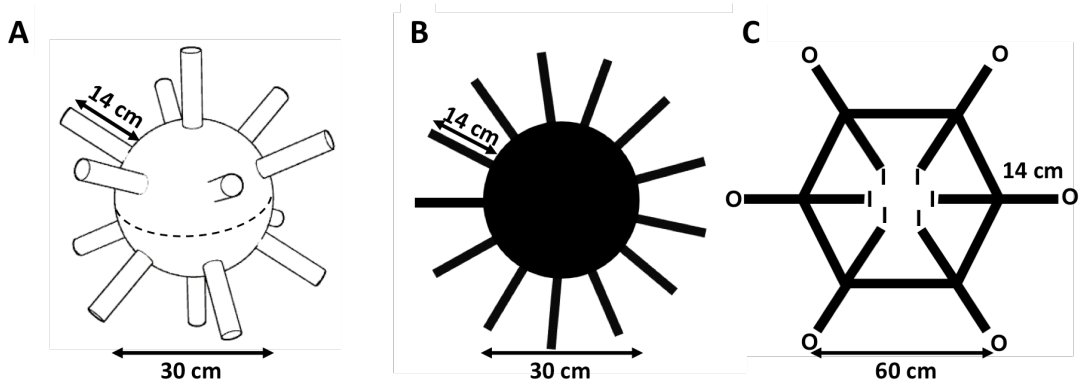


Figure 4.1 A) Schematic of the Radiolarian maze. The dashed line indicates the equator point of the radiolarian maze with arms above this line considered to be in the upper hemisphere of the maze and arms below this line in the lower hemisphere of the maze. There were seven upwardly oriented arms in the upper hemisphere (one upright and six angled at 30° upwards) and seven downwardly oriented arms (one facing vertically downwards and six angled 30° downwards). B) Schematic of the classic maze showing 13 equally spaced arms radiating from a central circular body. C) Schematic of the hexagon maze showing arms radiating from the apices of a hexagonal track. Six arm radiated outwards ('O' labels) and six arms radiated inwards ('I' labels).

The radiolarian maze (Fig. 4.1A) was constructed from lightweight materials and coated with papier-mâché followed by crèpe bandage, to provide grip. The central section comprised a 30 cm diameter sphere from which radiated 14 equidistantly placed cylindrical arms, each 14 cm in length and 3.5 cm in diameter. The arrangement of arms was such that there were seven upwardly oriented arms, six of which were angled at 30 degrees upwards and one vertically oriented, and seven arms that were oriented downwards, with six arms angled at 30 degrees downwards and one vertically oriented. The maze was suspended by nylon line in the centre of an empty 19-inch rack, with the lowermost arm 30cm above the floor of the rack.

The classic maze (Fig. 4.1B) was a 13-arm version of the standard Olton radial arm maze, constructed using MDF and covered with crèpe bandage so as to maintain

consistency with the radiolarian maze. The central section comprised a 30 cm diameter circle from which radiated 13 evenly spaced arms, each 14 cm in length and 3.5 cm in diameter. The maze was raised 30 cm above a table, and was placed in the centre of the experimental room.

The hexagon maze (Fig. 4.1C) had twelve arms. This maze was designed with the intention of providing animals with a two-dimensional apparatus that was more similar to the radiolarian maze than the classic maze. The hexagon maze was considered to have a greater similarity to the radiolarian maze because its structure contained six arms extending towards the centre of the maze and six arms extending outwards from the hexagonal track – similar to the number of upwards oriented and downwards oriented arms on the radiolarian maze. This design meant that mice had a comparable number of movement choices when moving between arms for the radiolarian and classic mazes. The maze constituted a hexagonal ring, with 30 cm sides each 3.5 cm in width, with six 14 cm arms, with a width of 3.5 cm, extended outwards and six 14 cm arms extended inwards from the corners of the ring. Thus, on returning from an arm excursion mice would have four choices – turn left, turn right, go straight ahead or turn back. This maze was therefore more geometrically similar to the radiolarian maze than was the classic maze. The maze was covered in crèpe bandage, so as to maintain consistency with the radiolarian and classic mazes, and was again raised 30 cm above a table in the centre of the experimental room.

4.2.1.3 Habituation

For both experiments, subjects were habituated to their assigned maze for five days before training commenced. In the first two days no arms were baited and mice were allowed to freely traverse the maze for 15 minutes. In the final three days each mouse was introduced to the maze from each of the arms in turn. Once it made its way from an arm to the centre of the maze it was removed and placed on another randomly selected arm. This was repeated until all animals willingly navigated from each of the arms to the centre within one minute.

4.2.2 Experiment 1 – Working memory task methods

4.2.2.1 Subjects and training

Each cohort of mice (n = 8 per cohort) was trained on one of the three mazes only.

Once habituation was completed, the working memory phase of training began. For the working memory task, all of the arms of each of the mazes were baited with condensed milk. Mice were required to retrieve food from all arms of the maze. An arm visit was only recorded when an animal's head reached the end of an arm. The number of re-entry errors (repeated visits to an already-depleted arm) and omission errors (number of unvisited arms) and order of visits were scored manually by two experimenters, who sat in opposing corners of the experimental room. Mice were removed from the maze after either 15 minutes or once they had collected the food reward from all arms of the maze, whichever was soonest. Mice were trained on this task for one trial per day for at least seven days or until the number of omission and re-entry errors had reached a three-day plateau, defined as a non-significant difference between the final three days of trials (using repeated measures ANOVA).

4.2.2.2 Analysis

Paired t-tests comparing the first three trials to the last three trials were used to assess learning. Values for task latency, the total number of visits, the number of omission errors and the rate of re-entry (working memory) errors as a percentage of total visits were compared between mazes using repeated measures ANOVA.

For the movement pattern analysis on the radiolarian maze, the lower vertically projecting arm was removed from analysis as this arm was only ever visited by two mice, and was consistently the last to be visited. For comparison of movements within and between the upper and lower hemispheres of the maze, seven arms comprised the upper section– the vertically projecting arm at the top of the maze and the six upwardly projecting arms on the upper hemisphere of the maze. The six

arms projecting downwards in the lower hemisphere of the maze comprised the lower section. The percentages of total visits that could be assigned to within- and between hemisphere movements were then calculated as a proportion of chance for each movement type, with a chance level of 50% for both within-hemisphere and between-hemisphere movements, as there was an overall 6.5/13 chance of movement between hemispheres (due to the 7 arms upwardly projecting arms, and the 6 downwardly projecting arms), and a 6.5/13 chance of movement within hemispheres.

Further analyses of movement patterns tested for neighbouring-arm biases. In the radiolarian maze, on the upper hemisphere the animals could visit one of seven neighbouring arms (two horizontally adjacent, two diagonally adjacent, one directly below the current arm, one vertically oriented arm, or an immediate revisit to the just-visited arm). When on the lower hemisphere animals could visit one of six neighbouring arms (two horizontally adjacent, two diagonally adjacent, one directly above the current arm, or an immediate revisit).

In the classic maze, 3 out of 13 arms were neighbouring: the two immediately adjacent arms, and the just-visited arm.

In the hexagon maze, 6 out of 12 arms were neighbouring: the arm opposite the previously visited arm, two diagonally adjacent arms, two adjacent arms which were within the same subset of arms (i.e. moving from an outwards projecting arm to one of the two adjacent outwards projecting arms), and the just-visited arm. Arm visits were expressed as a proportion of chance.

4.2.3 Experiment 2 – Reference memory task methods

4.2.3.1 Subjects and training

Each cohort of mice (n = 8 per cohort) was trained on one of the two mazes only. Six baited arms were assigned to each mouse. In the radiolarian maze, only arms on the

two central sections of the maze were baited (three upwardly projecting and three downwardly projecting). The two vertically oriented arms were not baited for the reference memory task. In the hexagon maze, three of the outer six arms and three of the inner six arms were baited. The six goal locations in each maze were specified in allocentric co-ordinates, such that the baited arms for each mouse were defined by their relation to extramaze cues. Each maze was rotated horizontally by 180 degrees either every trial or every other trial to control for intramaze cues, such as tactile cues and olfactory cues related to scent marks left by the mice. Mice were required to visit the same arms with respect to the room cues for each trial, rather than visiting the same physical arms. For each maze, two trials were carried out per day over 25 days, with the first session commencing at 1pm and the second at 3pm. Each trial lasted for five minutes or until all six rewards had been retrieved, whichever was sooner.

Performance was scored as working memory errors (visits to already-visited arms) and reference memory errors (visits to never-baited arms). The order of arm visits, number of total visits, number of omission errors (baited arms that were not visited), task latency, and the total number of erroneous visits (commission errors) were also collected.

4.2.3.2 Probe trials

Two five-minute probe trials were carried out the day after completion of the reference memory task, in order to rule out the (remote) possibility that the animals had used olfaction to find the food. All arms were left unbaited, and the maze was rotated 180 degrees between probe trials to control for intramaze cues. Reference memory errors, re-entry errors, commission errors, omission errors, task latency, order of arm visits and the total number of visits were scored.

4.2.3.3 Analysis

Values for task latency, the total number of visits, number of omission errors, and the percentages of reference memory, working memory and commission errors were analysed. Paired t-tests comparing the first three and last three days of training were used to assess learning. A between-subjects analysis compared the rate of learning for each of these variables between mazes.

4.3 Results

4.3.1 Experiment 1 – Working memory task

4.3.1.1 Radiolarian maze

The first and last three training trials were compared to assess learning. The four variables analysed were the total number of visits, task latency, omission errors, and re-entry errors, measured as a percentage of the total. Paired t-tests showed no difference in the total number of arm visits between the first three trials (16 ± 2 visits) and final three trials (17 ± 1, $t_{(7)} = -0.520$, p =.619), but task latency decreased significantly from 842 ± 40 seconds to 496 ± 68 seconds ($t_{(7)} = 5.129$, p = .001).

Omission errors (failures to enter one of the arms) decreased significantly from an average of 3.9 ± 1.2 errors on the first three trials to 0.4 ± 0.4 errors on the final three trials ($t_{(7)} = 3.816$, p = .007; Fig. 4.2A). Re-entry errors declined significantly from an average of 44.7 ± 7.9% to 24.5 ± 2.9% ($t_{(7)} = 2.538$, p = .039; Fig. 4.2B).

In light of findings from previous studies of a bias towards horizontal movements in three-dimensional environments (Jovalekic et al., 2011; Grobéty & Schenk, 1992), the movement patterns of mice on the radiolarian maze were also assessed. The percentage of the total number of visits that were within the same hemisphere of the maze were calculated and expressed as a proportion of chance (chance = 50%). Within-hemisphere movements did not exceed chance (proportion of chance:

1.04 ± 0.04, $t_{(7)}$ = 0.756, p = .474), but there was a significant preference for visiting neighbouring arms regardless hemisphere (mean 1.4 ± 0.05, $t_{(7)}$ = 8.20, p <.001).

In conclusion, mice showed good working memory performance on the radiolarian maze, showing a decrease in task latency, omission errors and re-entry errors. Thus, they were able to represent the spatial aspects of the task and track these across trials, even though the arms were distributed in 3D space, and each horizontal arm position occurred at two vertical locations. Mice did not appear to use stereotyped choice strategies and did not show differences between horizontal and vertical movement patterns.

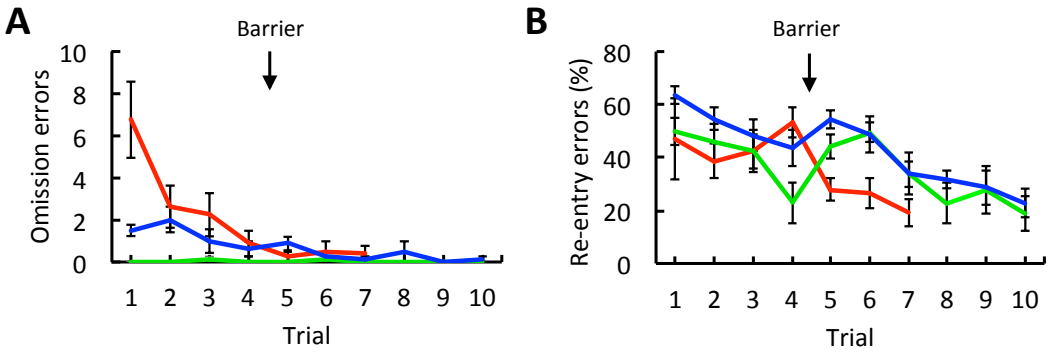


Figure 4.2 Comparison of working memory task learning between the radiolarian maze (red), classic maze (green) and hexagon maze (blue). A) Number of omission errors and B) Rate of arm-re-entry errors as a percentage of the total number of visits. Arrows represent introduction of barriers to the classic maze.

4.3.1.2 Classic maze

The performance of a different cohort of mice was tested on a 13-arm version of a standard radial maze, in order to develop a benchmark of standard two-dimensional performance against which to compare the radiolarian maze findings. Mice received ten days of trials.

Observation of the animals during learning revealed a marked propensity for the mice to adopt stereotyped movement patterns, showing a strong preference for visiting neighbouring arms, unlike the patterns typically shown by rats in a similar

apparatus (Olton, Collison & Werz, 1977). Over the first four trials the proportion of visits to neighbouring arms increased from 0.9 ± 0.1 on the first trial to 3.0 ± 0.3 on the fourth trial. One-sample t-tests for all ten trials combined revealed a significantly above-chance preference for visiting neighbouring arms (mean 2.3 ± 0.18, $t_{(7)} = 7.28$, $p < .001$).

To thwart use of an adjacent-arm rule, barriers were introduced at the entrance of each arm on the fifth day to try and reduce stereotyped behaviour. While this initially reduced the proportion of neighbouring arm visits to 1.4 ± 0.3 on the fifth trial, animals quickly returned to this stereotyped behaviour, reaching a peak proportion of 3.4 ± 0.3 neighbouring arm visits by trial ten.

There was no difference in the number of total visits between the first three trials (27 ± 2) and the final three trials (19 ± 2, $t_{(7)} = 2.254$, $p = .059$). There was, however, a decrease in task latency from the first three trials (412 ± 49 seconds) to the final three trials (210 ± 49 seconds, $t_{(7)} = 3.505$, $p = .010$).

Owing to the stereotypy discussed above, mice were able to complete the working memory task with zero omission errors from the first trial, with an average of 0.04 ± 0.04 omission errors first three trials and an average of 0 ± 0 omission errors on the final three trials (Fig 4.2 A). These values were not significantly different ($t_{(7)}$=1.000, p=.351). Re-entry errors between the first three trials (45.9 ± 4.9%) and the final three trials (23.1 ± 6.0) almost approached significance ($t_{(7)}$=2.350, p=.051; Fig 4.2 B).

In summary, mice completed the working memory task on the classic maze without omission errors and with no decrease in re-entry errors, while movement pattern analysis indicated a high tendency to choose neighbouring arms in a stereotyped manner, which accounted for the error pattern. To overcome this problem and assess true spatial learning, a second two-dimensional radial arm maze was created to reduce the affordance of the maze for stereotypy and make it structurally more

similar to the radiolarian maze, while retaining the two-dimensional layout. Results from this “hexagon maze” are described next.

4.3.1.3 Hexagon maze

The hexagon maze is more akin to the radiolarian maze in which the animals had multiple neighbouring arms to choose from and we hoped it would prevent, or at least reduce, the application of simple rules. Mice received ten days of trials on this maze, and analysis proceeded as before.

There was a significant decrease in the total number of arm-visits ($t_{(7)}$ = 5.667, p = .001), from 26 ± 2 visits on the first three trials to an average of 17 ± 1 on the final three trials. There was also a decrease in task latency from 776 ± 31 seconds to 269 ± 91 seconds ($t_{(7)}$ = 6.396, p < .001). Omission errors decreased from an average of 1.5 ± 0.4 to 0.2 ± 0.2 ($t_{(7)}$ = 4.651, p = .002; Fig. 4.2A). Analysis of re-entry errors revealed that, unlike in the classic maze, there was evidence of spatial learning, with a significant decrease in the percentage of re-entry errors from an average of 55.5 ± 2.0% to 27.8 ± 3.9% ($t_{(7)}$ = 6.905, p <.001; Fig 4.2B). Nevertheless, movement pattern analysis found that despite the change in maze design, there remained a significant preference for visiting neighbouring arms (mean 1.5 ± 0.03, $t_{(7)}$ = 17.13, p < .001).

4.3.1.4 Comparison between mazes

Repeated measures ANOVA were used to compare the learning between the three radial arm mazes. The two error types (omission errors and re-entry errors) were first compared between mazes, followed by comparison of neighbouring-arm visits.

Comparison of omission errors between mazes for the first three vs. last three trials revealed a significant reduction in the number of omission errors ($F_{(1,21)}$ = 25.389, p < .001). Analysis of between-maze effects revealed a significant difference between mazes ($F_{(2,21)}$ = 5.342, p = .013; Fig 4.2A), in which Bonferroni adjusted post-hoc comparisons revealed significantly fewer omission errors on the classic

maze than the radiolarian maze (p = .012), with no differences between the hexagon and classic maze (p = .638) or hexagon and radiolarian mazes (p = .190). For re-entry errors, repeated measures ANOVA also showed a significant overall reduction ($F_{(1,21)}$ = 28.801, p < .001) with no difference between mazes ($F_{(2,21)}$ = 1.517, p = .242; Fig 4.2B).

Finally, comparisons of the neighbouring arm bias revealed a significant difference between mazes ($F_{(2,21)}$ = 21.026, p <.001), with Bonferroni-corrected independent-groups *t*-tests (only p values <0.017 were considered to be significant) revealing a stronger preference for visiting neighbouring arms in the classic maze than the radiolarian ($t_{(7.97)}$ = 4.951, p = .001) and hexagon mazes ($t_{(7.39)}$ = 4.347, p = .003) and no difference between the radiolarian and hexagon mazes ($t_{(14)}$ = 2.281, p = .039; Fig. 4.3 A-B).

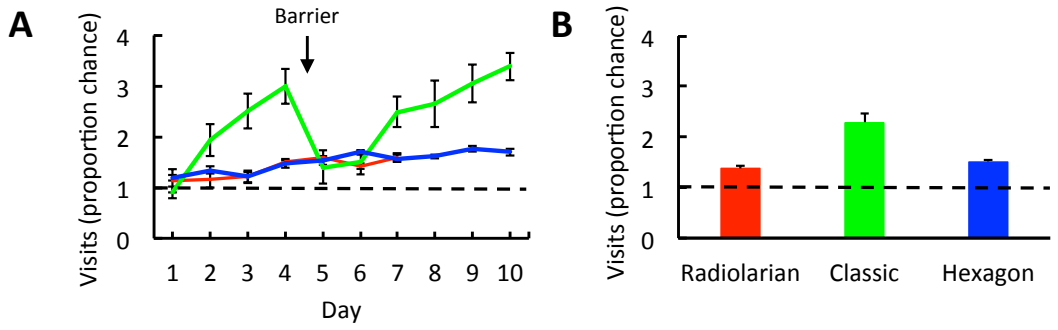


Figure 4.3 Movement patterns during the working memory task. A) Neighbouring-arm visits on the radiolarian (red), classic (green) and hexagon mazes (blue) for each day of trials, represented as a proportion of chance. Arrows represent the time points at which barriers were added to the classic maze B) Task average of neighbouring arm visits represented as a proportion of chance for each of the three mazes. Chance levels are represented by a horizontal dashed line.

4.3.1.5 Summary

Together, these data show that mice can hold a short-term working-memory representation of reward locations on the hexagon and radiolarian mazes, and that their ability to do so increases with experience of the task. Both the hexagon maze

and radiolarian maze yielded similar results for reductions in working memory errors, and for neighbouring arm biases, suggesting that the greater geometric complexity of the mazes compared to the classic maze can reduce the behavioural biases observed on the latter.

The ability of mice to form long-term spatial memory representations of three-dimensional space was assessed next.

4.3.2 Experiment 2 – Reference memory experiment

This experiment compared long-term spatial memory, in addition to working memory, between the 3D radiolarian maze and its 2D counterpart, the hexagon maze. Sixteen naïve mice, split into two cohorts of n=8 each, were trained on either the radiolarian maze or hexagon maze over 25 consecutive days, with two trials per day. Both cohorts of mice were able and motivated to move around their designated maze by the end of habituation. The classic maze was not used for the reference memory task, due to the strong neighbouring-arm bias observed in Experiment 1.

4.3.2.1 Radiolarian maze

In the radiolarian maze, total arm visits did not decrease between the first three days (9 ± 1) and the last three days (10 ± 1, $t_{(7)}$ = -0.768, p = .468) but there was a reduction in task latency from 295 ± 3 seconds to 168 ± 22 seconds ($t_{(7)}$ = 5.841, p = .001).

Omission errors decreased significantly from an average of 2.3 ± 0.3 errors on the first three days of trials to 0.3 ± 0.2 on the final three days of trials ($t_{(7)}$ = 8.064, p <.001; Fig. 4.4A). Commission errors (percentage of all visits that were erroneous) declined significantly from 57.1 ± 2.8% on the first three days of trials to 37.7 ± 4.0% on the final three days of trials ($t_{(7)}$ = 4.393, p =.003; Fig 4.4B). These types of errors were further split into two component parts – re-entry errors and reference

memory errors (visits to arms that had never been baited). The percentage of re-entry errors decreased significantly from 15.3 ± 2.5% to 7.0 ± 2.0% ($t_{(7)}$ = 2.7, p =.032; Fig. 4.4C), while reference memory errors decreased from 56.0 ± 2.6% to 31.5 ± 2.9% ($t_{(7)}$ = 7.141, p <.001; Fig 4.4D). There was thus clear evidence of 3D spatial learning: working memory performance improved slightly and reference memory improved considerably across the course of training.

4.3.2.2 Hexagon maze

Mice on the hexagon maze showed a significant reduction in the total number of visits between the first three days (13.0 ± 0.9) and the last three days (7.0 ± 0.3; ($t_{(7)}$ = 5.341, p = .001) and a significant reduction in task latency from 271 ± 14 seconds to 104 ± 12 seconds ($t_{(7)}$ = 14.878, p <.001).

Omission errors decreased from 1.5 ± 0.3 on the first three days to 0 ± 0 on the final three days ($t_{(7)}$ = 5.029, p = .002; Fig 4.4A). Commission errors decreased from 60.2 ± 3.0% to 15.52 ± 2.9% ($t_{(7)}$ = 11.546, p <.001; Fig 4.4B). Of these, the percentage of re-entry errors decreased from 29.4 ± 2.2% to 3.6 ± 1.7% ($t_{(7)}$ = 8.943, p <.001; Fig 4.4C), while the percentage of reference memory errors decreased from 41.9 ± 2.3% to 12.1 ± 1.7 ($t_{(7)}$ = 5.44, p = .001; Fig 4.4D).

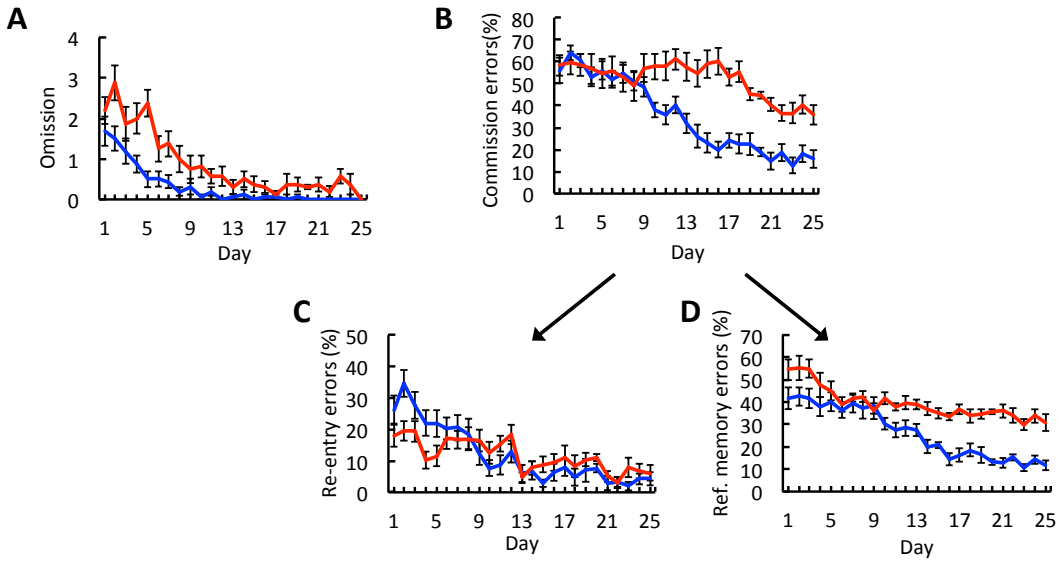


Figure 4.4 Comparison of learning rates between radiolarian maze (red) and hexagon maze (blue). A) Omission errors B) Commission errors C) Working memory errors D) Reference memory errors.

4.3.2.3 Probe trials

Probe trials were then conducted to rule out the use of olfactory cues in solving the task; it was expected that probe trial performance should differ from performance on the first day but not from performance on the last day, so these were examined with repeated-measures ANOVA comparing trial type (training/probe) against day (first/last). For the radiolarian maze, there was a main effect of trial type on the percentage of reference memory errors ($F_{(2,14)} = 11.736$, p = .001). Bonferroni-corrected paired *t*-tests (in which only values with a p < .017 were considered significant) found a significantly lower rate of reference memory errors on the final day when compared to the first day ($t_{(7)} = 3.31$, p = .013) and a significantly lower rate of reference memory errors on the probe day when compared to the first day ($t_{(7)} = 4.03$, p = .005). As expected, there was no difference between the final day of trials and the probe trials ($t_{(7)} = 0.99$, p = .354).

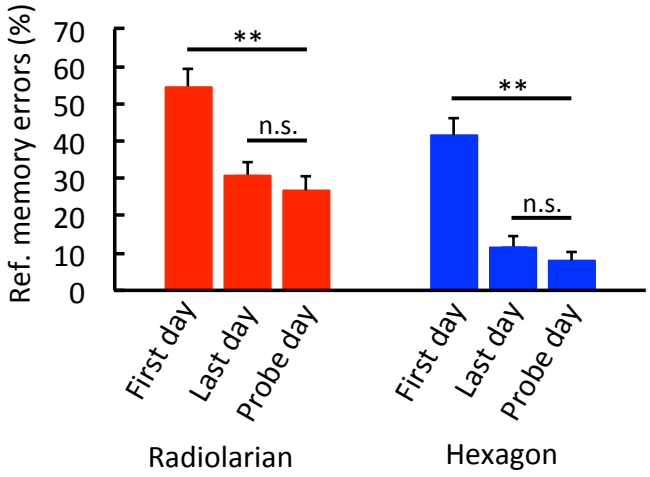


Figure 4.5 Probe trials to rule out reward detection (** denotes p < .01; n.s. denotes non-significant)

For the hexagon maze, there was a main effect of trial type on the percentage of reference memory errors ($F_{(2,14)} = 26.868$, p < .001). Bonferroni-correct paired *t*-tests (p < .017 considered significant) showed a significantly lower rate of reference memory errors in the final day compared to the first day ($t_{(7)} = 5.44$, p = .001) and in the probe trials when compared to the first day ($t_{(7)} = 5.34$, p = .001), with again, no difference in probe trials compared to the final day of trials ($t_{(7)} = 1.44$, p = .194; Fig 4.5).

Overall, probe trial performance was similar to performance by the end of training for both the radiolarian maze and the hexagon maze.

4.3.2.4 Comparison between mazes

Learning between the radiolarian and hexagon mazes was assessed by examining the interaction component of a repeated-measures ANOVA comparing the first and last three days of trials. For total visits, there was no overall between-maze effect ($F_{(1,14)} = 1.646$, p = .220) but there was a significant interaction ($F_{(1,14)} = 20.03$, p = .001), reflecting a decrease in the total of number of visits in the hexagon maze but not in the radiolarian maze. For task latency, there was a significant between-maze effect ($F_{(1,14)} = 1.409$, p = .017), with mice taking less time to complete the reference memory task on the hexagon maze than on the radiolarian maze. There

was no interaction effect on the reduction in task latency between mazes ($F_{(1,14)}$ = 2.72, p = .122).

Omission errors showed significant between-maze effects ($F_{(1,14)}$ = 4.659, p = .049), in which mice exhibited both fewer omission errors and shorter trial times in the hexagon maze than the radiolarian maze, with no interaction ($F_{(1,14)}$ = 2.02, p = .178; Fig 4.4A).

Analysis of commission errors found a significant between-maze effect in which mice made fewer errors in the hexagon maze than the radiolarian maze overall ($F_{(1,14)}$ = 7.621, p = .015). Additionally, the interaction with trial block was highly significant ($F_{(1,14)}$ = 18.509, p = .001) due to a greater reduction in the hexagon maze than the radiolarian maze (Fig 4.4B). We further split the commission error data into its component parts, re-entry and reference memory errors. For re-entry errors, there were significant between-maze effects overall ($F_{(1,14)}$ = 6.525, p = .023) and a significant interaction ($F_{(1,14)}$ = 16.738, p = .001), whereby re-entry errors decreased more on the hexagon maze (Fig 4.4C). For reference memory errors, mice committed fewer errors in the hexagon maze than in the radiolarian maze overall ($F_{(1,14)}$ = 36.036, p < .001), but there was no interaction ($F_{(1,14)}$ = 1.724, p = .210; Fig 4.4D).

Together, these data suggest that mice made fewer errors and learned more effectively on the hexagon maze than on the radiolarian maze.

4.3.2.5 Summary

In summary, mice were able to learn a spatial reference memory task on both a 3D maze (the radiolarian maze) and a 2D maze (the hexagon maze). There were, however, differences in learning between mazes, with mice exhibiting more re-entry errors, and more reference memory errors on the three-dimensional maze than on the two-dimensional maze. These results are further discussed below.

4.4 Discussion

Motivated by previous behavioural studies of spatial memory in three-dimensional space, together with the well-replicated studies of two-dimensional spatial memory on the radial arm maze, a three-dimensional radial arm maze (the radiolarian maze) was developed. The ability of mice to form and maintain representations of reward locations distributed throughout three-dimensional space was tested.

Mice were able to learn both the working memory and reference memory tasks on the radiolarian maze, indicating that they could form and use representations of three-dimensional reward locations, maintaining these representations both within trials and across days. They were, however, less able to do this than on a comparable two-dimensional radial maze variant (the hexagon maze). Mice are therefore able to represent 3D space, but with more difficulty than 2D space. Discussion of the experimental results will begin with an examination of the findings, followed by a discussion of how the radiolarian maze may have been represented.

4.4.1 Spatial performance in two vs. three dimensions

In experiment 1, a working memory task was used to ascertain whether mice are able to represent the spatial layout of the three radial maze variants, and to use this representation alongside a working memory of recent behaviours to refrain from visiting previously visited reward locations. In the classic maze, mice exhibited a strong propensity for visiting neighbouring arms, and the results were not necessarily informative with regard to spatial encoding. The discussion is therefore restricted to learning on the radiolarian and hexagon mazes. In these two mazes, the percentage of re-entry errors declined significantly across the course of the experiment. The rate of decline was comparable for both mazes, indicating that mice are equally able to hold short-term representations of two-dimensional and three-dimensional space.

In the reference memory task (experiment 2), mice showed a significant decrease in both working and reference memory errors in both the hexagon and radiolarian mazes. The rate of reduction of reference memory errors was comparable between the mazes; however, the total number of both working and reference memory errors was significantly greater in the radiolarian maze than the hexagon maze. The percentage of reference memory errors asymptoted at ~30% in the radiolarian maze, compared to ~12% in the hexagon maze. These results suggest that there may be an increased difficulty in the formation, retention and/or use of long-term representations of locations distributed in three-dimensional space.

Nonetheless, the finding that mice can learn both working and reference memory tasks on the radiolarian maze suggests some ability to represent the vertical component of 3D space. The possible representations of the radiolarian maze, alongside their implications for successfully completing the experimental tasks are discussed below.

4.4.2 How is the radiolarian maze represented?

There are two possibilities for the differences in learning between the radiolarian and hexagon maze. First, the physical demands of moving on in three-dimensional environments like the radiolarian maze are considerably greater than in two-dimensional environments. This increased difficulty in moving around the maze may have diverted cognitive resources, such as attention, away from the spatial components of the task. The second is that the cognitive mechanisms for representing vertical space may either be at a lower resolution than for horizontal space space, or that they are computationally more complex and therefore take longer to acquire and use effectively.

While considerable effort was taken to create analogous three-dimensional and two-dimensional radial mazes, there remained some key differences between the mazes that may have resulted in the differences in learning rate between the mazes. The radiolarian maze, as a result of its three-dimensional structure, had

sections in which the animal needed to climb vertically, and sometimes slightly inverted, towards goal positions. Although mice are competent climbers, and regularly climb in an inverted position in their own cages, it is possible that the increased motor demands of moving on the radiolarian compared to the hexagon maze contributed to the differences in learning rates between the mazes.

One potential effect of the increased motor demands on the radiolarian maze is that animals paid more attention to their movement on the radiolarian maze than they did on the actual position of rewarded goal locations. This diversion of attention could impact on the ability of animals to encode the position of rewards relative to the extramaze cues in the room. While the presence of this effect could not be tested within the current experimental setup, the comparable percentage of working memory errors between the two mazes indicates that mice were at least able to pay sufficient attention to their position on the radiolarian maze to recall in the short-term the arms that they had previously visited.

Nevertheless, the effects of the increased motor demands could be tested in the future with the introduction of climbable barriers or ramps to the arms of the hexagon maze. In such a maze, the position of food rewards would be kept in the same horizontal plane to ensure that the goal locations could be described in a two-dimensional co-ordinate framework, but animals would also be required to carry out more energetically costly movements to reach those goals than in the original hexagon maze.

The representational explanations for the findings of this study are next discussed with reference to the mechanisms for representing three-dimensional space that were introduced in Chapter 2. Did mice use a volumetric, planar or multi-planar representation of the radiolarian maze?

First, mice might have created an integrated volumetric map for representing the three-dimensional structure of the radiolarian maze. In such a representation, each goal location would have unique spatial co-ordinates, described by x, y, and z co-

ordinates, or each arm may be represented by a unique vector. In successful completion of the task, mice could visit each of these unique locations and recall the co-ordinates of previously visited locations. Such a map would provide the richest detail in aiding navigation on a 3D surface, and presumably would not have resulted in the diminished reference memory performance seen in the radiolarian maze. On the other hand, the formation of a fully volumetric map may have been more cognitively challenging than the 2D map required for successful task completion on the hexagon maze.

One possibility that can likely be discounted based on the present results is that mice use a single purely-horizontal planar cognitive map. If the mice were using such a map, they would be expected to (a) confuse arms between the upper and lower arms on the radiolarian maze and (b) solve the task in a planar way, visiting one layer of arms first before proceeding to the second layer, as seen in Jovalekic et al. (2011). The equally fast learning of mice on the working memory task makes arm confusion on the radiolarian maze unlikely, while movements between layers in the radiolarian maze occurred at an equal proportion to within-layer movements.

This does not, however, mean that the representation of space is not planar. Mice might instead use and integrate multiple planar maps. For example, mice may hold separate representations for vertical and horizontal locations, with one map representing height, and the other representing horizontal displacement. As such, in completion of the radiolarian maze tasks, mice would be required to integrate their memory for the horizontal position of rewards with their memory for the vertical position of rewards. Such a system might explain their ability to complete the tasks on the radiolarian, as well as the difference in reference memory between the radiolarian and hexagon mazes. It is possible that the integration of two separate maps is computationally more difficult, making it harder to establish memory of a constellation of different reward locations.

Another way of looking at a multi-planar map is to consider the representation of the upper and lower layers of the radiolarian as two separate horizontal maps, with the decision to move between the layers based upon a binary decision (i.e. is the reward on the upper layer or lower layer). Again, using such a map system requires the integration between the upper and lower layer maps in the radiolarian maze, while only one map would be need in the hexagon maze.

Finally, a possible intermediate between one-volumetric vs. multi-planar maps is a surface coding one, in which mice form one large planar map in which the spherical surface of the radiolarian maze is "unwrapped" and represented in two-dimensional co-ordinates. In such a map, the absolute vertical space is disregarded, and the arm position is specified relative to the surface of the sphere. This would rely on metric information – distance and direction – being applied to the curved plane of locomotion of the mouse. While this cannot be discounted with behavioural experimentation alone, it seems unlikely that a surface-coding map would have resulted in the difference in reference memory between the hexagon and radiolarian maze as both mazes would be represented by a two-dimensional metric.

4.5 Conclusions and future work

An understanding of how animals represent and use three-dimensional space is needed if we are to gain a comprehensive view of the mechanisms underlying spatial cognition. The present experiment therefore investigated whether mice could learn working and reference memory tasks on a 3D version of the radial arm maze (the radiolarian maze). The findings show that mice are able to learn these tasks, but with greater difficulty than on a 2D analogue of the radiolarian maze.

Future work is needed to address two questions brought about by the present findings: (a) how is the radiolarian maze represented, and (b) why is learning less efficient in the radiolarian maze than its 2D analogue? The differences in learning efficiency may have come about due to increased motor task demands on the

radiolarian maze, which diverted attention or other cognitive processes away from the spatial task. This could be tested with modifications to the hexagon maze, in which the introduction of climbable barriers on the arms could be used to increase motor demands on the 2D maze.

On the other hand, the lower learning efficiency on the radiolarian maze may be explained by the mechanisms used to represent the radiolarian maze. Understanding whether this representation is volumetric, planar or multi-planar or otherwise will require more detailed study of the neural processes underlying spatial cognition in three-dimensional space. The experiment detailed in the next chapter therefore tested whether head direction cells, considered to be the neural basis for judging spatial orientation, respond to movement on a three-dimensional structure in a planar, multi-planar, or volumetric manner.

Chapter 5 Updating of head direction cells in a three-dimensional environment

5.1 Introduction

The study detailed in Chapter 4 showed that mice are able to represent a three-dimensional maze and that they learn to utilise this representation to guide their behaviour towards rewarded goal locations. While the behavioural data give some indication of the manner in which the maze is represented (i.e. it appears that mice do not use a single horizontally-oriented reference frame), studies of the neural processes underlying this representation are needed to determine whether the representation of three-dimensional structures is planar, multi-planar, or volumetric (detailed in Chapter 2). The study presented in this chapter therefore tested how head-direction cells, thought to be the neural basis for judging orientation, behave during movement on a three-dimensional structure. Furthermore, understanding the functioning of the HD system in three-dimensional space can further inform the understanding of place and grid cell activity in three-dimensional environments.

Recently, HD cells have been reported in bats, with the HD cells recorded as animals climbed on a tilted surface and during flight. Unlike the findings from studies with rats, some of these cells were modulated by pitch rotations or roll rotations, and some were even conjunctively modulated by two or more of the three Bryan rotations (as discussed in Chapter 2). The cells that were conjunctively modulated by yaw and pitch rotations encoded for azimuth angle as well as elevation angle, thus providing an orientation vector in 3D space (although only in instances that the animal was in an upright position).

Studies of rodent HD cells in three-dimensional environments have provided somewhat different results, with HD cell firing directions appearing to be anchored to the animal's plane of locomotion. Indeed, as an animal moves from a horizontal surface to a vertical plane, the PFD of HD cells is maintained (Stackman & Taube, 2000). That is, a cell with a PFD facing towards a climbing wall will be maximally active when an animal faces upwards on the wall, and a cell with a PFD pointing away from the climbing wall would only exhibit firing as the animals faces downwards on the wall (Stackman & Taube, 2000; Taube et al., 2013). In essence, the firing of HD cells as animals climb between horizontal and vertical planes indicates that the vertical plane may be treated as an extension of the horizontal plane.

However, the reason for this continuation of PFD between horizontal and vertical planes is ambiguous. One possibility is that the HD system is unresponsive to the pitch rotation carried out by animals as they traverse between horizontal and vertical planes, and thus treat the two planes as the same entity. A second possibility is that HD cells are actively updated between differently oriented planes and are thus sensitive to non-yaw rotations. Although the recordings of Taube et al. (2013) did not indicate that rodent HD cells represent orientation volumetrically, it remains possible that HD cells encode for both elevation and azimuth angle during movement on structures with multiple vertical surfaces.

To test whether the HD system represents orientation in a planar, multi-planar or volumetric manner, an apparatus was developed on which HD cells were recorded as animals moved between differently oriented vertical planes (Fig 5.1). More specifically, HD cells were recorded on two opposing climbing walls (East and West walls). Rats climbed from a horizontally oriented starting box onto a starting wall (South wall) before transitioning to either the East or West walls where they foraged for food. Importantly, the transition from the South wall to the recording walls can only be achieved through pitch or roll rotations, meaning that any change in HD firing behaviours between the East and West walls would be driven by non-

yaw rotations. Such an effect of non-yaw rotations of rodent HD cell firing has not been previously reported.

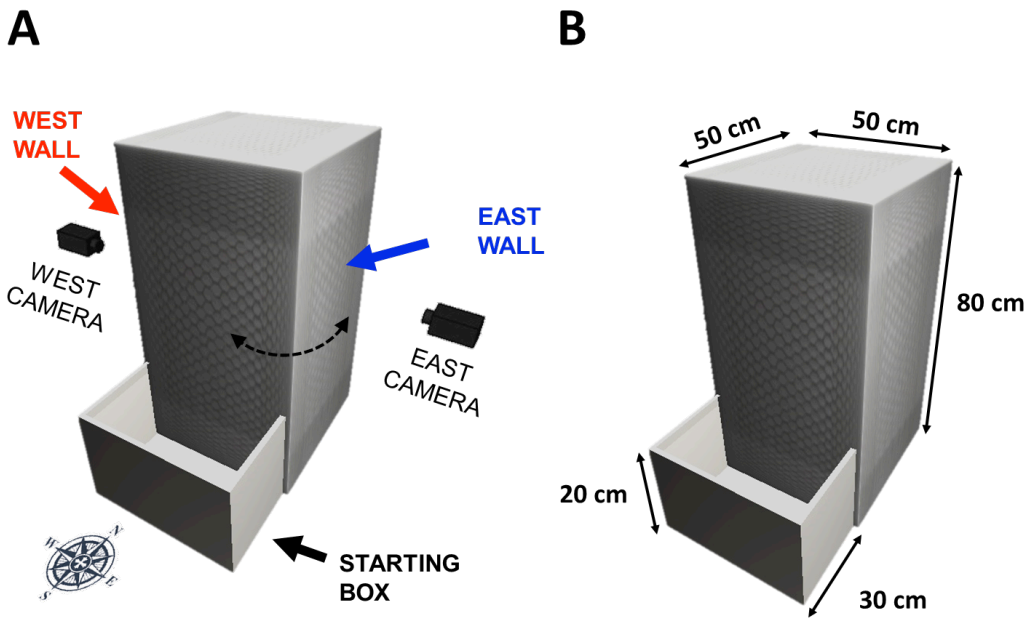


Figure 5.1 Climbing apparatus. A) Experimetal setup B) Dimensions of the climbing apparatus. Animals climbed from the starting box onto a South wall, before traversing to the East or West walls to forage for food. HD cells and the animal's heading direction were recorded on the East and West walls.

5.1.1 Pilot studies

A series of pilot studies informed the final design of the climbing apparatus and recording protocols described above. In the first set of pilot studies HD cells were recorded from mice and rats as they carried out roll rotations around the earth-vertical axis. Animals were required to climb a 200cm corkscrew ladder that forced a roll rotation of 450° (Fig 5.2A). In a second set of pilot experiments HD cells were recorded from mice as they carried out pitch rotations around the inside wall of a circular arena (Fig 5.2B). In both sets of experiments the animals' head direction was tracked using a ceiling-mounted camera. The two sets of pilot studies were both designed to test whether HD cells are responsive to non-yaw rotations around the earth-vertical axis. This section will describe the progression of the experimental

designs used to test the functioning of HD cells in three-dimensional space, and will provide reasoning for the use of the final experimental apparatus and protocol.

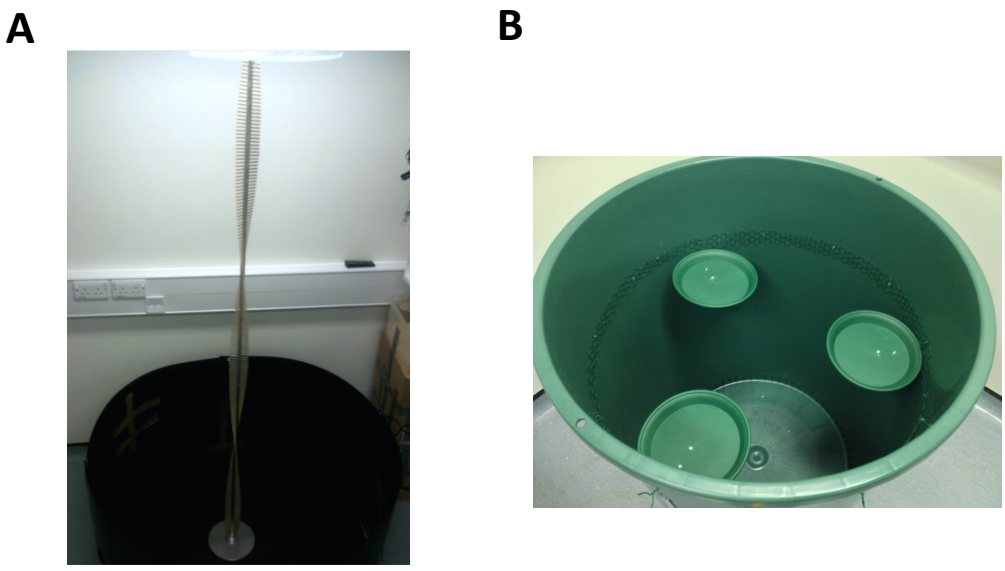


Figure 5.2 Pilot study apparati. A) The corkscrew ladder was developed to test the modulation of rat and mouse HD cells by roll rotations of the head around the earth vertical axis. The corkscrew ladder was 200cm and forced a rotation of 450° as animals climbed up the ladder. B) The cylindrical climbing apparatus was developed to test the modulation of HD cells by pitch rotations of the head. Mice climbed from one of three starting points (green discs) 360° around a wire mesh ladder.

5.1.1.1 Corkscrew Ladder

The corkscrew ladder was designed to test whether roll rotations of the head around the earth-vertical axis modulate HD cell activity in the same way that yaw rotations of the head have been shown to. In the first iteration of the corkscrew ladder experiment rats were required to climb 200cm up a ladder to retrieve a food reward. The angle of the climbing rungs on the ladder was offset incrementally such that they forced a roll rotation of 450° around the earth vertical axis as the animals climbed the height of the ladder. The heading direction of rats was recorded using an overhead camera.

Rats were initially used in this experiment because the only previous study of HD cell activity in mice (Yoder & Taube, 2009) had noted wider tuning curves and

greater instability of mouse HD cells compared to rat HD cells. Secondary to this, all previous studies of HD cell activity during vertical locomotion had used rats (Stackman et al., 2000; Calton & Taube, 2005) and thus there was reason to believe that rats would be able to successfully complete task of climbing the corkscrew ladder.

Rats were trained on the corkscrew ladder task using a method of successive approximation, in which they were initially trained to climb from the base of the maze to a height of 30 cm to retrieve food reward. After five successive climbs to the required height the distance to the goal was increased by 15-20 cm until the rat climbed to the top of the ladder willingly and without the need for encouragement. Even after three weeks of training for two hours per day, rats regularly failed to complete the full 450° roll rotation around the ladder, with the animals taking frequent rests while climbing the ladder. During these rest epochs animals tended to reorient their heads to be in-line with the horizontal plane rather than the vertical plane, and would carry out scanning behaviours that involved yaw rotations of the head around the vertical axis.

The inconsistency of the behaviour of rats on the corkscrew ladder made it very difficult to retrieve sufficient sampling of heading directions for the recording of HD cells. Moreover, recordings of HD cells on the corkscrew ladder that were carried out may have been confounded by the animals reorienting themselves in the horizontal plane during their frequent resting epochs.

As a result of the behavioural issues discussed above, and based upon the willingness of mice to move on the radiolarian maze (presented in the previous chapter), the corkscrew ladder was adapted for use by mice. Rather than using climbing rungs, a Velcro track was wrapped around the central ladder pole. This track rotated 450° around the 200cm height of the corkscrew ladder.

As with the training of rats on this apparatus, mice were trained using successive approximation to climb the height of the ladder. Unlike the rats, mice were quickly able to climb the full height of the ladder, both upwards and downwards. However, upon recording of HD cells on this apparatus, during which the microdrive attached to the head of the mice was connected to the recording system via a 2 metre long cable, mice were regularly unable to complete climb the full height of the corkscrew ladder. The added weight of this cable and the microdrive on the animal's head appeared to be major contributory factors in the fatigue of mice during climbing experiments.

Again, as with the behaviour of rats, even successful climbs of the corkscrew ladder were regularly broken up by resting epochs in which the mice would move their head back to a horizontal position and scan the room. Yaw rotations of the head during resting epochs and roll rotations of the head could not be distinguished using this recording apparatus, as the rotation of the animals was tracked using only two LEDs, unlike the four LED set up used by Finkelstein et al. (2015). This meant that any modulation of head direction activity while animals climbed the corkscrew ladder could not be confirmed to be a result of roll-only rotations.

One final issue with the design of the corkscrew maze was the alignment of the recording camera with the maze itself. The heading direction of the animals was tracked from above, with the camera placed on the ceiling and directed toward the floor. While this setup is regularly used and is highly successful for tracking animals moving on a single horizontal plane, there were several issues when recording animals climbing directly towards the camera. First, the camera could not be sufficiently well focussed to track the head-mounted LEDs for the full 200cm height of the corkscrew ladder, meaning that the accuracy of the measurements of the LED position at the base of the corkscrew ladder differed to that at the midpoint of the ladder and at the top of the ladder. Second, the effect of foreshortening of the cameras view resulted in a larger discrepancy in the measured distance between the two head-mounted LEDs at the top of the ladder than at the base of the ladder.

This effect was so great that in order for the tracking system to reliably distinguish between the two LEDs at the base of the ladder the camera has to be set to a zoom level that prevented both LEDs from being recorded by the camera at the top of the ladder. The pitch rotation experiment, discussed next, was designed to alleviate this issue of foreshortening and to provide a task that was achievable by mice during recording.

5.1.1.2 Pitch rotation experiment

In the pitch rotation experiment (5.2 B) mice climbed along a chicken-wire track around the inner perimeter of a cylindrical arena. The mice were required to climb 360° from one of three starting points on the cylinder. Prior to recording, mice were first trained to climb between the three starting platforms to retrieve a food reward. They were trained to do this until they could successfully climb between the platforms five times within a twenty-minute training session. Following this they were trained to climb two thirds of the way around the apparatus between platforms and then to climb a the full 360° around the apparatus.

Once implanted with microdrives and attached to the recording setup mice were able to initially complete a single full rotation around this track. However, over the course of a five-trial session they became fatigued and resorted to long periods of rest, both on the starting platform and on the climbing track. As with the rest periods on the corkscrew ladder, mice would resort to aligning their head with the horizontal plane and carrying out yaw rotations.

Mice also used a range of climbing techniques; they would either carry out roll rotations by shimmying around the climbing track with their head facing upwards, or they would carry out pitch rotations by climbing with their body axis parallel with the horizontal plane. Again, because only two LEDs and one ceiling-mounted camera were used, it was not possible to determine how exactly the head was aligned with the climbing track, and therefore we could not conclude that any modulation of head direction activity was modulated by non-yaw rotation.

5.1.1.3 Development of the final apparatus design

While the issues of foreshortening were prevented with the development of the pitch rotation climbing apparatus, there remained significant issues with the tracking of the animal's head direction during climbing. The issues of tracking for both of the pilot study apparati were related to the fact that as the animals moved around the two climbing apparati they were constantly changing their plane of locomotion. In standard two-dimensional recording environments the tracking camera is usually placed perpendicular to the animals' plane of locomotion, meaning that yaw rotations of the head can be consistently monitored. This was not possible in the two pilot studies, as it would require the camera's focal point to be moved around the apparati in accordance with the animals' movement.

The development of the apparatus used in the final experiment took these tracking issues into account, while still maintaining the requirement for animals to carry out non-yaw rotations around the earth vertical axis. HD cells were recorded on two vertically oriented planes (the East and West walls) by cameras that were aligned perpendicular to the plane of locomotion. This allowed for consistent tracking of yaw rotations on the two walls, much like the tracking set-ups used in horizontal planes. The design of the apparatus also meant that animals could not access the East or West walls without first carrying out non-yaw rotations from the horizontal plane (starting box) to the South wall, and then from the South wall to one of the two recorded walls. This design therefore enabled the comparison of head-direction cell modulation between two different vertical planes after animals had carried out non-yaw rotations of the head, thus allowing us to determine whether the HD signal uses a planar, multi-planar or volumetric representation of orientation.

The final decision to use rats as opposed to mice for the final experiment was driven by the development of a new environmental enrichment apparatus within the lab (set up by Giulio Casali and Kate Jeffery) that had been used to successfully encourage climbing in rats from weaning age. Moreover, while mice had been shown to be proficient climbers in the radiolarian experiment and during training in

the pilot studies, the added weight of the implanted microdrives (~1.5-2g) seemed to increase levels of fatigue while climbing during recording sessions.

5.1.2 Hypotheses

The three hypothesised representation mechanisms (planar, multi-planar and volumetric) predict different outcomes for the preferred direction of firing of HD cells on the climbing apparatus used. First, based on the findings of Finkelstein et al. (2015), HD cells may use a volumetric global reference frame, in which the PFD of HD cells on the East and West walls of the apparatus would point in the same direction relative to the external world. For example, an HD cell firing upwards and right on the East wall would fire upwards and left on the West wall. Relative to the external world, the cell would fire towards the North with an upward pitch irrespective of the animal's plane of locomotion (Fig 5.3A). This will be referred to as the volumetric hypothesis.

Second, HD cells may be unresponsive to rotations between different planes. In this case, HD cells would treat each plane as the same entity, and as such, a cell's PFD would be in the same direction relative to each wall (i.e. a cell firing upwards and right on the East all would fire upwards and right on the West wall). The reference frame being used by HD cells could be considered to be using a purely local reference frame, with their PFD being defined relative to the animal's plane of locomotion alone (Fig 5.3B). This will be referred to as the planar hypothesis.

The third possibility is that HD cells remain anchored to the animal's plane of locomotion, but that the orientation of the plane of locomotion relative to the azimuth further serves to update the PFD of HD cells between walls. In this case, HD cells would still be modulated by yaw rotations relative to the plane of locomotion, but their PFD would rotate accordingly with the angular displacement between climbing walls. As the East and West climbing walls are displaced by 180 degrees, there would be an expected PFD rotation of 180 degrees between walls (i.e. a cell firing upwards and right on the East wall, would fire downwards and left on the

West wall). Note that such a system would not provide consistent orientation relative to the local reference frames, nor to a three-dimensional global reference frame (Fig 5.3C). This will be referred to as the multi-planar hypothesis.

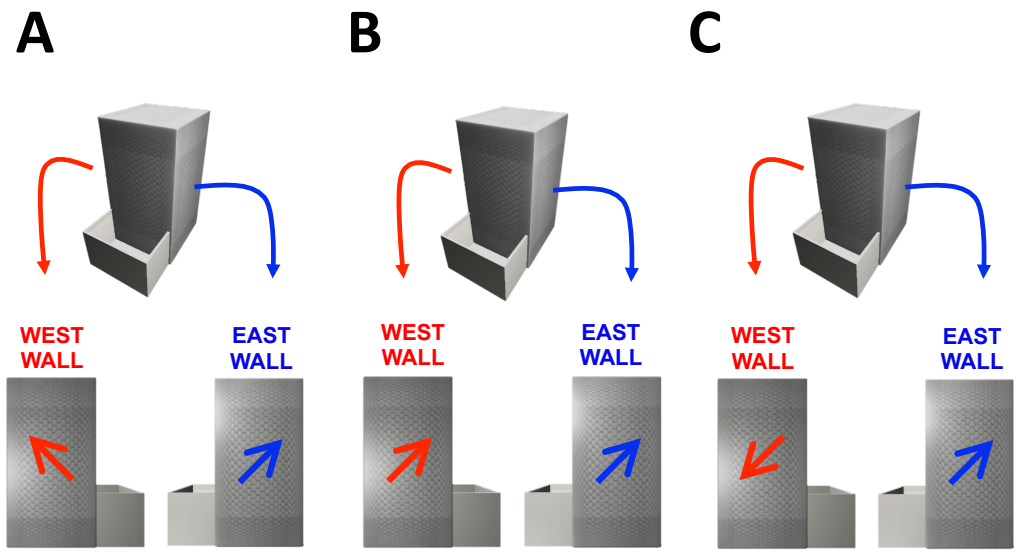


Figure 5.3 Three hypotheses for HD activity on the climbing apparatus. A) The volumetric hypothesis predicts that HD cells will fire in the same global direction (e.g. North and upwards). For example, a cell pointing upwards and right on the East wall would fire left and upwards on the West wall. B) The local hypothesis predicts that HD cells will not be updated by non-yaw rotations and will exhibit them same PFD relative to their plane of locomotion. For example, a cell firing upwards and right on the East wall would fire upwards and right on the West wall. C) The multi-planar hypothesis predicts that the HD system is updated by the position of the plane of locomotion relative to the external azimuth. This would result in a 180° rotation between East and West walls. A cell firing upwards and right on the East wall would fire downwards and left on the West wall.

The findings of the current study indicate that rats do not hold a volumetric or single-planar representation of orientation: rather they use a multi-planar system in which HD cell firing rotates accordingly with the rotation between differently oriented planes. It is concluded that the rodent HD system is optimised to update the firing direction of HD cells during movements on three-dimensional structures in a manner that allows for a consistent translation of the HD cell firing direction to the horizontal plane without the accumulation of heading errors. These results show that animals have the ability to represent orientation on three-dimensional

structures, and thus begin to explain the ability of mice to successfully complete the working and reference memory tasks on the radiolarian maze, and show that the HD system provides one of the required components for successful navigation of three-dimensional environments.

5.2 Materials and methods

5.2.1 Subjects

Three adult male Lister Hooded rats (weighing 300-350g at the time of surgery) were raised from weaning age (21 days) in a large aviary cage (Fig 6.3), enriched with climbing apparati. Animals were raised in this environment to enhance their climbing abilities before pre-training. The walls of the cage were covered in chicken wire, on which the rats could climb. Rats were initially housed with their litter in groups of 4-8 rats, and individually housed in standard cages after surgery. Light conditions were set to a reversed light-dark cycle, with simulated dawn and dusk, each lasting one hour, at 11pm and 11am respectively. After a seven day recovery period after surgery, rats were placed on a restricted diet sufficient to maintain 90% of their free-feeding weight. All procedures were licensed by the UK Home Office subject to the restrictions and provision contained in the Animals (Scientific Procedures) Act 1986.

5.2.2 Apparatus

5.2.2.1 Aviary cage

All rats were raised from weaning age in a large parrot cage measuring 219 cm (length) x 158 cm (width) x 220 cm in height. The climbing apparati in the cage included a large lattice structure (60 x 60 x 60cm), a conical climbing apparatus, suspended walkways, ramps, ropes suspended from the roof of the cage, and raised platforms on which food was placed. The whole cage was covered with chicken wire, on which the rats could climb with ease. Observation of rats within

the first day of being placed in the parrot cage revealed that rats tended to start climbing the various apparati within hours of being placed in the cage, and within days would generally spend the majority of their time on the raised platforms. An image of the cage (Rainforest Cages, Sky Pet Products Ltd, Northamptonshire), and rats within the cage can be seen in Figure 5.4.

5.2.2.2 Screening apparatus

Screening for HD cells took place in a 70x70 cm square screening box with 50cm high walls, placed in the centre of the experimental room. The screening box contained one orienting cue placed in the centre of one of the four walls – the position of this cue remained constant for all screening trials. There were also distal cues available to the rats from the experimental room. These included a door behind the cue location, and a set of shelves running along the wall opposite to the door. All efforts were made to ensure that the cues available to animals remained consistent across training session and animals.

Figure 5.4 The aviary cage. Top - the large aviary cage in which rats were raised (Image from Sky Pet Products). Bottom – Two rats climbing on the chicken wire mesh wrapped around the inner walls of the cage, with a raised platform to their right (left) and one rat using some of the environmental enrichment (right). Rats were well accustomed to climbing throughout the cage prior to surgery and experimentation.

5.2.2.3 Experimental apparatus

The experiment was carried out in a different room to screening trials. The experimental apparatus was enclosed by black curtain, so as to remove the availability of extramaze cues to the animal and thus prevent the anchoring of HD cells towards salient cues in the experimental room.

The experimental apparatus was a cuboidal structure (80 cm in height and 50 cm in width) covered in chicken wire, to aid the animals' climbing. Attached to the bottom of the climbing structure was a starting box measuring 50 x 30 cm from which rats would start all trials. Twenty centimetre high walls surrounded the side of the starting box. The wall to which the starting box was attached was defined as the South wall. Walls (30 cm in height) were attached to the top of each of the sides of the climbing apparatus to prevent the animals from climbing atop the apparatus.

5.2.3 Pre-surgery training

All rats received at least five days of pre-surgical training on the climbing apparatus. This training included two days of 15-minute long habituation to the starting box of the apparatus. Following these two days rats were encouraged to climb onto the South wall to receive food reward, and once they were willing to do this regularly, were encouraged to climb around to the East and West walls of the apparatus for food reward. This was repeated for at least three days, or until rats willingly climbed from the starting box to the South wall, and around to the East and West walls with ease. Video stills of a rat during training can be seen in Fig. 5.5.

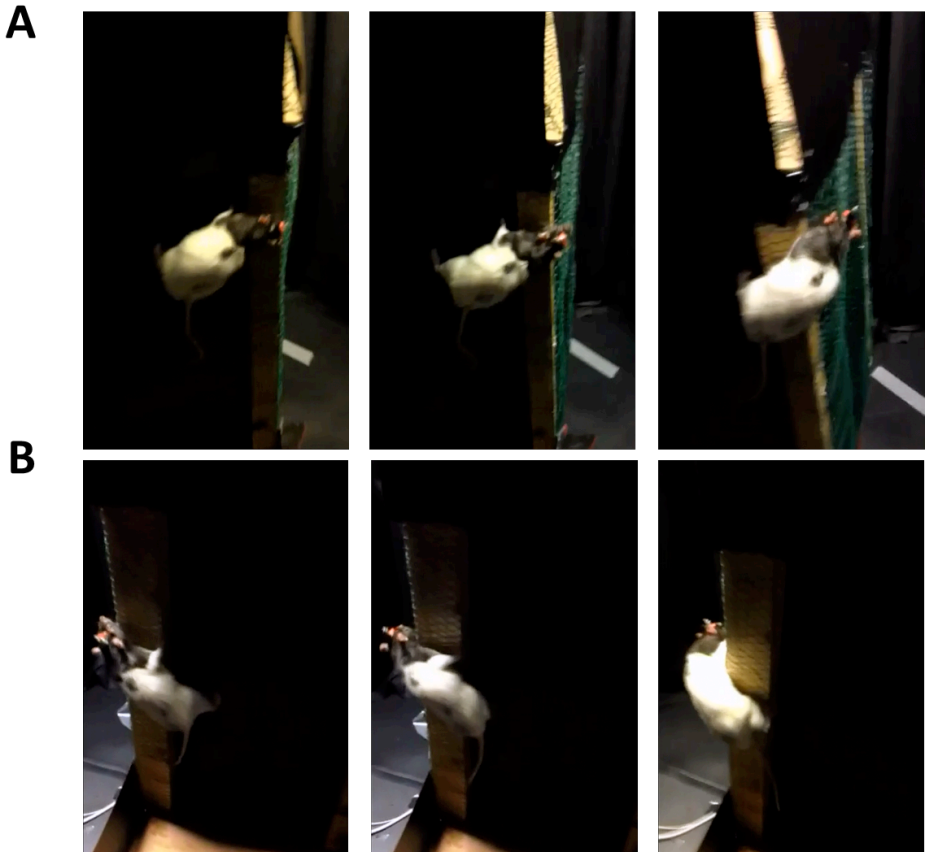


Figure 5.5 Pre-surgery training. Video stills of a rat on the climbing apparatus. A) The rat is climbing from the South wall to the East wall of the apparatus. The rotation carried out is a pitch rotation of the head. B) The rat is climbing from the South wall to the West wall of the apparatus.

5.2.4 Surgery

All rats were implanted after the completion of pre-training with moveable microelectrodes. Rats were either implanted with 16 individual electrodes (n = 2) or four tetrodes (n=1). Descriptions of electrode and tetrode manufacture, as well as the surgical procedure can be found below. Rats were implanted in the anterodorsal nucleus of the thalamus (ADN). This region of interest was selected as it has previously been shown to be the region containing the highest yield of HD cells (Taube, 1995), with a yield of ~60% compared to around 25% in the PoS (Taube et al., 1990a) and ~10% in RSC.(Chen et al., 1994). The bregma co-ordinates

used for surgeries were -1.8 AP, ±1.4ML, -2.1DV. All animals were given at least one week to recover from surgery before screening commenced.

5.2.4.1 Electrodes and microdrives

All animals were implanted after pre-surgery training with moveable platinum-iridium microelectrodes. These microelectrodes were housed within 16-channel microdrives (Axona Ltd., St. Albans, UK). The microdrives allowed for incremental progression of microelectrodes into the brain, using a precision screw (Fig 5.6). One full counter-clockwise turn of the screw would progress the microelectrodes ventrally in the brain by 200µm. Two animals were implanted with 16 individual electrodes, while one was implanted with four tetrodes.

Tetrodes were constructed from four interwoven 17µm diameter platinum-iridium (H-ML insulated) wires (California Fine Wire, USA). During production of tetrodes ~7.5mm of each wire was left unwoven, and ~5mm of insulation from these four strands was removed through heating with a paraffin flame. Each tetrode was then passed through a 12-15 mm long 21-gauge cannula, which was attached to the screw of the microdrive, until the full interwoven section of the tetrode had entered the cannula. The remaining, insulation-stripped, unwoven strands of the tetrode were then wrapped around wires connected to the microdrive, such that each strand was attached to a single channel of the microdrive. The strands of each tetrode were wired systematically, such that a given tetrode was attached to a specific set of four channels of the microdrive. The wires of each tetrode were then secured to each individual microdrive wire using conductive silver paint. Once all tetrodes had been connected to the microdrive, the area was covered with non-conductive nail varnish to protect the electrodes.

Microdrive wiring for individual electrodes followed a similar protocol. Initially, 20 individual 17µm diameter platinum-iridium (H-ML insulated) wires were cut to 6cm in length. They were then held together at one end using saline to create a temporary adhesion of electrodes through surface tension. This bundle was then

advanced into the same 21-gauge cannula as was used for tetrode microdrive construction. The electrodes were then glued together at the tip exiting the microdrive. Less than 1 mm of the electrodes was glued. The now-glued bundle was then retracted such that 4cm of electrodes extended outwards from the microdrive. The electrodes were then separated and stripped of insulation using a paraffin flame. Electrodes were then advanced forwards so that the insulation-stripped section (~5mm) of the electrodes could be individually wrapped around the wires of the microdrive. Twenty, rather than 16, electrodes were used as an insurance against the potential of damage to any one of the electrodes. Once the 16 channels of the microdrive had been wired, any remaining unconnected wires were trimmed. As with tetrode wiring, each wire was secured using silver paint, followed by nail-varnish to protect and insulate the electrodes.

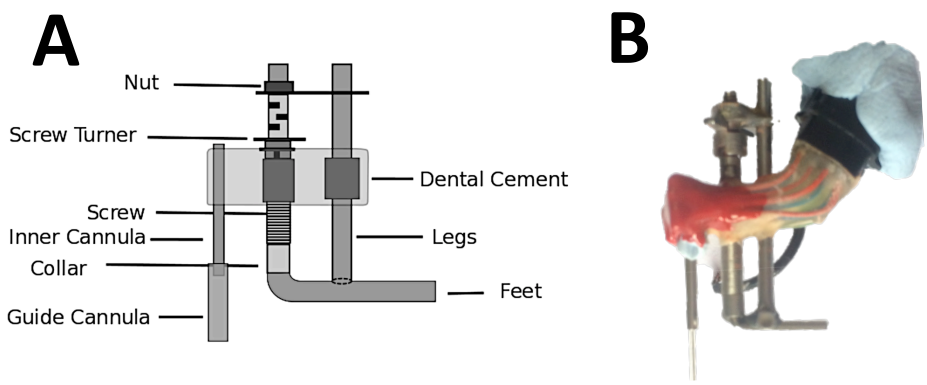


Figure 5.6 Microdrive mechanism. A) Schematic of the microdrives used in experiments B) Image of a wired microdrive with the guide cannula retracted to expose electrodes prior to surgical implantation. The guide cannula was lowered into place over the brain surface once the electrodes had been inserted into the brain.

The following description of the final stages of microdrive construction applies to both types of microdrive (single-electrode and tetrode microdrives), and for ease of explanation both types of wire bundle will be referred to as electrodes for the remainder of this section.

Just prior to surgical implantation, electrodes were cut to a desired length of ~8mm. As well as the length of the electrodes, it was also important to ensure that the final cut was as clean as possible, leaving each electrode with a circular, highly conductive surface from which to record neural activity. Following this final cut, each electrode was tested for conductivity individually, by passing a current through each electrode into a saline solution. The formation of bubbles at the end of each electrode, as a result of electrolysis at the cathode and anode, indicated that a current could be passed through the wires, and that the wiring of the electrodes had been successful. Microdrives were only accepted for surgical implantation if all channels passed this "bubble test".

5.2.4.2 Surgical procedures

Prior to surgery, the operating table and stereotactic frame were sterilised by spraying them with a solution of chlorhexidine gluconate and ethanol (Hydrex, Adams Healthcare, UK). All surgical tools and surgical gowns were sterilized in an autoclave, apart from burr drill bits, which were sterilized in a hot-bead sterilizer to prevent rusting. Animals were initially anaesthetised in an anaesthetic container with an isoflurane and oxygen solution at 3L/min. Once sedated, animals were injected subcutaneously with Carprieve (Carpofen) for analgesia.The injected solution was a 1:10 solution of Carprieve (4 mg/kg of 5% Carpofen solution) to injectable sterile water, and animals were given 0.1ml of this solution for each 100 grams they weighed. Their heads were also shaved to expose the surgical site.

Once animals were suitably anaesthetised to a point at which there was no reflex to a toe-pinch they were placed in the stereotaxic frame used for surgery. Initially, the nose of the animal was placed in a gas mask attached to the stereotaxic frame, with their teeth placed over a horizontal bar. The animals were then secured in place using ear-bars, in which each ear-bar was gently placed into the ear canals of the animal. Once ear-bars were secured in place, a nose bar was gently lowered over the animal's nose to further hold the animal in place. The body temperature of

animals was kept stable using water-flow heat pads and eyes were protected using carbomer (Viscotears, Novartis Pharmaceuticals Ltd).

Once firmly secured in the stereotactic frame, an iodine solution was spread over the surface of the surgical area (the top of the animal's skull). At this point, the surgeon robed up into a sterile gown and sterile gloves. The iodine solution was then removed using cotton buds and sterile saline, and an incision was made down the length of the centre of the animal's skull. Sterile cotton buds were then used to remove any blood from the incision site, and to remove any connective tissue still attached to the skull. Haemostats were then attached to the upper and lower corners of each side of the incision site, such that the skull could be exposed. If necessary, each side of the incision was further spread by pushing away the skin using cotton buds so that bregma and lambda were exposed. Stereotaxic co-ordinates were then used to ensure that the skull was flat by confirming that the dorso-ventral height of bregma and lambda were the same. Stereotaxic co-ordinates were also used to confirm that the skull was straight in the anterior-posterior axis, by ensuring that bregma and lambda were at the same medio-lateral distance.

Once this was confirmed, further measurements were made to mark the position of the electrode implant site. Six holes (1mm in diameter) were then drilled in the skull: two in the frontal bone, one in the same side of the parietal bone as the implant site, two in the contralateral parietal bone and one in the interparietal bone. Once drilled, the skull was cleaned with sterile saline, and screws with a 1.2mm diameter thread and 1.6mm diameter head were screwed into the surface of the skull, such that some of the screw thread and head were still exposed. Prior to surgery, one of the screws had a wire soldered to its head, and was used to electrically ground the animal. This screw entered deeply into the frontal bone of the skull, to ensure a connection between the base of the screw thread and the brain surface.

The marked implant site was then drilled with a 2.7mm trephine bit, and the dura at the implant site was removed using the bent end of a sterile needle. The implant co-ordinates were then measured in stereotaxic co-ordinates using the electrodes of the microdrive. The microdrive was held in a crocodile clip above the skull and the electrodes were aligned such that they were perpendicular to the surface of the skull. Initially, bregma was re-measured with the electrodes, before the electrodes were moved to the correct anterior-posterior and medio-lateral co-ordinates of the implant site. The electrodes were then lowered to the surface of the brain, and advanced ventrally by 2.1mm to the desired position within the brain. The guide cannula was then lowered to protect any exposed length of electrodes. Any region of the brain still exposed was covered in sterile Vaseline. The microdrive was then attached to the skull using dental acrylic, which was spread around the surface of the exposed skull, and built upwards to attach to the legs of the microdrive and to cover the majority of the guide cannula. The ground wire of the microdrive was then soldered to the wire connected to the ground screw, with the connection secured with dental acrylic. Once the dental acrylic was completely dried the animal was removed from the stereotaxic frame and allowed to wake up naturally in a heated chamber.

Post-operative care included the provision of pain relief in the form of meloxicam (Metacam), which was administered in jelly at a concentration of 0.15ml meloxicam for 0.85ml jelly per portion (0.2 mg/kg). Animals received one portion of this jelly per day for the first three days after surgery. For one week after surgery animals were given *ad libitum* food and water. Food was given to the animal in the form of pellets and a water-pellet mash. Animals were individually housed after surgery and throughout the experiments.

5.2.5 Screening procedures

Recording was carried out using multichannel recording equipment (DacqUSB) supplied by Axona Ltd. (St Albans, Herts) in the screening apparatus described above.

5.2.5.1 Screening trials

Recordings commenced at least one week after surgery. Multichannel recording equipment (DacqUSB, Axona Ltd., St. Albans, UK) was used for recordings. Animals were connected to a pre-amplifying unit via a lightweight cable attached to the microdrive via a head-stage that modified the signal with AC-coupled, unity gain operational amplifiers. The signal was amplified ~15,000 times and bandpass filtered between 500Hz and 7kHz. Recording thresholds were set to ~70% above baseline activity levels, to detect neural spiking activity without the collection of background electrical activity. The timing of spikes above the threshold from all channels was collected, with activity from 200ms preceding the peak amplitude of a spike to 800ms following the peak amplitude being stored at 50Hz. The activity of channels from any given tetrode was referenced against the activity of a single channel from another tetrode. This means that the activity of one electrode was subtracted from that of another, allowing for the reduction of common noise, such as chewing artifacts or background electrical noise in the recording room. Background electrical noise was also reduced through the grounding of the experimental apparatus to the recording system. As well as single unit activity, local field potentials (LFP) were recorded from one of the electrode channels.

During screening sessions, the position and heading direction of the animal were recorded using an overhead camera. Two LEDs of different sizes were mounted atop the headstage with a separation of 5cm. The tracking software of DacqUSB isolated the two different LEDs and recorded the pixel position of each LED at 50Hz throughout the recording. The position of the animal in the environment was estimated as the average pixel position in x and y co-ordinates between the two LEDs, and the animal's heading-direction was determined by measuring the angular displacement between the two LEDs.

During screening, the digital oscilloscope was monitored for spiking activity. If spiking was detected a recording session, of at least five minutes, would follow. This recording served as the baseline of HD cell activity in a horizontal two-dimensional

environment. If spiking activity was not detected, the tetrodes would be lowered, using the screw of the microdrive, by 50-100µm. Animals were encouraged to move through the environment to forage for food rewards of either cereal, rice, or condensed milk droplets. If no head direction cell activity was present (methods described below) the electrodes would be lowered, and the rats would not be screened again for at least three hours (to allow for the electrodes to settle in their new position within the brain). If HD cells were found, the animals would progress onto the experimental procedures described later in this section.

5.2.5.2 Cell isolation

Cluster cutting software (Tint, Axona Ltd.) was used to isolate single units from the multi-channel recordings. Irrespective of whether tetrodes or single electrodes were used, recordings were loaded to Tint in groups of four channels. In the case of tetrodes, all four channels of a given tetrode were loaded simultaneously. The software then plotted the peak amplitudes of spikes from each channel against the peak amplitudes of the other channels from a given tetrode. This resulted in six separate cross-channel scatter-plots of spike amplitudes (Fig 5.7). The amplitudes of spikes differ between electrodes due to the difference in distances between a cell and a given electrode, with cells more proximal to the electrode appearing to exhibit higher spiking amplitudes. This leads to the clustering of spiking amplitudes of single cells, allowing for the isolation of different cells.

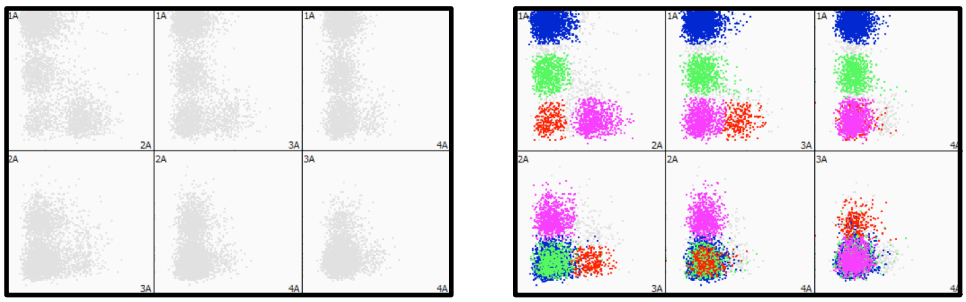


Figure 5.7 Left – Peak-to-peak amplitude plot of spiking activity from one tetrode using Tint software. Each grey dot represents a single above-threshold spiking event. The spiking activity of one cell typically forms a single cluster that can then be isolated. *Right* – Isolated cells from the cluster space are then assigned a cell number (colour).

Confirmation of whether a given selected cluster was a result of neural activity was done through inspection of the average waveform of a cluster (Fig 5.8). A selected cluster was considered to be a single cell's activity if the waveform of the cell had the characteristics of an action potential (i.e. an initial peak followed by a refractory period). This was further determined by assessing the temporal autocorrelogram of the cell, with neurons exhibiting a short gap in spiking (1-2ms), indicating the presence of a refractory period.

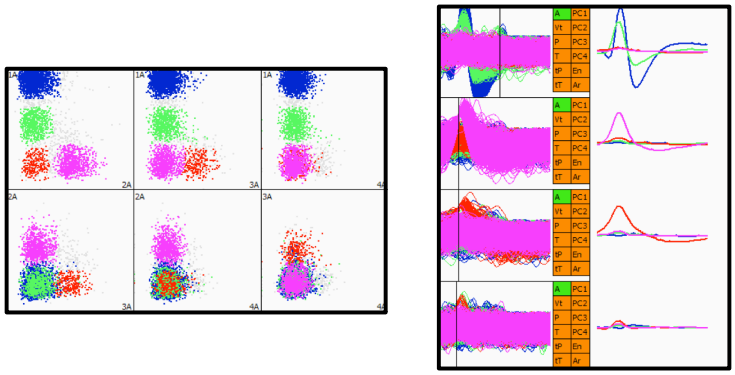


Figure 5.8 Waveforms of isolated cells. Left – identified cells. *Right* – Waveforms of the four isolated cells for each tetrode channel. Note the difference in amplitude for the different cells in each channel.

5.2.5.3 HD cell identification

Once single cells had been isolated, the heading direction of animals was correlated to the neural spiking activity of each cell. The heading direction of animals was computed from the angular distance between the LEDs on the headstage, and was grouped into 64 directional bins. The directional firing of cells was determined by first plotting the spiking rate (spikes per second) the animal spent in each of the directional bins. This data was smoothed using a Gaussian kernel of 5 directional bins.

Several parameters were then used to describe the directional firing of cells. These were: firing direction, peak firing rate, the angular standard deviation of cell spiking, and the mean resultant vector of the cell's firing. The computation of each of these

parameters is described below. Cells were considered to be directional if they had a peak firing rate over 1Hz, exhibited more than 100 spikes during the five minute screening session and had a significant Rayleigh vector score (described below).

Firing direction

The preferred firing direction of an HD cell was defined by the mean direction of firing (Fig 5.9). The mean was used rather than the mode because it accounts for the spiking activity at all binned heading directions, rather than just for one, and thus provides a more suitable estimate of the cell's firing behaviour as a whole. Moreover, the calculation of the Rayleigh vector score used to describe a given cell's sensitivity to orientation (described below) also uses the firing behaviour of cells in all directional bins.

The mean firing direction was calculated using the CircStats Toolbox (Berens, 2009) in Matlab (Mathworks, Natick, MA, USA). The calculation of mean directional firing was as follows:

(1) Calculate the circular representations of spiking activity trigonometrically by first converting the binned heading direction values into cosine and sine values for each directional bin. Then, using the formulae given for grouped data by Batschelet (1981), calculate the mean cosine ('X') and sine ('Y') values of the cell's firing rate. These 'X' and 'Y' values are subsequently used in the calculation of mean firing direction, angular standard deviation and in the calculation of the Rayleigh vector.

$$X = \frac{\sum_{i=1}^{n} n_i \cos\emptyset_i}{\sum_{i=1}^{n} n_i}$$

$$Y = \frac{\sum_{i=1}^{n} n_i \sin\emptyset_i}{\sum_{i=1}^{n} n_i}$$

Where n_i is the firing rate of the cell in directional bin i and $\emptyset_i$ is the angle of the centre of bin i.

(2) Calculate the mean firing direction from 'X' and 'Y' values calculated above. $\emptyset$ is the mean firing direction in radians.

$$\emptyset = \arctan(X/Y)$$

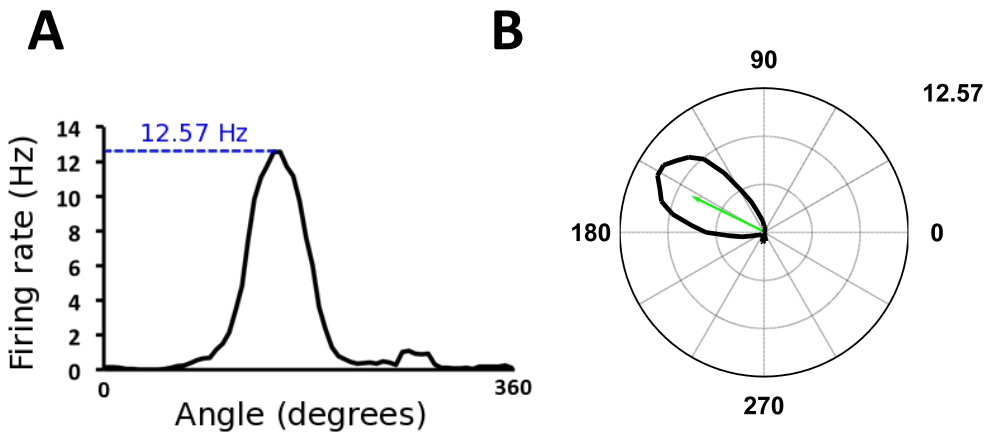


Figure 5.9 Firing rate and directions of HD cells. *A) Line chart of an HD cell showing the tuning function of the cell over 360° of azimuth (black). The peak firing rate of the cell is shown in blue B) Polar plot showing the mean firing direction (green) of the same cell.*

Firing rates

The peak firing rate was defined as the firing rate of a cell in the directional bin with the highest firing rate (Fig 5.9 A). The mean firing rate was calculated as the mean of the firing rates from the 64 directional bins.

Mean resultant vector length

The mean resultant vector length, otherwise known as the Rayleigh vector, was calculated to determine whether a cell's spiking activity significantly deviated from a circular distribution. This analysis was used as a tool for classification of directionally modulated neural activity, as well as a description of a cell's directional modulation between different conditions of experiments. Cells with significant Rayleigh vector lengths were considered to be HD cells if they also had a peak firing rate over 1Hz and emitted more than 100 spikes in a screening trial. The mean resultant vector was calculated using the X and Y values calculated above:

$$r = \sqrt{x^2 + y^2}$$

Rayleigh vector values range from 0 to 1, with values closer to 0 indicating that the cell activity is not directionally modulated and fires equally in all directions, and values closer to 1 indicating directionally modulated cells.

Z-scores and their associated significance values could then be calculated to determine whether the firing of a given cell was significantly directional, using the following equation:

$$z = nr^2$$

Where n is the total number of directional bins and r is the Rayleigh vector score calculated above.

Angular standard deviation

The angular standard deviation was used as a measure of the tuning width of cells. This measure was primarily used as a comparison of tuning widths between cells, as

opposed to a direct measurement of tuning widths, as the tuning widths of HD cells throughout the recorded brain regions are well documented. The angular standard deviation was calculated as follows:

$$S = \sqrt{-2\,ln\,r}$$

Where *ln* is the natural log, *r* is the Rayleigh vector score and the angular standard deviation of firing (S) is represented in radians.

5.2.6 Experimental recording procedures

All recordings were carried out in the experimental room described above. The recording setup was in general very similar to that described for the screening procedures; however two cameras were used during the experimental recordings. Cameras were aligned to face the East and West walls for all sessions. One cell was also recorded with the animal climbing on the South wall. The camera signal was passed through a time-base corrector (Datavideo TBC-5000, Datavideo UK Limited, Derbyshire, UK) before being relayed to the DacqUSB System Unit. The time-base corrector relayed the image from each camera on alternating frames, such that the images from each camera were relayed to the DacqUSB system at a frequency of 25Hz, as opposed to the usual 50Hz frequencies used in single-camera experimental setups. The implication of this reduced sampling rate was that the sampling of an animal's position and direction was at half the rate for each climbing wall, requiring interpolation of orientation and position over larger time scales than in baseline trials. In baseline trials, position and direction samples were taken every 20ms, while position and directional sampling occurred every 40ms for each climbing wall.

Recordings were run continuously in sessions of up to 20 minutes: however sometimes multiple sessions were required to ensure adequate directional coverage on the walls. At the start of each recording session the rat was placed in the starting box of the apparatus, and would then climb onto the South wall of the

apparatus, before climbing around to either the East or West walls. The paradigm used was a free-choice foraging paradigm, in which rats were able to choose to climb to either the East or West walls to receive food reward. At times, the rat was encouraged – with a small trail of food reward – to climb to one particular wall in order to increase the sampling of given wall. Once on the recording walls, the rat was also further encouraged to carry out yaw rotations using food reward placed on the end of a cotton bud in order to gain as much sampling of downwards facing head directions as possible – a direction that rats seldom sample without encouragement.

5.2.7 Experimental data analysis

5.2.7.1 Analysis of basic firing properties

The basic firing properties were then calculated for baseline trials (taken from the 5-minute screening session recordings) and climbing trials. These basic firing properties were: Rayleigh vector length, angular standard deviation of firing, mean firing rate and peak firing rate. The Rayleigh vector length was calculated as described above, where a value of 0 represents a uniform circular distribution of cell firing, and a value of 1 would result from a cell firing only within one directional bin i.e. highly directionally selective.

The mean firing direction and angular standard deviation of head direction cells was calculated as described above.

5.2.7.2 Concatenation of climbing trials

As climbing sessions were run using continuous recording, it was necessary to separate climbing trials from each camera, and then to concatenate all of the climbing trials for each given wall. This was done by stepping through the path of the animal recorded by each camera and splitting the recorded file into climbing epochs (using Dacq File Splitter, Axona Ltd). As, at times, there could be ambiguity

with regards to whether the animal had carried out a full rotation onto the climbing walls, positional data was only included after the animal had been tracked by a camera for 2 seconds, and was cut off two seconds prior to the camera being unable to track the animal (the animal had moved back to another climbing wall, to which the current camera was not aligned). After splitting these climbing trials into individual climbing epochs for each wall, the trials were joined. At this point, the electrophysiological data for each wall was analysed separately, with pre-defined HD cells identified using Tint cluster cutting software, before being analysed in MATLAB. The steps taken in the concatenation procedure are shown in Fig. 5.10 on the next page.

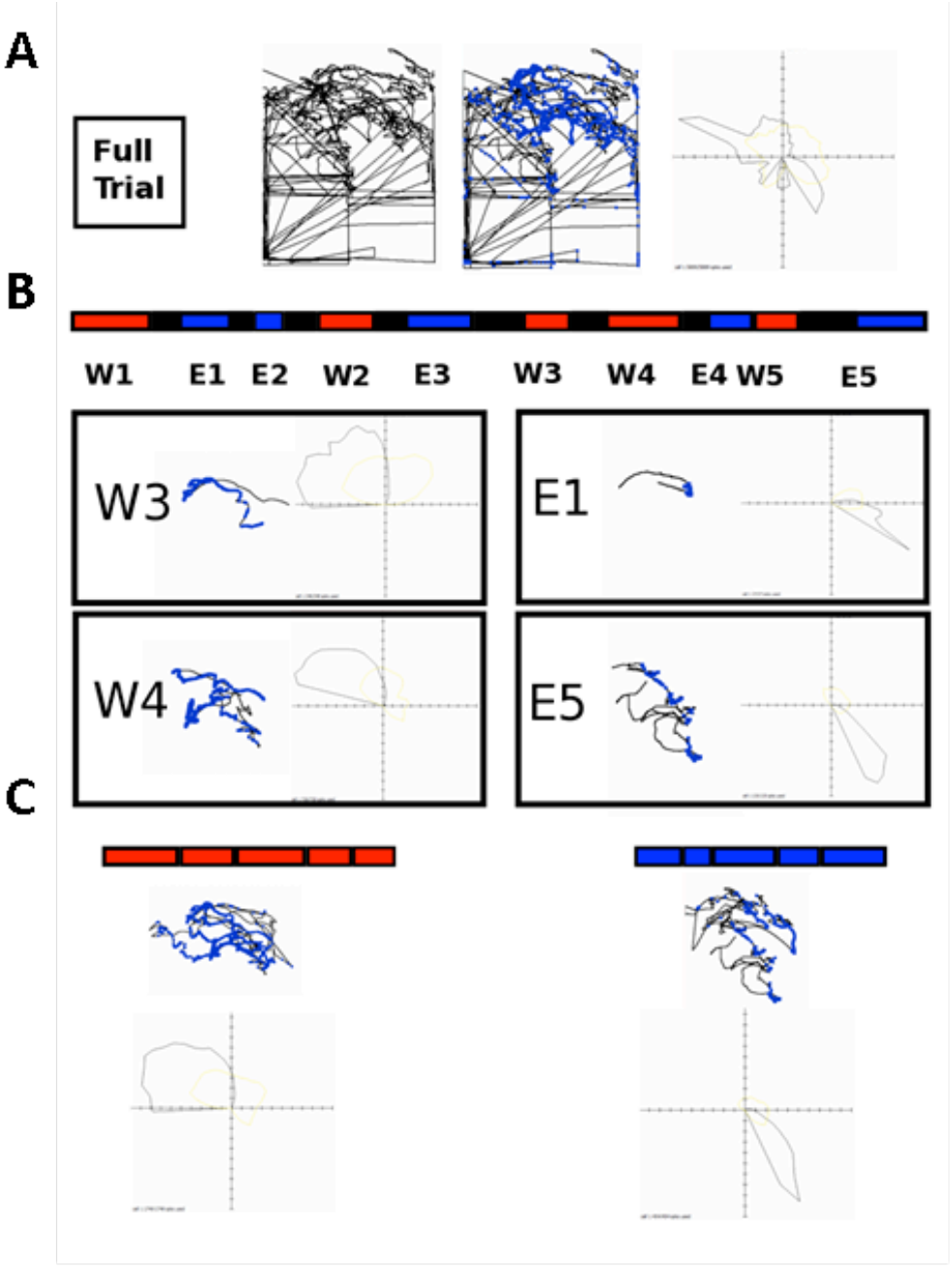


Figure 5.10 Concatenation of climbing trials. A) Raw tracking data created from combining the images of the two alternating recording cameras, showing the path of the animal (left), spike plots overlaying the animal's path (center) and polar plot of directional specific cell activity (right). These data needed to be separated for the analysis of HD cell firing on each of the climbing walls. Sharp straight lines show instances in which the Tint software interpolated tracked positions between the two recorded walls. B) Top - separated trials for the West wall (red), East wall (blue) and non-tracked positions when the animal was in the starting box or South wall (black). Bottom – example spike plots and their associated polar plots for individual climbing epochs. C) Spike plots and their associated polar plots for the concatenated climbing trials on each wall (left – West wall, right – East wall). All spike plots show spiking events (blue dots) overlaying the animal's path (black line).

5.2.7.3 Comparisons between climbing trials

Having concatenated the climbing trials for each wall, the direction of firing, Rayleigh vector lengths, peak firing rates and angular standard deviations were compared for each cell between climbing walls, and baseline trials (5 minute recordings in the screening box).

The directionality of firing was compared using rotational cross correlations of the East and West wall polar plots. These rotational cross correlations followed four steps:

(1) Determining the firing rate in each of the 64 directional bins for the climbing and baseline trials.
(2) Shifting the firing rate for the wall at bin-by-bin increments (repeated 64 times). This is done in a wraparound manner, with the 64^{th} value replaced at the position of the first value after each shift.
(3) Carrying out correlations of the firing rates between the wall and baseline trial at each step. Pearson's r values approaching 1 indicate high positive correlations, values of zero indicate no correlation, and values approaching -1 indicate highly negative correlations.
(4) Determining the rotational value that indicates the highest positive correlation.

5.2.7.4 Inferential statistics

Rayleigh vector lengths, peak firing rates and angular standard deviation were compared between the baseline and climbing trials using repeated measures ANOVA. Post-hoc Bonferroni-corrected pairwise comparisons were used to test differences between specific trial types.

Circular V-tests (Batschelet, 1981) were used to determine whether the rotations between climbing trials supported the mutli-planar hypothesis which predicted that

the PFD of HD cells rotates by 180° between the East and West climbing walls. The angle of rotation between climbing trials was defined as the angle at which the rotational cross-correlations yielded the peak positive correlation. This test was carried out using the following equation:

$$V' = nycos(\emptyset) + nx\sin(\emptyset)$$

Where $\emptyset$ is the predicted direction, 'X' and 'Y' are the cosine and sine values calculated from the angular data using Batschelet's formulae (as outlined in section 5.2.5.3). In this case, the 'X' and 'Y' values are calculated using the frequency of peak correlation values in each angular bin, as opposed to firing rate of cells in each bin.

H_0 = the bearings are randomly distributed with respect to the predicted direction

H_1 = the bearings are non-randomly distributed with respect to the predicted direction i.e. the cells are firing in the predicted direction. In the case of the multiplanar hypothesis, the cells rotate their PFDs by 180° between the climbing walls.

For the case of comparisons between East and West walls, the predicted rotational direction was 180 degrees.

5.2.8 Histology

At the end of the experiment, which was usually determined to be the point at which electrodes were considered to have moved beyond the region of interest, or when head direction cells had not been observed for a matter of weeks, animals were deeply anaesthetised with isoflurane before being administered with an

intraperitoneal injection with a lethal dose of sodium pentobarbital (~1.5ml). Once breathing had ceased, animals were transcardially perfused with saline (~300ml) followed by the same amounts of 4% paraformaldehyde.

Once perfused, brains were removed from the animals and placed in ~50ml of 4% paraformaldehyde for at least one week. Prior to sectioning, each brain was placed in 30% sucrose solution for at least 24 hours, or until the brain floated in solution. This allowed for removal of water from the brain tissue for cryoprotection. Brains were sliced at 40µm thicknesses coronally using a freezing microtome. Sections were then wet-mounted onto gelatinized or Superfrost Plus (Thermo Scientific, U.S.A) and left to dehydrate before staining. Once dehydrated, they were stained in Thionin solution.

Once stained, slides were left in Histo-Clear (National Diagnostics, U.S.A.) for 10 minutes before cover slides were secured over the slices using O.C.T™ mounting medium (Tissue-Tek®, U.S.A.). The slides were then observed under a Leica DM750 microscope in order to determine the site of electrode tracks within the brain.

5.3 Results

Nineteen head direction cells were recorded from three rats as they foraged for food between differently oriented climbing walls. Observations of the animals' behaviour on the climbing walls revealed that rats carried out roll and pitch rotations of the head as they moved between walls. While this could not be quantified, a representative example of the animals' climbing behaviours can be seen in Fig 5.5 and Supplementary Movie 1 (on the attached DVD). The basic firing properties of HD cells were first compared between climbing and baseline trials, followed by analysis of the PFD of the HD cells between the climbing walls.

5.3.1 Basic firing characteristics

Of the 19 HD cells recorded, defined by their baseline firing in a horizontal arena, 100% were active on at least one wall while 16 were directionally modulated on both walls. The other 3 cells were directionally modulated on one of the two walls but were not active on the opposing wall. The reason for this, discussed in more detail later, was that during recording of these three-cells the animal did not sample the direction in which the cells were expected to fire according to the multi-planar hypothesis. Histological analysis revealed that electrodes entered the ADN in all three animals (Fig 5.11).

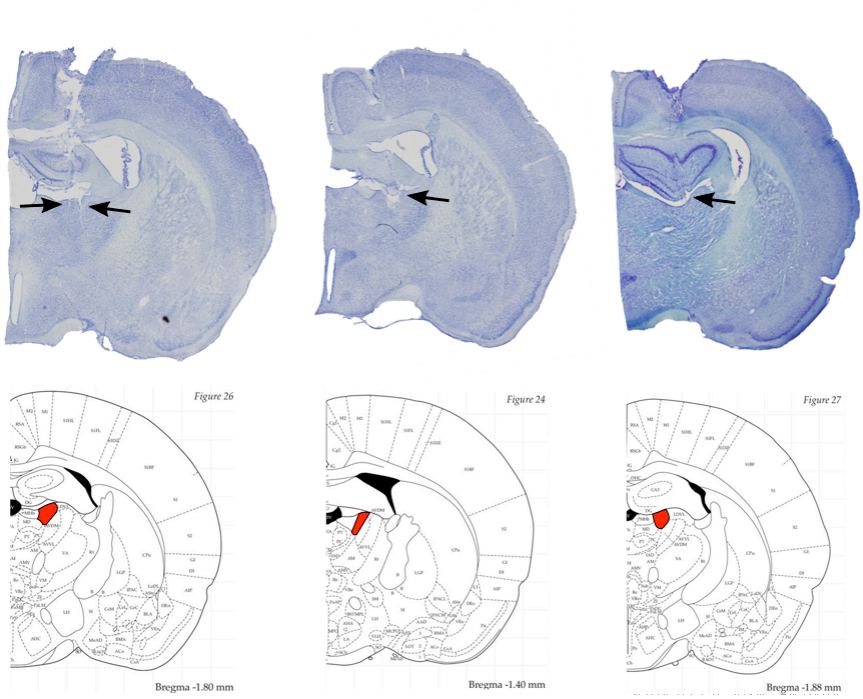


Figure 5.11 Histology for the three rats. Top – Stained coronal slices for each rat. Arrows indicate the position of the electrode tracks. Bottom – Sections from the Paxinos rat brain atlas showing the posterior location of the slices. The ADN is highlighted in red. Note: desired A-P co-ordinates were -1.8mm from bregma.

The basic firing characteristics of the 16 cells active on both walls were first compared between climbing trials and baseline trials (recorded in a horizontal environment in a different experimental room). Polar plots of one HD cell in the baseline and climbing trials can be seen in figure 5.12. Polar plots of all cells in the baseline and climbing trials can be found in Appendices 1 and 2.

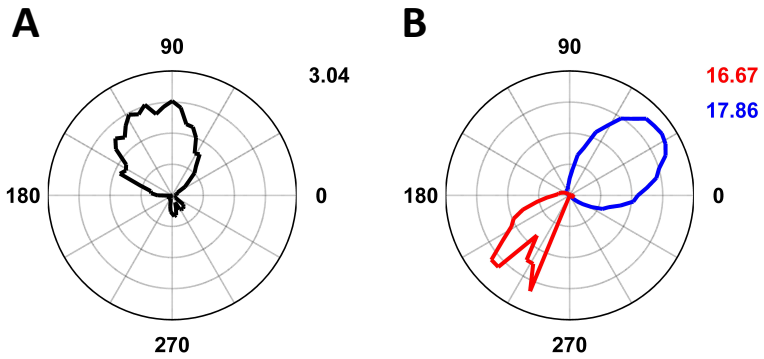


Figure 5.12 Polar plots of HD cell activity A) Baseline trial B) Climbing trials on the West wall (red) and East wall (blue). Numbers at the top right of each image show the peak firing rates of the cell in Hz.

A repeated measures ANOVA of Rayleigh vector values revealed no significant difference in the extent of directional modulation between baseline trials and climbing trials (Baseline = 0.75 ± 0.03, West = 0.68 ± 0.05, East = 0.70 ± 0.04; $F_{(2,30)} = 0.832$, $p = .45$; Fig 5.13A), indicating that HD cell firing is similarly modulated by yaw rotations during movement on horizontal and vertical planes.

Comparisons using repeated measures ANOVA showed a significant difference in peak firing rates between the baseline and climbing trials (Baseline = 30.5 ± 5.9 Hz, West = 19.8 ± 3.5 Hz, East = 24.1 ± 4.6 Hz; $F_{(2,30)} = 5.47$, $p < 0.01$; Fig 5.13B). Post-hoc Bonferroni-corrected paired t-tests revealed significantly lower firing rates on the West wall climbing trials compared to baseline trials ($t_{(15)} = 2.949$, $p = .01$), but not on the East wall ($t_{(15)} = 1.807$, p = .09). There was no difference in peak firing rates between the East and West walls ($t_{(15)} = 1.746$, $p = .10$). Comparisons of mean firing rates, however, did not reveal any difference in the mean firing rates of HD cells between climbing trials and baseline trials (Baseline = 5.6 ± 1.0 Hz, West = 4.1 ± 0.9 Hz, East = 4.5 ± 0.9 Hz; $F_{(2,30)} = 1.951$, $p = .16$; Fig 5.13C)

Repeated measures ANOVA showed no difference in angular standard deviations between trials (Baseline = 0.69 ± 0.05, West = 0.75 ±0.07, East = 0.74 ±0.06; $F_{(2,30)} = 0.568$, $p = 0.57$; Fig 5.13D), indicating comparable tuning widths for baseline and climbing trials.

These data show that, in agreement with previous research (Taube et al., 2013), rodent HD cells remain modulated by yaw rotations during locomotion in the vertical plane, with cells exhibiting single-peaked Gaussian tuning curves responsive to orientation relative to the animal's plane of locomotion.

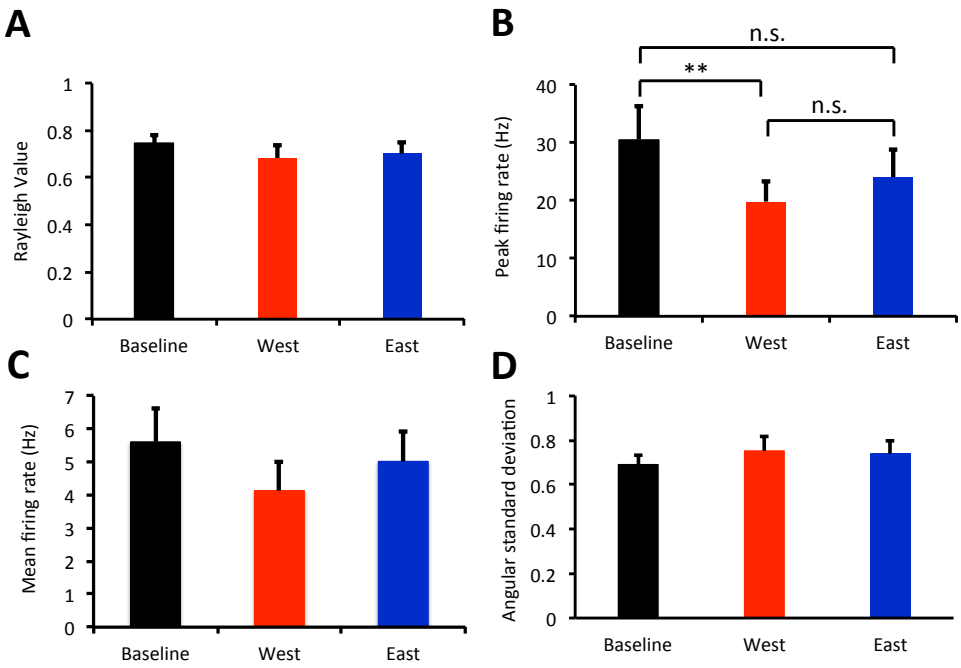


Figure 5.13 Basic firing characteristics of HD cells are maintained during climbing. A) The directional selectivity of HD cells is maintained during climbing as Rayleigh vector values are comparable between baseline and climbing sessions. B) There is a drop in firing rate between baseline and climbing trials, explained by a drop on firing rate on the West wall, but not the East wall. C) There is no difference in mean firing rate between climbing trials and the baseline trials. D) There is no effect of the vertical plane on the tuning widths of HD cells, as shown by comparable angular standard deviation values between baseline and climbing trials. (** denotes p values <.01, n.s. denotes non-significant values of p > .05).

5.3.2 HD cells of the rodent ADN are not volumetrically modulated

The defining characteristic of an HD system using a three-dimensional global reference frame is that HD cells would fire in the same global direction irrespective of an animal's plane of locomotion. Using an earlier example, a cell firing upwards

and right on the East wall would be expected to fire upwards and left on the West wall. Relative to the external world, such a cell would be considered to be firing upwards and North.

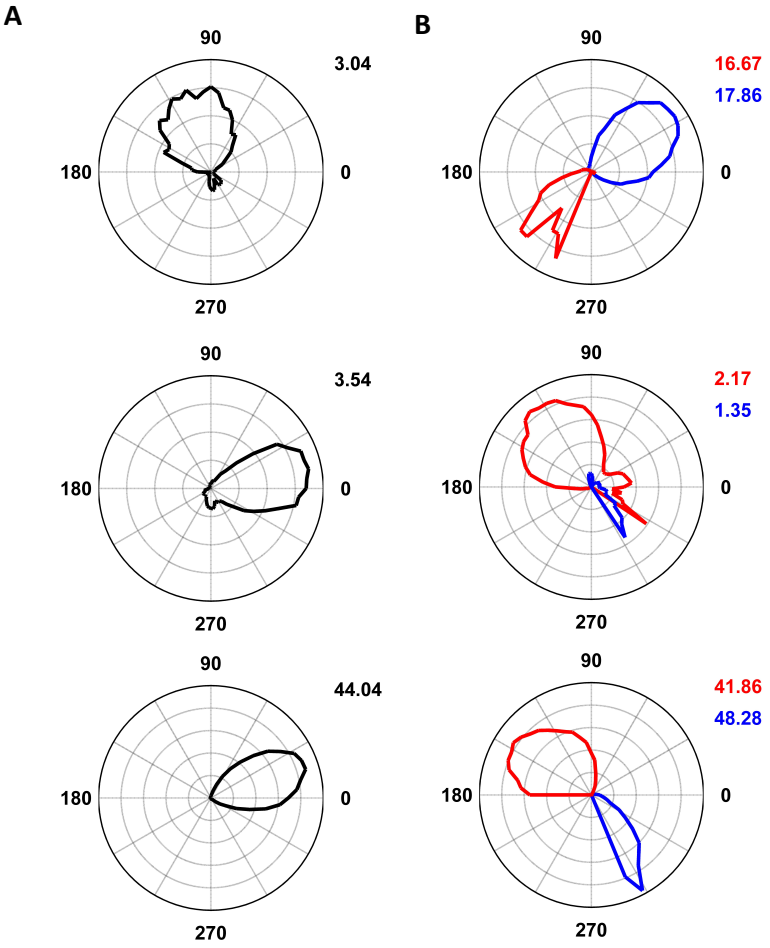


Figure 5.14 Non-volumetric encoding of orientation. A) Baseline polar plots of three cells B) Climbing trial polar plots for the same cells showing the directional modulation of these cells on the East wall (blue) and West wall (red). Note that when the cell fires upwards on one wall, it fires downwards on the opposing wall. This result is inconsistent with the volumetric hypothesis prediction that HD cells would fire in the same elevation angle and azimuth angle on the two climbing walls.

Visual inspection alone confirms that the firing of HD cells in rats must not be informed by a three-dimensional global metric. For those cells with a vertical component on the walls (i.e. those cells that did not fire straight to the left or right

of either wall) the vertical component of the firing switched direction by 180° between East and West walls. That is, a cell that fired upwards on the East wall fired downwards on the West wall, and vice versa. Some examples of these cells can be seen in Figure 5.14.

5.3.3 HD cells of the rodent ADN are updated by non-yaw rotations of the head

The firing directions of cells were next compared between the two climbing walls to test whether the rodent HD system uses a planar or multi-planar reference frame. For the planar hypothesis, it was predicted that cells would not rotate their firing direction between climbing walls due to an inability to process non-yaw rotations between walls. For the multi-planar hypothesis, it was predicted that HD cells would rotate their firing direction relative to each wall by 180°, indicating that HD cells were sensitive to the amount of rotation between the East and West walls.

These predictions were tested using rotational cross correlations between the polar plots of HD cells on the East and West climbing walls. For the rotational cross correlations, the East wall polar plot was rotated one directional bin at a time and correlated with the West wall polar plot at each step. The PFD rotation between the East and West walls was determined to be the amount of rotation of the East wall polar plot that maximised its correlation with the West polar plot (Fig 5.15). According to the multi-planar hypothesis, HD cells would determine their firing direction relative to each climbing wall according to the orientation of that wall in the room, and in order to do this they must be informed by non-yaw (i.e. pitch, or perhaps roll, depending on how the rat turned the corner between walls) rotations between walls. As the angular displacement between the two walls was 180 degrees, the predicted rotation of preferred firing direction for cells using a multi-planar system was 180°. The rotational cross correlations for all cells can be viewed in appendix 3.

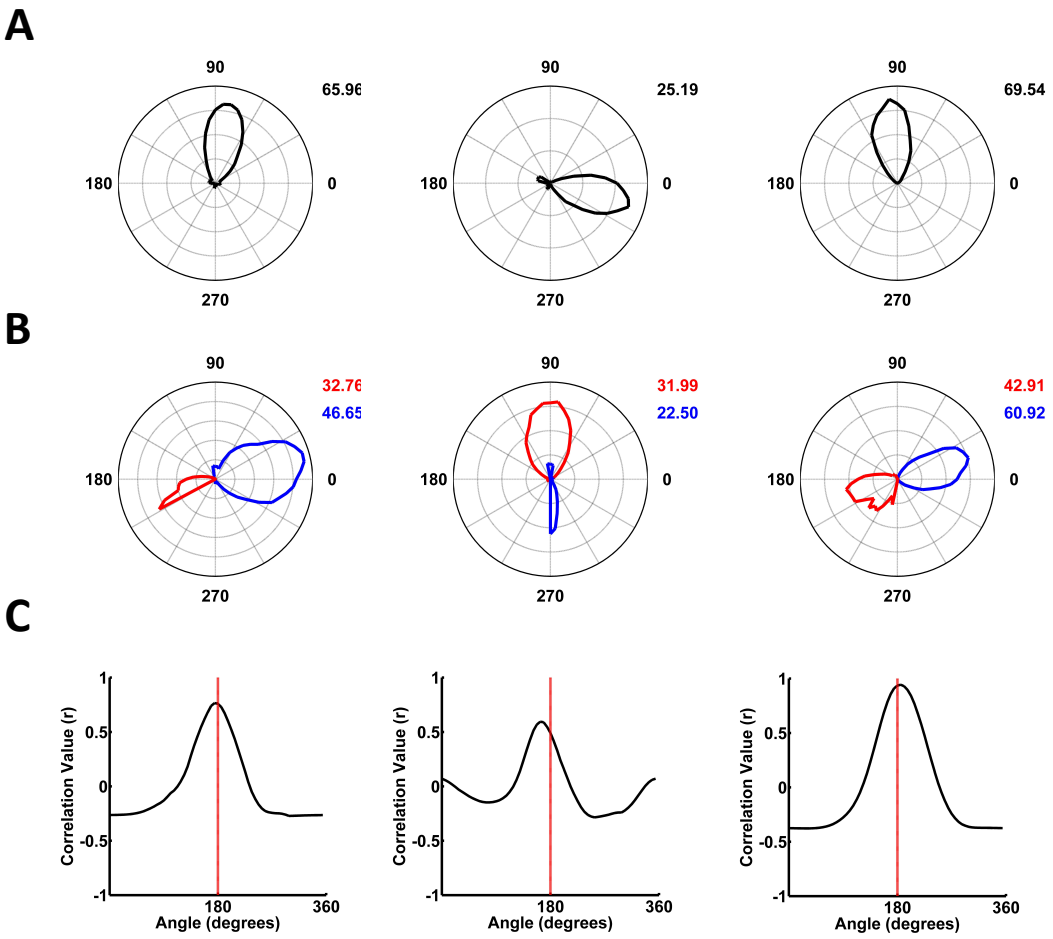


Figure 5.15 Multi-planar representation of orientation. A) Polar plots of baseline trials in a horizontal environment for three representative cells. B) Polar plots of climbing trials, showing firing directions for the East wall (blue) and West wall (red) for the same 3 cells. C) Rotational cross correlation analysis for the three cells showing the correlation between East and West wall polar plots after varying degrees of rotation of the East polar plot. Red line represents the expected peak correlation value for the multi-planar hypothesis.

A circular V-test confirmed significant clustering of peak cross correlation angles at a rotation angle of 180° ($V_{(1,15)}$ = 12.78, p<.001; Fig 5.16). This indicates that HD cells are actively updated by non-yaw rotations between differently oriented planes. Further support for this result comes from one cell that was recorded on the South wall as well as the East and West walls. The PFD of this cell on the South wall was rotated by +90° relative to the East wall, and -90° relative to the West wall (Appendix 2).

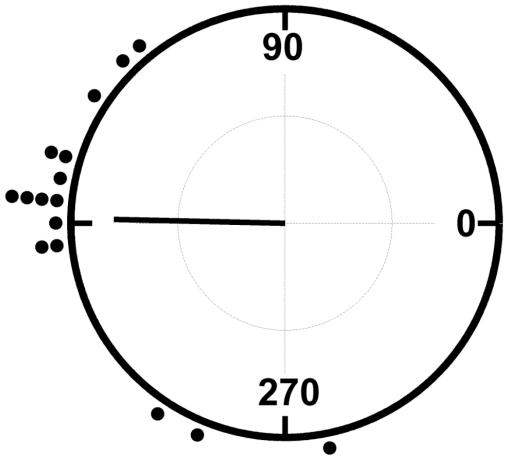


Figure 5.16 Consistent updating of HD cells between climbing walls. Circular histogram showing the peak correlation values (black dots) for the 16 cells that were active on both walls. There is a significant clustering of peak correlation values at 180°, as shown by the resultant mean vector length (black line). Rotation correlation values of 0 indicated no rotation between the two walls, while values of 90 degrees and 270 degrees indicated a clockwise and counter-clockwise deviation, respectively, from the 180° rotation predicted for the multi-planar hypothesis.

Furthermore, the reason that three cells were not active on one wall is that the rat did not adequately sample the direction that was 180 degrees opposed from their preferred firing direction on the opposing wall. These three cells (cells 2, 4 and 5) were recorded conjunctively during one climbing session. Each of them exhibited directional firing on one wall only, with mean firing directions of 25° and 74° for cells 2 and 4, respectively, on the West wall, and 115° for cell 5 on the East wall. According to the predictions of the multi-planar hypothesis, these cells were expected to fire 180° away from these angles on the opposing walls (cell 2 at 205° and cell 4 at 254° on the East wall and cell 5 at 295° on the West wall, Fig 5.17 A-B). Analysis of the sampling directions show that the animal did not sample these heading directions during climbing trials, and based upon the multi-planar hypothesis could not have been expected to fire (Fig 5.17C).

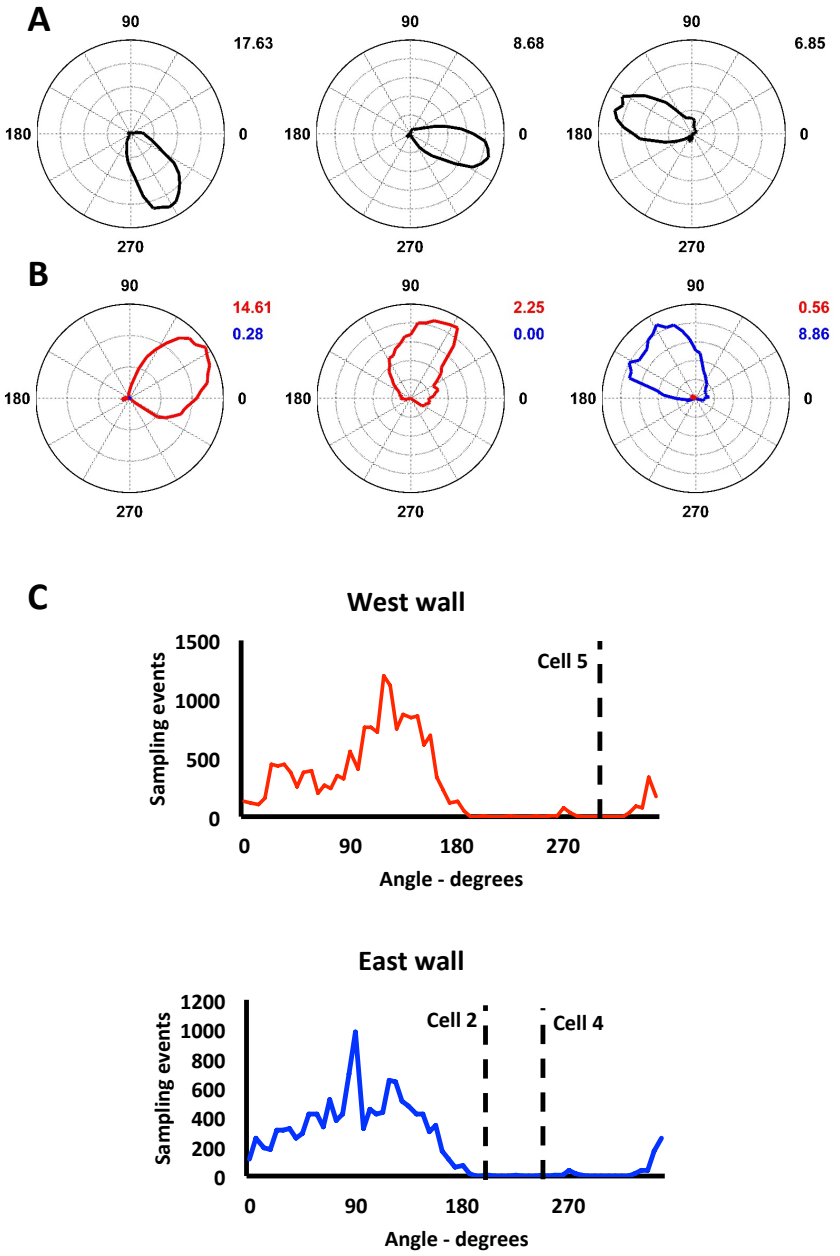


Figure 5.17 Non-sampling of expected firing directions for three cells. A) Baseline polar plots for the three cells that did not fire on both walls B) Climbing trial polar plots for these cells (Red – West wall, Blue – East wall) C) Heading angles sampled by the animals for the West (top) and East (bottom) walls, with the black dashed lines showing the expected firing direction of the cells according to the predictions of the multi-planar hypothesis.

5.4 Discussion

This study aimed to determine whether the HD system represents orientation in a planar, multi-planar or volumetric manner. The hypotheses predicted that there would either be no rotation of HD cell PFD between the East and West climbing walls (planar hypothesis), a maintenance of a volumetrically-defined PFD on both walls (volumetric hypothesis), or a 180° rotation of HD cell PFDs between the East and West climbing walls (multi-planar hypothesis). The results indicated that HD cells are actively updated by rotations between differently oriented planes in a manner consistent with the multi-planar hypothesis. This form of representation allows animals to remain oriented in a way for them to consistently translate their heading orientation from vertical surfaces to the horizontal plane without the accumulation of heading errors (discussed later in this section). The findings of the study are examined below, followed by a discussion of the ways in which such a multi-planar system might aid navigation in three-dimensional environments.

5.4.1 The activity of rodent ADN HD cells is best described by the multi-planar hypothesis

This experiment was designed to disambiguate three potential mechanisms for encoding orientation in three-dimensional space. Either the rodent HD system is two-dimensional and modulated purely by yaw rotations in an animal's plane of locomotion (planar hypothesis), or the system is sensitive to non-yaw rotations. If the system is sensitive to non-yaw rotations, HD cells could be expected to either encode for global orientation, with both elevation and azimuth angle components (volumetric hypothesis), or the direction in which they are modulated relative to their locomotor plane could be informed by the position of that plane relative to the global azimuth (multi-planar hypothesis).

The findings of this study show that HD cells do not use either a purely local or a purely global reference frame. Rather, HD cells remain modulated by yaw rotations

relative to the animals' plane of locomotion, but are further informed by the position of that plane in the azimuth. This resulted in a 180° rotation of the PFD of HD cell's between the East and West walls.

5.4.2 How might this system be useful for navigation?

In geometry, the best way to define orientation in three-dimensional space uses two directional components – angle in the azimuth and elevation angle. Using such a system, any directional heading in three-dimensional space can be uniquely described by two values. However, it is clear from the results showing the change in preference for upwards vs. downwards firing between walls that HD cells in rats do not define 3D orientation in this way. How, therefore, is a multi-planar system useful for navigation?

This question can be addressed if we consider the firing direction of HD cells on a spherical surface, with reference to the concept of the Berry-Hannay angle (Berry, 1988; Hannay, 1985). In his study of particle physics and quantum mechanics, Berry (1984) noted that a spinning particle moving around a sphere acquires a change in its spin direction once it returns back to its starting point. Berry described this acquired rotation in a simple thought experiment:

> Take a pencil, lay it on the north pole of a globe and point it in the direction of any of the meridians: the lines of longitude that radiate from the pole. Move the pencil down along the line to the equator and, keeping it perpendicular to the equator, slide it to another line of longitude. Move the pencil back to the north pole along the new meridian, and you will find that although the pencil has been returned to its starting point and at no time was rotated, it no longer points along the original line of longitude. (Berry, 1988)

The resultant discrepancy between the pencil's original pointing direction and its final pointing direction is known as the Berry-Hannay angle. Now, if we replace

“pencil” with “HD cell firing direction”, we can consider how the multi-planar representation of orientation is useful for navigation of three-dimensional structures.

First, as shown in Figure 5.18A, if HD cells used a single-planar reference frame, and did not rotate their PFD between vertical planes, their PFD at each point on the equator of a sphere, relative to the tangent plane for each location, would be the same. For example, a cell with a PFD pointing upwards with the animal at 0° on the equator would also have a PFD pointing upwards when the animal is at 90° on the equator. If, as in Berry’s description of anholonomy, we then translate the position of the animal from each of these equatorial locations towards the pole, the cell incurs a Berry-Hannay angle. In this case, because the two starting positions of the animal on the equator differ by 90°, the Berry-Hannay angle incurred at the pole of the sphere would also be 90° (Fig. 5.18A).

Now, consider the same issue with regards to the findings of this study in which the PFD of HD cells rotates accordingly with the angular displacement between walls. A cell with a PFD pointing upwards with the animal at 0° on the equator of a sphere would have a PFD pointing to the right (relative to the tangent plane) with the animal at 90° on the equator. Translating the heading direction from each equatorial position to the pole of the sphere does not result in a Berry-Hannay angle problem (i.e. the PFD of the cell is the same at the pole irrespective of the starting position on the equator; Fig 5.18B).

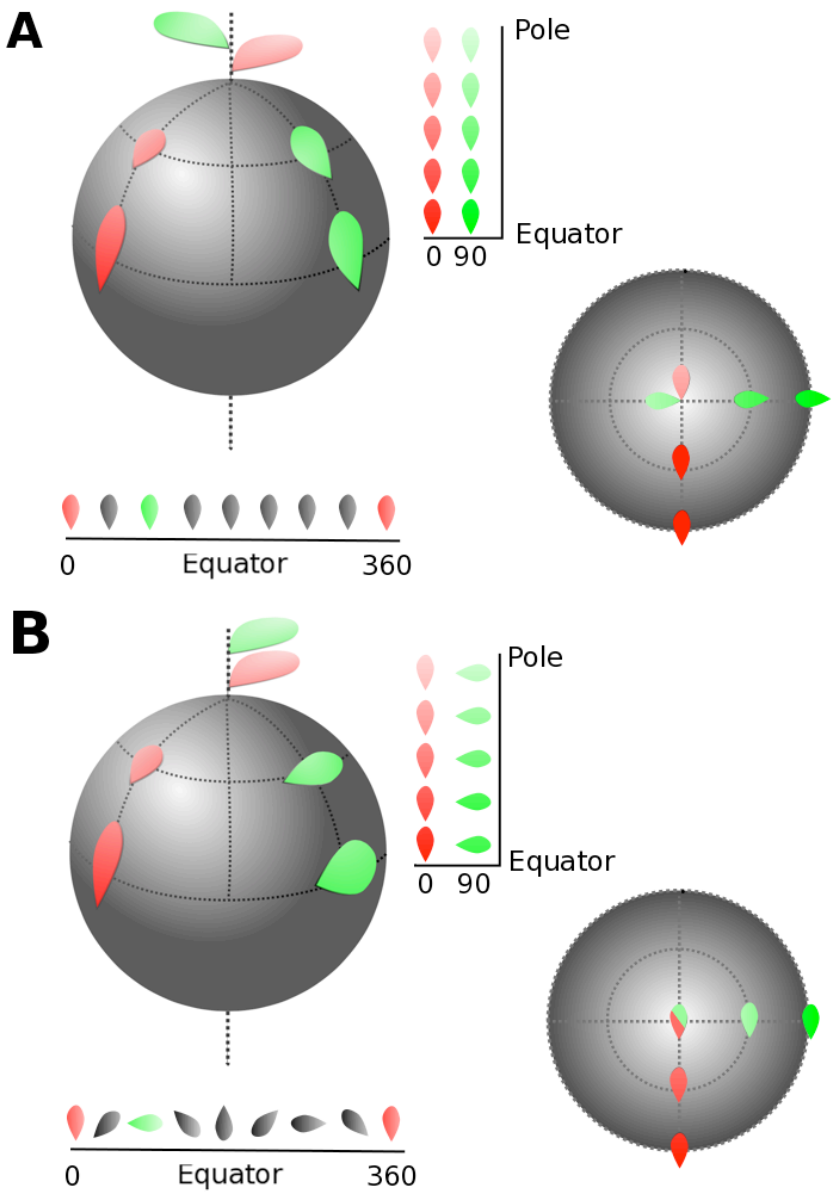


Figure 5.18 Representation of the expected behaviour of cells on a sphere if HD cells used a single-planar reference frame (A) or a multi-planar reference frame (B). Left – illustration of HD cell activity on a sphere. Red teardrops illustrate the PFD of a stylised HD cell at varying points on the meridian at 0° in longitude. Green teardrops show the expected firing of the same cell at 90° of longitude for several points along the meridian. Bottom-left – The expected firing of the same cell on the tangent-plane of equidstant points around the equator, showing the cell at 0° (red) and 90° (green). Centre-top – the expected firing of the cell at different points along the meridian for both 0° and 90° longitude. Bottom-right – polar view of the spheriical illustration. Note for a single-planar reference frame (A) there is no rotation of the PFD of the cell along the equator, resulting in a discrepancy in the PFD of the cell at the pole of the sphere. This is known as a Berry-Hannay angle. For a multi-planar reference frame (B) the PFD of the cell rotates for each of the tangent planes around the equator, in a manner that allows for a translation back to the horizontal plane at the pole, without the accumulation of a Berry-Hannay angle.

The multi-planar system therefore allows the HD system to maintain a consistent horizontally defined orientation, irrespective of the plane of locomotion. Such a system would allow for animals to remain oriented relative to the azimuth as animals rotate around complex curved and multi-planar surfaces.

5.4.3 Conclusions and future work

For a full understanding of the neural encoding of orientation, it is important to understand how animals represent orientation in all three-dimensions: accordingly, the present experiment investigated whether the rodent HD system represents orientation on a three-dimensional structure in a planar, multi-planar or volumetric manner. The findings indicate that HD cells are actively updated by non-yaw rotations, but their activity does not represent orientation in volumetric space, as cells firing upwards on one wall fired downwards on the opposite wall, meaning that their firing direction relative to volumetric allocentric co-ordinates differed between walls. Rather, the direction of HD cell firing is updated so as to always provide orientation information that can be translated back to the horizontal plane without accumulation of heading errors.

These findings are distinct from results obtained from recordings of bat HD cells (Finkelstein et al., 2015). HD cells in bats are modulated by pitch and roll rotations, as well as yaw rotations, allowing bats to represent orientation in volumetric space. It is possible that the differences in behavioural ecology between bats and rats have driven the evolution of the HD system in different ways. As bats are able to fly in volumetric space they might require an equally high-resolution representation of elevation angle (pitch) as they do azimuth angle (yaw). Rats, on the other hand, are surface-dwelling mammals with the majority of their time spent on horizontally oriented surfaces. While they are able to climb on vertical surfaces with relative ease, these behaviours are most commonly simple movements between two horizontal surfaces. The multi-planar representation by HD cells found in this study allows rats to simply translate their heading direction from the vertical back to their

most-used horizontal representation without the accumulation of errors. While this does not allow for judgement of absolute orientation in volumetric space, it does provide a simple mechanism for maintaining orientation in the rat's most commonly used behavioural space (the horizontal plane) during movement on three-dimensional structures.

The question remains how the rodent HD system implements this multi-planar representation. The current experiment was carried out in a well-lit environment; rats therefore had visual access to intramaze cues (such as the position of the starting box). It is possible that these cues were sufficient to provide the HD system with the relative orientation of the two walls with respect to the starting box, thus indicating the position of the walls relative to the azimuth. This could be tested with recordings of HD cells on the apparatus during dark conditions. If the same results are acquired during dark conditions as in the current experiment, visual cues would be concluded to be unnecessary for the multi-planar representation of orientation. Information must therefore be provided to the HD system through idiothetic cues – from the vestibular system, or through proprioception and motor-efference copy. This would suggest the existence of non-yaw modulated cells, which would likely receive input from the vertical semicircular canals. Pitch-modulated cells have previously been reported in the LMN of the rat brain (Stackman et al., 1998), but as discussed previously, these cells only appeared to be active after rotations of over 80°. Further recordings of these cell types using similar tracking techniques and the ring-like apparatus used in Finkelstein et al. (2015), may provide further insight into the capability of the rodent brain to represent non-yaw rotations of the head.

While it is not yet clear whether the multi-planar orientation of HD cells in rats is driven by allothetic or idiothetic cues, the results of the above study show that HD cells in rats are able to account for a series of complex egocentric rotations, allowing them to always maintain accurate orientation relative to the azimuth, whether or not they are actually moving and rotating in the horizontal plane.

Further studies of place and grid cells in similar environments could test whether the rodent cognitive map as a whole uses a multi-planar representation.

Chapter 6 General discussion

The overarching aim of this thesis was to ascertain whether rodents are able to represent three-dimensional space, and if so, to describe how the neural substrates of spatial cognition support this representation. Behavioural and electrophysiological techniques were used to address these questions.

The main findings of the experimental work undertaken show that rodents are able to distinguish between reward locations distributed in three-dimensional space (Chapter 4), but with less accuracy than two-dimensional space. Subsequently, electrophysiological recordings indicated that the head direction system uses a multi-planar representation that allows animals to maintain a representation of orientation during vertical locomotion that can always be translated back to the horizontal plane without the accumulation of errors (Chapter 5). Such a representation can explain how mice were able to orient themselves during movement on the radiolarian maze. A discussion of the findings from each of these studies is outlined below. These complementary findings will next be discussed alongside previous research to argue that the rodent cognitive map is served by a multi-planar, rather than a volumetric, representation of three-dimensional space. Finally, suggestions of future work to test the multi-planar hypothesis will be presented.

6.1 Memory of a three-dimensional world

Findings from the behavioural experiments discussed in Chapter 4 illustrated that rodents (in this case, mice) are able to form both short- and long-term representations of locations distributed throughout three-dimensional space.

Based upon a wealth of research using the apparatus, a three-dimensional version of the radial arm maze was developed (the radiolarian maze), and mice were tested on working memory and reference memory tasks. Learning on these tasks was

compared to learning on a two-dimensional analogue of the radiolarian maze. The ability of mice to represent the radiolarian maze was comparable with the two-dimensional maze (the hexagon maze) for the working memory task, but not for the reference memory task. Mice exhibited more working memory and reference memory errors on the radiolarian maze during the reference memory task than on the hexagon maze.

There are two potential reasons for the diminished learning in the radiolarian maze; either the physical demands of moving over the spherical surface led to the reallocation of cognitive resources away from the spatial components of the task towards its more procedural aspects, or, there might be a limitation in the ability of mice to represent three-dimensional space.

With the current study, it was not possible to discount the explanation that the physical demands of moving on the radiolarian maze impaired the animals' completion of the reference memory task, although it does seem somewhat unlikely given the comparable learning rates on the working memory task. Nonetheless, this potential reason for animals' diminished learning on the RM task could be tested with the introduction of climbing barriers to the hexagon maze. These barriers would introduce physical demands while moving on the hexagon maze that were not present in the original study. This design was not implemented in the reported study because of the risk that the introduction of climbing to the hexagon maze would also introduce a three-dimensional component to the behaviour of the animals that might affect the ability of mice to represent the hexagon maze. However, if such a maze were to be designed, one could ensure that the barrier heights were the same for all arms of the maze and that the reward locations at the end of the arms of the maze were all in the same horizontal plane. This would increase the physical demands on the mice while still ensuring that the hexagon maze, unlike the radiolarian maze, could be solved using a purely two-dimensional representation of the reward locations. If learning were to still be diminished on the radiolarian maze compared to this modified hexagon maze it

would indicate that representational rather than physical-demand explanations best explain the differences in learning between three-dimensional and two-dimensional structures.

With regards to the representational findings, it appears that a volumetric representation of the maze could not explain the discrepancy in learning between the radiolarian and hexagon mazes. If mice had a system for describing co-ordinates in the x, y and z axes with the same resolution, one would expect mice to be equally able to encode and recall locations distributed in three-dimensions as those distributed in two. On the other hand, it is also unlikely that the representation of the radiolarian maze was horizontal-planar, as mice did not show a behavioural bias for visiting arms on a layer-by-layer basis, nor did they appear to confuse reward locations between layers as learning on the working memory task was comparable to the hexagon maze.

The best representational explanation for the findings of this set of behavioural experiments is that the mice use multiple planar maps to represent rewards locations. In this case, mice may either possess two separate horizontal maps for each layer of arms, or they might possess a map for the vertical plane, and a separate map for the horizontal plane. In the radiolarian maze mice would have to access both of these maps and integrate them before determining which arms remained baited, whereas in the hexagon maze only one map (horizontal planar) would be needed to determine the location of rewards. The increased cognitive load of integrating two separate maps may explain the differences in learning between the two mazes.

Testing whether rodents use a multi-planar representation of the radiolarian maze rather than a volumetric or horizontal-planar representation could not be done using behavioural experiments alone. Instead, electrophysiological recordings of grid and head direction cells would be needed to determine how neurons encoding for distance and direction behave in three-dimensional environments. As such, the

electrophysiological experiment in Chapter 5 was designed to test the behaviour of head-direction cells on a multi-planar surface. The findings of this experiment are discussed next.

6.2 Orientation in a three-dimensional world

6.2.1 Overview

The findings presented in Chapter 5 indicated that head direction cells in rodents use a multi-planar reference frame to define their firing direction on vertical surfaces. HD cells were recorded as rats climbed between differently oriented vertical walls, and their preferred firing directions were compared between these walls.

The data show that HD cells rotated consistently by 180° between two opposing walls. This result is inconsistent with the presence of a volumetric representation of orientation, as cells were shown to be active as the animal faced upwards on one wall, but downwards on the opposing wall (i.e. cells did not fire in the same global elevation angle on the two climbing walls). This result is also inconsistent with a purely locally defined representation of orientation (e.g. cells firing upwards relative to the local plane), as cells fired in different directions relative to the animal's plane of locomotion.

Rather, the data indicated that HD cell firing is anchored to the animal's plane of locomotion, but is further informed by the position of that plane relative to the external world. This indicates that the HD system in rats is likely to be multi-planar. Interrogation of this multi-planar representation, in combination with the theory of anholonomy and Berry phase, further reveal the potential functional significance of reorienting a cell's firing direction based upon the position of the climbing walls. Such a system would allow rodents to always remain oriented relative to the azimuth, even when the animal is moving on non-horizontal surfaces.

6.2.2 Limitations

While the findings of this study suggest the presence of an orienting mechanism in rodents that might allow for accurate navigation over multi-planar surfaces, there are a number of caveats concerning the strength of conclusions that can be made from these results alone.

First, there are several inherent limitations to chronic in-vivo electrophysiology that preclude a wider generalisation of the results of this study. Perhaps the most major of these limitations is the inability to look at the functioning of neural activity at a wider population level. This is a result of a trade off between the ability to record neural activity at a high spatio-temporal resolution and the ability to record wider brain functioning, either within the same region of interest, or in multiple regions of the brain. Unfortunately, when using single-unit electophysiology the number of neurons that can be recorded simultaneously in a given brain region is constrained by the number of microelectrodes that can be inserted into the brain, which is itself constrained by the size and weight of the electrode microdrives that are fixed to the head.

Historically, in-vivo chronic electrophysiology has allowed for the identification of numerous cells with specific cognitive and perceptual function, including but not limited to the neurons associated with navigation, mirror neurons (Di Pellegrino et al., 1992) and orientation selective neurons in the visual cortex (Hubel & Wiesel, 1959). The technique of chronic extracellular recording in freely moving animals is likely to continue to adapt over time with the development of lightweight and more compact microdrive assemblies, which will in the future allow for the recording of a larger number of neurons per animal as well as a larger number of simultaneously recorded. Further to this, the development of miniaturised head-mounted fluorescence microscopes has allowed for the simultaneous Calcium imaging of a larger number of neurons (~200), albeit with a lower temporal resolution than electrophysiological recordings.

A related limitation to electrophysiological recordings is the ability to record from multiple brain regions at a time. The implantation of multiple microdrives in distinct brain regions has become more commonplace in recent years (Peyrache et al., 2015 for example), however this approach was not used in this study due to concern about the effect of the added weight of an extra microdrive on animals' movement on the three-dimensional recording apparatus. As described in chapter 3, there are a variety of distinct brain regions containing HD cells, and while many of the features of HD cells are remarkably similar between brain regions there remains the possibility that HD cells in different regions encode for three-dimensional space differently. One cannot therefore conclude that HD system as a whole conforms to a multi-planar representation of orientation.

A final inherent limitation to chronic extracellular electrophysiological recordings is that of the cells recorded in a single brain region only a small amount of neurons are ever identified as cells to which a behavioural, cognitive or perceptual process can be attributed. For example, of the cells recorded in the postsubiculum, retrosplenial cortex and ADN only ~25%, ~6% and 60% can be identified as HD cells, respectively. For the most part, we are currently unaware of the functioning of the other neurons within these regions. The non-HD cells of these regions may be interneurons that work to moderate the activity of HD cells, or as reported recently non-HD cells in the RSC may be modulated by egocentric actions, route locations and non-directional allocentric space (Alexander & Nitz, 2015). It should be noted that the study presented in chapter 5 focused only on cells that had been identified as HD cells in a horizontal environment.

Together, these limitations of electrophysiological recordings alongside the complexities of developing a three-dimensional apparatus on which HD activity in 3D could be reliably tested resulted in the relatively small number of HD cells that were recorded. The following discussion of the results outlined chapter five must therefore be approached in mind of the caveat that the HD cells tested were a only

small percentage of all HD cells in the ADN and were recorded from only one brain region.

6.2.3 Formation of a multi-planar representation

If the rodent HD system does conform to a multi-planar representation of three-dimensional space, there remains the question as to which input signals to the HD system drive the formation of this representation, and why this representation differs to that of bats.

Understanding how the multi-planar representation might be formed must first begin with determining whether the observed rotation of HD cell firing directions can occur without the use of visual cues. If it can, the mostly likely candidate for informing the rats of their position comes from the pitch and roll-sensitive vertical semicircular canals of the vestibular apparatus. Pitch modulated cells have been found in the LMN of the rodent brain (Stackman & Taube, 1998), while roll-modulated cells have not yet been reported in the rodent brain. The pitch cells of the LMN have firing behaviours that are distinct from the classic yaw-modulated head direction cells and also are distinct from pitch-modulated cells found in bats (Finkelstein et al., 2015) – rather than being Gaussian tuned, they appear to have a rapid ramping of activity only when animals are pitched up or down by 80-90 degrees. Nevertheless, the mechanisms for detecting pitch and roll appear to be present in the rodent brain, and it is likely that pitch and roll-modulated cells do indeed exist in some form in the rodent brain.

Another candidate for informing rats of their position on the climbing apparatus is the cerebellum. Sensory inputs to the cerebellum include vestibular information projecting from the vestibular nuclei, visual projections and proprioceptive information (see review in Rondi-Reig et al., 2014). Apart from the contribution of vestibular signals discussed previously, proprioceptive information from the neck could be important in detecting non-yaw head rotations. This information might then be integrated with vestibular information within the cerebellum. This system

could provide information relative to an allocentric reference frame through processing of the gravity vector, as well as providing information relative to the animals' egocentric head-rotations (Rochefort et al., 2013). Indeed, the cerebellum has previously been shown to be important for navigation in the absence of visual cues. Rochefort et al., (2011) showed that disruption of long-term depression at the cerebellar-Purkinje cell synapse impaired mice on a path integration task and disrupted the ability of place cells to use self-information cues.

The reason for the difference between the representation of orientation in rats and bats could be that the development or evolution of a volumetric representation of orientation is driven by the behavioural ecology of the animals. Bats can move freely in volumetric space, and might therefore need to represent elevation angle with equal resolution as their representation of azimuth angle. Rats, on the other hand, spend the majority of their lives on planar surfaces, so might therefore be better suited to maintaining orientation relative to the azimuth, and forgoing the extra computational demand of a volumetric representation. In short, forming a volumetric representation is surplus to the navigational needs of the rat, and would require an unnecessary diversion of cognitive resources away from other neural systems.

6.2.4 The multi-planar framework and ring-attractor dynamics

As discussed in chapter 3, computational models of head-direction cells commonly use a ring-attractor network to describe the transfer of activity between HD cells. Briefly, the ring-attractor model of HD cells states that input from visual and rotation sensitive cells excites HD cells near to the currently active HD cell. Further excitation by the currently active HD cell to cells with similar preferred firing directions and inhibition to those cells with dissimilar preferred firing direction allows for a smooth transfer of activity between HD cells.

Ring-attractor models of HD cells have been used described HD cell activity in simple two-dimensional planes, and have also led to a variety of predictions about the presence of angular head velocity encoding cells, but can they be used to describe the multi-planar framework for updating HD cells during movement on complex three-dimensional work?

To answer this question one must first determine whether a ring attractor model can account for HD cell firing on a vertical plane, and if so, how would the ring-attractor network behave during movement between vertical planes, and finally, if the ring attractor dynamic were affected by movement between planes, what mechanism would drive this change in dynamic.

Previous work by Taube et al., (2013) and the work presented in chapter 5 of this thesis have shown that HD cells remain modulated by yaw rotations of the head when animals move on vertical surfaces. Within the ring attractor models it is these yaw rotations of the head that are thought to drive the rotation sensitive cells (or angular head velocity cells) which in turn drive the transfer of activity between HD cells. It is possible that the ring-attractor is defined by the animal's plane of locomotion, as has been suggested by Stackman, Tullman & Taube (2000), with HD cell activity driven by yaw rotations irrespective of the animal's plane of locomotion.

So, how might the ring-attractor behave during rotations between vertical planes? Recent modelling work by Hector Page (personal communication) suggests that the ring attractor itself is rotated during movement between vertical planes. For example, the cells that are active when an animal faces North in the starting box of the climbing apparatus used in chapter 5 would be active when the animal faces upwards on the South wall, and on the East wall it would be active when the animal faces to the right. For this to happen, Page suggests that the ring attractor is rotated by an amount equal to the rotation of the animal between vertical planes. As the

animal has moved 90° between walls, the ring attractor has also rotated by 90° causing a rotation of the preferred heading direction of cells between walls.

One potential mechanism for the rotation of the ring attractor is that the HD network is sensitive to rotations of the animal's dorso-ventral axis around the gravity vector as well as their well-documented modulation by rotations around the dorso-ventral axis of the head (i.e. modulation by yaw rotations of the head). It is not yet understood how HD cells might process the gravity vector, but there are a variety of candidate mechanisms that could drive the system proposed by Page, including but not limited to processing by the gravity sensitive otolith organs of the vestibular system, integration of otolith and semi-circular canal signals with the cerebellum, or perhaps input from pitch-sensitive cells in the LMN (Stackman & Taube, 1998).

6.3 Is the rodent cognitive map multi-planar?

A huge wealth of literature has revealed the remarkable ability of animals – from insects to humans – to accurately navigate two-dimensional environments. The most prominent theory explaining this ability is that animals possess an internal representation of the world around them, known as a cognitive map. The studies discussed in this thesis asked how this cognitive map represents three-dimensional space. An overview of previous studies alongside the findings presented in this thesis has revealed that the rodent cognitive map is not volumetric, nor is it a simple horizontal planar map. Rather, the experiments detailed in chapters four and five indicate that the cognitive map is likely to be multi-planar, with a function of maintaining horizontal orientation, even during movement on vertical planes.

The directional information provided by the HD system, however, is only one component of the information required to form a functional representation of space. The metric information provided by grid cells and the spatial localisation of place cell firing has not yet been tested as animals climb on vertical surfaces. It is

interesting to speculate on the behaviour of these subtypes of neurons and whether they too use a multi-planar map.

Grid cells require input from the HD system to exhibit their characteristic periodic firing (Winter, Clark & Taube, 2015), and the orientation of grid cell firing patterns is thought to be provided by directional information relayed to the medial entorhinal cortex from the postsubiculum. We can therefore consider the effects of the multi-planar HD cell representation on grid cell firing characteristics – in particular, the orientation of the grid-pattern relative to the locomotor plane.

Based upon the findings presented in Chapter 5, one might expect that as animals move from horizontal surfaces to vertical surfaces the orientation of the grid pattern would be maintained, as has been reported for HD cells (Stackman et al., 2000; Taube et al., 2013). Indeed, we found a continuation of grid orientation was seen on a 40° slope (Hayman et al., 2015). Upon movement between differently oriented vertical planes (e.g. the East and West walls of the climbing apparatus used in Chapter 5) we might expect the orientation of the grid pattern to rotate accordingly with the rotation of HD cells between planes. Such an effect could not be studied using walls with an angular separation of 180° as in the study presented in Chapter 5, as the grid cell pattern has a rotational symmetry at 60°, 120° and 180°. However, if grid cells were recorded between the two walls with an angular separation of 90° one might expect a reorientation of the grid pattern by 90° (this would in fact be seen as a 30° rotation due to the grid pattern's rotation symmetry).

Casali & Jeffery (2015) have recently reported an increase in the spacing between grid fields as rats climb on a vertical plane. As well as angular information, grid cells are thought to use information from linear acceleration to define the spacing (phase) of grid fields. The effect reported by Casali & Jeffery (2015) might have arisen due to difficulties in processing linear acceleration information when the head is oriented upwards. It is, as yet, unclear how the linear acceleration components of the vestibular apparatus (the utricle and saccule) process

acceleration when the head is upright. One possibility is that the utricle, which measures horizontal linear acceleration during horizontal movement, is less able to measure acceleration during vertical movements due to the effect of gravity on the utricle endolymph, causing a constant downwards displacement of the cell hair membranes within the utricle.

A multi-planar representation of space would also hold implications for the behaviour of place cells in vertical planes. The predicted behaviours of place cells are more complex, however, as they have been shown to be highly sensitive to context as well as position. The behaviour of place cells, therefore, could be affected by the judgement of whether different climbing surfaces are contextually different (as in Jeffery & Anderson, 2006) or whether the surfaces are considered to be repeating environments (as in Derdikman et al.; Spiers et al., 2014) – albeit between differently oriented surfaces. The findings of the experiments in Chapter 5 indicate that the rodent HD system is sensitive to non-yaw rotations. If the cognitive map in general is similarly sensitive to these rotations, place cells might treat differently oriented vertical planes as different contexts. If this were to be the case, one might expect place cells to exhibit remapping behaviours between two orthogonal climbing walls. As with previous studies, some place cells may not be active on both walls (global remapping), some might exhibit a repositioning of their fields relative to the plane of locomotion, while some might not exhibit any change in the location, or change in firing rate of cells between different planes. Similar effects may also be seen for instances in which animals move from the horizontal plane to vertical plane.

On the other hand, if place cells treat the horizontal planes and vertical planes as differently oriented repeating surfaces, based on the multi-planar firing of HD cells, one might expect place fields on vertical surfaces to be in the same relative position as they are on the horizontal surface. For example, a cell firing on the floor at the left corner nearest the vertical plane might fire at the top left corner of the vertical plane. If this were to be true, the multi-planar hypothesis, based on findings from

HD cells reported in Chapter 5, could also predict the firing of place cells as animals move between two orthogonal vertical planes. As HD cells rotate by 90° between two orthogonal planes, we might expect the position of place fields to rotate by the same amount. For example, a place cell firing at the top left corner of a South-facing wall would be expected to fire at the top-right corner of an East-facing plane.

It must be noted that while the multi-planar activity of the HD system provides a functional mechanism within which to maintain horizontal orientation, it is unclear how the potential place cell remapping events described above would aid navigation. Nevertheless, the multi-planar hypothesis for the representation space provides several predictions for the behaviour of place and grid cells, all of which could be tested with relative ease using apparatus similar to that used in Chapter 5.

6.4 Implications for episodic memory

The multi-planar hypothesis provides predictions for spatial memory and navigation of three-dimensional structures but how might the findings of this thesis impact on our understanding of wider hippocampal function such as episodic memory?

Episodic memory, defined by Endel Tulving in 1972 (Tulving, 1972), was described as a form of mental time travel that allows us to revisit and replay past episodes of our lives in our minds. Simply, episodic memories can be reduced to the memory of the *what*, *where*, and *when* of an event in our lives (Tulving, 1984). A model of episodic memory by Michael Hasselmo suggests that episodic memories are built along a spatio-temporal trajectory, by which memories of events are intimately linked with their spatial and temporal context (Hasselmo, 2009; Hasselmo, Giocomo & Brandon 2010; Hasselmo, 2012). Hasselmo (2012) argues that the network of place, grid and head-direction cells provides this spatio-temporal trajectory onto which memories of events are attached. Place cells are thought to map individual points along the trajectory, while the internal membrane dynamics of grid cells as well as their modulation by theta-phase provides a potential metric for time as well as space.

Further support for this model has been provided by evidence for the replay of place cell firing sequences during REM sleep (Louie & Wilson, 2001; Foster & Wilson, 2006). Further to this, recently discovered hippocampal time cells might also play a role in defining the temporal context of episodic memories (MacDonald et al., 2011; Kraus et al., 2013; *for review,* see Eichenbaum, 2014). Another key feature of episodic memories that can be explained by the spatio-temporal trajectory model is that they have a perspective, either in the point of view of the person remembering or from a third-person perspective (Conway, 2009). Hasselmo (2012) argues that HD cells provide the information required for recalling perspective and orientation.

If episodic memories are indeed formed by the anchoring of events to place, grid and HD cell defined spatio-temporal trajectories, it is important that the brain maps the world accurately. As stated at the beginning of this thesis we live in and navigate through three-dimensional environments, and events which we may need to remember might occur in non-planar non-horizontal environments. If we, therefore, are unable to represent the trajectories of our movements over three-dimensional terrains, we may be unable to form accurate episodic memories of events occurring on non-horizontal spaces.

The findings of this thesis indicate that the neural mechanisms for encoding space might, at least in rodents, have developed to encode three-dimensional space in a multi-planar rather than a volumetric or simple horizontal-only framework. As shown in figure 5.18, the proposed multi-planar framework for HD cells allows for a mapping of head direction that can be consistently translated back to the horizontal plane without the accumulation of heading error. This functionality would allow for a consistent directional component of the spatial trajectory described by Hasselmo, even when animals are navigating over complex three-dimensional terrains. Moreover, given the importance of the HD signal for the normal functioning of place (Calton et al., 2003) and grid cells (Winter, Clark & Taube, 2015), the multi-planar firing of HD cells may also further drive the place and grid cell mapping of the

spatio-temporal trajectory. While it has not yet been shown whether or not the multi-planar framework can be generalised to HD cells in other brain regions or to other spatially-modulated neurons, it remains possible that the findings of this thesis might also add to our understanding of the neural basis for episodic memories.

6.5 Final conclusions

In summary, this thesis produced two complementary novel findings. First, mice are able to hold short and long-term representations of goal locations distributed throughout three-dimensional space, albeit with greater difficulty than two-dimensional environments (Chapter 4). Second, the firing of head direction cells appears to fit within a multi-planar framework, which would allow for maintenance of orientation in the azimuth irrespective of the animal's plane of locomotion (Chapter 5). Together these findings indicate that the rodent cognitive map has evolved to provide a suitable multi-planar framework for navigation within the animals' most-used behavioural space, forgoing a more informative, but more computationally costly, volumetric representation of space.

Reference List

Aadland, J., Beatty, W. W., & Maki, R. H. (1985). Spatial memory of children and adults assessed in the radial maze. Developmental Psychobiology, 18(2), 163–172. http://doi.org/10.1002/dev.420180208

Alexander, A. S., & Nitz, D. A. (2015). Retrosplenial cortex maps the conjunction of internal and external spaces. Nature Neuroscience, 18(8), 1143–1151. http://doi.org/10.1038/nn.4058

Allen, G. V., & Hopkins, D. A. (1989). Mammillary body in the rat: Topography and synaptology of projections from the subicular complex, prefrontal cortex, and midbrain tegmentum. *The Journal of Comparative Neurology*, *286*(3), 311–336. http://doi.org/10.1002/cne.902860303

Amaral, D. G., & Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience*, *31*(3), 571–591.

Anderson, M. I., & Jeffery, K. J. (2003). Heterogeneous modulation of place cell firing by changes in context. *The Journal of Neuroscience*, *23*(26), 8827–8835.

Angelaki, D. E. (2004). Eyes on Target: What Neurons Must do for the Vestibuloocular Reflex During Linear Motion. Journal of Neurophysiology, 92(1), 20–35. http://doi.org/10.1152/jn.00047.2004

Angelaki, D. E., & Cullen, K. E. (2008). Vestibular System: The Many Facets of a Multimodal Sense. Annual Review of Neuroscience, 31(1), 125–150. http://doi.org/10.1146/annurev.neuro.31.060407.125555

Angelaki, D. E., & Hess, B. J. (1994). Inertial representation of angular motion in the vestibular system of rhesus monkeys. I. Vestibuloocular reflex. Journal of Neurophysiology, 71(3), 1222–1249.

Artal, P., Herreros de Tejada, P., Muñoz Tedó, C., & Green, D. G. (1998). Retinal image quality in the rodent eye. Visual Neuroscience, 15(4), 597–605.

Barlow, J. S. (1964). Inertial navigation as a basis for animal navigation. *Journal of Theoretical Biology*, *6*(1), 76–117.

Barry, C., Hayman, R., Burgess, N., & Jeffery, K. J. (2007). Experience-dependent rescaling of entorhinal grids. *Nature Neuroscience*, *10*(6), 682–684. http://doi.org/10.1038/nn1905

Bassett, J. P., & Taube, J. S. (2001). Neural correlates for angular head velocity in the rat dorsal tegmental nucleus. *The Journal of Neuroscience, 21*(15), 5740–5751.

Bassett, J. P., Tullman, M. L., & Taube, J. S. (2007). Lesions of the tegmentomammillary circuit in the head direction system disrupt the head direction signal in the anterior thalamus. *Journal of Neuroscience*, *27*(28), 7564–7577. http://doi.org/10.1523/JNEUROSCI.0268-07.2007

Batschelet, E. (1981). *Circular Statistics In Biology*. Academic, London

Berens, P. (2009) CircStat: A Matlab Toolbox for Circular Statistics, *Journal of Statistical Software*, Volume 31, Issue 10.

Berry, M. (1988). The geometric phase. *Scientific American*, *259*, 46–52. http://doi.org/10.1038/scientificamerican1288-46

Berry, M. V. (1984). Quantal phase factors accompanying adiabatic changes. *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, *392*(1802), 45–57. http://doi.org/10.1098/rspa.1984.0023

Blair, H. T., Cho, J., & Sharp, P. E. (1998). Role of the lateral mammillary nucleus in the rat head direction circuit: A combined single unit recording and lesion study. *Neuron*, *21*(6), 1387–1397. http://doi.org/10.1016/S0896-6273(00)80657-1

Blair, H. T., Cho, J., & Sharp, P. E. (1999). The anterior thalamic head-direction signal is abolished by bilateral but not unilateral lesions of the lateral mammillary nucleus. *The Journal of Neuroscience*, *19*(15), 6673–6683.

Blair, H. T., & Sharp, P. E. (1996). Visual and vestibular influences on head-direction cells in the anterior thalamus of the rat. *Behavioral Neuroscience*, *110*(4), 643-660. http://dx.doi.org/10.1037/0735-7044.110.4.643

Bostock, E., Muller, R. U., & Kubie, J. L. (1991). Experience-dependent modifications of hippocampal place cell firing. *Hippocampus*, *1*(2), 193–205. http://doi.org/10.1002/hipo.450010207

Brettler, S. C., Rude, S. A., Quinn, K. J., Killian, J. E., Schweitzer, E. C., & Baker, J. F. (2000). The effect of gravity on the horizontal and vertical vestibulo-ocular reflex in the rat. Experimental Brain Research, 132(4), 434–444.

Brown, A. C. (1874). The Sense of Rotation and the Anatomy and Physiology of the Semicircular Canals of the Internal Ear. Journal of Anatomy and Physiology, 8(Pt 2), 327–331.

Brun, V. H., Solstad, T., Kjelstrup, K. B., Fyhn, M., Witter, M. P., Moser, E. I., & Moser, M.-B. (2008). Progressive increase in grid scale from dorsal to ventral medial entorhinal cortex. *Hippocampus*, *18*(12), 1200–1212. http://doi.org/10.1002/hipo.20504

Buechner, S., Hoelscher,C., & Strube,G. (2007) Path choice heuristics for navigation related to mental representations of a building. In Vosniadou S., Kayser D., and Protopapas A. Proceedings of the European Cognitive Science Conference , 504, 9. 2007.

Burgess, N., Jackson, A., Hartley, T., & O'Keefe, J. (2000). Predictions derived from modelling the hippocampal role in navigation. *Biological Cybernetics*, *83*(3), 301–312. http://doi.org/10.1007/s004220000172

Butler, W. N., & Taube, J. S. (2015). The nucleus prepositus hypoglossi contributes to head direction cell stability in rats. *The Journal of Neuroscience*, *35*(6), 2547–2558. http://doi.org/10.1523/JNEUROSCI.3254-14.2015

Calton, J. L., Stackman, R. W., Goodridge, J. P., Archey, W. B., Dudchenko, P. A., & Taube, J. S. (2003). Hippocampal place cell instability after lesions of the

head direction cell network. *The Journal of Neuroscience*, *23*(30), 9719–9731.

Calton, J. L., & Taube, J. S. (2005). Degradation of head direction cell activity during inverted locomotion. *Journal of Neuroscience*, *25*(9), 2420–2428.

Casali, G. & Jeffery, K. (2015) Neural representation of space by place and grid cells on the vertical plane in rats. Abstract from *British Neuroscience Association: Festival of Neuroscience 2015. Edinburgh, Scotland.*

Chen, L. L., Lin, L.-H., Green, E. J., Barnes, C. A., & McNaughton, B. L. (1994). Head-direction cells in the rat posterior cortex. *Experimental Brain Research*, *101*(1), 8–23.

Cho, J., & Sharp, P. E. (2001). Head direction, place, and movement correlates for cells in the rat retrosplenial cortex. *Behavioral Neuroscience*, *115*(1), 3–25.

Creem, S. H., & Proffitt, D. R. (1998). Two memories for geographical slant: Separation and interdependence of action and awareness. *Psychonomic Bulletin & Review*, *5*(1), 22–36. http://doi.org/10.3758/BF03209455

Daunicht, W. J., & Pellionisz, A. J. (1987). Spatial arrangement of the vestibular and the oculomotor system in the rat. Brain Research, 435(1-2), 48–56.

Davies, K. T. J., Bates, P. J. J., Maryanto, I., Cotton, J. A., & Rossiter, S. J. (2013). The Evolution of Bat Vestibular Systems in the Face of Potential Antagonistic Selection Pressures for Flight and Echolocation. PLoS ONE, 8(4), e61998. http://doi.org/10.1371/journal.pone.0061998

Doeller, C. F., Barry, C., & Burgess, N. (2010). Evidence for grid cells in a human memory network. Nature, 463(7281), 657–661. http://doi.org/10.1038/nature08704

Eichenbaum, H. (2014). Time cells in the hippocampus: a new dimension for mapping memories. Nature Reviews Neuroscience, 15(11), 732–744. http://doi.org/10.1038/nrn3827

Ekstrom, A. D., Kahana, M. J., Caplan, J. B., Fields, T. A., Isham, E. A., Newman, E. L., & Fried, I. (2003). Cellular networks underlying human spatial navigation. Nature, 425(6954), 184–188. http://doi.org/10.1038/nature01964

Dekker, J. J., & Kuypers, H. G. (1976). Morphology of rats AV thalamic nucleus in light and electron microscopy. *Brain Research*, *117*(3), 387–398.

Etienne, A. S., Maurer, R., & Séguinot, V. (1996). Path integration in mammals and its interaction with visual landmarks. *The Journal of Experimental Biology*, *199*(1), 201–209.

Finkelstein, A., Derdikman, D., Rubin, A., Foerster, J. N., Las, L., & Ulanovsky, N. (2015). Three-dimensional head-direction coding in the bat brain. *Nature*, *517*(7533), 159–164. http://doi.org/10.1038/nature14031

Flores-Abreu, I. N., Hurly, T. A., Ainge, J. A., & Healy, S. D. (2014). Three-dimensional space: locomotory style explains memory differences in rats and hummingbirds. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1784), 20140301. http://doi.org/10.1098/rspb.2014.0301

Flores-Abreu, I. N., Hurly, T. A., & Healy, S. D. (2013). Three-dimensional spatial learning in hummingbirds. *Animal Behaviour*, *85*(3), 579–584. http://doi.org/10.1016/j.anbehav.2012.12.019

Florian, C., & Roullet, P. (2004). Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice. *Behavioural Brain Research*, *154*(2), 365–374. http://doi.org/10.1016/j.bbr.2004.03.003

Foster, D. J., & Wilson, M. A. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature*, *440*(7084), 680–683. http://doi.org/10.1038/nature04587

Fraiture, L. (2013). A history of the description of the three-dimensional finite rotation. *The Journal of the Astronautical Sciences*, *57*(1-2), 207–232. http://doi.org/10.1007/BF03321502

Fyhn, M., Molden, S., Witter, M. P., Moser, E. I., & Moser, M.-B. (2004). Spatial representation in the entorhinal cortex. *Science*, *305*(5688), 1258–1264. http://doi.org/10.1126/science.1099901

Geva-Sagiv, M., Las, L., Yovel, Y., & Ulanovsky, N. (2015). Spatial cognition in bats and rats: from sensory acquisition to multiscale maps and navigation. *Nature Reviews Neuroscience*, *16*(2), 94–108. http://doi.org/10.1038/nrn3888

Giocomo, L. M., Stensola, T., Bonnevie, T., Van Cauter, T., Moser, M.-B., & Moser, E. I. (2014). Topography of head direction cells in medial entorhinal cortex. *Current Biology*, *24*(3), 252–262. http://doi.org/10.1016/j.cub.2013.12.002

Glasauer, S. (2005). Vestibular and motor processing for head direction signals. In: Head direction cells and the neural mechanisms of spatial orientation (Wiener S. I. & Taube J.S., eds.), MIT Press, *pp 113-136*

Goodridge, J. P., & Taube, J. S. (1997). Interaction between the postsubiculum and anterior thalamus in the generation of head direction cell activity. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *17*(23), 9315–9330.

Goodridge, J. P., & Touretzky, D. S. (2000). Modeling attractor deformation in the rodent head-direction system. *Journal of Neurophysiology*, *83*(6), 3402–3410.

Grobéty, M.-C., & Schenk, F. (June 1992a). Spatial learning in a three-dimensional maze. *Animal Behaviour*, *43*(6), 1011–1020. http://doi.org/10.1016/S0003-3472(06)80014-X

Grobéty, M., & Schenk, F. (1992b). The influence of spatial irregularity upon radial-maze performance in the rat. *Learning & Behavior*, *20*(4), 393–400. http://doi.org/10.3758/BF03197962

Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature*, *436*(7052), 801–806. http://doi.org/10.1038/nature03721

Hannay, J. H. (1985). Angle variable holonomy in adiabatic excursion of an integrable Hamiltonian. *Journal of Physics A Mathematical General*, *18*, 221–230. http://doi.org/10.1088/0305-4470/18/2/011

Hartley, T., Burgess, N., Lever, C., Cacucci, F., & O'Keefe, J. (2000). Modeling place fields in terms of the cortical inputs to the hippocampus. *Hippocampus*, *10*(4), 369–379. http://doi.org/10.1002/1098-1063

Hasselmo, M. E. (2009). A model of episodic memory: Mental time travel along encoded trajectories using grid cells. Neurobiology of Learning and Memory, 92(4), 559–573. http://doi.org/10.1016/j.nlm.2009.07.005

Hasselmo, M. E. (2012). How We Remember: Brain Mechanisms of Episodic Memory. MIT Press.

Hasselmo, M. E., Giocomo, L. M., Brandon, M. P., & Yoshida, M. (2010). Cellular dynamical mechanisms for encoding the time and place of events along spatiotemporal trajectories in episodic memory. Behavioural Brain Research, 215(2), 261–274. http://doi.org/10.1016/j.bbr.2009.12.010

Hayman, R. M. A., Casali, G., Wilson, J. J., & Jeffery, K. J. (2015). Grid cells on steeply sloping terrain: evidence for planar rather than volumetric encoding. *Frontiers in Psychology,* 925. http://doi.org/10.3389/fpsyg.2015.00925

Hayman, R., Verriotis, M. A., Jovalekic, A., Fenton, A. A., & Jeffery, K. J. (2011). Anisotropic encoding of three-dimensional space by place cells and grid cells. *Nature Neuroscience*, *14*(9), 1182–1188. http://doi.org/10.1038/nn.2892

Healy, S. D., & Hurly, T. A. (1995). Spatial memory in rufous hummingbirds (Selasphorus rufus): A field test. *Animal Learning & Behavior*, *23*(1), 63–68. http://doi.org/10.3758/BF03198016

Henderson, J., Hurly, T. A., & Healy, S. D. (2001). Rufous hummingbirds' memory for flower location. *Animal Behaviour*, *61*(5), 981–986. http://doi.org/10.1006/anbe.2000.1670

Holbrook, R. I., & Burt de Perera, T. (2009). Separate encoding of vertical and horizontal components of space during orientation in fish. *Animal Behaviour*, *78*(2), 241–245. http://doi.org/10.1016/j.anbehav.2009.03.021

Holbrook, R. I., & Perera, T. B. de. (2011). Three-dimensional spatial cognition: information in the vertical dimension overrides information from the horizontal. *Animal Cognition*, *14*(4), 613–619. http://doi.org/10.1007/s10071-011-0393-6

Hölscher, C., Meilinger, T., Vrachliotis, G., Brösamle, M., & Knauff, M. (2006). Up the down staircase: Wayfinding strategies in multi-level buildings. *Journal of Environmental Psychology*, *26*(4), 284–299. http://doi.org/10.1016/j.jenvp.2006.09.002

Hubel, D. H., & Wiesel, T. N. (1959). Receptive fields of single neurones in the cat's striate cortex. The Journal of Physiology, 148(3), 574–591. http://doi.org/10.1113/jphysiol.1959.sp006308

Huterer, M., & Cullen, K. E. (2002). Vestibuloocular Reflex Dynamics During High-Frequency and High-Acceleration Rotations of the Head on Body in Rhesus Monkey. Journal of Neurophysiology, 88(1), 13–28.

Inglis, J. T., Shupert, C. L., Hlavacka, F., & Horak, F. B. (1995). Effect of galvanic vestibular stimulation on human postural responses during support surface translations. Journal of Neurophysiology, 73(2), 896–901.

Jacobs, J., Kahana, M. J., Ekstrom, A. D., Mollison, M. V., & Fried, I. (2010). A sense of direction in human entorhinal cortex. Proceedings of the National Academy of Sciences, 107(14), 6487–6492. http://doi.org/10.1073/pnas.0911213107

Jacobs, W. J., Laurance, H. E., & Thomas, K. G. F. (1997). Place Learning in Virtual Space I: Acquisition, Overshadowing, and Transfer. Learning and Motivation, 28(4), 521–541. http://doi.org/10.1006/lmot.1997.0977

Jeffery, K. J., Anand, R. L., & Anderson, M. I. (2006). A role for terrain slope in orienting hippocampal place fields. *Experimental Brain Research*, *169*(2), 218–225. http://doi.org/10.1007/s00221-005-0138-3

Jeffery, K. J., Donnett, J. G., Burgess, N., & O'Keefe, J. M. (1997). Directional control of hippocampal place fields. *Experimental Brain Research*, *117*(1), 131–142. http://doi.org/10.1007/s002210050206

Jeffery, K. J., Jovalekic, A., Verriotis, M., & Hayman, R. (2013). Navigating in a three-dimensional world. *Behavioral and Brain Sciences*, *36*(05), 523–543. http://doi.org/10.1017/S0140525X12002476

Jeffery, K. J., Wilson, J. J., Casali, G., & Hayman, R. M. (2015). Neural encoding of large-scale three-dimensional space—properties and constraints. *Frontiers in Psychology,* 927. http://doi.org/10.3389/fpsyg.2015.00927

Jovalekic, A., Hayman, R., Becares, N., Reid, H., Thomas, G., Wilson, J., & Jeffery, K. (2011). Horizontal biases in rats' use of three-dimensional space. *Behavioural Brain Research*, *222*(2), 279–288. http://doi.org/10.1016/j.bbr.2011.02.035

Kamikouchi, A., Inagaki, H. K., Effertz, T., Hendrich, O., Fiala, A., Göpfert, M. C., & Ito, K. (2009). The neural basis of *Drosophila* gravity-sensing and hearing. *Nature*, *458*(7235), 165–171. http://doi.org/10.1038/nature07810

Kammann, R. (1967). The overestimation of vertical distance and slope and its role in the moon illusion. *Perception & Psychophysics*, *2*(12), 585–589. http://doi.org/10.3758/BF03210273

Kelly, J. W. (2011). Head for the hills: The influence of environmental slant on spatial memory organization. *Psychonomic Bulletin & Review*, *18*(4), 774–780. http://doi.org/10.3758/s13423-011-0100-2

Knierim, J. J., & McNaughton, B. L. (2001). Hippocampal place-cell firing during movement in three-dimensional space. *Journal of Neurophysiology*, *85*(1), 105–116.

Knierim, J. J., & Zhang, K. (2012). Attractor dynamics of spatially correlated neural activity in the limbic system. *Annual Review of Neuroscience*, *35*(1), 267–285. http://doi.org/10.1146/annurev-neuro-062111-150351

Knight, R., Piette, C. E., Page, H., Walters, D., Marozzi, E., Nardini, M., Stringer, S., & Jeffery, K. J. (2014) Weighted cue integration in the rodent head direction system. *Phil. Trans. R. Soc. B*. 369 http://dx.doi.org/10.1098/rstb.2012.0512

Kraus, B. J., Robinson, R. J., White, J. A., Eichenbaum, H., & Hasselmo, M. E. (2013). Hippocampal "Time Cells": Time versus Path Integration. Neuron, 78(6), 1090–1101. http://doi.org/10.1016/j.neuron.2013.04.015

Krupic, J., Bauza, M., Burton, S., Barry, C., & O'Keefe, J. (2015). Grid cell symmetry is shaped by environmental geometry. *Nature*, *518*(7538), 232–235. http://doi.org/10.1038/nature14153

Kubie, J. L., & Muller, R. U. (1991). Multiple representations in the hippocampus. *Hippocampus*, *1*(3), 240–242. http://doi.org/10.1002/hipo.450010305

Lever, C., Burton, S., Jeewajee, A., O'Keefe, J., & Burgess, N. (2009). Boundary vector cells in the subiculum of the hippocampal formation. *The Journal of Neuroscience*, *29*(31), 9771–9777. http://doi.org/10.1523/JNEUROSCI.1319-09.2009

Levy, A., Kluge, P. B., & Elsmore, T. F. (1983). Radial arm maze performance of mice: acquisition and atropine effects. *Behavioral and Neural Biology*, *39*(2), 229–240.

Lorente De Nó, R. (1933). Vestibulo-ocular reflex arc. Archives of Neurology & Psychiatry, 30, 245–291.

Louie, K., & Wilson, M. A. (2001). Temporally Structured Replay of Awake Hippocampal Ensemble Activity during Rapid Eye Movement Sleep. *Neuron*, *29*(1), 145–156. http://doi.org/10.1016/S0896-6273(01)00186-6

MacDonald, C. J., Lepage, K. Q., Eden, U. T., & Eichenbaum, H. (2011). Hippocampal "Time Cells" Bridge the Gap in Memory for Discontiguous Events. Neuron, 71(4), 737–749. http://doi.org/10.1016/j.neuron.2011.07.012

Maguire, E. A., Nannery, R., & Spiers, H. J. (2006). Navigation around London by a taxi driver with bilateral hippocampal lesions. Brain: A Journal of Neurology, 129(Pt 11), 2894–2907. http://doi.org/10.1093/brain/awl286

McNaughton, B. L., Chen, L. L., & Markus, E. J. (1991). "Dead reckoning," landmark learning, and the sense of direction: a neurophysiological and computational hypothesis. *Journal of Cognitive Neuroscience*, *3*(2), 190–202.

Miller, G. A., Galanter, E., & Pribram, K. H. (1960) *Plans and the structure of behaviour*. New York: Holt, Rinehart & Winston, pp. 65.

Mittelstaedt, H., & Mittelstaedt, M.-L. (1982). Homing by Path Integration. In P. D. F. Papi & D. H. G. Wallraff (Eds.), *Avian Navigation* (pp. 290–297). Springer Berlin Heidelberg.

Mittelstaedt, M.-L., & Glasauer, S. (1991). Idiothetic navigation in gerbils and humans. *Zool. Jb. Physiol*, *95*(427-435).

Mittelstaedt, M.-L., & Mittelstaedt, H. (1980). Homing by path integration in a mammal. *Naturwissenschaften*, *67*(11), 566–567. http://doi.org/10.1007/BF00450672

Mizumori, S. J., Rosenzweig, M. R., & Kermisch, M. G. (1982). Failure of mice to demonstrate spatial memory in the radial maze. *Behavioral and Neural Biology*, *35*(1), 33–45.

Mizumori, S. J., & Williams, J. D. (1993). Directionally selective mnemonic properties of neurons in the lateral dorsal nucleus of the thalamus of rats. *The Journal of Neuroscience*, *13*(9), 4015–4028.

Moghaddam, M., Kaminsky, Y. L., Zahalka, A., & Bures, J. (1996). Vestibular navigation directed by the slope of terrain. *Proceedings of the National Academy of Sciences*, *93*(8), 3439–3443.

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*(2), 239–260. http://doi.org/10.1016/0023-9690(81)90020-5

Morris, R. G. M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681–683. http://doi.org/10.1038/297681a0

Müller, M., & Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proceedings of the National Academy of Sciences*, *85*(14), 5287–5290.

Muller, R. U., & Kubie, J. L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *The Journal of Neuroscience*, *7*(7), 1951–1968.

Nadel, L., & Hardt, O. (2011). Update on Memory Systems and Processes. Neuropsychopharmacology, 36(1), 251–273. http://doi.org/10.1038/npp.2010.169

O'Connor, R. C., & Glassman, R. B. (1993). Human performance with a seventeen-arm radial maze analog. Brain Research Bulletin, 30(1-2), 189–191.

Oda, S., Kuroda, M., Kakuta, S., & Kishi, K. (2001). Differential immunolocalization of m2 and m3 muscarinic receptors in the anteroventral and anterodorsal thalamic nuclei of the rat. *Brain Research*, *894*(1), 109–120. http://doi.org/10.1016/S0006-8993(01)01986-2

O'Keefe, J., & Burgess, N. (1996). Geometric determinants of the place fields of hippocampal neurons. *Nature*, *381*(6581), 425–428. http://doi.org/10.1038/381425a0

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Research*, *34*(1), 171–175. http://doi.org/10.1016/0006-8993(71)90358-1

O'Keefe, J., & Nadel, L. (1978). *The Hippocampus As A Cognitive Map*. Oxford University Press.

Olton, D. S., Collison, C., & Werz, M. A. (1977). Spatial memory and radial arm maze performance of rats. *Learning and Motivation*, *8*(3), 289–314. http://doi.org/10.1016/0023-9690(77)90054-6

Olton, D. S., & Paras, B. C. (1979). Spatial memory and hippocampal function. *Neuropsychologia*, *17*(6), 669–682. http://doi.org/10.1016/0028-3932(79)90042-3

Olton, D. S., & Samuelson, R. J. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology*, *2*(2), 97–116.

Papez, J. W. (1937). A proposed mechanism of emotion. Archives of neurology and psychiatry, 38(4), 725.

Paxinos, G., Watson, C., (2006). The Rat Brain in Stereotaxic Coordinates, 6th ed Academic Press, San Diego.

Pellegrino, G. di, Fadiga, L., Fogassi, L., Gallese, V., & Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. Experimental Brain Research, 91(1), 176–180. http://doi.org/10.1007/BF00230027

Peyrache, A., Lacroix, M. M., Petersen, P. C., & Buzsáki, G. (2015). Internally organized mechanisms of the head direction sense. Nature Neuroscience, 18(4), 569–575. http://doi.org/10.1038/nn.3968

Plotnik, M., Freeman, S., Sohmer, H., & Elidan, J. (1999). The effect of head orientation on the vestibular evoked potentials to linear acceleration impulses in rats. *The American Journal of Otology*, *20*(6), 735–740.

Proffitt, D. R., Bhalla, M., Gossweiler, R., & Midgett, J. (1995). Perceiving geographical slant. *Psychonomic Bulletin & Review*, *2*(4), 409–428. http://doi.org/10.3758/BF03210980

Ranck, J. B. (1986) Head direction cells in the deep cell layer of dorsal presubiculum in freely moving rats. In Electrical Activity of the Archicortex (G. Buzsaki and C.H. Vanderwolf, eds) 217-220. Budapest: Akademai Kiado

Restat, J. D., Steck, S. D., Mochnatzki, H. F., & Mallot, H. A. (2004). Geographical slant facilitates navigation and orientation in virtual environments. *Perception*, *33*(6), 667–687. http://doi.org/10.1068/p5030

Robertson, R. G., Rolls, E. T., Georges-François, P., & Panzeri, S. (1999). Head direction cells in the primate pre-subiculum. Hippocampus, 9(3), 206–219. http://doi.org/10.1002/(SICI)1098-1063(1999)9:3<206::AID-HIPO2>3.0.CO;2-H

Rochefort C., Arabo A., Andre M., Poucet B., Save E., Rondi-Reig L. (2011). Cerebellum shapes hippocampal spatial code. *Science*, 334, 385–389. 10.1126/science.1207403

Rochefort C., Lefort J. M., Rondi-Reig L. (2013). The cerebellum: a new key structure in the navigation system. *Front. Neural Circuits,* 7:35, 10.3389 /fncir.2013.00035

Rolls, E. T., & O'Mara, S. M. (1995). View-responsive neurons in the primate hippocampal complex. Hippocampus, 5(5), 409–424. http://doi.org/10.1002/hipo.450050504

Rolls, E. T. (1999). Spatial view cells and the representation of place in the primate hippocampus. Hippocampus, 9(4), 467-480.

Rolls, E. T., & Xiang, J. Z. (2006). Spatial view cells in the primate hippocampus and memory recall. Reviews in the Neurosciences, 17(1-2), 175-200.

Rondi-Reig L., Paradis A. L., Lefort J. M., Babayan B. M., Tobin C. (2014). How the cerebellum may monitor sensory information for spatial representation. *Front. Syst. Neurosci.,* 8:205. 10.3389/fnsys.2014.00205

Sagar, H. J., Cohen, N. J., Corkin, S., & Growdon, J. H. (1985). Dissociations Among Processes in Remote Memory. Annals of the New York Academy of Sciences, 444(1), 533–535. http://doi.org/10.1111/j.1749-6632.1985.tb37637.x

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B. L., Witter, M. P., Moser, M.-B., & Moser, E. I. (2006). Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science*, *312*(5774), 758–762. http://doi.org/10.1126/science.1125572

Schmidt, I., Collett, T. S., Dillier, F.-X., & Wehner, R. (1992). How desert ants cope with enforced detours on their way home. *Journal of Comparative Physiology A*, *171*(3), 285–288. http://doi.org/10.1007/BF00223958

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery & Psychiatry*, *20*(1), 11–21. http://doi.org/10.1136/jnnp.20.1.11

Seelig, J. D., & Jayaraman, V. (2015). Neural dynamics for landmark orientation and angular path integration. *Nature*, *521*(7551), 186–191. http://doi.org/10.1038/nature14446

Sharp, P. E. (2005). Regional distribution and variation in the firing properties of head direction cells. In Wiener, S. I. & Taube, J.S. *Head Direction Cells and the Neural Mechanisms of Spatial Orientation*, 3–15. MIT Press.

Sharp, P. E., Blair, H. T., Etkin, D., & Tzanetos, D. B. (1995). Influences of vestibular and visual motion information on the spatial firing patterns of hippocampal place cells. *The Journal of Neuroscience*, *15*(1), 173–189.

Shinder, M. E., & Taube, J. S. (2011). Active and passive movement are encoded equally by head direction cells in the anterodorsal thalamus. *Journal of Neurophysiology*, *106*(2), 788–800. http://doi.org/10.1152/jn.01098.2010

Skaggs, W. E., Knierim, J. J., Kudrimoti, H. S., & McNaughton, B. L. (1995). A model of the neural basis of the rat's sense of direction. *Advances in Neural Information Processing Systems*, *7*, 173–180.

Solstad, T., Boccara, C. N., Kropff, E., Moser, M.-B., & Moser, E. I. (2008). Representation of geometric borders in the entorhinal cortex. *Science*, *322*(5909), 1865–1868. http://doi.org/10.1126/science.1166466

Souman, J. L., Frissen, I., Sreenivosa, M. N., & Ernst, M. O. (2009). Walking straight into circles. *Current Biology, 19 (18),* 1538-1542. http://dx.doi.org/10.1016/j.cub.2009.07.053

Stackman, R. W. (2003). Passive transport disrupts directional path integration by rat head direction cells. *Journal of Neurophysiology*, *90*(5), 2862–2874. http://doi.org/10.1152/jn.00346.2003

Stackman, R. W., Clark, A. S., & Taube, J. S. (2002). Hippocampal spatial representations require vestibular input. *Hippocampus*, *12*(3), 291–303. http://doi.org/10.1002/hipo.1112

Stackman, R. W., Lora, J. C., & Williams, S. B. (2012). Directional responding of C57BL/6J mice in the Morris water maze is influenced by visual and vestibular cues and is dependent on the anterior thalamic nuclei. *The Journal of Neuroscience, 32*(30), 10211–10225. http://doi.org/10.1523/JNEUROSCI.4868-11.2012

Stackman, R. W., & Taube, J. S. (1997). Firing properties of head direction cells in the rat anterior thalamic nucleus: dependence on vestibular input. *The Journal of Neuroscience*, *17*(11), 4349–4358.

Stackman, R. W., & Taube, J. S. (1998). Firing properties of rat lateral mammillary single units: head direction, head pitch, and angular head velocity. *The Journal of Neuroscience*, *18*(21), 9020–9037.

Stackman, R. W., Tullman, M. L., & Taube, J. S. (2000). Maintenance of rat head direction cell firing during locomotion in the vertical plane. *Journal of Neurophysiology*, *83*(1), 393–405.

Steck, S. D., Mochnatzki, H. F., & Mallot, H. A. (2003). The role of geographical slant in virtual environment navigation. In C. Freksa, W. Brauer, C. Habel, & K. F. Wender (Eds.), *Spatial Cognition III* (pp. 62–76). Springer-Verlag Berlin, Heidelberg.

Suzuki, S., Augerinos, G., & Black, A. H. (1980). Stimulus control of spatial behavior on the eight-arm maze in rats. *Learning and Motivation*, *11*(1), 1–18. http://doi.org/10.1016/0023-9690(80)90018-1

Takeuchi, Y., Allen, G. V., & Hopkins, D. A. (1985). Transnuclear transport and axon collateral projections of the mammillary nuclei in the rat. *Brain Research Bulletin*, *14*(5), 453–468.

Taube, J. S. (1995). Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. *The Journal of Neuroscience*, *15*(1), 70–86.

Taube, J. S. (2007). The head direction signal: origins and sensory-motor integration. *Annual Review of Neuroscience*, *30*, 181–207. http://doi.org/10.1146/annurev.neuro.29.051605.112854

Taube, J. S., & Burton, H. L. (1995). Head direction cell activity monitored in a novel environment and during a cue conflict situation. *Journal of Neurophysiology*, *74*(5), 1953–1971.

Taube, J. S., & Muller, R. U. (1998). Comparisons of head direction cell activity in the postsubiculum and anterior thalamus of freely moving rats. *Hippocampus*, *8*(2), 87–108.

Taube, J. S., Muller, R. U., & Ranck, J. B. (1990a). Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. *The Journal of Neuroscience*, *10*(2), 420–435.

Taube, J. S., Muller, R. U., & Ranck, J. B. (1990b). Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. *The Journal of Neuroscience*, *10*(2), 436–447.

Taube, J. S., & Shinder, M. (2013). On the nature of three-dimensional encoding in the cognitive map: Commentary on Hayman, Verriotis, Jovalekic, Fenton, and Jeffery. *Hippocampus*, *23*(1), 14–21. http://doi.org/10.1002/hipo.22074

Taube, J. S., Wang, S. S., Kim, S. Y., & Frohardt, R. J. (2013). Updating of the spatial reference frame of head direction cells in response to locomotion in the vertical plane. *Journal of Neurophysiology*, *109*(3), 873–888. http://doi.org/10.1152/jn.00239.2012

Taylor, G. K., Holbrook, R. I., & Perera, T. B. de. (2010). Fractional rate of change of swim-bladder volume is reliably related to absolute depth during vertical displacements in teleost fish. *Journal of The Royal Society Interface*, *7*(50), 1379–1382. http://doi.org/10.1098/rsif.2009.0522

Thorndike, E. L. (1911), *Animal Intelligence*, Macmillan.

Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychological Review*, *55*(4), 189–208. http://doi.org/10.1037/h0061626

Tolman, E. C., & Honzik, C. H. (1930). “Insight” in rats. *University of California Publications in Psychology*. Vol 4, 215-232.

Tolman, C, E., F, B., & Kalish, D. (1946). Studies in spatial learning. I. Orientation and the short-cut. *Journal of Experimental Psychology*, *36*(1), 13–24. http://doi.org/10.1037/h0053944

Tolman, E. C., Ritchie, B. F., & Kalish, D. (1946). Studies in Spatial Learning. II. Place Learning Versus Response Learning. *Journal of Experimental Psychology*, *36*(3), 221.

Tsoar, A., Nathan, R., Bartan, Y., Vyssotski, A., Dell’Omo, G., & Ulanovsky, N. (2011). Large-scale navigational map in a mammal. *Proceedings of the National Academy of Sciences*, *108*(37), E718–E724. http://doi.org/10.1073/pnas.1107365108

Tulving, E. (1972). Episodic and semantic memory 1. Organization of Memory. London: Academic, 381(4).

Tulving, E. (1984). Precis of elements of episodic memory. Behavioral and Brain Sciences, 7(02), 223-238.

Ulanovsky, N. (2011). Neuroscience: How Is three-dimensional space encoded in the brain? *Current Biology*, *21*(21), R886–R888. http://doi.org/10.1016/j.cub.2011.09.031

van Groen, T., & Wyss, J. M. (1990a). The postsubicular cortex in the rat: characterization of the fourth region of the subicular cortex and its connections. *Brain Research*, *529*(1-2), 165–177.

van Groen, T., & Wyss, J. M. (1990b). The connections of presubiculum and parasubiculum in the rat. *Brain Research*, *518*(1-2), 227–243.

van Groen, T., & Wyss, J. M. (1990c). Connections of the retrosplenial granular a cortex in the rat. *The Journal of Comparative Neurology*, *300*(4), 593–606. http://doi.org/10.1002/cne.903000412

van Groen, T., & Wyss, J. M. (1992). Connections of the retrosplenial dysgranular cortex in the rat. *The Journal of Comparative Neurology*, *315*(2), 200–216. http://doi.org/10.1002/cne.903150207

Walsh, E. G. (1960). Perception of linear motion following unilateral labyrinthectomy: variation of threshold according to the orientation of the head. *The Journal of Physiology*, *153*(2), 350–357.

Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *189*(8), 579–588. http://doi.org/10.1007/s00359-003-0431-1

Winter, S. S., Clark, B. J., & Taube, J. S. (2015). Disruption of the head direction cell network impairs the parahippocampal grid cell signal. *Science (New York, N.Y.)*, *347*(6224), 870–874. http://doi.org/10.1126/science.1259591

Yartsev, M. M., & Ulanovsky, N. (2013). Representation of Three-Dimensional Space in the Hippocampus of Flying Bats. *Science*, *340*(6130), 367–372. http://doi.org/10.1126/science.1235338

Yoder, R. M., & Taube, J. S. (2009). Head direction cell activity in mice: robust directional signal depends on intact otolith organs. *The Journal of Neuroscience*, *29*(4), 1061–1076. http://doi.org/10.1523/JNEUROSCI.1679-08.2009

Zhang, K. (1996). Representation of spatial orientation by the intrinsic dynamics of the head-direction cell ensemble: a theory. *The Journal of Neuroscience*, *16*(6), 2112–2126.

Zoladek, L., & Roberts, W. A. (1978). The sensory basis of spatial memory in the rat. *Animal Learning & Behavior*, *6*(1), 77–81. http://doi.org/10.3758/BF03212006

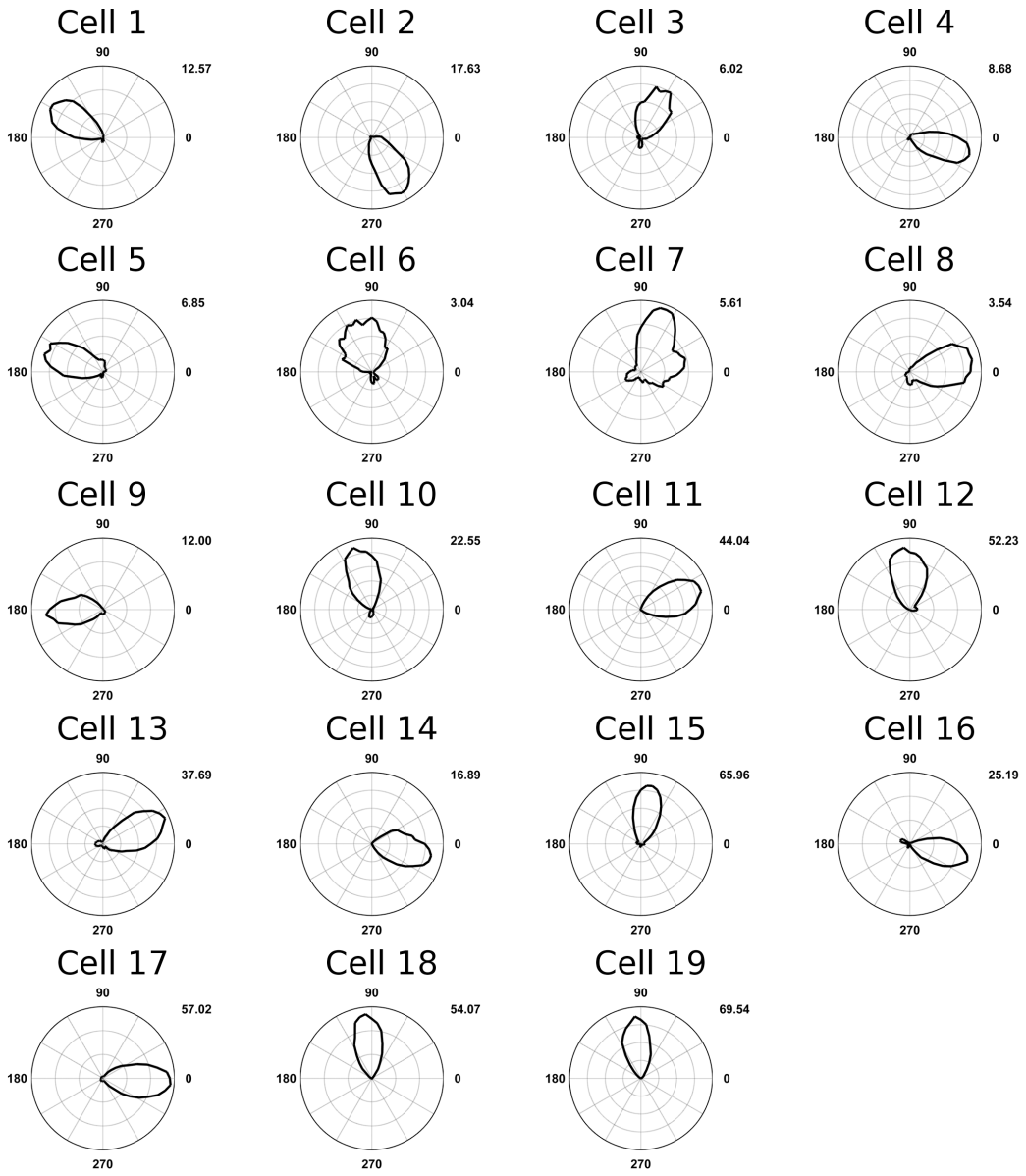
Zugaro, M. B., Arleo, A., Dejean, C., Burguiere, E., Khamassi, M., & Wiener, S. I. (2004). Rat anterodorsal thalamic head direction neurons depend upon dynamic visual signals to select anchoring landmark cues. *European Journal of Neuroscience*, *20*(2), 530–536. http://doi.org/10.1111/j.1460-9568.2004.03512.x

Zugaro, M. B., Berthoz, A., & Wiener, S. I. (2001). Background, but not foreground, spatial cues are taken as references for head direction responses by rat

anterodorsal thalamus neurons. *The Journal of Neuroscience*, *21*(14), RC154–RC154.

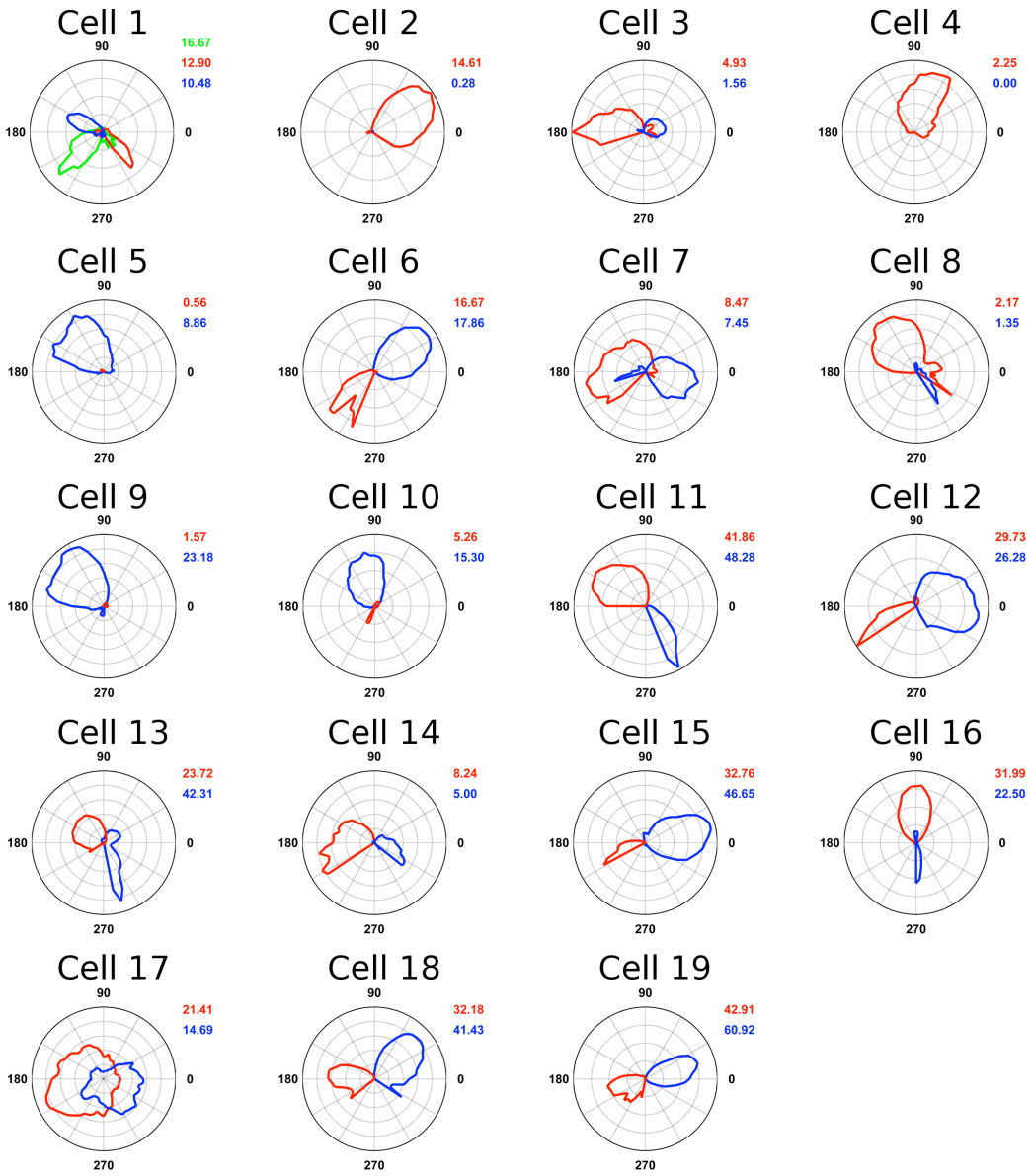
Zwergal, A., Schöberl, F., Xiong, G., Pradhan, C., Covic, A., Werner, P., … Brandt, T. (2015). Anisotropy of Human Horizontal and Vertical Navigation in Real Space: Behavioral and PET Correlates. Cerebral Cortex, 213. http://doi.org/10.1093/cercor/bhv213

Appendix 1



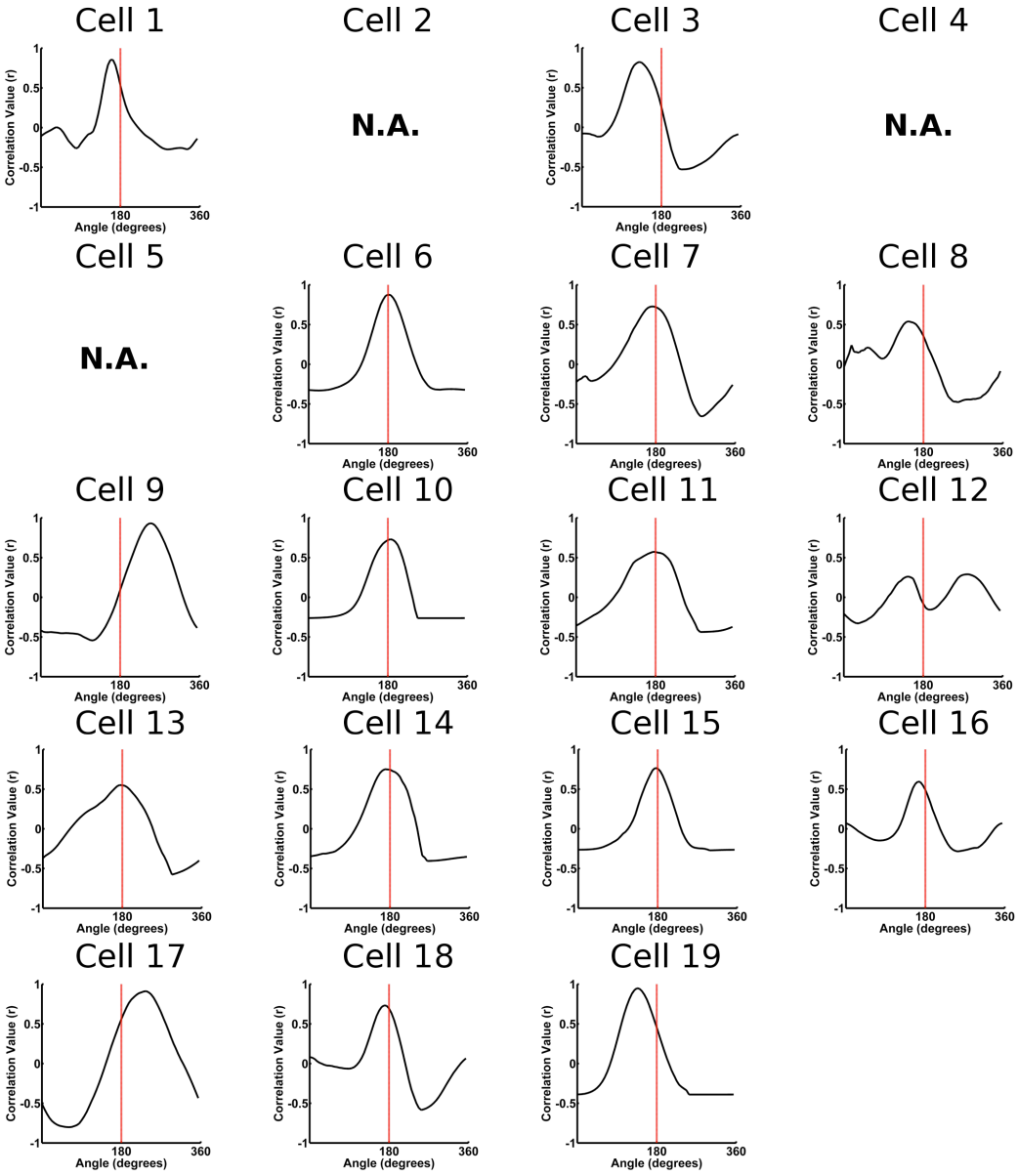
Appendix 1. Baseline trial polar plots for the 19 HD cells recorded in the electrophysiology experiment. Numbers at the top right of each polar plot show the firing rate of the cell in Hz.

Appendix 2



Appendix 2. Climbing trial polar plots for the 19 HD cells recorded in the electrophysiology experiment. The East wall polar plots and peak firing rates are shown in blue, and the West wall polar plots and peak firing rates are shown in red. Cell 1 was recorded on the South wall as well the East and West walls. The South wall polar plot is shown in green. Note that the South wall polar plot is rotated ~90 degrees counter-clockwise from the East wall polar plot, and ~90 degrees clockwise from the West wall polar plot.

Appendix 3



Appendix 3. East vs West wall cross-correlations of polar plots for the 16 cells that were directionally modulated on both walls. Cross-correlations were not carried out for cells that were only directionally modulated on one wall. Red dashed lines indicate the peak cross-correlation angle predicted by the multi-planar hypothesis.