Integration of sensory cues by the head direction system

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

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Abstract

Head direction (HD) cells fire as a function of the animal's heading direction, with each cell responding to a specific head orientation. This thesis describes the findings from single cell recordings of HD cells in rats. In particular, the thesis focuses on the manner in which these cells integrate landmark and path integration cues.

The first experimental chapter aimed to assess the degree to which HD cell firing is influenced by the geometry of an environment. The findings from this experiment suggest that the reliability of the path integration signal affects the degree to which HD cells integrate geometric cues. As the stability of the path integration signal increased (rat was oriented) the weighting of geometric cues decreased. Conversely, when the stability of the path integration signal decreased (rat was disoriented) the weighting of geometric cues increased. This finding, that the influence of a cue is inversely proportional to the variability of that cue, supports a Bayesian account of cue integration.

The second experiment therefore directly tested a Bayesian model of cue integration. HD cells and behaviour were simultaneously recorded during a conflict between path integration signals and landmark information. Behavioural findings did not support a Bayesian model, but recordings of HD cells did show evidence of cue weighting based on cue reliability. Interestingly, the reliability of the cues was not expressed in the cells' firing rates or tuning widths and therefore the reliability coding could occur before the signal is sent to the HD system.

The final experiment had three main objectives: To establish when HD cells switch from primarily using landmark information to mostly using path integration information. The second aim was to reveal whether HD cells in different areas of the HD circuit respond to these two sensory cues in a similar manner. The third aim was to provide an insight into how activity in the HD network propagates from one preferred firing direction to another.

HD cell firing demonstrated cue integration across the system with no differences in brain region. At small conflicts, HD cell firing suggested landmark dominance, whilst larger conflicts demonstrated a greater weighting of path integration. Preliminary findings also suggest that activity can sweep from one preferred firing direction to the next.

Publications

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List of Abbreviations

ADN: Anterior dorsal thalamic nuclei

AMN: Anterior medial thalamic nuclei

ATN: Anterior thalamic nuclei

AVN: Anterior ventral thalamic nuclei

CA: Cornu Ammonis

DG: Dentate gyrus

DTN: Dorsal tegmental nuclei

EC: Entorhinal cortex

HD: Head direction

LDN: Lateral dorsal thalamic nuclei

LMN: Lateral mammillary nuclei

MSTd: Dorsal medial superior temporal area

PoS: Postsubiculum

RSP: Retrosplenial cortex

S: Subiculum

V1: Primary visual cortex

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Chapter 1 General introduction

Navigation is a vital survival tool for all animals, allowing them to find food, locate potential mates and avoid predators. The remarkable ability of animals to perform complex navigational feats within challenging environments can be witnessed across many organisms from the Sahara desert ant (*Cataglyphis bicolor*) navigating across a featureless terrain, to the complex return flights performed by the homing pigeon (*Columba livia)*.

The ability of animals to navigate is thought to depend on the information provided by two types of cue, static external cues (such as landmarks) and dynamic self-motion cues (a process known as path integration). Landmark information is classified as an allothetic cue, as the information originates outside of the animal's body, i.e. in the environment. Path integration is a term given to a collection of internal processes that an animal uses to navigate. These internal or idiothetic processes include vestibular, motor and proprioceptive information (Gallistel, 1980). Integrating this information allows an organism to establish where they are and what is around them in space.

In order to understand how these organisms use this information to perform these complex navigational strategies, scientists have observed and manipulated both the behaviour and the biological systems underlying this behaviour. The experimental work in this thesis aimed to examine both behavioural responses to and the neural processes involved in the integration of idiothetic and allothetic spatial cues. In particular, cells that encode for an animal's heading direction were recorded in rats using electrophysiology. During recording, the rats were exposed to situations where their internal sense of direction (idiothetic cues) conflicted with directional cues within the environment (allothetic cues). Creating this cue conflict allowed for the opportunity to observe how the system that encodes for an animal's heading direction resolves a conflict in idiothetic and allothetic cues. Taking simultaneous recordings of neural firing and the animals' behaviour also reveals how the resolution expressed in the cells is reflected in the behaviour of the animal. This coupling of behavioural observations and recording of directionally sensitive cells is relatively infrequent and therefore these experiments allow for a rare insight into this relationship.

Previous recordings of these cells during a conflict between idiothetic and allothetic cues often show firing based on an integrated signal of these two cues. The site at which this cue integration occurs has yet to be fully understood and therefore part of this thesis aimed to determine whether or not cue integration occurs in the system that is responsible for

processing the animal's heading direction (Chapter 5) and whether this cue integration is the same across the HD network (Chapter 6). Current studies have also yet to determine what factors influence this process of cue integration and this is therefore another important focus of this thesis. Factors that have been examined in this set of experiments are the properties of the allothetic cue (Chapter 4), perceived reliability of each cue (Chapter 5) and the degree of cue conflict (Chapter 6). The findings from these three experimental chapters illustrate that HD cells use a combination of both idiothetic and allothetic information. The degree to which these cues are used depends upon the perceived reliability of the cues and the level of conflict between the cues.

Before this set of experiments are reported, a brief overview of the study of navigation is presented, followed by a more detailed account of the neural mechanisms that underlie navigation.

1.1 History of the study of navigation

Work by Tolman (1948) has been highly influential in the research field of navigation and his was the earliest work that provided convincing evidence that the ability to navigate requires specific cognitive processes allowing the animal to mentally represent space. Tolman was spurred on from his earlier incidental observations in the lab, where rats that had been trained on a simple alley maze climbed out of the starting box and made a direct route towards the goal box. This short-cut behaviour was directly tested by Tolman in order to establish whether rats could indeed mentally represent their environment. Rats were initially trained in the training apparatus (Fig 1.1) to run from point A to point B. The rats were then tested on the sun-burst maze where rats could choose between multiple routes. Tolman found that on the sun-burst maze, rats tended to choose the arm that provided a direct path to the goal location (point B). This path offered the shortest route from the centre platform to the area of space that the food box occupied during training. In order to perform such a shortcut and travel a route that had not been previously experienced or even seen by the rat, Tolman concluded that the rats must have produced a cognitive representation of the maze at the training phase.

Figure 1.1: Training and test apparatus adapted from Tolman (1948). *Left:* The training apparatus, where rats were trained to run from location A to location B. *Right:* The sun burst maze, where rats were tested. Most rats accurately chose the shortcut route highlighted in red (path 6).

Tolman (1948) termed such a spatial representation a 'cognitive map'. This concept of a mental representation of space and the cognitive map was later restated by O'Keefe and Nadel (1978) and is discussed later in this chapter. Support for the idea of the cognitive map also came from Morris (1981) using his newly developed and now highly popular water-maze. Inside the pool of cloudy water, rats could locate a hidden platform that could not be seen, smelt or heard. This meant that they could locate the platform without relying on any extramaze cues. Although this ability demonstrates a very accomplished skill in locating objects in space, it does not prove that they were using a cognitive map over an associative-based strategy. In other words, they may have used an internal based, path integration strategy (e.g. body turns, time of swimming) to find the platform. The flexibility of this learning shown in later manipulations however does not support a stimulus-response explanation. Rats could also locate this hidden platform when they were released from random sites in the pool that were not experienced at the training phase. A couple of years later, Morris et al (1982) replicated this initial work using rats that had undergone hippocampal aspiration lesioning, creating damage to the hippocampal formation (the hippocampus proper and neighbouring areas - the dentate gyrus and the subiculum). Compared to control rats, the lesioned rats performed poorly on the spatial task, implying that the hippocampal formation is essential in locating a platform that could not be seen,

smelt or heard. Since this study, many experiments have gone on to test the cognitive map hypothesis (for a review see McNaughton et al (2006))

Findings from Morris et al (1982) therefore support the idea that the hippocampus is not only important for the formation and retrieval of episodic memories (Scoville and Milner, 1957) but it also plays a particularly pertinent role in spatial memory. This idea came about because of the illustrious body of work which began with lesion work by Olton (1977) and single cell recordings by O'Keefe (1976). Olton (1977) reported that rats that had lesions of hippocampus formation were severely impaired in a radial arm maze task. Around the same time, the field was rapidly advanced by the discovery of place cells (O'Keefe, 1976), which provided very important implications for the processing of space and the role that the hippocampus plays in this process. The process of single cell recording or electrophysiology is described first, followed by the discovery of place cells.

1.2 Neural correlates of space

Due to the invasive nature of electrophysiology, recordings have mostly been done on small mammals such as rodents, although there has been notable work using humans (Ekstrom et al., 2003) which have allowed cross-species comparisons to be made. In brief, the implanting of electrodes allows for the detection of cell firing within the brain. Action potentials generated within an active cell will cause changes to the cell's membrane potential. The implanted electrodes can detect potential changes in the extracellular space between cells ('spikes') corresponding to action potentials. When this information about cell firing is coupled in time with the spatial behaviour of the animal, the function of the cell can sometimes be determined.

Renshaw et al (1940) pioneered the use of single cell recording when they successfully obtained recordings in the pyramidal cell layer of the hippocampus in cats and rabbits. Using microelectrodes they observed two types of activity in the hippocampus. The first activity they observed was a rapid cell discharge (typically the phase duration was around 1ms), which they interpreted as axon-like firing. This firing could persist for hours if the electrode position remained stable. They also reported finding this activity in clusters, which could only be captured using microelectrodes and not the larger gross electrodes. These gross electrodes however, could record the second type of activity they observed in the hippocampus - slow wave activity. This slow wave activity was monophasic and reported to

repeat over 4-7 seconds. This activity is now more commonly known as the hippocampal theta rhythm.

The frequency of theta activity (typically around 7 Hz) occurs when the rat is moving (Vanderwolf, 1969) or sleeping (Olmstead et al., 1973). One popular theory proposed by Petsche et al (1962) for the function of this theta activity is that it controls the timing of spiking activity within the hippocampus by acting as a periodic clock. The classic theory was later modified to incorporate the findings that the temporal relationship between pyramidal cell firing in the hippocampus and the phase of theta are dependent upon the behaviour of the animal (O'Keefe and Recce, 1993; Skaggs et al., 1996). The theta cycle is also thought to play an important role in hippocampal plasticity, as it is believed that theta provides temporal control over long-term potentiation (Larson et al., 1986). The role of theta activity in the hippocampus has drawn large attention from the field (Burgess and O'Keefe, 2005) and it remains a highly debated topic.

Leading on from the work by Renshaw et al (1940), O'Keefe and Dostrovsky (1971) successfully recorded neurons in the dorsal CA1 region of the hippocampus of freely moving rats, providing the first indication that single cells can code space. From this preliminary work, O'Keefe (1976) implanted 12 rats with either a single, pair or quartet of electrodes. Single cell recordings of CA1 were taken when the rats were exploring an elevated radial arm maze. As a result, he discovered that a population of cells in this region of the brain coded for space. From the fifty neurons recorded, twenty-four pyramidal cells showed bursts of activity when rats walked through particularly locations in the environment (Fig 1.2). The area in which this activity occurred was termed a place field. Each cell, referred to as a place cell, differed in terms of their place field location. Thus an ensemble of place cells can map out an environment, providing a neural candidate for a cognitive map.

Proposed by O'Keefe and Nadel (1978), the cognitive map theory of hippocampal function primarily suggests that the hippocampus constructs a three-dimensional Euclidean framework onto which spatial memories are stored. More specifically, there are five main suppositions of this cognitive map theory. Firstly, when spatial memories are encoded, they are 'mapped' or placed in association with existing traces. For example, the allocentric locations of landmarks are stored in the hippocampus in a 'map-like' structure during exploration. The second supposition relates to the mechanisms by which this stored information is used to navigate. From the discovery of place cells, it was predicted that different components of the spatial signal (place, direction and distance) are encoded

separately by different neurons. The later discover of these neurons strongly supports the idea of a cognitive map and is discussed later in this chapter. The third supposition highlights the role of evolution in creating the specialised function of the cognitive map. Fourthly, the site at which these spatial maps are stored is restricted only to the hippocampus formation. Finally, in humans it is believed that although the right hippocampus is primarily involved in spatial function, the left hippocampus deals with storage and recall of episodic memories and linguistic processing (O'Keefe and Nadel, 1978).

The cognitive map theory therefore proposes a number of strong claims about the encoding, storage and retrieval of spatial memories. In essence, the theory implies that there is something special or unique about spatial processing. Thus the proposal of the cognitive map and the discovery of place cells which led to this theory, has not only been highly influential to the field of memory research, but it has in itself created a field dedicated to understanding the neural mechanisms underlying spatial memory. This thesis aimed to further examine these neural mechanisms that are thought to underlie a cognitive map. More specifically, this thesis aimed to assess how spatial cues are weighted, integrated and expressed by head direction cells. These cells are one class of specialised neuron which responds specifically to spatial information. These spatial cells will be now be discussed in the order in which they were discovered.

1.3 Place cells

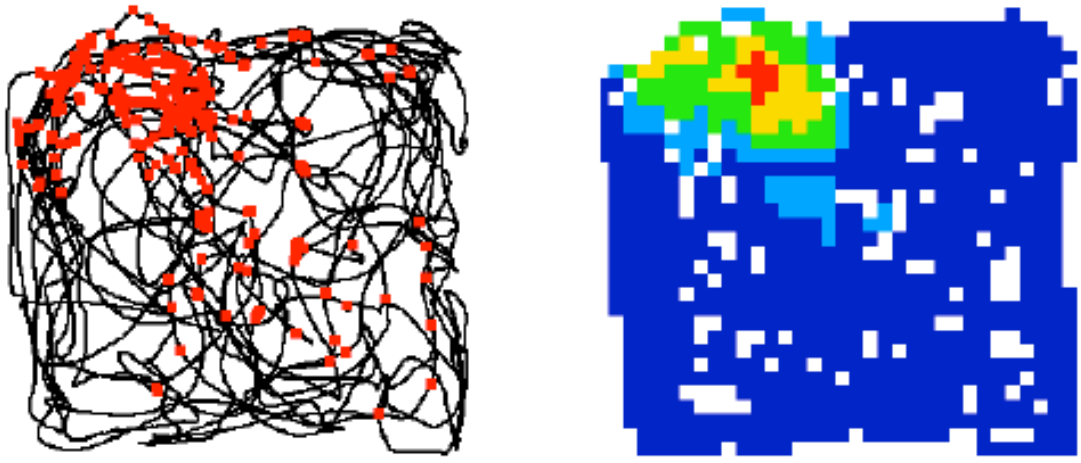

Figure 1.2. One place cell recorded in the CA1 region of the hippocampus as a rat explored a 60 x 60cm box. *Left* The firing of this cell (spikes shown red) superimposed on the bird's eye view of the rat's path (black lines). *Right* The corresponding firing rate map, where hot colour (red) represents high firing and cool colours (blue) represent low firing. Illustration supplied by Caswell Barry.

The first spatially modulated cell and arguably most important component of the cognitive map are place cells. Since the discovery of place cells (O'Keefe, 1976), a wealth of studies has recorded these cells from CA1 and CA3 regions of the hippocampus to investigate place cell properties. Measuring the size and location of the place field, as well as the firing rate of the cell, provides a useful tool for measuring which types of information place cells utilise. By examining these quantitative properties, the phenomenon of remapping has been established. For example, recordings from multiple cells in multiple environments have revealed that cells active in one environment (A) will not necessarily be active in another (B) (Kubie and Muller, 1991). Moreover, from the remaining cells which are active across both environments, the spatial relationship between these place fields are not necessarily preserved. The situation where all cells that fire in environment A either become silent in environment B or change the location of their firing field is referred to as complete remapping (Bostock et al., 1991). The function of complete remapping is thought to enable the system to differentiate environments that are deemed sufficiently different (Muller et al., 1996). The antithesis of this would be null remapping, where all the cells that fire in environment A also fire in environment B, and thus these environments are presumed too similar to require separate representations in the hippocampus. There is also an intermediary state of remapping termed partial remapping where some, but not all cells that fire in environment A will also fire in environment B and the place fields of these cells will retain the same relationship with each other (Muller et al., 1996). Finally, comparing place fields from one environment to another can demonstrate rate remapping, where cells fire in both instances, but the rate of firing changes, either increasing or decreasing across environments.

The phenomenon of place cell remapping on its own is an interesting concept as it indicates how populations of place cells can represent multiple environments and how they can quantify similarities and differences between environments. The striking question is then, which factors of an environment deem it to be sufficiently similar or sufficiently different to the place cell system? The shape of the environment is thought to be an important factor in place cell remapping (Muller and Kubie, 1987) and the size of the environment too (O'Keefe and Burgess, 1996). The influence of the shape of an environment on head direction cells is examined in the first experimental chapter of this thesis.

As mentioned, these place cells make an ideal neuronal candidate for the cognitive map. However, on their own, a population of place cells can only provide a map that indicates

one's position in relation to other features, e.g. walls. However, if the navigator does not know which way the map is orientated, this cognitive map is rendered useless. In other words, to navigate successfully a mammal not only needs to know its location, it also needs to know about the direction in which they are travelling. Neurones believed to encode for directionality were initially discovered by Ranck (1984).

1.4 Head direction cells

Since the observations made by Ranck (1984) the lab continued to record cells that encoded for directionality and in 1990, Taube et al published the first quantitative measurements of these head direction (HD) cells (Taube et al., 1990a; Taube et al., 1990b). Sixteen rats were implanted with electrode bundles in an area of the brain termed the postsubiculum (PoS). A total of 239 cells were isolated from the recordings, and from this, 61 units were classified as HD cells. In order to determine whether a cell could be classified as an HD cell, a directional tracker was used. This consisted of two LEDs attached to a headstage, one large LED and one small. The larger of the two LEDs allowed a camera to track the location of the animal's head. The relative angle between the two LEDs allowed the camera to track the direction that the animal's head was pointing. When this directional information was coupled with the timing of cell firing, cells that fired as a function of the animal's heading direction could be identified.

Taube et al (1990a) found that each HD cell is specific to a single head orientation, and when the head is directed to the cell's preferred firing direction, the cell fires at maximum. This peak firing rate can be measured, and the cells that were recorded in this study had a mean peak firing rate of 36 Hz. The preferred firing direction was only in reference to the animal's head, the body of the animal was not a determinant of cell firing. Collectively these cells were thought to encode for all possible heading directions. This was supported by histograms illustrating that HD cell ensembles have an even distribution of preferred firing directions. Taube et al (1990a) also found that the preferred firing direction of cells was not related to the anatomical position of the cell. For example, two cells that were simultaneously recorded (and therefore were located very close to each other) had a very large angular difference in their preferred firing direction.

Figure 1.3: Plot of a HD cell adapted from Taube et al (1990a). The plot illustrates each parameter of a HD cell; preferred firing direction, peak firing rate and directional firing range.

Taube et al (1990a) also found that the firing plots of these cells resembled a Gaussian shape, with the firing rates of neighbouring head directions decreasing in a linear fashion with an increase in angular deviation from the preferred firing direction (Fig 1.3). They found that after approximately 45° either side of the preferred firing direction, the cell no longer fired. This 90° window of activity is referred to as the cell's firing range (Wiener and Taube, 2005). If the animal's head was kept within this directional range, the cell would constantly fire (i.e. show no adaptation) and would only cease when the animal's head moved out of this range. This firing range did however vary across cells, as did the firing rate of the cells. No clear reason was identified as to why the directional firing ranges and peak firing rates varied across cells. These two variables were also shown to be unrelated to each other. For example, cells that demonstrated a higher firing rate did not necessarily have a larger firing range (Taube et al, 1990a).

Taube et al (1990a) also reported that the HD cell firing was independent of the animal's location in the cylinder. The preferred firing direction and firing rate remained stable regardless of the animal's location in the arena. HD cell firing was also independent of the animals' ongoing behaviour, such as grooming, eating or rearing. Interestingly, cells fired in their preferred firing direction regardless of whether the rat was moving or stationary, suggesting that motion cues were not required for cell firing. The orientation of the head

(pitch and roll) was another factor that was independent of the HD cell firing in the PoS recordings. As HD cells therefore only encode the mammals' directional heading in the horizontal plane, they can be likened to a compass (Robertson et al., 1999), although activity is thought to be independent of geomagnetic fields (Zugaro et al., 2003).

As the focus of this thesis is on HD cells, a greater discussion of these neurons occurs in the next chapter. In summary though, the combination of place and HD cells could provide the information needed to know one's location on a cognitive map, and the orientation of this map. However, in order to navigate successfully, a map must indicate a scale. A map not drawn to scale and thus no indication of distance is only useful to a point, and could certainly not support the impressive navigational abilities demonstrated by Tolman (1948) and Morris (1981). The relatively recent discovery of grid cells then (Hafting et al., 2005) provided an ideal candidate to perform this very role.

1.5 Grid cells

Grid cells are found in the medial portion of the entorhinal cortex (Hafting et al., 2005) and encode for metric distance across an entire environment. The patterns of grid cell firing take on the form of a regular grid of equilateral triangles (Hafting et al., 2005) and can therefore be analogous to a sheet of graph paper laid out on the environment (Jeffery and Burgess, 2006) (Fig 1.4).

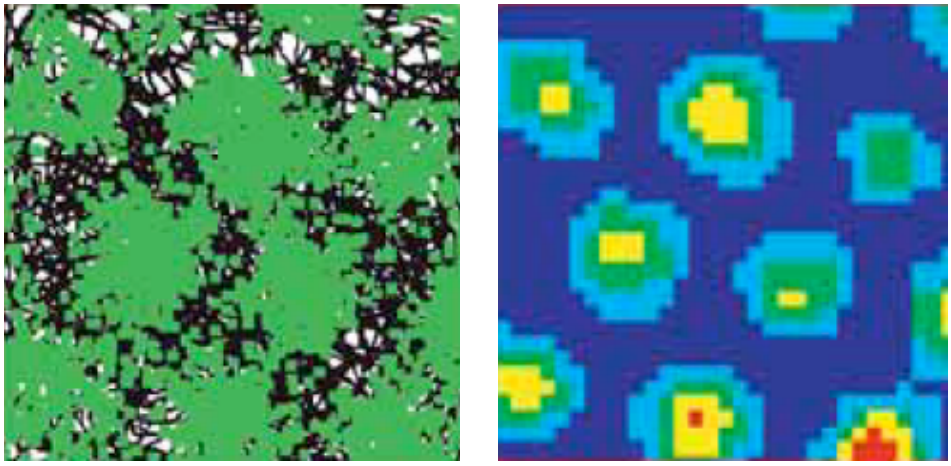

Figure 1.4. One grid cell recorded in the MEC by Barry et al (2007). *Left* The firing of this cell (spikes shown in green) superimposed on the bird's eye view of the rat's path (black lines). *Right* The corresponding firing rate map, where hot colours (red) represents high firing and cool colours (blue) represents low firing.

As with place cells and HD cells, grid cells have certain characteristics that vary from cell to cell. These include the spacing between each firing field, the orientation of the grid, the position of the grid (the offset) and the firing rate of the cell. For example, Sargolini et al (2006) showed that the speed at which the rat is travelling is positively correlated with the firing rate of some grid cells. Additionally, the scaling of grid cell grids has shown to differ across the medial entorhinal cortex (Hafting et al., 2005). Brun et al (2008) found that the scaling of grids increases (firing fields become further apart) from dorsal-medial locations to ventral-lateral locations, where grid spacing is approximately 39cm to 73cm. Together these findings suggest that grid cells can encode for both distance and speed, thus allowing them to play an important role in path integration.

This role of grid cells in path integration is only useful if the signal also expresses the direction in which the animal is travelling. The role of head direction cells in path integration is explored in greater detail in the next chapter (2.6). Additionally, cells that encode for both metric distance and heading direction have also been discovered (Sargolini et al., 2006). These conjunctive cells were recorded in layers III and VI of the medial entorhinal cortex and further implicate the role of the entorhinal cortex in path integration.

Grid cells are not only thought to compute path integration signals, but they are also thought to be sensitive to environmental landmark cues (e.g. wall distance). Barry et al (2007) implanted six rats in the MEC and recorded grid cell activity whilst the rats explored boxes of varying scales. Rats were either exposed to a square or rectangular environment in the first instance. In the square condition, the two axes of the environment were either compressed to form a smaller square, or only one of the axes was compressed to form rectangles. Similarly in the rectangle condition, one of the axes was either stretched or compressed to form a large or small square respectively. These manipulations caused the grid cells to re-scale. They found an overall re-scaling of 48% in the direction of the manipulation, 6% in the opposite direction. This re-scaling also decreased over sessions, suggesting grid cells are affected by experience. It is therefore generally thought that landmark or boundary information will set the initial position or offset of the grid cell, but firing is maintained by path integration information, allowing the grid cell population to encode for speed and distance. The importance of boundaries have also been illustrated by recordings of boundary vectors cells in the subiculum (Lever et al., 2009).

The study by Barry et al (2007) also raises interesting questions as to whether or not grid cells are sensitive to the geometry of an environment. Place cells are thought to be sensitive

to geometry, but manipulations of geometric features only caused remapping in place cells after repeat exposure, over many days or weeks (Lever et al., 2002). In order for geometry to cause rapid remapping in place cells, pre-exposure to different geometric environments that also had distinct contextual cues (colour and texture) were required for cells to later remap when only geometry differed (Wills et al., 2005).

The role that geometry plays in HD cell firing is explicitly tested in the first experimental chapter of this thesis (Chapter 4), with a discussion of the current literature in the next chapter. The next chapter will also provide an overview of the current head direction literature, discussing the anatomy of the brain regions in the HD network (Chapter 2.2), the connectivity of these areas (2.3) and the current theories speculating how HD cells in the system form a network (2.4). The discussion will then focus on how HD cells respond to allothetic (2.5) and idiothetic cues (2.6) with particular attention given to cue integration, which is the overarching theme of this thesis. The idea of cue integration is introduced below, and will be discussed at a neural level in the next chapter (2.7).

1.6 Cue integration

Allothetic and idiothetic information conveying an animal's sense of direction arises in different modalities, producing distinct and separate spatial signals. Along with these signals, each multisensory cue carries a degree of error or uncertainty. In order to use both types of information and reduce the degree of uncertainty, these multisensory cues need to be combined to produce a single, reliable signal. The process by which cues are integrated in an optimal fashion can be described by a Bayesian model (Yuille and Bulthoff, 1996).

Two years after his death in 1761, a theoretical framework by Thomas Bayes was presented to describe real-world outcomes using probabilities and prior beliefs. This framework was later extended to describe a cue integration model (Yuille and Bulthoff, 1996), which essentially predicts that multiple cues of differing reliabilities are integrated in a manner that provides an optimal outcome. In essence, a cue has a perceived reliability, which is termed inverse variance and defined as $1/\sigma^2$ (where σ is the standard deviation). Calculating a cue's inverse variance assumes that each cue is normally distributed and unbiased. Each cue has its own inverse variance and therefore two cues (A and B) would have an inverse variance of $1/\sigma^2_A$ and $1/\sigma^2_B$ respectively. Based on the inverse variance of each cue (i.e. the cue's perceived reliability) a weighting is given. This weighting determines the amount of influence that each cue has, when computing an optimal estimate. The greater the cue's weighting,

the more influence that cue has in determining the optimal estimate. These weightings (w) for cue A and cue B are calculated using the following formula:

$$w_A = \frac{1/\sigma_A^2}{1/\sigma_A^2 + 1/\sigma_B^2}$$

$$w_B = \frac{1/\sigma_B^2}{1/\sigma_A^2 + 1/\sigma_B^2}$$

From the formula it can be seen that calculating the weighting of one cue requires the reliability of the other cue to be taken into account. This is because the sum of weightings will always equal 1. Consequently, when the reliability of one cue increases, the weighting of that cue will increase whilst the weighting of the other cue will decrease. The sum of weightings is calculated using the following formula to produce an optimal estimate:

$$\sigma_{AB}^2 = \frac{\sigma_A^2 \sigma_B^2}{\sigma_A^2 + \sigma_B^2}$$

In addition to these weightings, the Bayesian model also takes into account top down processing based on expectations. For example, previous knowledge about the reliability of a cue can influence the weighting of that cue. The Bayesian model also accounts for any intrinsic bias that a system may have. For example, humans have an innate bias towards giving a stronger weighting to visual information, above and beyond its actual reliability (Battaglia et al., 2003). One of the main advantages of this relatively simple mathematical model is that it produces testable predictions. Advocates of the model (Yuille and Bulthoff, 1996) argue that Bayesian inference can predict many findings from psychophysical experiments. These experiments manipulate the reliability of two cues and then place these cues in conflict, in order to determine whether the subjects weigh that cue in a Bayesian fashion.

Alais and Burr (2004) adopted this method to examine the 'ventriloquist effect'. This effect refers to the situation where the perceived source of auditory information is manipulated through visual information. Human participants were asked to locate blobs of light (of varying visual acuity) and auditory clicks (of varying quality) that either provided unified or

conflicting spatial information. They found that performance of participants was crucially dependent of the visual acuity of the light blob. When the width of the light blob was small, localisation of the auditory stimuli was biased by the light location (the ventriloquist effect). When the width of the light blob was large, localisation of the auditory cue was not affected by the light position and instead was dominated by the click location. When the width of the light blob was intermediate, localisations were usually based on an average of visual and auditory information.

This study demonstrates how successful spatial localisation is dependent upon the reliability of a visual cue when integrating both visual and auditory cues. The findings from Alais and Burr (2004) can therefore be explained by a Bayesian model of cue integration. This model has received similar support from other types of cue integration, such as stereo and texture information (Knill and Saunders, 2003). Van Beers et al., (2002) tested the ability of participants to spatially locate their hand by integrating visual and proprioceptive information. Using a mirror, the experimenters were able to manipulate the reliability of visual information. This manipulation produced search patterns that were similar to those predicted by a Bayesian model. Participants tended to use visual information to locate their hand but when the reliability of the visual information was reduced, searches were based predominantly on proprioceptive information.

These studies have illustrated that the Bayesian model of cue integration can be successfully applied to the spatial domain. The application of this Bayesian principle to spatial localisation has recently been extended further to explain the more cognitively complex process of human navigation. More specifically, Nardini et al. (2008) examined how the process of cue integration during navigation develops. They tested adults and children aged 4-5 years and 7-8 years. Testing took place in a dark room which contained illuminated objects. The participant's task was to retrieve a selection of these objects and take them into the centre of the room. After a delay in the centre of the room, the participant then had to place the objects back in their original location. To successfully navigate around the dark room and replace the objects in the correct positions, the participant could rely on two types of information. The first type of information was visual, as they could use the location of the remaining illuminated objects to navigate. Second, the participant could use their own path integration to relocate the object's position in the room. In order to manipulate the reliability of the visual information, the light emitted from the objects was switched off. Additionally, participants were sometimes disoriented to decrease the reliability of the path

integration signal. The experiment was thus formed of three condition; visual cues available, path integration available and both cues available. When both cues were available, they could either be in agreement or in conflict (visual objects were rotated by 15°)

Nardini et al (2008) first found that performance on this task improved with age. Additionally, adults performed optimally when both cues were available but children showed optimal performance in the visual-only condition. Further analysis of the cue conflict condition revealed that whereas adults integrated visual and path integration cues to produce near optimal navigation, children did not. Instead, the children appeared to switch their strategy from using exclusively visual information to using only path integration. The authors therefore concluded that the ability to integrate visual and path integration cues requires developmental processes.

These studies have shown that humans can integrate spatial cues, often in an optimal manner (Van Beers et al., 2002; Knill and Saunders, 2003; Alais and Burr, 2004) although prior biases are also apparent (Battaglia et al., 2003). In comparison to these human studies, there are relatively few studies examining the integration of spatial cues in laboratory animals (Fetsch et al., 2009) and even less is known about this cue integration at a neural level. The next chapter introduces studies which have looked at how animals integrate allothetic and idiotheic spatial cues at a behavioural and neural level. This discussion highlights how the literature provides interesting, but so far inconclusive results regarding cue integration at a neural level. This thesis therefore aimed to take recordings of HD cells during situations where allothetic and idiothetic spatial cues conflicted. It is generally considered that the HD signal consists of an integration of allothetic and idiothetic cues (Taube et al., 1990b; Goodridge et al., 1998; Stackman et al., 2003; Bassett et al., 2005) (the next chapter discusses this in more detail). However, there is currently a lack of understanding as to how HD cells resolve cue conflicts, where this resolution occurs and what factors influence cue integration. The experimental chapters in this thesis aimed to address these currently unanswered questions.

Chapter 2 Head Direction Cell Introduction

Before the areas of the HD cell pathway are discussed, a brief overview of the hippocampal formation is described to situate these HD cell areas within a broader navigation circuit

2.1 Hippocampal formation

In rats, the hippocampus forms a pair of 'C' shapes and occupies around 50% of the total brain volume. As with many regions of the brain, the anatomical categorisation will vary slightly depending on different sources. One popular model of the hippocampal anatomy is proposed by Amaral and Witter (1995). According to their work, the rat hippocampal formation consists of the hippocampus proper, CA1, CA2, CA3 and neighbouring regions, entorhinal cortex, dentate gyrus and subiculum (Fig 2.1). This field categorisation was based on the pioneering works of Ramon y Cajal and Lorente de No (Brivanlou et al., 2004). In their work, they described the connections within the hippocampal formation as a "trisynaptic circuit", where connections are mostly unidirectional. The trisynaptic circuit describes the major input from the entorhinal cortex to the dentate gyrus, with cells of the dentate gyrus in turn projecting to the CA3 field of the hippocampus. Cells of the CA3 field in turn provide a major input to the CA1 field.

Figure 2.1: Left hippocampus from a rat highlighted in red (A), in dorsal-ventral horizontal sections (B) and in anterior-posterior coronal sections (C) showing the anatomical positions of CA1, CA3, entorhinal cortex (EC), dentate gyrus (DG) and the subiculum (S). Diagram from Amaral and Witter (1989)

2.2 Areas of the HD cell pathway

From the initial recordings taken in the postsubiculum (PoS) by Taube et al (1990a), recordings of HD cells have occurred in different brain regions which are directly or indirectly connected to the PoS (Sharp et al., 2001a). As a result of this further investigation, it is now thought that the head direction pathway involves multiple brain structures comprising the Papez circuit (Taube and Bassett, 2003). These areas include the thalamic nuclei, the dorsal tegmental nuclei the mammillary bodies (namely the lateral mammilary nuclei) and the retrosplenial cortex. The structure of these brain areas, along with the characteristics of the HD cells recorded in these areas are discussed below.

2.2.1 Postsubiculum and the subicular complex

The postsubiculum is part of the subicular complex, which in turn is part of the hippocampal formation (Amaral and Witter, 1995). The subicular complex has been divided up in a number of different ways, to form between three and five sub regions. These regions include; the subiculum proper, presubiculum, parasubiculum, prosubiculum and of course the postsubiculum (Fig 2.2). Initially termed by Brodmann, the postsubiculum is considered by some scientists not to be a sub region in its own right, but rather the dorsal section of the presubiculum (Amaral and Witter, 1995). Evidence supporting the idea that the postsubiculum is distinct from the presubiculum comes from the finding that they have differing projections. For example, the postsubiculum projects to deeper layers of the entorhinal cortex (layers IV-VI) than the presubiculum (layers I and III) (van Groen and Wyss, 1990). The distinction of the prosubiculum has also been debated and is not clearly defined in the rat brain (Slomianka and Geneser, 1991).

Figure 2.2: Line drawing of a horizontal view of a rat brain, illustrating the location of the postsubiculum, the presubiculum, the parasubiculum and the entorhinal cortex. Taken from Stafstrom (2005)

HD cells have been recorded in the parasubiculum (Boccara et al., 2010) but the majority of HD recordings in this region have occurred in the postsubiculum, where around 25% of cells in the postsubiculum are thought to be HD cells (Taube et al., 1990a). The postsubiculum has six layers which are divided into two groups; the external laminae (layers I-III) and the internal laminae (layers IV-VI) (van Groen and Wyss, 1990). The cells in layer III are relatively small and organised in rows that are parallel to the pial surface. The cells in layer II are much larger in comparison and are densely packed (Taube et al., 1990b) into clusters (van Groen and Wyss, 1990). HD cells are typically found in the deeper layers of the PoS, i.e. the external laminae region (Taube et al., 1990a). These deeper layers (IV-VI) contain smaller pyramidal cells (van Groen and Wyss, 1992).

2.2.2 Thalamic nuclei

The majority of studies investigating HD cells have taken recordings from thalamic regions. From these, a few recordings have been taken in the lateral dorsal thalamic nucleus (LDN) (Mizumori and Williams, 1993) but the vast majority of these HD recordings have occurred in the anterodorsal thalamic nuclei (ADN), where 60% of cells are thought to code for direction (Taube, 1995). The anterior dorsal thalamic nuclei (ADN) along with two other regions; the anterior ventral nuclei (AVN) and the anterior medial thalamic nuclei (AMN) make up the

anterior thalamic nuclei (ATN). This nucleus is located at the most rostral or anterior section of the thalamus and ventrally from the hippocampus and dentate gyrus (Fig 2.3) (Taube, 1995). Neurons in the ADN are medium sized cells, typically measuring 15µm (Dekker and Kuypers, 1976; Oda et al., 2001). Although the majority of HD cells in the network can be found in the ADN, it is thought that HD cells also exist in the AVN (Taube, 1995).

Figure 2.3: Line drawing of a coronal partial view of a rat brain, illustrating the location of the anterior dorsal thalamic nucleus (*AD*) in relation to the CA3 region of the hippocampus and the dentate gyrus (*DG).* Adapted from Taube (1995)

Taube (1995) used a similar paradigm to his experiment in 1990, and reported that the ADN contained HD cells with characteristics equivalent to those found in the postsubiculum. A total of 60 HD cells were recorded from eight rats that were implanted in the ADN. Theta cells were also recorded in this area; these were cells that spiked at a 5-8 Hz frequency when the animal was moving. As with the HD cells found in the PoS, HD cells in the ADN also demonstrated firing at a single preferred firing direction, with a peak firing rate at this preferred firing direction. This HD cell firing also showed a Gaussian distribution, where the firing range of these cells was similar to cells in the PoS. Taube (1995) also concluded that the peak firing rates of cells in the ADN were similar to those in the PoS. However, more recent findings (Yoganarasimha et al., 2006) suggest that neurones in the ADN tend to have a larger peak firing rate and a narrower tuning width in comparison to those in the postsubiculum.

Based on these observations, Blair and Sharp (1995) implanted a group of rats in the ADN and an additional group of rats in the PoS to compare HD cells in the two areas. The main

finding from this study was that cells in the ADN but not in the PoS showed anticipatory firing. That is, the cell's peak firing direction, on a directional firing plot shifted left (anti-clockwise) when the rat moved their head in a clockwise movement, but shifted right (clockwise) during anti-clockwise movements. Furthermore, they found that cell activity in the ADN increased with an increase in speed of the rat's head turn or angular head velocity. In other words, the angular separation between the mean firing directions of a clockwise turn versus an anticlockwise turn is greater when the turning speed is fast as opposed to slow. This implies that the HD cells in the ADN code for the future position of the rat's head. This anticipatory firing may also indicate the direction and temporal flow of information in the HD circuit. The flow of information through the HD circuit is discussed later on in this chapter (2.3).

Many of these findings were replicated by Taube and Muller (1998) when they formally examined data from previous studies that recorded HD cells from either the ATN or the PoS. Thirty three HD ATN cells were compared to thirty one HD PoS cells. Analysis of the peak firing rates showed that there was a trend for ATN cells to have a slightly higher firing rate, but this was not statistically significant. The directional firing ranges were also similar for the two regions. Both sets of HD cells showed an even distribution of preferred firing directions. Similar to Blair and Sharp (1995) this study found that HD ATN cells showed a peak firing rate at a positive time shift whereas PoS cells showed a peak firing rate with a zero time shift, illustrating the anticipatory firing of HD ATN cells. However, they also found that not all HD ATN cells show anticipatory firing and some HD cells in the PoS did. Again, like Blair and Sharp (1995), this study also found that the angular head velocity affected the mean or preferred firing direction of the cells. As well as the preferred firing direction, angular head velocity has also been shown to increase the peak firing rate of HD cells. One area which contains this type of HD cell is the dorsal tegmental nuclei (DTN)

2.2.3 Dorsal tegmental nuclei

Bassett and Taube (2001) implanted rats in the DTN and found that 75% of HD cells showed a correlation between firing rate and angular head velocity. More precisely, some of these modulated cells had a firing rate that was positively correlated with angular head velocity when they turned their head in either direction. However, the peak firing rate of other cells only showed a positive correlation with angular head velocity when the head turned in only one direction. Interestingly around 40% of these angular head velocity modulated cells were also modulated by linear velocity. In other words, the velocity of travel was correlated with

the firing rate of some HD cells. Finally, Bassett and Taube (2001) found that a small percent (16%) of angular head velocity cells were also sensitive to the pitch of the rat's head. This sensitivity to head pitch has also been shown in the lateral mammillary nucleus (LMN) an area in the mammillary bodies known to contain HD cells (Stackman and Taube, 1998).

2.2.4 Mammillary bodies

The lateral mammillary nuclei (LMN) along with the medial mammillary nuclei form the mammillary bodies, which is a collection of nuclei bordering the hypothalamus in rats. The LMN is a single nucleus that does not appear to have any subdivisions (Gurdjian, 1927; Rose, 1939; Allen and Hopkins, 1988). Stackman and Taube (1998) reported that out of the twenty LMN cells that they recorded, fourteen were sensitive to the pitch of the animal's head, with 90% of these cells firing maximally when the rat's head was angled upwards, towards the ceiling. Additionally, they found that like DTN cells (Bassett and Taube, 2001) the peak firing rate of cells in the LMN was modulated by angular head velocity, where faster head turns led to higher firing rates of HD LMN cells. Interestingly, like the DTN (Bassett and Taube, 2001) this effect was lateralised, with cells displaying higher firing rates for one direction of turn. Moreover, this asymmetry was correlated with the hemisphere where the HD cell was located. They found that HD cells recorded in the right hemisphere had higher firing rates for clockwise head turns and HD cells recorded in the left hemisphere had higher firing rates for anti-clockwise head turns.

Around the same time, this lateralisation of HD cells in the LMN was also shown by Blair et al (1998). They simultaneously recorded HD cells from both the LMN and the ADN in order to compare the two brain regions in the HD pathway. Rather than observing a lateralisation of firing rate during head turns (Stackman and Taube, 1998) they found an effect on the tuning width of LMN HD cells. More precisely, they found that HD cells recorded in the right hemisphere had narrower tuning widths for clockwise head turns and HD cells recorded in the left hemisphere had narrower tuning widths for anti-clockwise head turns. Taken together, the findings from Stackman and Taube (1998) and Blair et al (1998) suggest that LMN HD cells encode for both heading direction and angular motion, where cells show a greater degree of processing for contraversive directions (higher peak firing rates and narrower tuning curves respectively).

Angular head velocity modulation is therefore expressed in the HD cells from both the LMN (Stackman and Taube, 1998; Blair et al., 1998) and the DTN (Bassett and Taube, 2001).

Interpretation of this expression has led many to conclude that the LMN and the DTN are areas where path integration information is initially encoded before being sent to other areas in the HD network (McNaughton et al., 1991; Redish and Touretzky, 1996; Sharp et al., 2001a). It should be noted that HD cells in the ADN also show angular head velocity modulation (Blair and Sharp, 1995), but that far fewer cells show this modulation in comparison to the LMN and none of them code for the direction of the head turn (Stackman and Taube, 1998). This weaker modulation suggests that the ADN receives a path integration signal from the LMN (Blair et al., 1998). This is supported by the finding that HD cells in the ADN have significantly shorter anticipatory firing intervals in comparison to the LMN (Blair et al., 1997; Blair et al., 1998; Taube and Muller, 1998). The path by which information flows through the HD system will be discussed later on in this chapter.

2.2.5 Retrosplenial cortex

Finally, HD cells have also been recorded in an area neighbouring the postsubiculum, the retrosplenial cortex (Fig 2.4). The retrosplenial cortex (RSP) is also situated in the caudal part of the cingulated cortex (Hopkins, 2005). The RSP is divided into the granular and dysgranular cortex (Wyss and van Groen, 1992). The granular cortex can further be divided into granular 'a' region and granular 'b' region (Wyss et al., 1990). Head direction cells have been found in both the granular and dysgranular regions of the retrosplenial cortex (Chen et al., 1994b; Cho and Sharp, 2001). However, only around 10% of cells in both areas were classified as HD cells (Cho and Sharp, 2001).

Figure 2.4: Line drawing of a coronal view of a rat brain, illustrating the location of the retrosplenial cortex in relation to the postsubiculum, the CA1 region of the hippocampus and the dentate gyrus (DG). Taken from Taube et al (1990a).

HD cells in the RSP do show anticipatory firing, but like HD cells in the ADN, the anticipatory time interval is much shorter than cells recorded in the LMN (Sharp et al., 2001b). On the whole, Sharp and Cho (2001b) observed that HD cells in the RSP had narrower tuning widths than other areas in the HD pathway. They also found that as well as HD cells, the retrosplenial cortex also contained speed modulated cells. These cells tended to fire more with an increase in the rats' running speed. Studies that have taken recordings of HD cells in the RSP are relatively uncommon and this may be a reflection of the low percentage of HD cells in that area. Nevertheless, HD cells do exist here and have very similar properties to HD cells in the rest of the HD circuit. The connectivity between these areas in the HD circuit will now be discussed.

2.3 Connectivity of the HD circuit

There is a general agreement that the HD signal originates in the LMN (Blair et al., 1998) or the DTN (Bassett and Taube, 2001) after receiving a path integration signal from vestibular and motor brain areas (Brown et al., 2005; Biazoli, Jr. et al., 2006). This signal is then thought to project to the ADN, which in turn projects to the PoS (which also projects to the LMN) and RSP (Clark et al., 2010a) where visual information is integrated into the signal (Fig 2.5). For this reason, connectivity with the DTN and LMN will be discussed first, followed by the

connections between the LMN, ADN and PoS. Finally the connections between the ADN, PoS and RSP will be explored.

Figure 2.5: A schematic diagram taken from Clark et al (2010a). The diagram shows the flow of allothetic and idiothetic information in the HD system.

Reciprocal connections between the DTN and the LMN have been illustrated by a number of studies (Takeuchi et al., 1985; Allen and Hopkins, 1989). These reciprocal connections are illustrated in Figure 2.6, in particular it can be seen that the ventral part of the DTN specifically projects to the LMN. The descending projections from the LMN to the DTN were illustrated by Allen and Hopkins (1990). They injected a tracer (wheat germ agglutinin-horseradish peroxidase conjugate) into the medial and lateral mammillary nuclei and observed the widespread descending LMN projections to the DTN.

Figure 2.6: Distribution of projections from the ventral portion of the dorsal tegmental area (DTNv) to the lateral mammillary bodies (LM), indicated by the square symbols. Taken from Weiner and Taube (2005)

The ascending LMN projections have also been examined and clearly demonstrate that the LMN has extensive projection to the ADN (Powell and Cowan, 1954; Seki and Zyo, 1984). These projections are illustrated in Figure 2.7, where the connections between the LMN and ADN are bilateral.

Figure 2.7: Distribution of projections between the lateral mammillary bodies (LM) and the antereodorsal thalamic nuclei (AD), indicated by the square symbols. Taken from Wiener and Taube (2005)

The LMN also receives strong projections from the postsubiculum in a topographically organised fashion (Meibach and Siegel, 1977; Allen and Hopkins, 1989; Shibata, 1989; van Groen and Wyss, 1990). More specifically, projections terminate in horizontally orientated layers (Kishi et al., 2000), so that more dorsal regions of the PoS will project to more dorsal regions of the LMN. The projection from the PoS to the LMN does not appear to be reciprocal in nature (Clark et al., 2010a). There are however, reciprocal connections between the PoS and the ADN (Taube et al., 1996). This reciprocal projection is distinct from the other reciprocal projections between the subiculum and the thalamus. Van Groen and Wyss (1990) showed that the PoS has dense projections which terminate in the ADN, but the presubicular has projections which terminate in the AVN. The lateral dorsal thalamic nucleus (LDN), an area where HD cells have been recorded (Mizumori and Williams, 1993), also receives projections from the PoS (Allen and Hopkins, 1989) but to a lesser extent than the ADN (van Groen and Wyss, 1990).

The PoS also has strong reciprocal connections with the RSP (van Groen and Wyss, 1992). In particular, van Groen and Wyss (1992) found that efferent projections of the RSP dysgranular region and both granular regions (Rga & Rgb) are profuse to the PoS and less so

to the LDN. They also noted that these efferent projections were particularly sparse to the ADN. This led them to conclude that the HD signal sent from the RSP to the ADN would have to go via the PoS. The afferent projections are similar to the efferent projections, with the RSP receiving projections from the PoS and LDN (van Groen and Wyss, 1992; van Groen and Wyss, 2003). Interestingly however, the RSP subregions which receive these projections appear to be more selective. For example, the granular 'a' region of the RSP does not receive direct projections from the PoS, but rather the subiculum proper instead (van Groen and Wyss, 1992). Finally, the PoS also projects to the entorhinal cortex, that contains grid cells (Hafting et al., 2005) and HD cells (Sargolini et al., 2006; Wills et al., 2010). Interestingly developmental work has shown that HD cells in the entorhinal cortex exist in 'adult-like proportions' in rats aged postnatal day 16 (Wills et al., 2010; Langston et al., 2010). This developmental work suggests that an HD signal exists before the rat can perform an extensive exploration of their environment. The connections between the entorhinal cortex and the PoS have led some authors to consider the PoS to be a gateway region between the HD circuit and the hippocampus proper (Hopkins, 2005).

2.3.1 Lesion studies examining connectivity

The technique of lesioning areas of the HD pathway has been used to understand how the directional signal travels through the network. One of the main studies to implement this technique was performed by Goodridge and Taube (1997). They systematically lesioned, and simultaneously recorded HD cells from either the ADN or the PoS. They found that when the ADN was lesioned, HD firing in the PoS was completely abolished. This indicates that the ADN is required for normal HD cell firing in the PoS. Moreover, it implies that the HD signal is processed in the ADN first, and then this signal is sent to the PoS. Interestingly, when the PoS was lesioned, HD cell firing continued in the ADN but crucially, these lesions disrupted the control that the visual landmarks had over HD cells in the ADN. Similar lesion experiments have shown that this is also true when the retrosplenial cortex is lesioned and recordings are taken from the ADN (Clark et al., 2010a).

Together these findings suggest that visual landmarks are firstly processed in the PoS and the RSP, and then this information is sent to the ADN. These lesion studies therefore have important implications for the route by which signals portraying allothetic and idiothetic information travels though the system. One possibility may be that different areas of the HD system, are differentially influenced by idiothetic and allothetic cues (Mizumori and

Williams, 1993). This hypothesis is examined in the final experimental chapter of this thesis, where recordings of HD cells are taken in multiple areas of the HD pathway.

2.4 Theories of HD cell firing

Studies that have compared the properties of HD cells from different areas in the HD network have illustrated that there are subtle differences in firing characteristics between these brain structures. There is however an overriding consensus that firstly, all HD cells have only one preferred firing direction per cell and second, the angular relationship between any two HD cells' preferred firing direction always remains fixed. These fundamental attributes are thought to be the crucial contributing factors that allow HD cell ensembles to set a heading direction signal and to then update this signal as the animal's head moves through space. It is these two functions of establishing and updating a directional signal that allows the system to code for the animal's 'sense of direction' during navigation.

The first attempt to model the setting and updating of the animal's heading direction was by McNaughton et al (1991). The essential idea of this model was to combine information about the current heading of a rat and angular head velocity information. As this model was constructed only a year after the first formal recordings of PoS HD cells, that were not sensitive to angular head velocity (Taube et al., 1990a; Taube et al., 1990b), it was assumed that a set of linearly independent vectors were needed to combine the current heading direction and angular head velocity information. This combination would therefore allow the vector to express the resulting head direction. This information which is essentially born out of a path integration signal is then updated with landmark information. These vectors (or angular head velocity HD modulated cells) that are sensitive to both direction and angular head velocity were of course later discovered (Stackman and Taube, 1998; Blair et al., 1998; Bassett and Taube, 2001) lending support to this initial model. However, the model by McNaughton et al (1991) was unable to explain all of the characteristics of HD cells, most notably the Gaussian shape of the HD tuning curves as the model was based on linear associations. The model could also not account for later findings that showed differences in HD cells across different brain regions (Touretzky, 2005), such as anticipatory firing.

Since this initial theory, one class of model that has dominated the field is the dynamic attractor network hypothesis, which can indeed explain the Gaussian firing of HD cells. The main principle of a dynamic system is that the state of that system evolves over time. The

mechanism by which this dynamic activity copes with noise involves the action of 'attractors'. Attractors are stable states which act as memories for the system (Touretzky, 2005). When noise enters the system, the main characteristic is the tendency for the system to return back to these stable states. These attractors can be discrete but in the case of modelling space where space states are not discrete, continuous attractors are favoured. For example, a rat can position its head in any direction and thus a stable state needs to occur at any position in the system. In order to model directional states (0-360°), HD attractor networks are typically arranged in a one dimensional circle (Skaggs et al., 1995; Zhang, 1996). Within this ring attractor, neural units or HD cells are assigned to a position on this ring. HD cells with neighbouring preferred firing directions will be positioned next to each other in this virtual state space. Thus, positioning of neuronal units is not dictated by the physical location of HD cells within the brain (Taube et al, 1990a).

These neural units are linked together through both excitatory and inhibitory connections (Boucheny et al., 2005). Essentially, it is hypothesised that an HD cell will excite HD cells with neighbouring preferred firing directions but will simultaneously inhibit HD cells with distant preferred firing directions (Goodridge and Touretzky, 2000). Thus cells which represent similar directions will fire at the same time, but cells which represent opposing directions will never fire at the same time, and this relationship between cells would remain constant. So, if a rat is facing north, HD cells which represent north will fire, but those representing, south, east and west will not.

The stable activity within the network is represented by a 'bump' or 'activity packet', which is a Gaussian-like distribution similar to that expressed by the firing of the HD cells themselves (Sharp et al., 2001a), where the cell's activity is a weighted linear combination of its inputs (Touretzky, 2005). When a rat's head is maintained in a particular direction, the HD cell with that preferred firing direction will continue to fire without any adaptation (Taube et al, 1990a). This ability for the HD system to continue in a stable state is due to the coupling of excitatory and inhibitory connections within the ring. For example, if two distinct and conflicting inputs are of equal magnitude, the bump of activity won't move (Fig 2.8a). When the rat then moves its head to a new direction, the network detects this movement (i.e. angular head velocity) and shifts the bump of activity to the new direction. The bump of activity will only move when the magnitudes of these two inputs are unequal, where the larger of the inputs is favoured (Fig 2.8b).

Figure 2.8 Computational plots to show how HD activity responds to inputs of equal magnitude (A) and unequal magnitude (B). When the inputs are equal, the bump of activity remains stable. When the inputs are unequal, the bump of activity shifts in the direction of the input which is greater in magnitude. Taken from Touretzky (2005).

Skaggs et al (1995) proposed the first attractor network hypothesis of HD network activity. Although no formal calculations were presented and no results were simulated, the paper did describe a model which consists of a ring of HD cells in an attractor state. Using the principles of a dynamic attractor network, bumps of activity were able to propagate around the ring of HD cells by integrating angular head velocity information. The basic concepts proposed by Skaggs et al (1995) of an attractor network and the integration of angular head velocity information led to a number of subsequent models that did explicitly state the equations involved, consequently producing simulated data (Zhang, 1996; Goodridge and Touretzky, 2000; Xie et al., 2002).

Zhang (1996) also proposed a one dimensional dynamic attractor model that formed a ring based on the preferred firing directions of each cell. During a static attractor state, all the

weightings between connections or cells are equal to reflect the finding that HD cells continues to fire when the orientation of the rat's head is maintained in the cell's preferred firing direction (Taube et al., 1990a). When the rat's head moves, a vestibular signal is sent to the HD system, causing an asymmetry in the weightings (odd-weight component) between connections. As illustrated in Figure 2.8, this causes the bump of activity to shift in the direction signalled by the vestibular input. The Zhang (1996) model can also account for the effects of acceleration and anticipatory firing. The strength of this odd-weight component is directly determined by the speed of shift, i.e. the speed at which the rat's head moves. Moreover, if the strength of this odd-weight component is also proportional to the angular acceleration of the head, then the heading direction perceived by the network will be faster than the rat's actual head movement (Fig 2.9). This would result in the anticipatory firing observed in some HD cells in the ADN.

Figure 2.9: Illustrates that if the odd-weight component is determined by the speed at which the rat's head moves plus the angular acceleration of the head, then the firing rate of the internal direction will constantly move faster than the true head direction. This would produce anticipatory firing in HD cells which receive information about the angular acceleration of the head. Taken from Zhang (1996).

Therefore the Zhang (1996) model can explain some of the regional differences in HD cells, namely angular head velocity cells and the anticipatory firing of HD cells. However, this model cannot explain all of the variations in HD cells across the network. For example, it is thought that the effect of passive rotation on HD cell firing rates differs across the network. The lack of coherence between vestibular, motor and proprioceptive signals resulting from passive rotation caused a significant reduction in the peak firing rate of HD cells in the ADN

(Taube, 1995). However, there was no effect of peak firing rate on HD cells recorded in the LDN (Mizumori and Williams, 1993). The findings from passive rotation do need to be approached with caution however, as it is thought that stress factors may play and important role (Shinder and Taube, 2011) and is discussed in greater detail later in this section (2.6). Even so, findings which show that HD firing in the PoS was abolished by lesions to the ADN, but firing in the ADN was not abolished if the PoS was lesioned (Goodridge and Taube, 1997), is not captured by the Zhang (1996) model. Models that can arguably capture more regional variance in HD cells across the system are those that propose separate models for each brain region.

Goodridge and Touretzky (2000) suggested such a model where activity in the PoS, the LMN and the ADN are described separately. Essentially, activity in the PoS reflects the animal's actual heading direction through attractor dynamics (similar to those previously described). The signal from the PoS is then sent to the LMN, where the signal is encoded by two populations of cells. One population represents clockwise movements and one represents anticlockwise movements. At this point, the signal is also modulated by angular head velocity. This information is conveyed by HD cells in the LMN which produce higher firing rates for one direction of turn (Bassett and Taube, 2001). The HD signal is then believed to be projected to the ADN where Goodridge and Touretzky (2000) suggest an attractor dynamic does not exist. Instead, firing in the ADN is solely determined by external input from the LMN. The input from both the clockwise and the anticlockwise inputs from the LMN can explain why anticipatory firing occurs in the ADN. For example, if the amount of offset between the clockwise and anticlockwise signal was 15°, HD cells in the ADN with a preferred firing direction of 90° would receive inputs from a clockwise LMN cell which fires maximally at 75° and an anticlockwise LMN cell which fires maximally at 105° (Goodridge and Touretzky, 2000). Simulated data therefore showed that there was a positive correlation between the offset in the LMN and the degree of anticipation in the ADN. Interestingly, the simulated anticipation was typically shorter than that shown in actual data, which led the authors to conclude that other mechanism in ADN firing are involved. Even so, this model that describes a dual LMN input is able to explain the distortion of tuning curves in ADN cells. For example, when the animal is still, HD ADN cell tuning curves are often bimodal in shape. When the animal moves its head, the tuning becomes unimodal, but the curve is often skewed in the direction opposite to the turn. In summary, the model by Goodridge and Touretzky (2000) can explain a good deal of data. However, in its current state, their model cannot explain the findings by Blair et al (1998) who found that LMN HD cell lateralisation

was expressed in the width of tuning curves. Another model which describes local networks is by Redish et al (1996) and will be discussed in the sixth chapter.

2.4.1 Attractor network transition dynamics

An important question which emerges from these models is how this packet of activity propagates around the ring. Two potential mechanisms could be involved in the transition of HD cell activity. One possible mechanism is that the bump of activity travels or sweeps from the current stable state around the ring and stops at the new stable state (Fig 2.10a). Alternatively, the bump of activity does not travel over intermediate states, but instead jumps from one stable state to the next (Fig. 2.10b). It is important to note however that these mechanisms do not need to be mutually exclusive as it may be that the system adopts both types of mechanism (Zhang, 1996; Johnson et al., 2007).

Figure 2.10 The two possible mechanisms for the propagation of activity in the attractor ring. A: Illustration of activity sweeping across intermediate directions. B: Illustration of activity jumping from one preferred firing direction to the new direction. Taken from Zugaro et al (2003).

Zugaro et al (2003) attempted to address the question of transition dynamics in the HD system by moving a light cue back and forth between two locations separated by 90°. During this movement, the rat's head was maintained in the preferred firing direction by training them to drink from a reservoir. They found that the establishment of a new preferred firing

direction was highly rapid (~80ms). Due to this short latency period, it was concluded that the cells' activity jumped from one preferred firing direction to another. However, as acknowledged later by Zugaro (2005, cited in (Wiener and Taube, 2005) there is still a lively debate as to whether this jump in activity is present in the HD network. An alternative explanation is that the cell's activity sweeps across intermediate directions in order to reach the new preferred firing direction (Redish et al., 1996). The final experiment in this thesis therefore aimed to address this debate of whether a cell's activity jumps from one direction to another, or if the activity sweeps across intermediate directions.

A dynamic model based on an attractor network hypothesis therefore appears to explain many characteristics of HD cells across the network. Some of these characteristics include firing ranges, anticipatory firing and sensitivity to angular head velocity. These models also aim to capture how the network deals with incoming and constantly changing directional cues. There are two main types of information utilised by the head direction system: landmark cues (allothetic) and path integration (idiothetic). Studies that have examined the influence of allothetic cues will be described first, followed by a description of the literature focussing of the processing of path integration in HD cells.

2.5 Allothetic inputs

In the initial body of HD cell work by Taube, Muller and Ranck, Jr. (1990b) they examined the degree of control that landmark cues can have over HD cells. In order to examine this, they attached a white cue card to the wall of a grey cylinder. The card covered a 100° area of the wall. The animal was allowed to forage in the cylinder for 8 minutes, whilst cell activity was recorded. The animal was then removed from the apparatus and placed back in their home cage. The flooring in the cylinder was replaced and the cue card was rotated by either 90°, 180° or 270°. The rat then returned to the room and was placed back in the cylinder for a further 8 minutes to forage. The rat was then removed again, the floors replaced and the cue card rotated back to the original position for a further 8 minutes of foraging. Taube et al (1990b) found that the preferred firing direction of PoS HD cells rotated between each session, accompanied by an absence of change to the peak firing rate and directional firing range of the HD cell. Firstly, it can be inferred from these findings that the rat assumed the cue card was a stable landmark cue as the rotation of preferred firing direction was almost the same degree to which the card was rotated. There was however a small, but notable discrepancy between the rotation of the cell and the rotation of the cue card (ranging from

6-48°). This rotation of the cells suggests that the rat (or at the very least the HD cells) were using the cue card to orientate themselves. However, the under-rotation shows that they were also using another directional cue to orient.

This cue integration was more apparent when the experimenters rotated the cue card whilst the rat was still exploring the circular environment. During these manipulations, HD cells demonstrated a consistent under-rotation. This suggests that the cells were using the cue card, along with other directional cues to orient. These cues could consist of odour cues, auditory cues, extramaze visual cues and path integration cues. Even with this under rotation, this series of experiments clearly showed that HD cells utilise information from visual landmarks. This exertion of landmark control over HD cells has also been found in the ADN (Taube, 1995), the LMN (Stackman and Taube, 1998) and the RSP (Chen et al., 1994a).

From these early studies, the characteristics of these visual landmarks have been subsequently manipulated in order to establish what aspects of these landmarks causes them to have such a dominant control over HD cell firing. Zugaro et al (2001a) manipulated the distance between visual landmarks and a perceived environmental boundary from the rat, to observe any impact that landmark proximity may have on HD cell firing. Rats implanted in the ADN explored a raised circular arena that was placed in the centre of a curtained enclosure. In the proximal background condition, the circular arena had black walls which prevented the rat from seeing the curtain beyond the arena. In the distal background condition, these walls were removed so the rat could view the space beyond the circular arena, including the curtains. In both conditions, an array of three landmarks was placed on the periphery of the circular arena. In order to establish the influence that these landmarks have over the firing of HD cells, the objects were rotated by 120°. The configuration of these landmarks was always preserved. Thus, in the proximal background condition the landmarks could arguably be viewed by the rat as 'background' landmarks. In the distal background condition, the landmarks may have been viewed as 'foreground' landmarks.

Results showed that landmark rotations in the proximal background condition caused HD cells to rotate by a similar amount (rotations ranged from 99-134°). Interestingly however, cells in the distal background condition on average only shifted by 6°. Together these findings suggest that background but not foreground landmarks exert control over HD cell firing. Several explanations for this difference in landmark control were given. For example, in the distal background condition, rats were able to view the landmarks from multiple

perspectives and maybe these landmarks were thus viewed as less reliable than the curtain which is arguably more salient than the landmarks. Alternatively, HD cells may simply use the most distal feature in an environment to anchor to. In the proximal condition this was the landmarks and in the distal condition this was the curtain. Regardless of interpretation, the findings demonstrate the complex relationship between visual landmarks and HD cell firing.

This complexity has also been demonstrated through an extensive series of experiments by Goodridge et al (1998) who examined the effects of the type of landmark cue (visual, auditory or olfactory), the temporal relationship between landmarks and HD cell firing, and the prior experience of these landmarks on HD cell firing. In order to investigate these variables, they used a similar paradigm to Taube et al (1990b). In this modified paradigm, recordings of HD cells from the ADN and PoS were taken, whilst rats explored a cylindrical environment containing directional landmarks. Before these recordings, the rats were pre-exposed to this cylinder once a day for 1-2 weeks. This pre-exposure either consisted of an empty cylinder, a cylinder with a cue card attached to the wall or an empty cylinder coupled with the presence of a directional auditory cue. At the time of recording, the rat was exposed to one or more test manipulations depending upon the type of pre-exposure they were subject to. These manipulations included: blindfolding the rats that were pre-exposed to the cue card or the auditory cue, rotating an auditory or olfactory cue for those who were pre-exposed to the auditory cue, and rotating a visual cue (the cue card) to those that had not been pre-exposed to the cue card.

During the blindfold session, the authors replicated previous findings (Taube et al., 1990b) demonstrating that HD cells continue to fire in the absence of visual information with a stable peak firing rate and directional firing range. The direction at which the cells fired however, drifted over time (approximately 23°). Additionally they found that rotating the cylinder, in particular the floor caused the HD cells to rotate. This suggests that HD cells are able to use non-visual cues to orient. This was further explored by deliberately rotating a directional odour cue (peppermint extract). When the odour was rotated by 90° the cells rotated on average by 45°, illustrating a moderate influence over HD cell firing. Interestingly, when the odour returned to its initial position the HD cells showed little response. This lack of control was also seen with rotations of an auditory 'clicking' cue. They found that HD cells did not rotate with the rotation of the auditory cue. However, they did find that changing the location of the 'click' caused cells to randomly change their preferred firing direction.

Taken together, these findings suggest that non-visual cues are utilised by the HD system, but not to the extent that visual cues are used.

The influence of unfamiliar visual cues (cue card) showed that for the initial 8 minute exposure, the preferred firing direction of the cells rotated by a similar amount to the rotation of the cue card. The subsequent 8 minute session caused cells to rotate by an amount similar to the cue card rotation. When the rats were only left to forage for 1 minute, cell ensembles rotated in about half of the sessions. These landmark manipulations illustrate that an 8 minute exposure to a visual landmark is enough time for the landmark to exert a strong control over HD cells. A reduction in this exposure time reduces the influence that these visual landmarks have over HD cell firing, whereby a 1 minute exposure allows a strong control over HD cell firing but only on some occasions.

2.5.1 Geometric cues

All of the above studies examining the influence of visual allothetic cues on HD cell firing have used landmarks (most notably a cue card). However, visual landmarks are not the only type of allothetic cues used in navigation. The geometry of an environment is another important allothetic cue used in both human (Hermer and Spelke, 1994; Hermer and Spelke, 1996; Kelly et al., 2008; Lee and Spelke, 2008) and animal navigation (Cheng, 1986; Esber et al., 2005; Graham et al., 2006; McGregor et al., 2006). One important reason not to overlook the influence of geometry on HD firing is because geometric cues are thought to be processed differently to landmark information during orientation tasks (Cheng, 1986; Zugaro et al., 2001a; Doeller et al., 2008).

Cheng (1986) was one of the initial researchers to demonstrate the importance of geometric shapes during place finding tasks in rat populations. Cheng used rectangular environments with distinct feature panels in all corners and the goal location in one corner. It was found that during place finding tasks, rats made equal amounts of correct choices and rotational errors (orienting to the corner diagonally opposite the correct corner). It was concluded that the rats primarily used a purely geometric module, which then also provided a basis for coordinating the locations of non-geometric cues. This idea was later extended to other mammals, including humans (Hermer and Spelke, 1994). However, more recent studies using similar tasks (Graham et al., 2006) suggest that the processing of geometric cues does not occur in a single designated module. Further investigation into how these cues are

processed has lead to an argument over the degree to which mammals use geometric information.

One influential behavioural study by Pearce et al. (2004) used an innovative method to examine which features of the environment rats use in a submerged platform task. The rats were initially trained in a rectangular pool with a submerged platform in one corner. They were then placed in a kite shaped pool, where one corner corresponded to the correct corner in the rectangular pool and one corresponded to the diametrically opposite corner of the rectangular pool. They found that the rats that were consistently trained showed a strong preference for swimming to the corner that was geometrically equivalent to the correct corner in the rectangular pool. Local processing explanations were given for the findings, one of which was termed the 'individual wall' strategy, where the rat learns which way to turn after swimming down a short or long wall.

In response to findings like these, Cheng and Gallistel (2005) suggested that using a purely local strategy is not cognitively efficient and that in comparison, a combination of both local and global processing requires a smaller cognitive load. A global strategy which they believed provided a compelling explanation for the Pearce et al. (2004) data is the 'Principal Axis'. This principal axis provides global information which 'points the animal to the approximate region in which local processes take over'(Cheng and Gallistel, 2005). However, subsequent work suggests that this principal axis explanation is unwarranted. McGregor et al (2006) trained rats to find a submerged platform in a right-angled corner at the base of a pentagon. They were then tested to see if they preferred to search in corners that were geometrically similar to the previously correct corner in a rectangular environment. When the experimenters kept the geometric (local) shape of the corner the same but manipulated the principal axis of the arena, the rats showed a transfer of knowledge. However, when they kept the principal axis of the arena the same (global cues) but manipulated the geometric shape of the corner, the rats showed a lack of transfer of knowledge.

A recent study examining the spatial reorientation in chicks (Pecchia and Vallortigara, 2010a) has further highlighted the complexity surrounding geometric based orientation. When four landmarks were arranged in a rectangle inside a circular maze, they found that chicks relied upon the individual landmarks to reorient as oppose to the geometric array which they formed (rectangle). If however the four landmarks occupied the four corners of a rectangular maze they did appear to use geometric information (searching to the correct and geometrically equivalent corner).

This idea that geometric processing is susceptible to non-geometric information has been highlighted by a number of recent studies (reviewed in Cheng and Newcombe (2005). The literature now suggests that when geometric cues are placed in conflict with other sources of information, such as landmarks or self-motion cues (path integration) (Margules and Gallistel, 1988; Hermer and Spelke, 1996; Garrad-Cole et al., 2001; Wall et al., 2004; Esber et al., 2005; McGregor et al., 2006; Pecchia and Vallortigara, 2010a; Reichert and Kelly, 2011) the extent to which navigating animals use geometric information is variable, and governed by numerous factors including the species (Gray et al., 2005), the size of the environment (Chiandetti and Vallortigara, 2008) the amount of experience in the environment (Kelly et al., 1998) and whether the animal or subject is disoriented (Margules and Gallistel, 1988; Lew et al., 2006; Lourenco and Huttenlocher, 2006; Batty et al., 2009).

To a somewhat lesser extent, the impact of geometric cues on HD cells during orientation has also been studied. Taube et al (1990b) compared the preferred firing direction of cells in a circular arena, a square arena and a rectangular arena. Their findings were generally mixed, but the rotations of HD cell firing suggested that HD cells are sensitive to some aspect of geometry. This idea that geometric cues have a modest but notable influence on HD cell firing was later supported by Golob et al (2001), when they found that some HD cells rotated in multiples of 90° when rats were moved from a square to a rectangular environment. It is important to note that this study was not directly designed to assess how geometric cues are processed by the HD system. The primary aim of Golob et al (2001) was to see if behaviour correlated with HD firing. Thus, unlike the behavioural studies that directly addressed the issue of geometric processing (Margules and Gallistel, 1988; Hermer and Spelke, 1996; Garrad-Cole et al., 2001; Wall et al., 2004; Esber et al., 2005; McGregor et al., 2006; Pecchia and Vallortigara, 2010a; Reichert and Kelly, 2011), it is difficult to determine what extent the HD system utilises geometric cues.

A recent study by Clark, Harris and Taube (2010b) did directly address the issue of geometric processing by recording HD cells of rats exposed to rotations of either a rectangular or a trapezoidal environment. The rotation of these environments were done out of view of the rats. Whilst this was happening the rats were deliberately disorientated. They found that for both the rectangular and the trapezoidal environments, the firing of the HD cells rotated to a degree which indicated that the cells were using the geometric information provided by the environments. This influence was more apparent for the more geometrically salient trapezoid.

It is important to note, that the majority of these electrophysiology studies, including Clark et al (2010b), used a disorientation procedure to examine the influence of geometry on HD firing. However, behavioural studies have shown that the state of disorientation interacts with the effect of geometric cues (Margules and Gallistel, 1988; Lew et al., 2006; Lourenco and Huttenlocher, 2006; Batty et al., 2009). Given these findings, the first experimental chapter in this thesis aimed to determine which aspects of geometry are used by the HD system by manipulating the geometric complexity of an environment. The second aim was to estimate the impact that the reliability of a path integration signal (disorientation versus orientation) would have on this processing of geometric cues. Finally, cue card conditions were used to determine whether geometric cues are processed differently to landmark information during orientation tasks (Cheng, 1986; Zugaro et al., 2001a; Doeller et al., 2008).

In summary, these studies examining the influence of allothetic cues on HD firing have shown that the HD system is strongly reliant on external cues. However, all of these studies to some extent have shown that this reliance is variable, depending upon the type of allothetic cue, the location of that cue in the environment, the prior experience of that cue and the duration of exposure to that cue. Even with this uncertainty in allothetic cues, studies have reported that HD cells continue to fire and exhibit no changes to the firing rate of the cell or to its firing range (Taube et al., 1990b; Goodridge et al., 1998). Moreover, HD cells maintain their firing in the dark, albeit with a drift in preferred firing direction (Taube et al., 1990b). The ability for HD cells to maintain a stable firing during these situations suggests that to some degree HD cells can rely on information that originates within the animal.

2.6 Idiothetic inputs

Information originating within the organism is known as idiothetic information. The term given to the internal processes that allows an animal to navigate is termed path integration. Path integration consist of vestibular, motor and proprioceptive information (Gallistel, 1980). The importance of path integration in place cell firing was highlighted in a study by Sharp (Sharp et al., 1990) who showed that the starting position for an animal can be an orienting cue for place cells.

One study which eloquently demonstrates the importance of path integration in HD cell firing is Taube and Burton (1995). As part of their study, HD cells were recorded in the PoS and the ADN as rats moved around in a dual-chamber apparatus where a cylinder (familiar environment) was connected to a rectangular chamber (novel environment) via a 'U' shaped

passageway. The familiar environment contained a familiar landmark array, whereas the novel environment contained an unfamiliar landmark array. Rats should therefore not be able to orient using the unfamiliar landmarks in the same way that they could orient using the familiar landmarks. They found that the majority of HD cells maintained a constant preferred firing direction across exposure to all three sections of the apparatus, with only small shifts in firing (6°- 30°). They concluded that the only way that constant firing could occur is if the rats reliably used their path integration signal.

The main two problems however with this design is that the dual apparatus was located within the same room (and thus the rat may have used extramaze cues to navigate) and the distance the rat had to travel on the maze was relatively small. To overcome these problems, Yoder et al (2011) used a similar protocol except they used a dual apparatus design where each half was located in a separate room and was joined together by a 11m track. Additionally, they also used a T-maze that had fourteen choice points in order to make the path integration task more demanding. Even with these additional demands, the experimenters still found that HD cell firing would remain relatively stable.

The term path integration refers to three internal signals; vestibular, motor and proprioceptive information (Stackman et al., 2003). Vestibular information denotes the acceleration of the animal's head and is detected by the vestibular end organ, located in the inner ear. These organs are lined with sensory hair bundles that are able to detect changes in acceleration. When these sensory hair bundles respond to acceleration, they cause a change to the firing rate of vestibular neurons (Glasauer, 2005). One type of vestibular end organ is the semicircular canals that detect angular head acceleration. The other type of vestibular end organ is the otoliths that detect linear head acceleration. Proprioception relates to the sense of knowing the position of one's own body in space. This knowing is thought to be a result of a combination of the motion and orientation signals originating in the inner ear, and stretch receptors located in muscles throughout the body (Sherrington, 1907). Proprioception, along with vestibular information is also integrated with a motor signal. The cerebellum has been shown to play a vital role in the integration of motor information (Ito, 2002) through feedback loops (Kawato and Gomi, 1992).

A path integration signal is therefore composed of a number of distinct sets of information, originating from different locations throughout the body. Studies recording HD cells have therefore aimed to investigate the relative importance of each component of the path integration signal during HD cell firing. Indications that motor and proprioceptive

information are important in HD cell firing were demonstrated by Stackman et al (2003). They found that an HD signal became unstable when a rat was transported between rooms in an open box. Additionally, the importance of vestibular information was also demonstrated by Stackman and Taube (1997) who injected sodium arsanilate into the vestibular end organ, lesioning the area. Recordings from the ADN showed that HD cell firing was abolished in lesioned rats.

A commonly used technique to separate out a path integration signal into its components is to passively rotate the animal. This passive rotation disrupts motor and proprioceptive cues, whilst keeping vestibular cues intact. Taube et al (1990b) used this technique by wrapping a rat in a towel, rotating the animal in the horizontal plane and recording HD cells from the PoS. They found that there was a significant reduction in peak firing rate of HD cells during this passive rotation. The preferred firing direction and tuning widths of these cells remained relatively stable. This reduction in peak firing rate during passive rotation has been replicated in PoS HD cells (Golob et al., 1998) and in the ADN (Taube, 1995; Knierim et al., 1995). This reduction, but not a complete abolition of HD cell firing suggests that a combination of reliable motor, proprioceptive and vestibular signals are required for normal HD firing.

However, this finding does not appear to be universal. For example, passive rotation when rats were not tightly restrained (either loosely restrained or trained to maintain a stable head direction through drinking water) a reduction in firing rate was far less apparent (Zugaro et al., 2001b; Bassett et al., 2005). The discrepancy between these two findings could be attributed to the stress caused by tightly restraining the animal reduces the firing rate of HD cells. Alternatively, the studies which did not find a reduction in peak firing rate failed to keep the animal's head stationary.

Shinder and Taube (2011) aimed to address this discrepancy by familiarising the rat with a head restraint device in the aim to reduce stress caused to the animal. They did not find any changes to the firing rate of HD cells. Based on the findings from their 'hand held' condition however, this lack of firing rate change was not due to an absence in stress levels. The authors concluded that vestibular information is required to generate the HD signal, whilst motor, proprioceptive and allothetic cues are then required to update this signal. These collective findings suggest the path integration signal as a whole is needed for successful HD firing, but each component may play a different role in this process.

In summary, the HD system relies on both allothetic and idiothetic cues. In fact, there seems to be a consensus across the field that the integration of these two cue classes is crucial (Taube et al., 1990b; Goodridge et al., 1998; Stackman et al., 2003; Bassett et al., 2005). An intriguing question which arises from this integral relationship is how are these two cues integrated? For example, placing these cues in conflict with each other could uncover whether or not the HD system relies on these two cues equally, or whether there is an intrinsic bias. Many experiments have tested the effects of allothetic and idiothetic information on HD cell firing, yet relatively little is know about the relationship between these two cues. The response of HD cells to conflicting allothetic and idiothetic information is therefore a central theme to all three experimental chapters in this thesis.

2.7 Cue integration at a neural level

As previously discussed, human studies have shown that integration of spatial cues often occurs in an optimal manner (Van Beers et al., 2002; Knill and Saunders, 2003; Alais and Burr, 2004). The application of Bayesian inference to navigation has also been examined using non-human subjects but to a far lesser extent. Fetsch et al (2009) investigated the perceived heading in rhesus monkeys. Similar to Nardini et al. (2008), the subjects were required to integrate visual cues (optic flow) with path integration (in particular, vestibular information). Manipulating the reliability of these cues was achieved by using a virtual reality set-up, with motion platform and projector. The monkeys were presented with an initial heading direction for two seconds, after which they had to estimate their heading direction (signalled with a saccade). During this initial presentation, motion and optic flow could be manipulated slightly to produce a slight leftwards or rightwards trajectory. These two cues were manipulated using a Gaussian function to produce a psychophysical paradigm. An assessment of cue reliability was achieved by having conditions where only visual or vestibular information was available. Placing these cues in conflict then allowed the experimenters to determine whether the monkeys weighted the two cues according to their perceived reliability.

First, they found that performance using both available cues was better than using only one cue, indicating that they were integrating visual and vestibular information. Second, performance of the monkeys showed that they changed their cue weights as a function of cue reliability. Third and perhaps most strikingly, there was a general trend for subjects to overweigh vestibular information and under-weigh visual information. This could reflect the

problem of assessing the reliability of visual information in a visual-only condition. In the visual-only condition, some vestibular information would still be available; namely, the information indicating that the monkey is stationary. Alternatively, the overweighting of vestibular information could reflect an intrinsic bias in the heading direction system.

In addition to this behavioural work, Gu at al (2008) used a similar paradigm to examine the neural correlates that may underlie this Bayesian integration of visual and vestibular cues. They believed an ideal neural candidate would be located in the dorsal medial superior temporal (MSTd) areas. Neurons from this area in primates have very similar characteristics to the head direction cells found in rodents. MSTd neurons have been shown to have directional selectivity (increase firing for particular heading angles) for optic flow patterns that simulate self-motion (Tanaka and Saito, 1989; Duffy and Wurtz, 1991; Duffy and Wurtz, 1995; Schaafsma and Duysens, 1996). Specifically, the neurons in MSTd respond to changes in the translational velocity of visual stimulus or optic flow (Lee et al., 2007). Moreover, these cells still show this directional selectivity in the dark (Duffy, 1998; Gu et al., 2006), suggesting that vestibular information is utilised by these neurons. Thus, these cells might require the integration of vestibular and visual cues. Based on this prediction, Gu et al (2008) found that the width of the tuning curves for MSTd neurons were narrower when both cues were available in comparison to single cue only conditions.

The finding by Gu et al (2008) suggests that the increase in behavioural accuracy through cue integration is also expressed at a neuronal level. The next logical manipulation is to examine whether the weighting of these cues is also expressed at a neuronal level. Therefore the second experimental chapter in this thesis aimed to do this by recording HD cells in rats that had a stable path integration signal but were exposed to a directional light cue of varying reliability. Based on the work by Gu et al (2008) it would be predicted that a less reliable directional cue would produce an increase in the width of the HD cell tuning curve in comparison to a more reliable light cue.

2.8 Degree of cue conflict

Human studies assessing the applicability of a Bayesian model to spatial cue integration have found that the degree to which the spatial cues conflicted dramatically influenced cue integration. More specifically, if the perception of the two cues is too large, an integration of these cues will not occur (Cheng et al., 2007). Jack and Thurlow (1975) conducted a study using the 'ventriloquist effect' by simultaneously presenting visual information from one

location and auditory information from another location. It was found when the two stimuli were separated by more than 50° they were no longer perceived as one stimulus.

A small, but significant group of studies recording HD cells have addressed similar questions of cue integration by varying the degree of cue conflict. Goodridge and Taube (1995) allowed rats to first forage in a cylinder with a cue card attached to part of the cylinder wall. They then removed the rats, disorientated them and then returned to the cylinder. A conflict was introduced in one of two ways. If the cells had drifted by more than 30°, the cue card was returned to its original position. If the cells had not drifted, the cue card was moved 90° away from its original position. They found that for both protocols, the vast majority of cells illustrated a preference for landmark control. However, this control was significantly weaker during the 90° conflict, where some cells rotated as little as 6°.

Similarly, Knierim et al (1998) pitted visual information and path integration against each other, whilst recording HD cells from the ADN and place cells from CA1. They introduced a cue conflict of 45° or 180° (a couple of rats experienced 150° and 135°), by rotating the floors and walls of an environment whilst keeping a polarising cue card stationary. They found that HD cells and place cells rotated with the landmark rotation at 45°. At 180° however, this dominance was not observed as sometimes the HD cells rotated fully, some only rotated by half the predicted amount, and some failed to rotate at all. For those HD cells that did rotate with the cue card at a 180° cue conflict, they showed a delay in rotation. This delay suggests that the cells were using path integration information to update the directional signal and the landmark information provided more of a 'corrective' cue.

From this cue conflict study, Kneirim et al (1998) concluded that visual landmarks dominate the HD system during small conflicts (<45°) but path integration was the superior cue for larger conflicts. This finding supported previous work by Rotenberg and Muller (1997) who found that place cells would rotate when a cue card was rotated by 45° but remained stable when the cue card rotated by 180°. More stringently, Knierim (2005, cited in (Wiener and Taube, 2005) has subsequently suggested that there is a threshold of around 45°- 90°, where landmarks gain dominance over cells below this threshold. However, above 90° the landmark dominance is lost, producing variable results. These variable results during cue conflicts were also witnessed in a study by Blair and Sharp (1996).

Blair and Sharp (1996) also recorded HD cells from the ADN, whilst rats explored a cylindrical chamber. The walls and floors of this chamber could rotate together or separately to

produce a conflict. The wall was painted in a series of eight black and white stripes so that when the walls were rotated, visual motion cues were provided to the rat. As there were eight stripes, the walls were visually identical at 90° increments. The rotation of these stripes (or landmarks) only became apparent when the walls were rotated by 45°. This apparatus therefore meant that visual motion cues, vestibular cues and visual cues could all be manipulated and placed in conflict with each other. Rotating the wall by 90° would create a rotation of visual motion, a wall rotation of 45° would create a landmark rotation and rotating the floor would create a vestibular rotation.

This experiment showed that each of these three variables affected the firing direction of HD cells in the ADN. More specifically, when visual motion cues conflicted with vestibular cues, HD cells were more likely to use vestibular information. When this vestibular information conflicted with landmark information (producing a 45° cue conflict) cells showed a partial rotation. The majority of cells in this condition showed an intermediate shift in preferred firing direction, rotating on average by approximately 25°. They therefore concluded that during a cue conflict between vestibular information and visual cues, landmarks only have partial control over ADN HD cells.

This dominance of path integration information during a relatively large conflict with visual cues was also illustrated by Chen et al (1994a). This cue conflict was achieved by moving visual cues in an environment, whilst keeping the rat's path integration signal constant. Rats were exposed to an environment where four cue cards were placed around the maze (one white and three black cards). After the rats had explored the environment the lights were switched off and the set of cue cards were rearranged. During this time the rats remained of the maze with no disruption to their path integration signal. They found that the majority of cells failed to rotate with the white cue card and instead maintained a stable firing direction using their path integration signal.

Studies implementing a cue conflict (Chen et al., 1994a; Goodridge and Taube, 1995; Knierim et al., 1995; Blair and Sharp, 1996) have successfully highlighted the presence of an intricate relationship between idiothetic and allothetic cues. Using both a 45° and 180° cue conflict, Knierim et al (1998) aimed to quantify this interaction between visual and path integration cues. However, because only two levels of conflict were used for most rats, the experimenters were unable to establish exactly when the HD cells weighted path integration cues over landmark cues (only that this conflict was more then 45° and less than 180°). In

order to understand the exact nature of the relationship between these two cues, the conflict situations need to be studied in finer steps.

The final experimental chapter in this thesis therefore recorded HD cells during cue conflict situations where the conflict progressively increased. Using a similar design to the second experimental chapter, a conflict was created by moving the position of a light cue whilst the rat's path integration signal remained stable. At each level of conflict, the degree to which landmark cues and path integration influenced HD firing was estimated. This information could thus provide an insight into how the level of conflict between idiothetic and allothetic cues affects cue integration. Additionally, in this paradigm HD cells were recorded in multiple brain regions of the HD pathway. This was done to assess whether the findings from Knierim et al (1998) and Blair and Sharp (1996) from HD cells in the ADN could be generalised to other areas of the HD system. Mizumoir and Williams (1993) suggests that the path integration dominance found in ADN cells cannot be generalised to other areas of the HD network. Mizumoir and Williams (1993) recorded from HD cells in the lateral dorsal thalamic nuclei whilst rats ran in the dark on an eight-arm radial maze. During this time, the HD firing directions drifted considerably. However, when the lights were switched back on the cells fired in their original direction, even if this direction conflicted by 90°. It was therefore predicted that in the final experiment of this thesis, HD cells recorded in different areas of the HD pathway may rotate by different amounts during a cue conflict. Before this and the other two experiments are presented, the general methods used in each of the studies are described.

Chapter 3 General Methods

3.1 Subjects

All of the experiments used adult male Lister Hooded rats with an approximate average weight of 300g at the time of surgery. Before surgery, rats were housed in cages that typically consisted of two rats per cage. Immediately after surgery, all rats were housed individually in a holding room where the lights were on for 11 hours and off for 11 hours, with a 1 hour (x2) dawn/dusk simulation. One week after surgical procedures, rats were placed on a food-restricted diet that was sufficient to maintain 90% of free-feeding weight. They also had *ad libitum* access to water. All procedures were licensed by the UK Home Office subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act 1986.

3.2. Experimental room

All experiments were carried out in a room measuring 450 x 330cm. In the centre of the room was a circular rail of thick black curtains, which created an enclosure with a diameter of 250cm. All experiments were carried out inside the curtains. In the centre of this enclosure was a video camera, attached to the ceiling. The recording equipment was always situated in the eastern region of the room (Fig 3.1). The temperature and air flow of the room was matched to the conditions in the holding room. The lighting conditions in the experimental room varied between each experiment. The lighting conditions are therefore described separately in each experimental chapter.

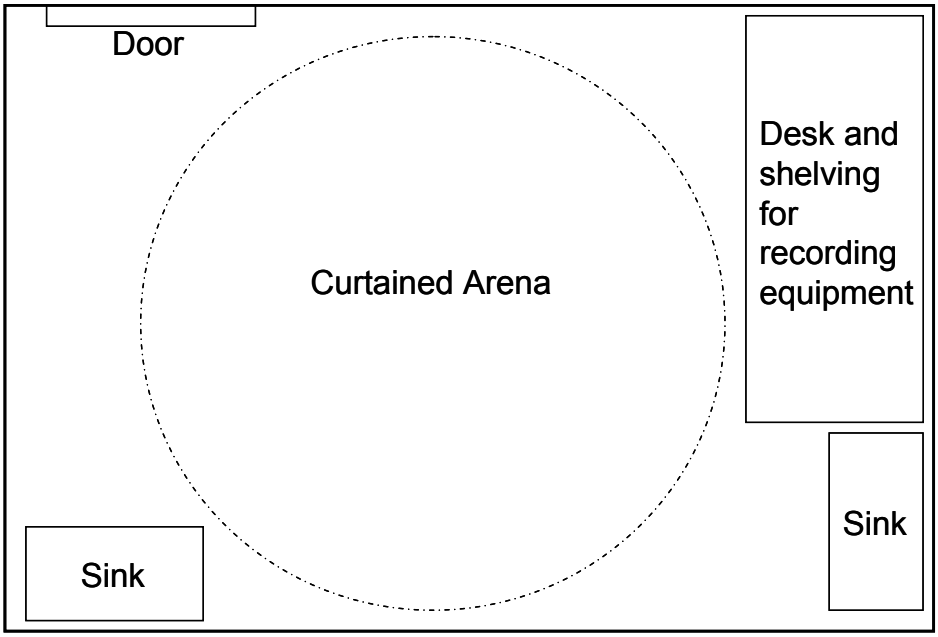

Figure 3.1: Schematic drawing of the experimental room

3.3 Electrodes and microdrives

All rats were implanted at the start of the experiment with moveable microelectrodes. These microelectrodes were held in assembly by a 16 channel microdrive (Fig 3.2) (Axona Ltd, St Albans, UK). The microdrive consisted of a precision screw held under spring tension. This allowed the electrodes to be lowered or raised with one full turn of the screw equal to an increment of 200µm (dorso-ventrally). The four tetrodes were constructed from four interwound 25-µm diameter platinum-iridium (H-ML insulated) electrodes (California Fine Wire, USA). For the top 5mm of each tetrode, the wires were separated and heated, stripping them of their insulation.

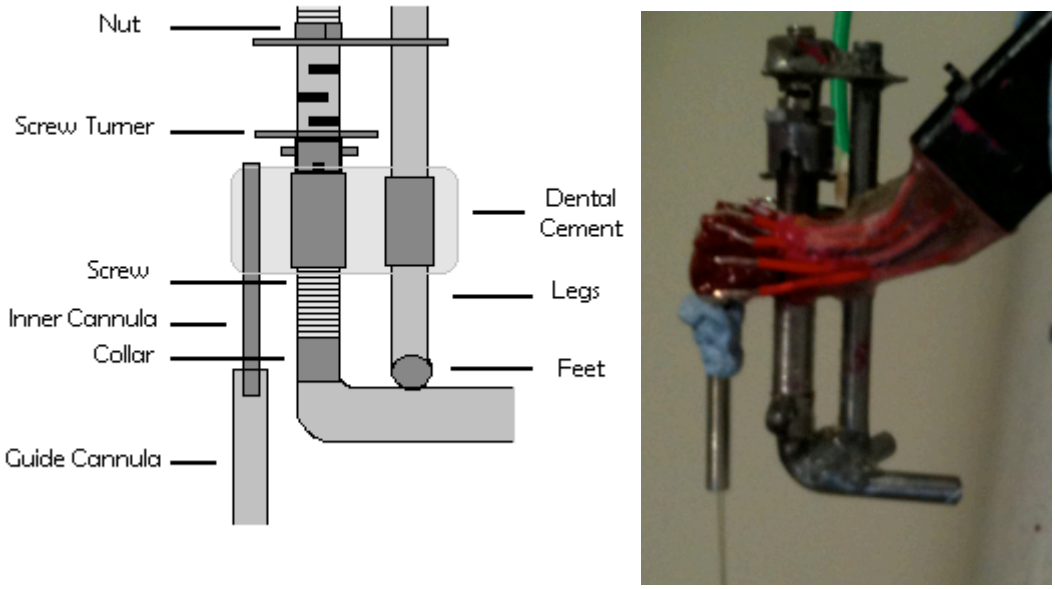

Figure 3.2: Schematic drawing of the microdrive. Along with a photograph of a wired microdrive

The four wire bundle was then threaded through an inner 21 gauge cannula (12mm in length) which was surrounded by a 19 gauge guide cannula (8 mm in length), until all of the interwound section of the bundle had been threaded through. The remaining separated electrodes were individually wrapped round each of the microdrive's wires, so that each electrode was connected to a single recording channel. These wires were secured using silver conductive paint. After all four tetrodes had been wired the entire area was covered in nail varnish to protect it. Just prior to surgery, the wires were cut so that the exposed length of wire measured 8mm. These wires were then placed in a saline solution whilst a current was passed through them. This was done by connecting a battery to each of the 17 pins on the microdrive (the pins which allow the microdrive to be connected to the recording equipment). The formation of bubbles at the end of each tetrode confirmed that a current could pass along the microdrive and through the electrodes. The seventeenth pin was checked to make sure that no bubbles were formed as this was connected to the grounding wire on the drive. Cross connections could also be checked during this procedure.

3.4 Surgical procedures

Before surgery commenced, sterile conditions were created by spraying surfaces with a solution of chlorhexidine gluconate and ethanol (Hydrex, Adams Healthcare, UK). Surgical

items and surgical clothing were autoclaved. Animals were anaesthetised with isoflurane and oxygen (3 L/min) inside an anaesthetic chamber. The animal's head was shaved to expose the surgical site and injected subcutaneously with 0.2ml of enrofloxacin (Baytril) and intramuscularly with 0.1ml of buprenorphine (Vetergesic). The anaesthetic gas was then switched to the mask on the stereotaxic frame. The animal's teeth were placed over a bar just in front of the anaesthetic mask. Once all pain reflexes ceased, the animal was placed on the stereotaxic frame with lambda and bregma in the horizontal plane, by inserting ear bars into each ear and gently securing a nose bar. The body temperature was kept stable using heat pads, and eyes were protected with carbomer (Viscotears, Novartis Pharmaceuticals Ltd).

Iodine was placed on the top of the animal's head before an inch long incision was made. Haemostats were attached to the skin surrounding the incision site (upper and lower left and right) in order to expose bregma and lambda. Stereotaxic measurements were then taken to ensure the skull was flat. Additional measurements were taken to mark the implant site. Seven holes were then drilled using a 1.0mm drill bit and 1.6mm diameter screws were then inserted into each hole. One of these screws was soldered to a ground wire to enable the animal to be electronically grounded. The entry hole for the electrodes was drilled using a 2.7mm trephine bit, and the circular section of the skull was removed.

The implant coordinates were re-measured by attaching the microdrive to the stereotaxic frame. The drive was adjusted so that the electrodes were lying at 90° from the skull's surface. The dura was incised at the implant site and the electrodes were carefully lowered by a specific depth measurement. The metallic guide cannula was pulled down over the remaining exposed wire. Sterile Vaseline (Lever Fabergé, Germany) was placed around the bottom of the guide cannula. In order to attach the microdrive to the skull, dental acrylic was placed on the skull, covering the microdrive's feet. Once the acrylic had set, the animal was removed from the anaesthetic mask and the Viscotears (Carbomer) was removed from the eyes. The animals were then monitored periodically until they awoke.

All animals were given meloxicam (Metacam) three days post surgery. The meloxicam was administered to the animals by adding 0.15ml to 0.85ml of jelly per portion. One portion was given once a day for three days post surgery. For one week after surgery they were given *ad libitum* access to food and water, during this time they were kept in their home cage to recover.

3.5 Single unit recordings

Recording commenced 1 week after surgery. Recording was done using multichannel recording equipment (DacqUSB Axona Ltd). The rats were connected to the recording device via lightweight wires attached to the microdrive. The potentials recorded on each of the 16 electrodes of the four tetrodes were passed through AC-coupled, unity gain operational amplifiers mounted on the rat's head (headstage) and fed to the recording system. The signal was amplified (~20 000 times), bandpass filtered (500 Hz – 7 kHz) and then collected and stored on a computer for offline analysis. Each of the four wires of one tetrode was recorded differentially with respect to one of the wires of one of the other tetrodes. In other words, the channel from one tetrode was referenced against a channel on another tetrode in order to subtract common noise (e.g. a chewing artefact). One of the recording channels was dedicated to acquiring the EEG signal. In order to determine the direction of the rat's head, a directional head-stage was used, where 2 LEDs of differing sizes (one large and one small) were positioned opposite each other. The angle of the lights with respect to the head was noted. The distance between the two lights was 8cm. This directional headstage was used along with a video camera attached to the ceiling, in order to track the rat's location and head direction. The tracking software could differentiate between the large and small lights and thus provided the information needed to infer the head direction of the rat in space. During screening, the oscilloscope was monitored to check for spiking. If no single cell activity was present, the electrodes were lowered (~50 μm). Screening for HD cells took place in a room separate from the actual experimental room, to minimize the learning of extraneous cues in the recording environment by the rats. During screening, rats were encouraged to sample the space by foraging for sweet cereal. If no head direction cells were found, rats were returned to their home cage for at least four hours before the next screening session. If head direction cells were found, one of the experimental protocols was run. The experimental protocol for each experiment is described in their respective chapters.

3.6 Data analysis

3.6.1 Cell isolation

Cluster-cutting software (Tint, Axona Ltd) was used to analyse the data offline. The peak-to-peak amplitude of one electrode was plotted against another electrode. Given the separation of the four electrodes within a tetrode, the activity of one cell is recorded by each electrode, but with varying amplitudes depending upon the proximity of the cell to the electrode. This means that spikes from one cell typically cluster on the peak-to-peak amplitude plot, which allowed cells to be isolated by hand (Fig 3.3). The waveform for the isolated spikes could be produced using the Tint software (Fig 3.4). A subjective visual examination of the waveform allowed confirmation of 1) an action potential and 2) identification of the same cell by comparing waveforms across trials and days.

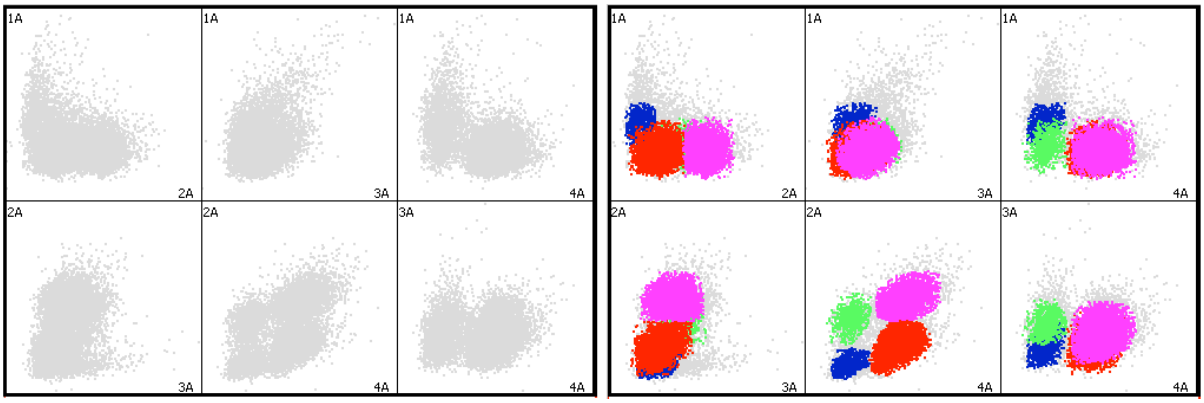

Figure 3.3: *Left* – Peak-to-peak amplitude plot from Tint Software. Each dot represents one spike, spiking from the same cell typically clusters in space, *Right* – allowing the clusters to be manually identified and cut into clusters, which allows spikes to be assigned a cell number (i.e. colour).

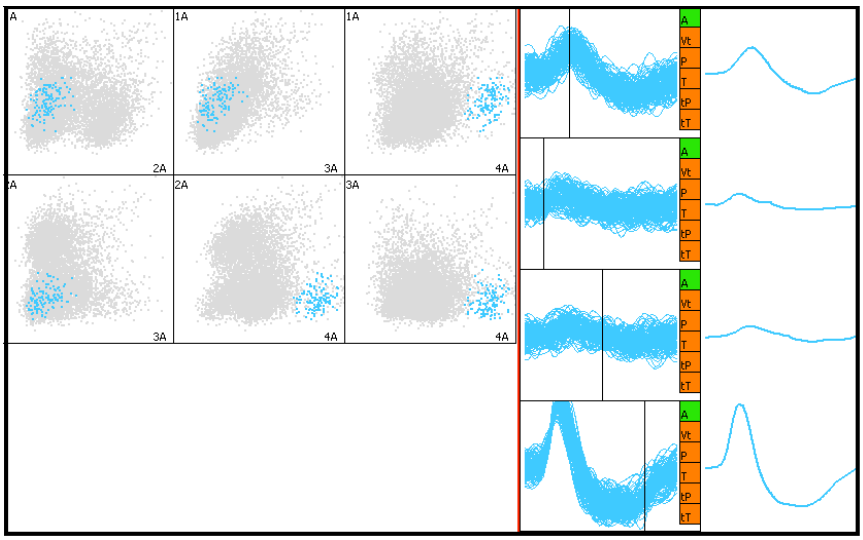

Figure 3.4: The right panel shows the waveforms of one cell isolated by Tint Software. Each waveform is produced by the four electrodes within a tetrode. The differing amplitudes of each waveform can be seen, with the bottom waveform showing the highest amplitude, therefore this electrode was the most proximal to the cell.

3.6.2 HD cell analysis

Once cells were isolated, polar plots were created in Tint in order to determine each cell's directional activity. This was done by plotting the spike rate against head direction, using a smoothing kernel of 5 bins (Left plot in Fig 3.5). Plotting this information on a linear graph using Matlab (The Mathworks, Natick, MA, USA) produced a HD cell tuning curve (Right plot in Fig 3.5).

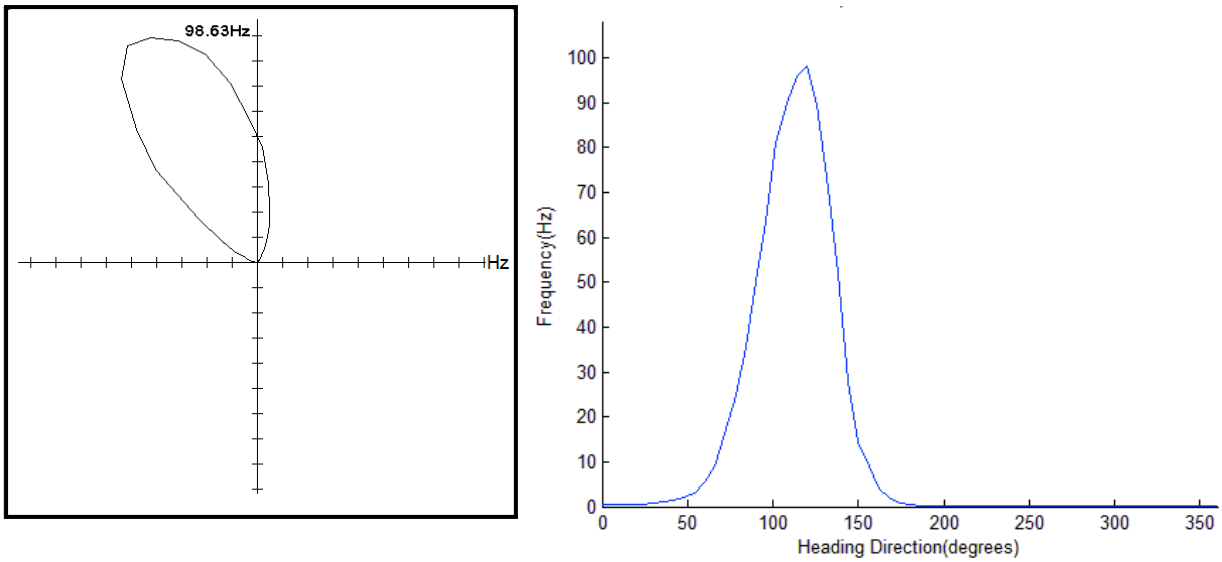

Figure 3.5: One HD cell plotted on a circular and linear plot. To produce these two head direction plots, the firing of one HD cell and the direction in which the animal's head was facing during the firing is recorded. This information is then smoothed using a smoothing kernel of 5°. This information can then either be plotted on a circular graph (*left)* to produce a polar plot, with firing rate on the polar axis (measured in Hz), or it can be plotted on a linear graph *(right)*, with head direction on the X axis (in degrees) and firing rate on the Y axis (in Hz).

Five parameters were used to assess the characteristics of each HD cell: mean firing direction, preferred firing direction, directional firing range, peak firing rate and resultant vector length. In order to calculate each cell's mean firing direction, peak firing rate and directional firing range, the animal's head direction was divided into sixty 6° bins and smoothed using a smoothing kernel of 5° in Matlab (The Mathworks, Natick, MA, USA). The preferred firing direction was defined as the directional bin with the highest average firing rate, and thus can be equated to the mode of the tuning curve (Fig 3.6). As the mode does not take into account all data points, the mean firing direction was also calculated. The peak firing rate was defined as the average firing rate of the preferred firing direction (Fig 3.6). All cells were required to meet a firing rate criterion whereby cells with a peak firing rate of less than 1.0 Hz across the session were excluded from further analysis.

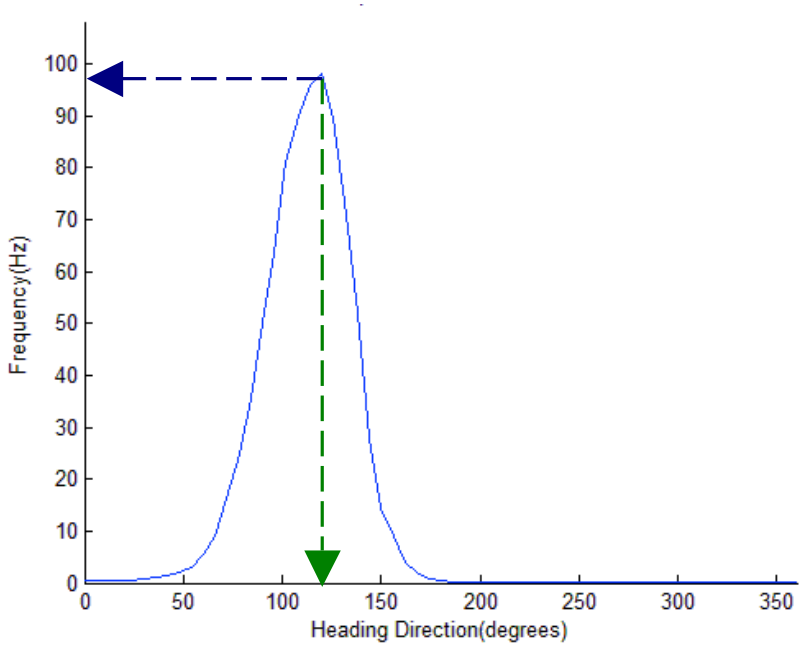

Figure 3.6: A linear plot of a HD cell tuning curve. The green dotted line indicates the cell's preferred firing direction, in this case 120°. The blue dotted line indicates the cell's peak firing rate, in this case 98 Hz.

In order to calculate the directional firing range, a triangle was fitted to a firing rate versus HD plot in Matlab. Using Taube, Muller, & Ranck, Jr., (1990a) criteria, left and right maximum, and left and right minimum data points were selected to form a triangular shape. A linear set of values were then extrapolated for the right and left legs. The base of the triangle (where the two legs intersect the x axis) was then taken as the directional firing range (Fig 3.7).

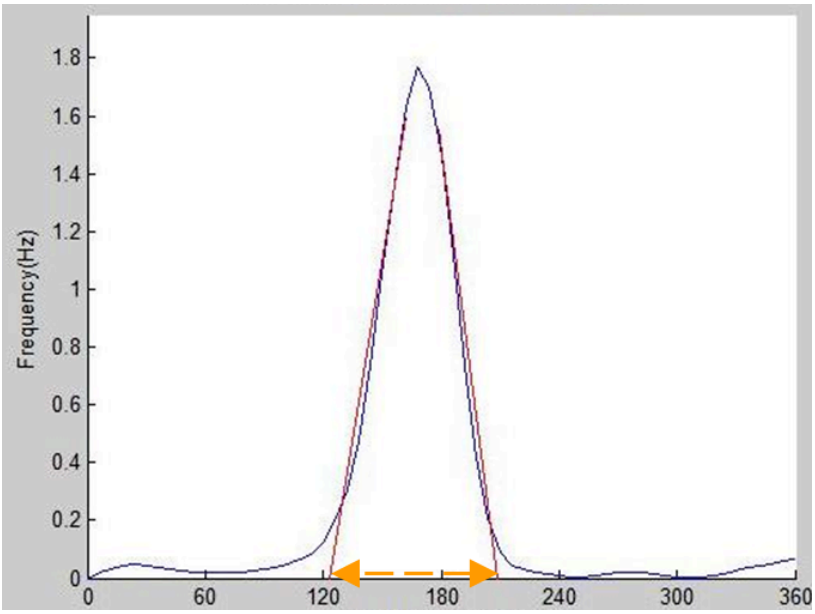

Figure 3.7: A linear plot of an HD tuning curve produced in Matlab. The two red lines are fitted to the tuning curve in order to assess the width of the tuning curve. The orange line indicates the directional firing range of this cell, i.e. where the two red lines intersect the X axis, in this case the directional firing range of this cell is 78°.

In order to assess the degree to which the data deviated from the circular mean, resultant vector lengths were calculated on unsmoothed data. Thus, resultant vector lengths ('R') measure the circular spread of data. In order to calculate the resultant vector length the circular mean value of the angular data is calculated. This is done by first converting the angular data from degrees into radians and then transforming the angular data into vectors:

$$r_i = \begin{pmatrix} \cos \alpha_i \\ \sin \alpha_i \end{pmatrix}$$

These vectors are then averaged to produce a mean resultant vector ('r'):

$$\bar{r} = \frac{1}{N} \sum_i r_i.$$

This value is then normalised to produce the length of this mean resultant vector ('R'):

$$R = \|\bar{r}\|$$

Resultant vector lengths ('R') range from 0-1. Resultant vector length 'R' values that are closer to 1 indicate less variance in the data set. HD cells that had an 'R' value of less than 0.3 within a given session were excluded (van der Meer et al., 2010). The HD cell in Figure 3.7 has an R value of 0.54. Inferential statistics were calculated using the CircStat Matlab toolbox (Berens, 2009). Details of these statistical tests are described in the methods section for each experimental chapter.

3.7 Histology

Once the experiments were finished, the rats were then deeply anaesthetised with isoflurane before they were injected with sodium pentobarbital until the animal's breathing ceased. They were then transcardially perfused using saline and then paraformaldehyde (4%). The brains were removed and stored in paraformaldehyde (4%) for at least 1 week before sectioning. The brains were sliced at 40 micrometers on a freezing microtome. The sections were then mounted and stained with Cresyl violet. This staining method firstly involved hydrating the sections by exposing them to solutions containing progressively more ethanol. They were then left in Histoclear for at least ten minutes. After this, they were re-hydrated in solutions which were progressively weaker in ethanol. They were then left in the Cresyl violet dye for a period of ~ 15 minutes, followed by an exposure to solutions containing progressively more ethanol. Finally they were left in Histoclear for 10 minutes. Cover slips were then glued to the upper surface of the slides and left to dry. The slides were then observed under a DM750 microscope (Leica, UK) in order to determine the site of the electrode track.

Chapter 4 Influence of geometric cues on HD cell firing

4.1 Introduction

A large number of behavioural studies have examined the influence of geometric cues during navigation (Cheng, 1986; Hermer and Spelke, 1994; Pearce et al., 2004; Cheng and Gallistel, 2005; Graham et al., 2006; McGregor et al., 2006; Pecchia and Vallortigara, 2010a; Pecchia and Vallortigara, 2010b). This body of work has suggested that although geometric features are used during navigation, their use is affected by the integration of non-geometric information (reviewed in Cheng and Newcombe (2005) including landmarks or path integration (Margules and Gallistel, 1988; Hermer and Spelke, 1996; Garrad-Cole et al., 2001; Wall et al., 2004; Esber et al., 2005; McGregor et al., 2006; Pecchia and Vallortigara, 2010a; Reichert and Kelly, 2011). It is also thought that the species (Gray et al., 2005), the size of the environment (Chiandetti and Vallortigara, 2008) the amount of experience in the environment (Kelly et al., 1998) and whether the animal or subject is disoriented (Margules and Gallistel, 1988; Lew et al., 2006; Lourenco and Huttenlocher, 2006; Batty et al., 2009) affects the influence of geometric cues. The contribution of geometric processing to heading computations thus remains poorly understood (Nardi and Bingman, 2009). This current study therefore used the HD signal to explore the role of geometric cues in heading determination (published in Knight et al, J Neurosci 2011). More specifically, this current study wanted to determine which aspects of geometry are processed by HD cells and whether geometric cues from multiple sources can be integrated by the HD system.

In order to process the overall shape of an environment, multiple geometric sources within the environment need to be integrated. For example, identifying the overall shape of an environment requires comparative judgements to be made about the length of walls or the angle of corners. There is a current debate in the behavioural literature as to whether animals can process the global geometry of an environment (see Chapter 2.5.1 for a detailed description). In order to shed new light onto the debate of local versus global geometric processing, this current study took recordings of HD cells during exposure to three distinctly polarized geometric environments; a trapezoid, an isosceles triangle and a teardrop shape (Table 4.1). The three environments varied in the degree to which rats needed to integrate geometric cues in order to orient. The trapezoid was mirror-symmetrical and therefore the

cells would need to either differentiate wall length, or simultaneously process corners and walls in order to use this geometric information to orient. This geometric ambiguity was reduced in the isosceles triangle as this environment contained both a unique wall length and a unique angle. For the cells to use geometric information in this environment they would either have to compare wall length or size of angle. The teardrop environment was therefore chosen because there was only one salient geometric feature, the apex. The cells could therefore use geometric information in this environment even if they could not make any comparative geometric judgements. Moreover, geometric processing should occur in the teardrop regardless of whether geometric processing occurs at a global (Cheng, 1986) or local (Pearce et al., 2004) level.

Rats were exposed to these environments in sessions which contained five trials in each. Between each trial the environments were rotated by either 90° or 180°. Previous HD cell findings have shown that disruption to path integration can prevent a rat forming a stable relationship between their internal sense of direction and their environment (Knierim et al., 1995), thus for the majority of this study (conditions 1-5, see Table 4.1) rats were not deliberately disorientated between trials. Consequently in these conditions, the rat's path integration signal would have conflicted with the information provided by the geometry. If HD cells use geometric information to orient, the HD cells' tuning curves would consequently rotate when the environment rotated. If however, the cells used the path integration signal or an uncontrolled extramaze cue, the cells would not rotate.

No.	Condition	Geometry	Path Integration	Visual Landmark
1st	**Trapezoid**		Orientated	Absent
2nd	**Triangle**		Orientated	Absent
3rd	**Teardrop**		Orientated	Absent
4th	**White Cue Card**		Orientated	Present
5th	**Grey Cue Card**		Orientated	Present
6th	**Disorientation**		Disorientated	Absent

Table 4.1. A breakdown of each of the six conditions, along with the type of geometric cues available in each condition (environments not drawn to scale), the manipulation to path integration, and whether or not visual landmark information was available.

Early work using these three 'geometry only' conditions revealed that HD cells did not reliably follow the geometry of the environment when the environments were rotated. The surprising failure of HD cells to rotate with the above enclosures could potentially be due to one of two reasons 1) HD cells cannot process geometric information, 2) HD cells can process geometric information but they chose instead to use a relatively stable path integration signal and/or uncontrolled extramaze cues. This later reason could be further extended to the idea that HD cells can use geometric information but were unable to in this situation as all the walls in the environments were black and the rats visual system was unable to detect the corners. In order to address the issue of visual saliency, fourth and fifth conditions were introduced (Table 4.1). An A3 white cue card was attached to the shortest wall of the isosceles triangle to ensure that HD cells do indeed use visually salient landmarks to orient. An A3 dark grey cue card was then attached to the triangle in order to match the visual contrast present in the geometry only conditions.

Findings from the fourth and fifth conditions would therefore provide information about the role of visually saliency in geometric and non-geometric processing. The findings from these two conditions however could not determine whether the cells were unable to use geometry or whether the cells chose not to use geometry when other (conflicting) cues were available. Thus a sixth condition (Table 4.1) was created to remove the arguably most accessible conflicting cue – path integration. In order to achieve this, the rat was disoriented between exposures to the triangle. This procedure of disorientation is typically used in behavioural experiments (Cheng, 1986; Wall et al., 2004; Pecchia and Vallortigara, 2010a), and has also been shown to modulate the learning of geometric cues (Batty et al., 2009).

4.2 Materials and methods

4.2.1 Subjects

Sixteen adult male Lister Hooded rats (weighing between 273 - 465 g at the time of surgery) were housed individually [11:11 light:dark, with 1 hour (x2) simulated dawn/dusk] on a food-restricted diet (sufficient to maintain 90% of free-feeding weight) with *ad libitum* access to water. All procedures were licensed by the UK Home Office subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act 1986.

4.2.2 Apparatus

The experiment was carried out in the experimental room described in the General Methods. Inside this room, a circular arena of 250cm diameter was surrounded by thick black curtains. The arena was lit directly above by lights emitting approximately 250 LUX of light, which is approximately the same light level as an indoor office. Adjacent to the light was a video camera, to record the location and orientation of the rat, and a small radio generating white noise. Recordings in this arena took place inside one of three geometric enclosures, which were constructed from hardboard, painted black and had no floor or ceiling (Fig. 4.1A). The enclosures comprised a trapezoid (trapezium), an isosceles triangle and a teardrop, chosen for the reasons outlined below.

Trapezoid: The trapezoid was regular (mirror-symmetric), with parallel walls of lengths 60 cm (short wall) and 120 cm (long wall), and angled end walls both of length 70 cm. The height of the walls was 60cm. This enclosure had two pairs of congruent angles of 65° and 115°, respectively. Thus, any ability of the directional system to use geometry to orient

would require the system to either discriminate wall length (since there was a unique shortest wall and a unique longest wall) or an ability to integrate information from more than one corner, or from a corner and a wall, simultaneously.

Isosceles triangle: The triangle had two equal long walls of length 122cm and a single short wall of length 60 cm, making a single unique angle of 30° at the apex and two congruent angles of 75°. The height of the walls was 60 cm. The presence of one very obvious, narrow corner makes this environment more polarised in terms of geometry in comparison to the trapezoid. Thus, it was predicted that the isosceles triangle would enhance the likelihood that HD cells would use this feature of the geometry to orient. However, if HD cells or the system utilised by these cells are unable to make comparative judgements about wall length or degree of angle, the three walls or three angles may be difficult to differentiate and thus unable to aid navigation. This may be particularly problematic if geometric processing does occur at a local rather than global level (McGregor et al., 2006)

Teardrop: The teardrop was therefore introduced to further remove geometric ambiguity by removing the two congruent angles. Thus, the teardrop possessed only a single corner, of angle similar to that in the triangle (30°). Thus, regardless of whether geometric processing occurs at a global or local level, if HD cells can use corners as a geometric cue, then the teardrop should provide unambiguous polarising information to them.

Triangle plus grey or white cue card: Cue cards were attached to the isosceles triangle in order to encourage HD cells to use the external cues to orientate and to provide a comparison to the geometry only conditions. Cue cards have been used extensively in previous HD cell studies and have overwhelming shown a strong ability for the cards to gain control over the cells (Taube et al., 1990b; Zugaro et al., 2000). Based on this previous work, it was expected that HD cells would reliably rotate with the cue cards in this experiment. In order to compare this landmark information to geometric information, a cue card of high contrast (white) or low contrast (dark grey) was introduced to the black walls of the isosceles triangle. This later card therefore matched the visual contrast of the corners in the geometric only conditions.

For each session, one of these enclosures was placed in the centre of the arena, directly onto two black plastic sheets which could be wiped and rotated between trials in order to scramble olfactory cues. The orientation of the environments on the first trial of each day was pseudo-randomised so that the orientation was never the same for consecutive days. In

order to maximise the chance of the rats relying on the geometric properties of the shape for orientation, no polarizing extramaze cues were placed anywhere in the in the arena. For cue card trials, either a white or a grey A3 card was taped to the inside of the isosceles triangle's shortest wall (Fig. 4.1A.).

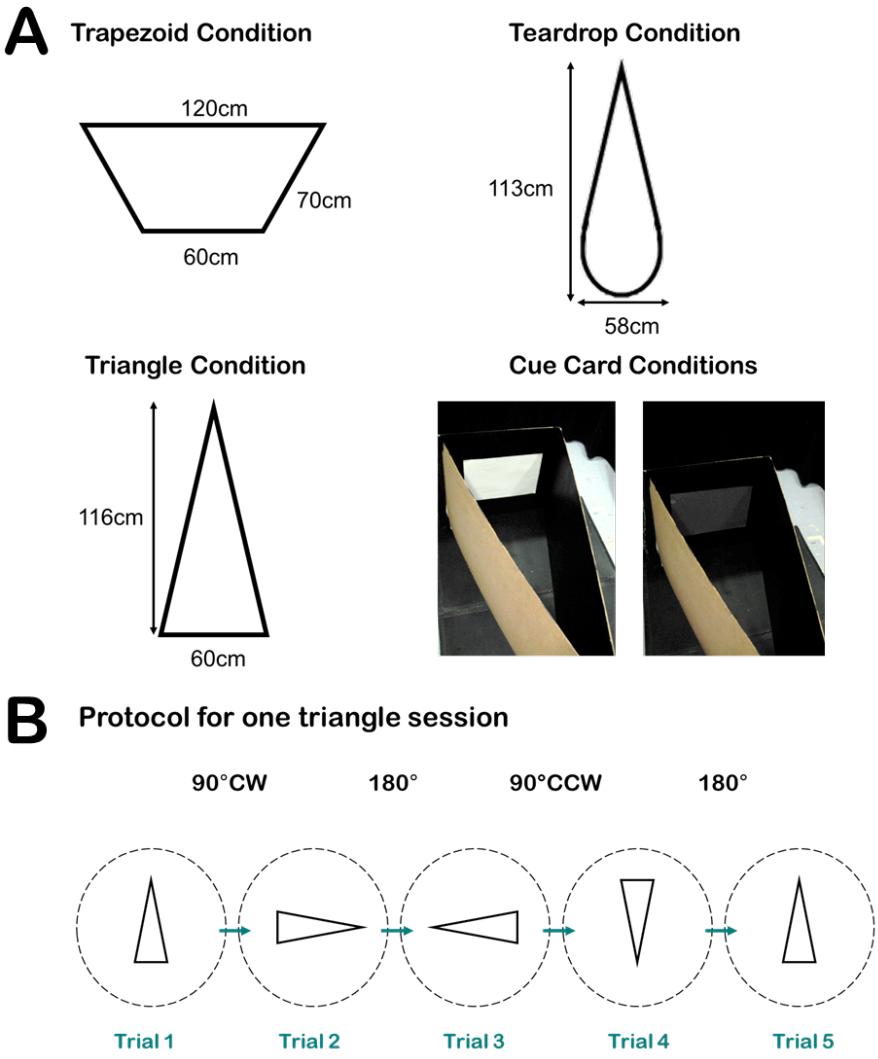

Figure 4.1 A: The dimensions of the environments used for each condition, along with photos of the A3 cue cards attached to the shortest wall of the isosceles triangle. All environments were constructed using hardboard and painted matt black. The height of all the environments measured 60cm. The teardrop shape environment was constructed from a flexible piece of hardboard inserted inside the isosceles triangle. **B:** An example of a protocol used in one session in the triangle condition. The outline of a triangular environment indicates how the environments were rotated between trials inside the curtained environment. Each session consisted of 2 x 180° and 2 x 90° (CW and CCW) rotations. In order to achieve this, 90° and 180° rotations had to be interleaved, which meant that four sequences of rotations were possible. These four sequences were pseudo-randomised.

4.2.3 Surgery and electrodes

All rats were implanted at the start of the experiment with moveable microelectrodes. Description of these microelectrodes and surgical procedures can be found in the General Methods section. The electrodes were lowered into the brain in areas known to contain HD cells: either the postsubiculum (PoS) (Bregma coordinates: 6.7 AP, 2.8 ML, 1.6 DV), the anterodorsal thalamic nucleus (ADN) (-1.8 AP, 1.4 ML, -2.1 DV), the retrosplenial cortex (RSP) (-5.5/-6.3 AP, 1.3/1.5 ML, -1.2/-2.3 DV) or the lateral mammilary nuclei (LMN) (-4.6 AP, 0.4 ML, 9.0 DV, 5°). Four HD cells were also recorded from two animals which were implanted for a separate experiment using coordinates for the CA1 region of the hippocampus (-3.0/-3.8 AP, 1.8/-2.2 ML, -1.5 DV), which has been shown to contain HD cells (Leutgeb et al., 2000). All animals were given at least one week to recover following the surgery.

4.2.4 Screening procedures

Recording commenced one week after surgery. Recording was done using multichannel recording equipment (DacqUSB, Axona Ltd). Screening for HD cells took place in a room separate from the actual experimental room, to minimize the learning of extraneous cues in the recording environment by the rats. A description of the screening procedures can be found in the General Methods.

4.2.5 Recording procedures

When an HD cell was found, the rat was taken into the experimental room in an opaque holding box. They were then placed on a raised holding box outside of the curtained enclosure in order to acclimatise to the room for five minutes. The rat was connected to the recording device outside of the curtained enclosure, then returned to the holding box and carried into the enclosure through one of three joins in the curtains and placed in a pseudorandom location on the floor. After a minute, the rat was taken out of the holding box and placed in the centre of the geometric enclosure. The point of entry into the enclosure was varied for each trial. The lightweight wires were clipped to two pieces of string suspended from the ceiling. The recording session, which comprised of five individual trials then commenced. Food was thrown into the environment in order to encourage rats to sample the space. During each trial the empty holding box was moved to a new location in the enclosure. Each trial lasted for 240 seconds, after which the wires were unclipped from

the string and the rat was removed from the environment. The rat was placed back in the holding box which now occupied a new location in the enclosure.

Whilst the rat was in the holding box, the two floor cards were flipped, shuffled and cleaned with water. The geometric enclosure was also flipped upside down and rotated by either 90° (clockwise or anti-clockwise) or 180°. Rotations were pseudorandom, so that each recording session contained a 90° clockwise rotation, a 90° anti-clockwise rotation and two 180° rotations, thus the shape aligned each of the compass axes once (N,S,E,W), except the last trial when the shape's orientation was the same as that in the first trial (Fig. 4.1B). In order to fulfil this requirement, there were only four possible rotation schedules (Table 4.2). These four schedules were also pseudorandomised so that the same schedule was not used on consecutive days and each schedule was carried out across all of the six conditions.

	Rotation 1 →	Rotation 2 →	Rotation 3 →	Rotation 4
Schedule 1	90°AC	180°	90°CW	180°
Schedule 2	180°	90°AC	180°	90°CW
Schedule 3	90°CW	180°	90°AC	180°
Schedule 4	180°	90°CW	180°	90°AC

Table 4.2: The four possible schedules for a session given that each session had to contained a 90° clockwise rotation, a 90° anti-clockwise rotation and two 180° rotations, where the shape aligned each of the compass axes once (N,S,E,W), except the last trial when the shape's orientation was the same as that in the first trial

Before the rat was placed back in the newly rotated environment, it either did or did not undergo a disorientation treatment. For five out of the six conditions the rat's path integration was not disrupted, because previous findings have shown that disruption to path integration can prevent a rat forming a stable relationship between their internal sense of direction and their environment (Knierim et al., 1995). However in the remaining

'disorientation' condition, the holding box was manually spun by the experimenter, whilst they walked around the curtained enclosure for approximately one minute.

Then, for all six condition types, the rat was then taken out of the holding box and placed back in the centre of the shape for a further 240 second trial. The protocol was repeated until the rat had been recorded in the five trials, at which point the session was complete and the rat was taken out of the curtained enclosure in the holding box, unplugged and returned to the home cage. The presentation order for each condition was pseudorandomised, ensuring that each of the six conditions was the first condition for at least one rat and the last condition for at least one rat (Table 4.3).

Rat	Location	1st Condition	2nd Condition	3rd Condition	4th Condition
R318	CA1	Triangle x 2			
R321	PoS	Trapezoid x 4	Triangle		
R330	CA1	Trapezoid			
R333	ADN	Trapezoid	Triangle		
R344	PoS	Triangle x 5	Disorientation	Trapezoid	
R371	PoS	White Card	Triangle	Trapezoid	
R388	RSP	White Card			
R404	RSP	Grey Card	Teardrop		
R409	ADN	White Card	Teardrop	Triangle	Grey Card
R410	ADN	Grey Card			
R420	ADN	Grey Card x 2	White Card x 2	Grey Card	Disorientation x 2
R1693	LMN	Triangle			
R1696	RSP	Grey Card	Teardrop x 2		
R1697	RSP	White Card	Teardrop		
R1704	RSP	Teardrop	White Card		
R433	PoS	Disorientation x 2			

Table 4.3 The presentation order of conditions for each rat. All conditions were presented first for at least one rat, and presented last for at least one rat in order to counterbalance any order effects. The table also presents the brain area where each rat was implanted.

4.2.6 Data analysis

Cluster-cutting software (Tint, Axona Ltd) was used to analyse the data offline. A description of the cell isolation process can be found in the General Methods. Each cell's mean firing direction, peak firing rate, directional firing range and resultant vector length for each trial was calculated using the procedures detailed in the General Methods. All cells were required

to meet a firing rate criterion whereby cells with a peak firing rate of less than 1.0 Hz across the session were excluded from further analysis. HD cells which had an 'R' value of less than 0.3 within a given session were also excluded (van der Meer et al., 2010). The HD cell in Figure 4.2A has an R value of 0.66 pre-180° rotation (solid line).

At this point in the analysis, any subsequent calculations were performed using the circular mean values of cells that had been recorded simultaneously, i.e., as an ensemble (ranging from 1 to 7 cells), as HD cells from a single animal always act in concert and react together to environmental changes (Taube et al., 1990b). All analysis was also done using the CircStat Matlab toolbox (Berens, 2009). In order to analyse the rotation of HD cells from one trial to the next, the circular mean direction of cells in the first trial was subtracted from the circular mean direction of cells in the second trial (Fig. 4.2) to provide a measure of how far the ensemble had rotated. This was repeated for each subsequent trial of the session so that each session produced four rotation estimates. These mean rotations were then subtracted from the predicted angle of shift (based on how much the environment had rotated) to produce absolute deviations from expected rotation. The mean vector length of absolute deviations for each condition was calculated. The Rayleigh test (Berens, 2009) was then used to determine whether these absolute mean deviations clustered around a particular direction. Circular one- and two-sample t-tests (Berens, 2009) were used to compare absolute mean deviation values against zero (the predicted deviation given perfect enclosure-following), and to compare absolute mean deviation values between conditions respectively . A mean absolute deviation which was not significantly different from zero indicates that the HD cells followed the rotation of the shape.

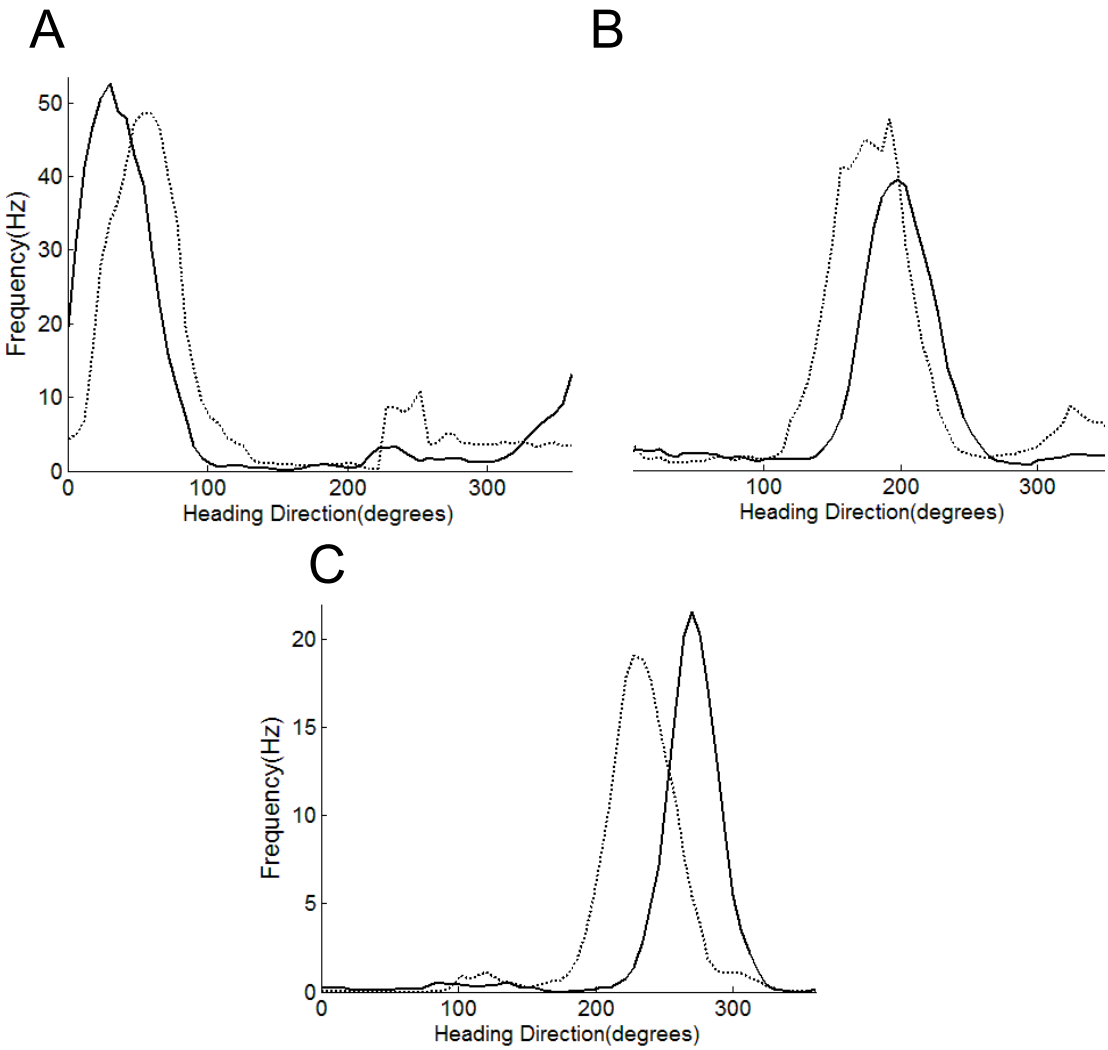

Figure 4.2: An example of one HD cell from the PoS (A) the ADN (B) and the RSP (C). **A**: PoS cell during a 180° rotation in a trapezoid trial. The plot shows the tuning curve of an HD cell pre-rotation (solid line) and post-rotation (dotted line). The HD cell for the pre-rotation session had an r value of 0.66. For each rotation a mean shift was calculated by taking the mean of the pre-rotation tuning curve and subtracting it from the mean of the post-rotation tuning curve. For this example, the mean shift was 22.16°. **B:** ADN cell during a 90° rotation in a teardrop trial, where the cell had a pre-rotation r value of 0.71. **C:** RSP cell during a 90° rotation in a teardrop trial, where the cell had a pre-rotation r value of 0.88. Statistics showed that HD cells in the ADN had significantly higher peak firing rates in comparison to PoS HD cells. There were no significant differences in directional firing ranges and r values of HD cells in the PoS, ADN and RSP.

4.2.7 Histological analysis

At the end of testing, the rats were then deeply anaesthetised with isoflurane before they were injected with sodium pentobarbital. They were then transcardially perfused using saline and then paraformaldehyde (4%). The brains were removed and stored in paraformaldehyde (4%) for at least one week before sectioning. The brains were sliced at 40 micrometers on a freezing microtome. The sections were then mounted and stained with

Cresyl violet (Fig. 4.3). A full description of this process can be found in the General Methods section.

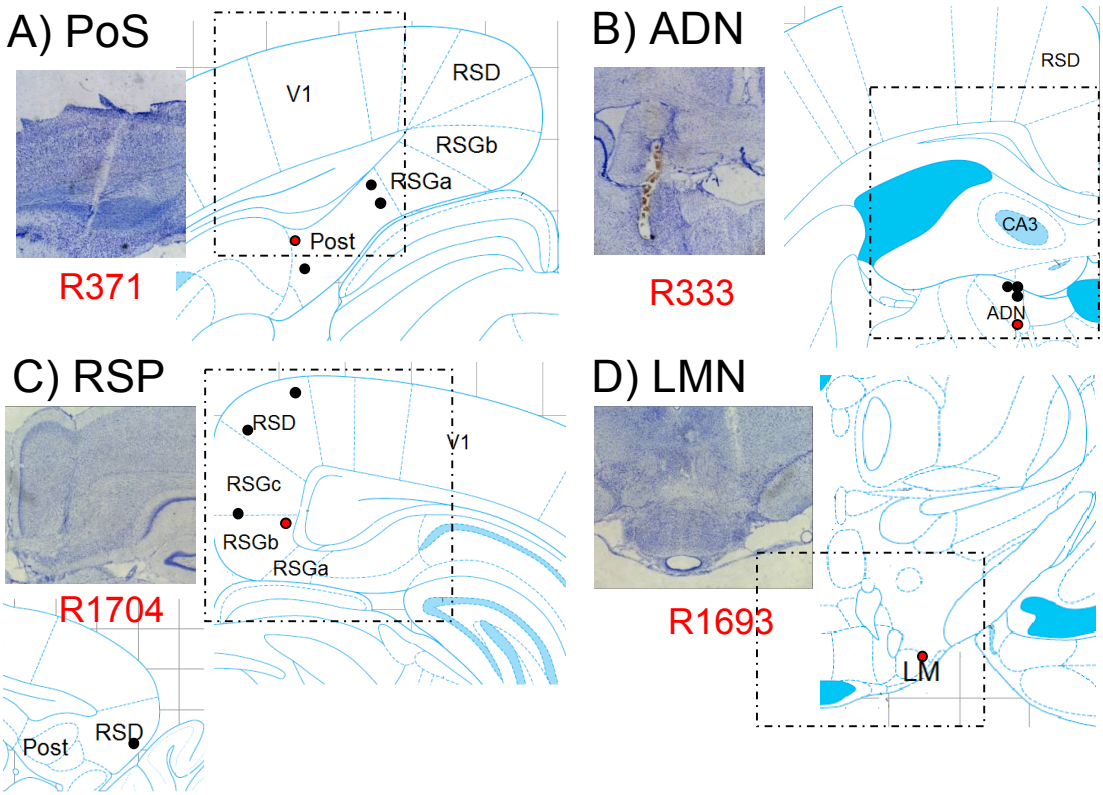

Figure 4.3: Histology from (A) postsubiculum (Post) (B) anterodorsal thalamic nuclei (ADN) (C) retrosplenial cortex (RSD and RSG) and (D) lateral mammillary nucleus (LM). An example slice is shown for each brain region, along with the corresponding red dot on the template (Paxinos and Watson, 2004). Each black dot represents the recording site for the remaining rats implanted in that region. The histology for the two rats implanted in the CA1 region of the hippocampus could not be recovered. They were however implanted using the following coordinates: anterior/posterior -3.5, medial/lateral, 2.0 dorsal/ventral -1.5. Place cells were also found in this region.

4.3 Results

A total of 56 individual head direction (HD) cells were recorded from 16 rats, during 47 sessions. Single ensembles, ranging from one to seven cells, of which there were 22 in total. Descriptive data from the individual neurons are presented first, followed by the inferential statistics performed on the ensemble data.

56 cells met the inclusion criteria, with a peak firing rate of more than 1.0 Hz and a mean vector lengths ('R') of more than 0.3 (van der Meer et al., 2010). Of these 56 cells,

histological analysis (Fig 4.3) showed that 24 were recorded in the postsubiculum (PoS), 16 were recorded in the anterodorsal thalamic nucleus (ADN), 12 were recorded in the retrosplenial cortex (RSP) and 1 was recorded in the lateral mammillary nucleus (LMN). The 3 remaining cells were recorded from rats implanted using CA1 coordinates (where HD cells are sometimes found; Leutgeb et al. (2000), although this could not be confirmed with histology. Overall, these 56 HD cells had an average peak firing rate of 21.6 Hz, and an average directional firing range of 109°. Circular statistical analysis determined the mean vector length, or 'R' value, which is a measure of the spread of the data ranging from 1 (perfectly concentrated) to 0 (randomly distributed around all directions). The mean 'R' value for the complete HD cell data set was 0.5.

Comparisons were made between these brain areas to determine whether there were significant differences in peak firing rate, directional firing range and 'R' values of the cells. As only one cell was recorded from the LMN and the three cells implanted with CA1 coordinates could not be confirmed, these areas were excluded from comparisons. Of the three parameters, peak firing rate ($F(2,49) = 6.04$, $p<0.05$) showed overall significant differences (Fig. 4.2). Post hoc tests (with Bonferroni corrections) at an alpha level of 0.05 found that cells in the ADN had significantly higher peak firing rates in comparison to the PoS (upper CI = 39.87, lower CI = 5.76).

During each session, rats were exposed to one of the environments over five trials. Between trials the environments were rotated by either 90° clockwise (CW), 90° anti-clockwise (AC), or 180°, during which (except for condition 6) care was taken to not disorient the rats (Knierim et al., 1995). There were no overall significant differences in peak firing rate for pre and post 90° rotations (21.6 Hz and 22.02 Hz respectively) ($t(1,204) = -0.468$, NS) or pre and post 180° rotations (21.77 Hz and 22.12 Hz respectively) ($t(1,204) = -0.563$, NS). There were also no overall significant differences in directional firing range for pre and post 90° rotations (108.6° and 108.5° respectively) ($t(1,204) = 0.059$, NS) or pre and post 180° rotations (109.1° and 108.7° respectively) ($t(1,204) = 0.144$, NS).

In the analyses to follow, rotations of 90 degrees clockwise and 90 degrees anti-clockwise were equated by a normalization process in which the anti-clockwise data were reflected in the 0-180 degree axis, to map them onto the clockwise data. This was done as no differences in ensemble behaviour between clockwise and anti-clockwise rotations were noted.

4.3.1 Condition 1: The trapezoid

An example of a session in the trapezoid is shown in Figure 4.4. Note that the firing direction of the cell did not follow the enclosure except on the first trial: thereafter, it resumed and maintained a stable orientation with respect to the room. A stable orientation could be due to a stable path integration cue or uncontrolled extramaze cues. Indeed, the possibility that rats were using extramaze cues during some trials, or more likely a combination of both extramaze cues and path integration cannot be ruled out. Efforts were made to minimise the influence of any extramaze cues in all conditions. For example, white noise was played and a black curtain surrounded the environment. However, they may have been distal odour cues or auditory cues which the rat, but not the experimenter could hear.

A total of 16 HD cells, comprising 5 ensembles, were recorded in the trapezoid from 5 rats, over 8 sessions. The data from the collection of sessions are shown in Figure 4.10A. The subsequent statistical analyses looked at how much the cells' firing directions remained locked to the room cues vs. rotated with the enclosure (the deviation-from-expected measure), and whether the resulting distribution was randomly scattered or clustered around a particular direction. Data from the 90° rotations is presented first, followed by the 180° rotations.

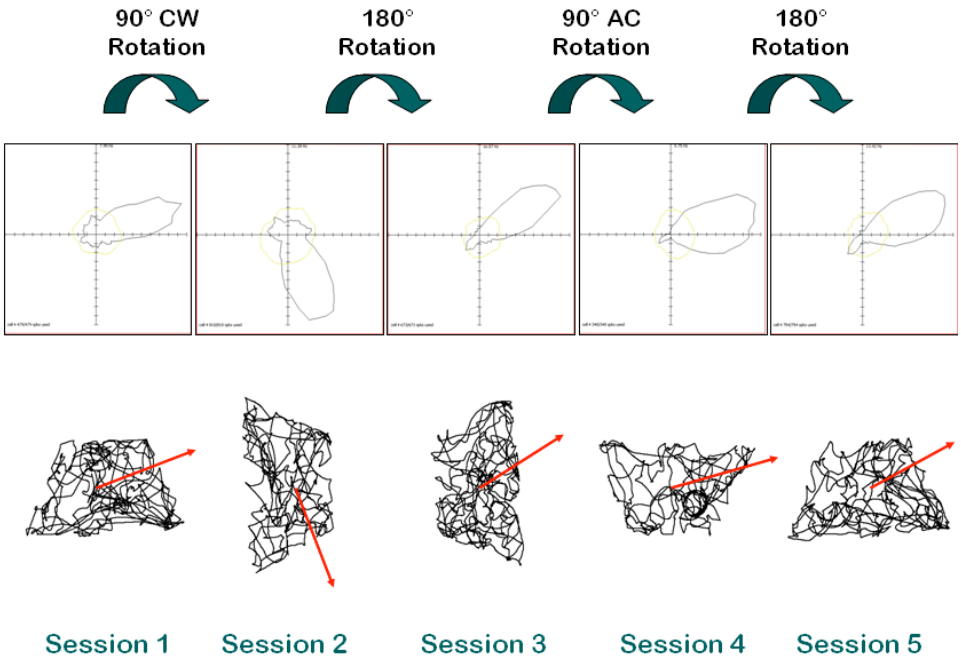

Figure 4.4: An example of the results produced during one trapezoid session. The polar plots indicate the firing direction of one cell across the five trials. The corresponding paths that the rat took for each trial are also shown along with a superimposed red line to illustrate how the rotation of the HD cell corresponded to the rotation of the environment.

Overall, for all ensembles during a 90° trapezoid rotation, the mean shift in firing direction with respect to the room beyond the curtains, was -1°, with a resultant vector length of 0.89 (Fig 4.10A). A Rayleigh test showed that these data significantly clustered around the -1° shift ($R=7.16$, $p<0.05$) and were not significantly different from zero (upper CI = 25.78, lower CI = -28.07), indicating a strong propensity for the cells to remain locked to the room cues. Correspondingly, a final deviation-from-expected value of 91° (+/- 26.33 SD), was significantly different from zero (upper CI = 118.0, lower CI = 64.2). Together, these results indicate that the rotations of the firing directions of the HD cells were significantly different from rotations of the trapezoid environment, and were not different from the direction predicted by the room cues.

For all ensembles during a 180° trapezoid rotation, the mean shift in firing direction was -14°, with a resultant vector length of 0.9 (Fig 4.10A). A Rayleigh test showed that these data significantly clustered around the -14° value ($R=7.21$, $p<0.01$) and were not significantly different from zero (upper CI = 8.59, lower CI = -36.1), indicating a strong propensity for the cells to remain locked to the room cues. Correspondingly, a final deviation-from-expected

value of -166° (+/- 25.45 SD) was significantly different from zero (upper CI = -143.8, lower CI = -188.5).

The overall conclusion from this phase of testing is that the trapezoid, despite its polarized geometry, failed to gain control of HD cells, which instead appeared to lock onto uncontrolled external cues.

4.3.2 Condition 2: The triangle

A total of 15 HD cells (8 ensembles) were recorded in the triangle from 7 rats, over 12 sessions. An example of a session in the triangle is shown in Figure 4.5. Note that in this example, which was representative, the firing direction of the cell did not rotate with the enclosure.

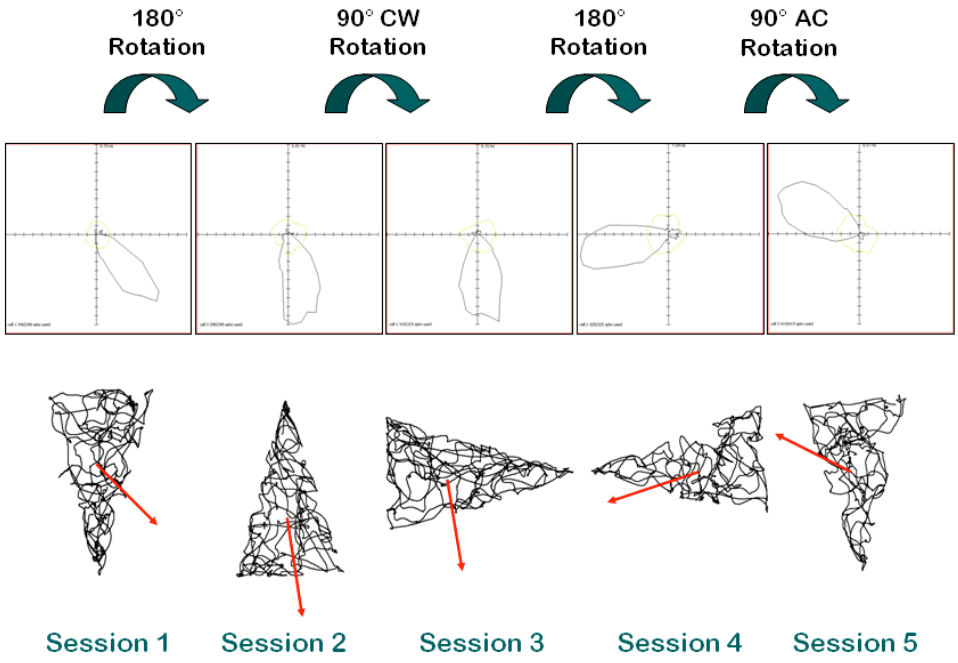

Figure 4.5: An example of the results produced during one triangle session.

Overall, for all ensembles during a 90° triangle rotation, the mean shift in firing direction was -13°, with a resultant vector length of 0.52 (Fig 4.10B). A Rayleigh test showed that these data significantly clustered around the 13° shift ($R=6.78$, $p<0.05$), and were not significantly different from zero (upper CI = 57.87, lower CI = -32.66), indicating, as with the trapezoid, a strong propensity for the cells to remain locked to the room cues. Correspondingly, a final deviation-from-expected value of 77° (+/- 56.0 SD) was significantly different from zero

(upper CI = 122.61, lower CI = 31.51). Together, these results indicate that the rotations of the firing directions of the HD cells were significantly different from rotations of the triangle environment, and were not different from the direction predicted by the room cues.

Similarly, for all ensembles during a 180° triangle rotation, the mean shift in firing direction was 2°, with a resultant vector length of 0.75 (Fig 4.10B). A Rayleigh test showed that these data significantly clustered around the 2° value ($R=9.72$, $p<0.01$), and were not significantly different from zero (upper CI = 29.22, lower CI = -25.21), indicating a strong propensity for the cells to remain locked to the room cues. Correspondingly, a final deviation-from-expected value of 178° (+/- 40.69 SD) was significantly different from zero (upper CI = 205.12, lower CI = 151.26).

One possible reason for the failure of cells to lock to the geometry of the triangle is that from the perspective of the rat, the corners look similar and the wall lengths are also hard to discriminate. This seems surprising, but nevertheless, if HD cells can process geometry the cells should rotate with the unambiguous teardrop enclosure.

4.3.3 Condition 3: The teardrop

A total of 12 HD cells (7 ensembles) were recorded in the teardrop from 5 rats, over 7 sessions. An example of a session in the teardrop is shown in Figure 4.6. Note that like the trapezoid and the triangle example, the firing direction of the cell did not rotate with the enclosure.

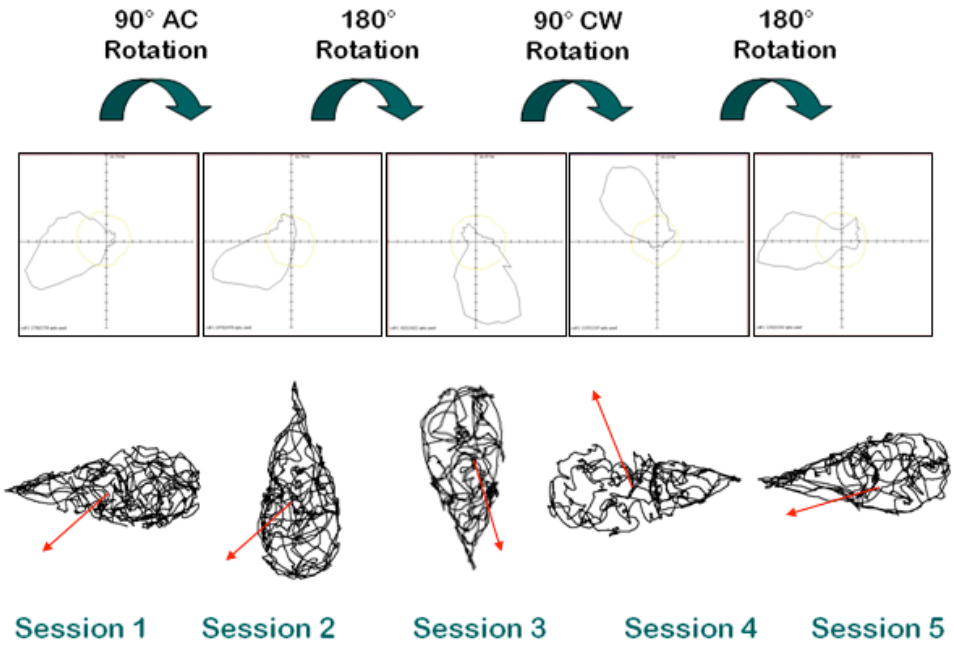

Figure 4.6: An example of the results produced during one teardrop session.

Overall, for all ensembles during a 90° teardrop rotation, the mean shift in firing direction was -23°, with a resultant vector length of 0.77 (Fig 4.10C). A Rayleigh test showed that these data significantly clustered around the 23° shift ($R=5.41$, $p<0.01$), and were not significantly different from zero (upper CI = 60.73, lower CI = -14.9), indicating a strong propensity for the cells to remain locked to the room cues. Correspondingly, a final deviation-from-expected value of 67° (+/- 38.62 SD) was significantly different from zero (upper CI = 104.85, lower CI = 29.79). Together, these results indicate that the rotations of the firing directions of the HD cells were significantly different from rotations of the teardrop environment, and were not different from the direction predicted by the room cues.

Similarly, for all ensembles during a 180° teardrop rotation, the mean shift in firing direction was 12°, with a resultant vector length of 0.9 (Fig 4.10C). A Rayleigh test showed that these data significantly clustered around the 12° value ($R=6.29$, $p<0.01$), and were not significantly different from zero (upper CI = 41.25, lower CI = -17.19), indicating a strong propensity for the cells to remain locked to the room cues. Correspondingly, a final deviation-from-expected value of 168° (+/- 25.75 SD) was significantly different from zero (upper CI = 197.1, lower CI = 139.23).

It appears that a failure of HD cell rotation in the geometric only condition means that HD cells cannot process geometric cues. However, it could be argued that in a situation where cue conflict is very strong (geometry conflicting with uncontrolled auditory and olfactory cues, as well as the rat's internal sense of direction), it may be that HD cells are unable to reliably use any local environmental cue. In order to assess whether or not HD cells can rotate in this situation, the condition where a salient landmark (a white cue card) was attached to the shortest wall of the isosceles triangle was analysed.

4.3.4 Condition 4: Triangle plus white cue card

A total of 15 HD cells (6 ensembles) were recorded from 6 rats during 7 sessions when a white A3 cue card was attached to the shortest wall of the triangle. An example of a trial in the white cue card condition is shown in Figure 4.7. Note that unlike the previous examples, in this example, which was representative, the firing direction of the cell reliably followed the triangle + white card.

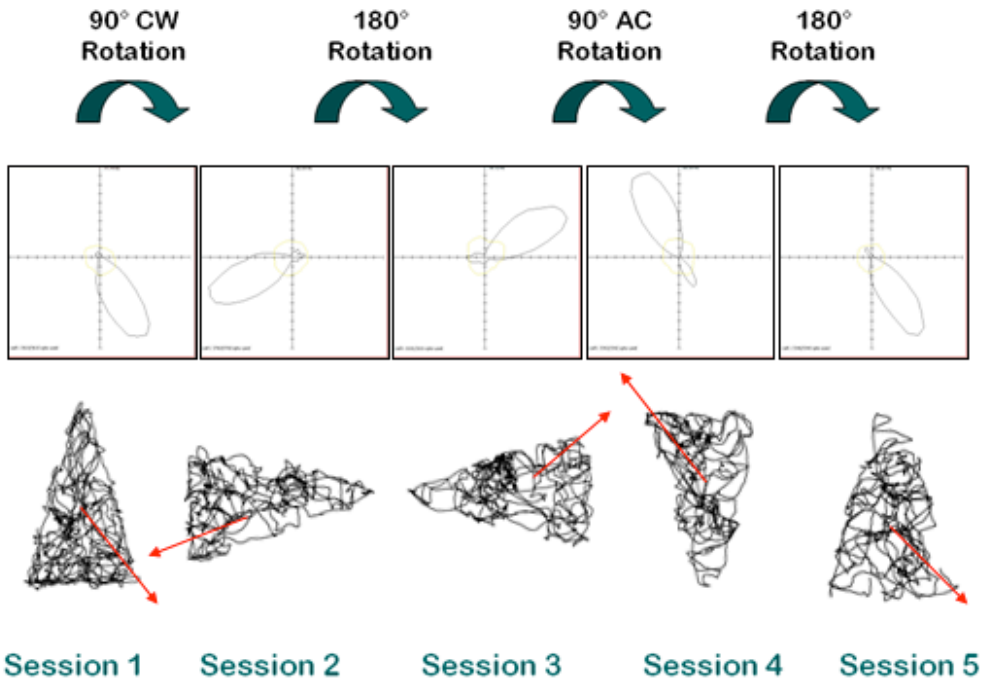

Figure 4.7: An example of the results produced during one white cue card session.

Overall, for all ensembles during a 90° triangle + white card rotation, the mean shift in firing direction was 85°, with a resultant vector length of 0.99 (Fig 4.10D). A Rayleigh test showed that these data significantly clustered around the 85° shift ($R=7.78$, $p<0.01$), and were significantly different from zero (upper CI = 92.82, lower CI = 77.35), indicating that the cells did not lock to the room cues. Correspondingly, a final deviation-from-expected value of 5° (+/- 9.62 SD) was not significantly different from zero (upper CI = 12.61, lower CI = -2.86), indicating that the cells locked to the triangle + white cue card.

Similarly, for all ensembles during a 180° triangle + white card rotation, the mean shift in firing direction was 173°, with a resultant vector length of 0.81 (Fig 4.10D). A Rayleigh test showed that these data significantly clustered around the 173° value ($R=6.5$, $p<0.01$), and were significantly different from zero (upper CI = 204.55, lower CI = 141.52), indicating that the cells did not lock to the room cues. Correspondingly, a final deviation-from-expected value of 7° (+/- 35.0 SD) was not significantly different from zero (upper CI = 38.96, lower CI = -24.64). Together these results show that the rotations of the firing directions of the HD cells were not significantly different from rotations of the triangle + white card environment, indicating that the firing directions remained generally aligned to the triangle + white cue card.

The visual saliency of the white cue card might explain the ability of the cue card but not the shape alone to control HD cells. In order to reduce the visual saliency of the cue card and thus make it visually more comparable to the geometry only conditions, a dark grey cue card was instead attached to the shortest wall of the isosceles triangle.

4.3.5 Condition 5: Triangle plus grey cue card

A total of 15 HD cells (7 ensembles) were recorded from 5 rats during 7 sessions when a grey A3 cue card was attached to the shortest wall of the triangle. An example of a trial in the grey cue card condition is shown in Figure 4.8. In this example the HD cell clearly rotates on two occasions, but fails to rotate on the other two trials. This combination of HD rotations and fixations were typical of sessions in this condition. As the data points in Figure 4.10 represent total shifts in each session, these inter-trial variations have been averaged out.

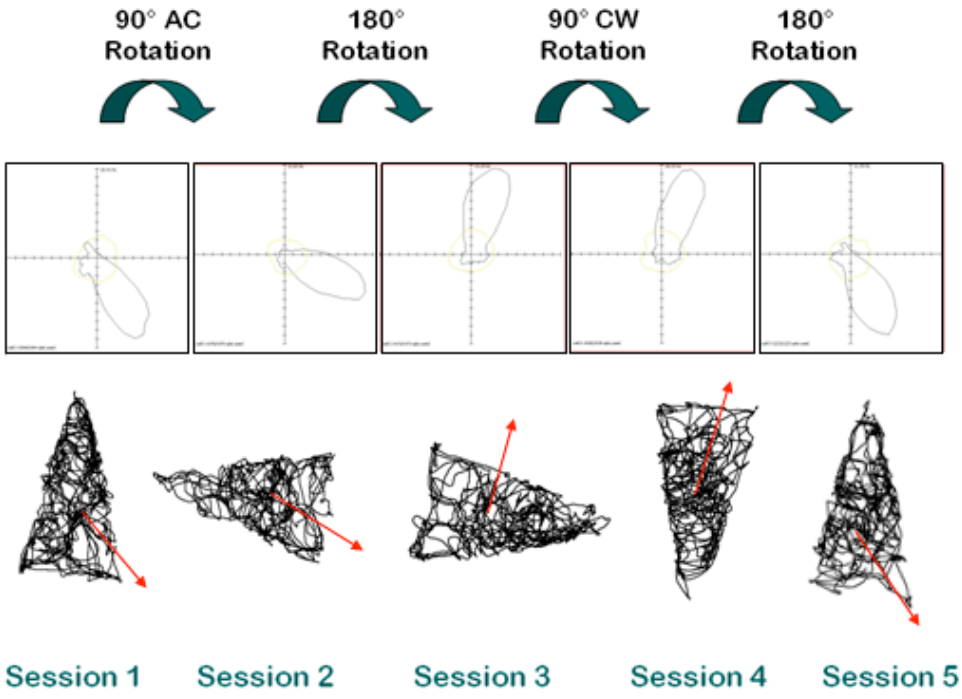

Figure 4.8: An example of the results produced during one grey cue card session.

Overall, for all ensembles during a 90° triangle + grey card rotation, the mean shift in firing direction was 41°, with a resultant vector length of 0.73 (Fig 4.10E). A Rayleigh test showed that these data significantly clustered around the 41° shift ($R=5.08$, $p<0.01$), and were not significantly different from zero (upper CI = 82.51, lower CI = -0.57). Note that the confidence intervals show the values to be just significant. However, a final deviation-from-expected value of 49° (+/- 42.46 SD) was significantly different from zero (upper CI = 91.1, lower CI = 7.45). Together, these results indicate that the rotations of the firing directions of the HD cells were significantly different from rotations of the triangle + grey card environment.

For all ensembles during a 180° triangle + grey card rotation, the mean shift in firing direction was -143°, with a resultant vector length of 0.11 (Fig 4.10E). A Rayleigh test showed that these data did not significantly cluster around the -143° value ($R=0.8$, NS), indicating that the cells in this condition showed large variations in the amount of firing direction rotation.

The cells during the grey cue card condition therefore failed to rotate with the grey card. However, cells would tend to rotate with the environment during one rotation, but never for

all five rotations within a session. One possible explanation for this is that, unlike a visually salient white cue card, which could arguably be seen by the rats from anywhere in the environment, information about the grey cue card might only be processed if the rat was close to the card, through the form of tactile, olfactory or weak visual cues. If the rat did not visit the card early on in a trial, this information might be processed after the HD circuit had established a stable orientation, resulting in a failure of the grey card to gain control over the cells. The paths of the rats in this grey cue card condition were examined to estimate when they first approached the cue card. The average time that a rat took to first approach the grey cue card, when the deviation-from-expected value for the trial was less than 20°, was 14.6 seconds. The average approach time when the deviation-from-expected for the trial was more than 20° was 15.0 seconds. An independent samples t-test showed that these approach time were not significantly different ($t(33)=0.055$, NS). These findings suggest that this proximity hypothesis is unlikely. However, no behavioural measures were taken during the trial so definitive conclusions cannot be drawn.

The failure of cells to rotate with the grey cue card suggests that one possible reason for a lack of HD rotation in the geometry only conditions is because of poor visual saliency. It could therefore be argued that HD cells can theoretically use geometric information but were unable to in this situation because the rats could not visually perceive the geometric cues. However, it may be that HD cells did not use geometric information because the system is simply unable to process such information. Alternatively, it may be that HD cells can use geometric cues but because the cells were receiving a reliable path integration signal, the conflicting information from the geometric cues was under-weighted or 'ignored'. Analysing the final condition could address this question.

4.3.6 Condition 6: Disorientation

In this condition, rats were disoriented by the experimenter manually spinning the holding box, while walking around the curtained enclosure for approximately one minute prior to recording.

A total of 10 HD cells (4 ensembles) were recorded from 3 rats during 6 disorientation sessions in the triangle. An example of a disorientation trial is shown in Figure 4.9. Note that like the white cue card example, in this representative example. The firing direction of the cell reliably followed the triangle.

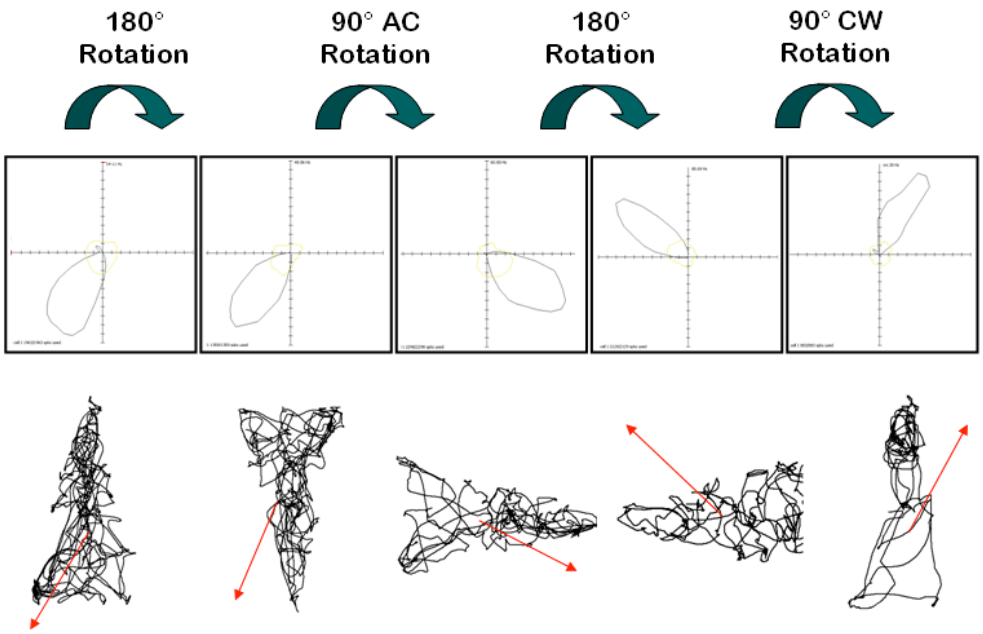

Figure 4.9: An example of the results produced during one disorientation session.

Overall, for all ensembles during a 90° disorientation + triangle rotation, the mean shift in firing direction was 73°, with a resultant vector length of 0.87 (Fig 4.10F). A Rayleigh test showed that these data significantly clustered around the 73° shift ($R=5.24$, $p<0.01$), and were significantly different from zero (upper CI = 107.14, lower CI = 39.53), indicating that the cells did not lock to the room cues. Correspondingly, the final deviation-from-expected value of 17° (+/- 28.87 SD) was not significantly different from zero (upper CI = 50.99, lower CI = -16.62). Together these results suggest that the rotations of the firing directions of the HD cells were not significantly different from rotations of the triangle.

Similarly, for all ensembles during a 180° disorientation + triangle rotation, the mean shift in firing direction was 175°, with a resultant vector length of 0.57 (Fig 4.10F). A Rayleigh test showed that these data significantly clustered around the 175° value ($R=5.24$, $p<0.05$), and were significantly different from zero (upper CI = 107.14, lower CI = 39.53), indicating that the cells did not lock to the room cues. Correspondingly, the final deviation-from-expected value of 5° (+/- 53.12 SD) was not significantly different from zero (upper CI = 50.99, lower CI = -16.62), indicating, that the firing directions remained generally aligned to the geometry of the triangle.

This indicates that when the rat was disorientated, the HD cells used the geometric features of the triangle. This consequently suggests that in the triangle condition (where no disorientation occurred) HD cells were potentially able to use the geometric features, but instead relied on a different stable cue.

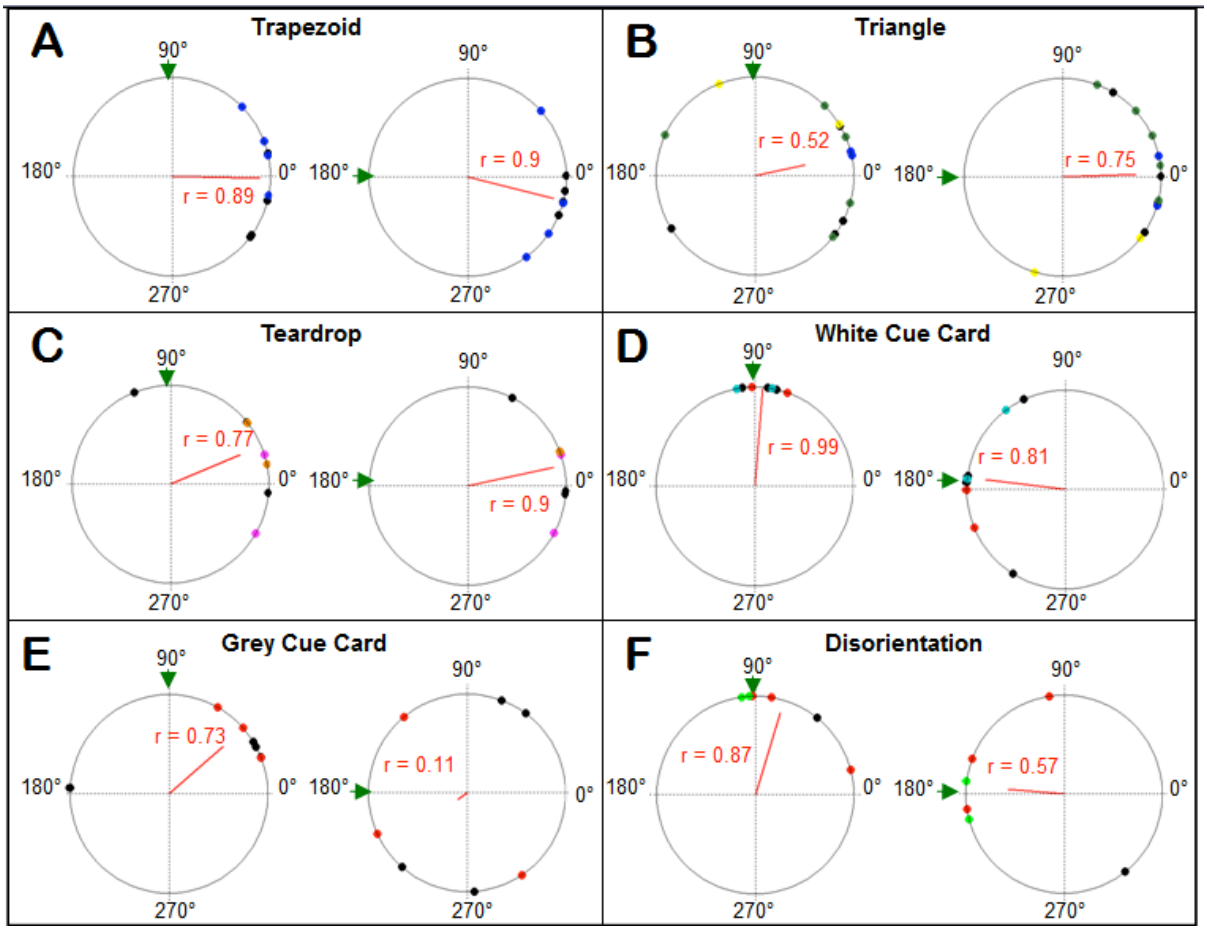

Figure 4.10: Circular plots showing the mean shift of HD cells in each condition, for 90 degree rotations (left plots) or 180 degree rotations (right plots). 90 degree rotations were normalised for direction by reflecting the data from anti-clockwise rotations onto clockwise rotations. The degree to which the environments were shifted (either 90° or 180°) is indicated by the green arrow. Each dot corresponds to the mean rotation for an ensemble of cells (ranging from 1-7 cells per ensemble) during one session. For all plots, the black dots represent cells from rats which only experienced that condition once. Coloured dots represent cells from rats which experienced that condition more than once, with different colours representing different rats. Red lines show the mean shift for each condition. The length of each red line represents the extent to which the data points are clustered. A longer line has an 'R' value nearer to 1, which indicates a greater clustering of data points. Note that in the 90° rotations for the grey cue card condition, cells would tend to follow the environment during one rotation but not both rotations in the session. As the data points represent one session this bimodal distribution is averaged out (~40°)

4.3.7 Comparison between conditions

It was predicted that because the conditions varied in the degree to which rats needed to process geometry in order to orient, the influence of geometry in each condition would also vary. More precisely, it was thought that the trapezoid would cause the HD cells to rotate the least, followed by the triangle, the teardrop, the grey cue card and finally the white cue card. Overall significant differences were found between all the conditions for 180° ($F(5,48) = 14.69$, $p<0.001$) and 90° ($F(5,48) = 4.34$, $p<0.01$) rotations. Circular t-tests were then used to do pair-wise comparisons between all the conditions. All comparisons are shown in Table 4.4, for 180° and 90° rotations. Due to the small R value in the 180° grey cue card condition the parametric test could not be used. However, sample sizes were too small to use the non-parametric test.

180°	Trapezoid	Triangle	Teardrop	White CC	Grey CC
Trapezoid					
Triangle	F(1,19) = 0.82				
Teardrop	F(1,13) = 3.15	F(1,18) = 0.29			
White CC	**F(1,14) = 85.23**	**F(1,19) = 58.31**	**F(1,13) = 69.41**		
Grey CC					
Disorien.	**F(1,12) = 6.76**	**F(1,17) = 23.54**	Sample sizes too small	F(1,12) = 0.01	
90°	**Trapezoid**	**Triangle**	**Teardrop**	**White CC**	**Grey CC**
Trapezoid					
Triangle	F(1,19) = 0.32				
Teardrop	F(1,13) = 1.57	F(1,18) = 0.14			
White CC	**F(1,14) = 61.77**	**F(1,19) = 10.09**	**F(1,13) = 15.27**		
Grey CC	F(1,13) = 4.36	F(1,18) = 0.33	F(1,11) = 0.54	F(1,13) = 6.34	
Disorien.	**F(1,12) = 19.62**	F(1,17) = 4.8	Sample sizes too small	F(1,12) = 1.02	F(1,11) = 1.86

Table 4.4. Table of statistics for each condition. Comparisons of absolute mean deviations (AMD) for each condition during 180° and 90° rotations of the environment. Significant differences with Bonferroni corrections are highlighted in bold.

Table 4.4 show that there were no significant differences between the absolute mean deviations in the trapezoid, triangle and teardrop conditions (the conditions in which firing directions failed to follow the enclosures). Similarly, there were no significant differences between the white cue card condition and the disorientation condition (the two conditions in which firing did follow the enclosures). Significant differences were found however with

all the pairwise comparisons between the geometry only conditions (trapezoid, triangle and teardrop) and the white cue card and with the majority of comparisons between the geometry only conditions and the disorientation condition, with the absolute mean deviations in the white cue card and disorientation conditions being significantly smaller (Fig 4.11).

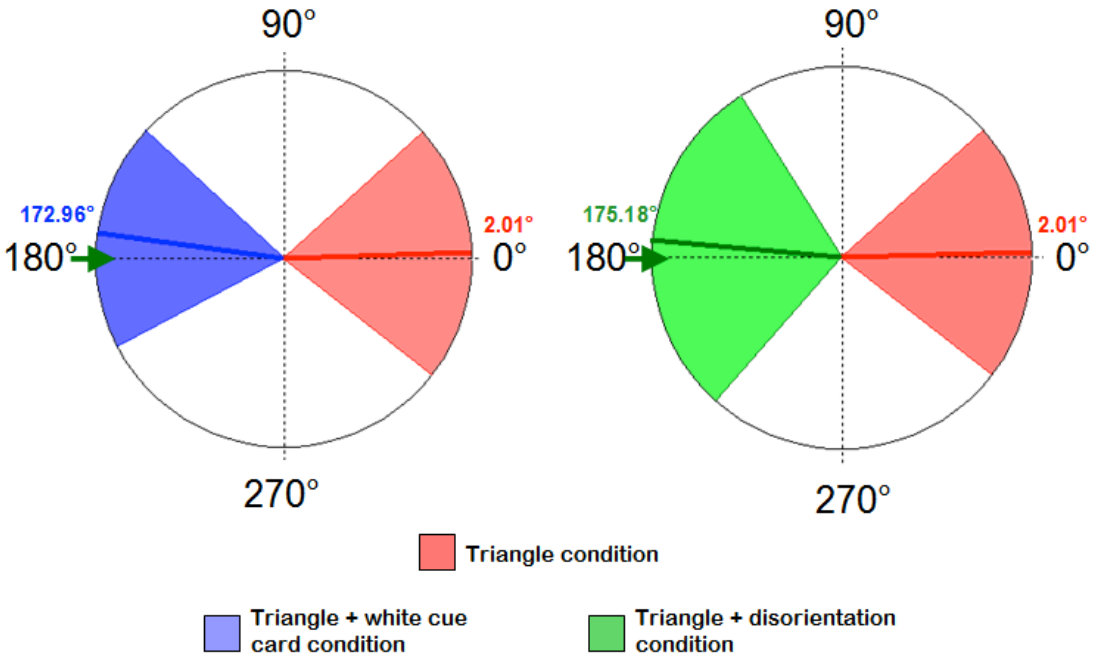

Figure 4.11: Circular plots showing the mean HD cell shifts (solid lines) and confidence intervals (shaded areas) during 180 ° rotations for the triangle condition (red), the white cue card condition (blue) where a white cue card was attached to the shortest wall of the triangle, and the disorientation condition (green) where rats were disoriented between exposures of the triangle. The two graphs show that adding a proximal landmark, or disorientating the rat respectively, produces significantly greater shifts in HD cells compared to a condition where geometric information was pitted against a stable path integration cue.

4.4 Discussion

4.4.1 General findings

This present study has illustrated how visual landmarks, path integration and geometric cues can influence HD cell firing. Ultimately however, there is an important interaction between these three cues that determine when these cues are utilised by the HD system and the

degree to which each cue is used. This study found that geometric cues could influence HD firing when the path integration signal was disrupted but not when this signal was relatively intact. However, this intact path integration signal could not override information provided by a visually salient landmark. This combination of findings suggests that the path integrator or a system heavily influenced by the path integrator, weights the relative influence of allothetic cues on the HD system. Before these findings are discussed, it is important to note that although efforts were made to minimise the influence of any extramaze cues in all conditions, it is certainly possible that rats were also using odour cues or auditory cues to orient.

4.4.2 Visual landmarks versus path integration

One of the main findings of this study was that tuning curves of HD cells rotated by the same amount as the triangular environment when a white cue card was attached to the shortest wall (even though this information conflicted with a stable path integration cue). This landmark control was present for both 90° and 180° rotations, suggesting that the degree of cue rotation did not impact on the cue card's control. This landmark dominance has been witnessed by other electrophysiology studies (Taube et al., 1990b; Knierim et al., 1998; Goodridge et al., 1998; Zugaro et al., 2000; Zugaro et al., 2003).

The visual saliency of the cue card was manipulated in this study, revealing the importance of visual information in HD cell firing. When the rats experienced the dark grey cue card against the black walls, HD cells did not significantly rotate with the environment. This suggests that the visual information provided by the white cue card caused the cells to rotate in that condition. It is however important to note that the white cue card might have actually increased the saliency of the triangle's geometry in this study. Support for this interpretation comes from Pearce et al (2006) who showed that features of an environment (colour) can block, overshadow and potentiate the impact of geometric cues on rats that have not been deliberately disoriented. Placing these two cues in conflict by attaching the cue card to one of the other walls could explicitly test this idea. Interestingly, studies that adopted a disorientation protocol found the opposite to Pearce et al (2006). Cheng (1986) found that feature information (landmarks) did not override the learning of geometric information when rats were disoriented between exposures. The discrepancy in findings between these behavioural studies lends support to the idea that the state of the path integrator determines the relative weighting of landmark and geometric cues.

The stability of the relationship between path integration and visual cues was previously examined by Knierim et al (1995). In their design they included two groups of rats, those that were disorientated before every exposure to the cue card during training and those that were not. They found that the cue card was much more likely to gain control in the non-disorientated training group. Knierim et al (1995) reasoned that this failure of cell rotation was due to the rats not establishing a stable relationship between their internal sense of direction and the cue card during training, thus the rat (or at least the HD cells) learnt not to view the cue card as stable. This reasoning can be applied to the current finding that HD cells in rats that were not disorientated, reliably followed the cue card. However, a lack of geometric control over orientated rats was also found, suggesting that the interaction between disorientation and landmark control found by Knierim et al (1995) cannot be extended to geometric features such as corners. It is however possible to reconcile the current findings with Knierim et al (1995) if geometric features are not considered to be landmarks.

4.4.3 Geometric information versus path integration

The HD cells did not follow rotations of the triangular environments when the cue card was absent, but path integration was stable. This lack of control was also apparent in the trapezoid and teardrop shape. It was predicted that the degree to which the geometric environments gained control over the HD cells, was dependent on the geometric ambiguity of each shape. For example, the trapezoid environment had the most geometric ambiguity, followed by the isosceles triangle and finally the teardrop. There was however no significant difference in absolute deviations between the trapezoid, triangle and teardrop conditions. The finding that the teardrop condition did not produce significantly smaller absolute deviations than the triangle or trapezoid condition also suggests that the HD cells were not anchoring to a local geometric feature, such as a corner (McGregor et al., 2006). However, it is important to note that most behavioural studies examining geometric processing use a disorientation protocol (Cheng, 1986; Maurer and Derivaz, 2000; Wall et al., 2004; Cheng and Gallistel, 2005; Skov-Rackette and Shettleworth, 2005; Gibson et al., 2007; Maes et al., 2009).

Behavioural studies that have examined the influence of disorientation on geometric processing do support the current findings. Lourenco and Huttenlocher (2006) found that when children were disorientated they could use geometric cues to find a target location, as opposed to the inability to use the same cues when they could rely on a stable path

integration signal. More recently, a behavioural study using rats (Batty et al., 2009) systematically manipulated orientation, landmark and geometric cues. They found that when a rat was orientated whilst a rectangular environment was rotated by 90°, rats were more likely to use path integration rather than the geometry. In further agreement with this current study, Batty et al (2009) also found that when the rats were disorientated during training, they were much more likely to use geometric cues.

By contract, the present finding that HD cells of oriented rats did not follow rotations of geometric environments appears to contradict studies that have also examined neuronal responses to changes in geometry. Golob et al (2001) coupled behaviour of oriented rats with HD cell recordings in order to examine the interaction between landmarks and geometry. HD firing of rats exploring a square environment with a cue card attached to one wall (baseline) was compared to firing when rats were in the same environment but had to locate the target corner. They found that HD cell firing remained stable 77% of the time, supporting the idea that landmarks provide a stronger orienting cue than geometry in non-disoriented rats. However, further manipulations where rats were exposed to a rectangular arena after the square box showed a larger influence of geometry on HD cells in comparison to this present study. When the arena was changed to a rectangle, cells rotated their firing directions on 12/13 occasions. In particular, the majority of shifts in firing direction were multiples of 90 degrees, suggesting that the cells' were using the corners of the arena to orient.

One explanation for this discrepancy is that the rats in Golob et al (2001) were extensively trained to locate a reward in one corner, making the corners highly salient. Rats explored the box by travelling along the walls, moving from one corner to the next with little exploratory movement across the box. Behavioural requirements of the task could cause rats to perceive the corners as landmarks or beacons to locate the reward. Additionally, rats were highly familiar with the square environment (204-250 trials) whereas rats in this study were not (recordings were taken during initial and then subsequent exposures).

Another apparent effect of environment geometry on HD cells was found by Dudchenko and Zinyuk (2006). They found that when rats were passively carried in a box from one room to another, HD cells were influenced by the geometry of a T-maze. The T-maze in the second room was oriented at an angle of 90° from the T-maze in the initial room. They found that the majority of cells rotated by around 90° when carried from the initial room to the second room. These findings show that the HD cells were using the apparatus in order to reorient.

However, it is not clear whether the geometric aspect of the maze was responsible for this effect. For example, the rats were always placed on the maze at the same location and were forced to run in the same direction relative to this start location. Sharp et al. (1990) found that when rats started from the same place in a maze, place cells used this information as an orienting cue. Furthermore, HD cells could have used the unique visual panorama witnessed from most locations on the T-maze in order to orient.

These present findings are in agreement with previous electrophysiology experiments that did deliberately disorient the animals between exposures to geometric environments. For example, Taube et al (1990b) found that when a square enclosure was changed to a rectangle, HD cells in disoriented rats rotated their firing directions. More recently, Clark, Harris and Taube (2010b) recorded HD cells of rats exposed to rotations of either a rectangular or a trapezoidal environment, between each exposure the rat was disorientated. They found that both geometric environments influenced the firing of the HD cells, with the more geometrically salient trapezoid showed a greater control over HD cell firing in comparison to the rectangle. In contrast to this current experiment, Clark et al (2010) did find that when they introduced stable landmarks, the HD cells still rotated with the trapezoid environment. This discrepancy could be because the landmarks were distal, extramaze cues, whilst landmark in this current experiment was proximal. Previous work has shown important differences between using distal and proximal cues (Zugaro et al., 2001a). One additional difference is that, unlike Clark et al (2010), the landmark in the present study rotated with the geometric environment. As previously mentioned, it could be that in this present study the landmark facilitated the processing of geometric information.

4.4.4 Path integration mediating allothetic information

Taken together, these findings suggest that the state of the path integrator determines the weighting of allothetic cues. When the signal from the path integrator was relatively stable (albeit with some mis-orientation, as there was slight disagreement between the path integration signal and the environmental cues when the shape was rotated) HD cells only rotated with the geometric environments when a landmark was present. In contrast, when the path integration signal was severely disrupted (during deliberate disorientation) the HD cells did use only geometric information. Since HD cells in disoriented rats still fired strongly and with tight tuning curves, this process of weighing the importance of allothetic information presumably arises outside of the HD system.

4.4.5 Conclusion

The findings from this present study agree with previous studies that have examined the role of path integration, visual landmarks and geometric features in orientation. It was found that when rats were not disoriented, HD cells did not use the highly polarised environmental geometry to orient. Generally, the firing directions remained stable between trials, indicating that the HD system did not reset (Redish and Touretzky, 1996). When a white cue card was affixed to a wall of the geometric enclosure the cells now reliably followed the enclosure, showing that the landmark could influence the cells where geometry did not. When rats were strongly disoriented, HD cells now rotated with the enclosures, indicating that both the HD system and the path integration system reset. This demonstrates that HD cells *can* process geometry when other cues are disrupted. It therefore appears that while geometric cues can influence orientation, this influence is relatively weak, which may explain why behavioural studies have found conflicting effects of geometry on spatial orientation (Cheng, 2008).

One of the most interesting findings that emerged from this experiment is that there is delicate relationship between path integration and differing allothetic cues during HD cell firing. Indeed it seems that path integration signals are ignored by the HD system if a visually salient landmark is present, but heavily relied upon if geometric information is present. Thus, the stability of the path integration signal appears to affect the degree to which different allothetic cues are integrated. This idea that the influence or weighting of cues is determined by the reliability of that cue and competing cues supports a Bayesian account of cue integration. As the reliability of the path integration cue increases (orientation) the weighting of geometric cues decreases. Conversely, when the reliability of the path integration cue decreases (disorientation) the weighting of geometric cues increases.

Based on this finding, the following two chapters aimed to investigate this cue integration further, by placing path integration and allothetic cues in conflict and assessing the extent to which these two cues influence HD cells. As geometric cues were found to have only a very weak control over HD cells during a relatively stable path integration cue, it was felt that a salient landmark cue that did have control over HD cells during intact path integration signal would be more suitable for subsequent testing. The following two studies therefore aimed to see whether in a cue conflict situation, the HD system responds exclusively to either a visual light cue or path integration, or whether the system integrates these two cues. Moreover, if the HD system does integrate these two cues, the additional objective was to

assess what factors other than the stability of a path integration signal influences the weighting of idiothetic and allothetic information during cue integration.

Chapter 5 Bayesian integration of allothetic and idiothetic cues

5.1 Introduction

As previously discussed, everyday navigation relies on the utilisation of two cue types; path integration (idiothetic cues) and landmark information (allothetic cues). Relying on at least two different multisensory cue types requires a degree of cue integration. The process by which cues are integrated in an optimal fashion can be described by a Bayesian model (Yuille and Bulthoff, 1996). This Bayesian model has been experimentally tested using both humans (Van Beers et al., 2002; Knill and Saunders, 2003; Alais and Burr, 2004; Nardini et al., 2008) and non-human subjects (Gu et al., 2008) (see Chapter 2.6.1 for a more detailed description). The findings from the previous experiment in this thesis also supports a Bayesian account of integration as the stability of the path integration signal affected the degree to which the HD system integrated this cue with geometric cues.

Another study that has directly assessed whether neurons integrate information in a Bayesian fashion was Gu et al (2008). They recorded cells in the dorsal medial superior temporal (MSTd) area and found that the width of the tuning curves for MSTd neurons were narrower when both visual and vestibular cues were available in comparison to single cue conditions. This finding suggests that the increase in behavioural accuracy through cue integration is also expressed at a neuronal level. Gu et al (2008) however did not examine whether the *weighting* of these cues is also expressed at a neuronal level.

This current study therefore aimed to assess whether the weighting of these cues is expressed at a neuronal level by simultaneously recording behaviour and HD cells. A behavioural experiment was initially performed in order to replicate previous behavioural findings (Van Beers et al., 2002; Knill and Saunders, 2003; Alais and Burr, 2004) which support the role of Bayesian cue integration in spatial localisation and navigation. In this current study, rats were required to locate a food reward in a relatively cue deprived environment. By using this environment it was hoped that rats would use either path integration or a light cue or both to locate the food. In order to test the Bayesian model, the reliability of the light cue was manipulated. In some training trials a torch produced a light which had a diameter of 2cm with sharp boundaries. In the remaining training trials the

torch produced a diffuse light which had a diameter of 5cm with blurred boundaries. To assess whether the rats weighted these two cues based on their reliability, probe trials were conducted where either the accurate or the diffuse light conflicted with the rats' path integration signal. Like Van Beers et al.,(2002), this current study only manipulated the reliability of one cue. During a trial, path integration was not deliberately interrupted.

Based on the findings from previous psychophysical studies and from the predictions of a Bayesian model, it was hypothesised that during training rats would be more accurate at locating the food when the more reliable light was on. Additionally, during the probe trials it was predicted that search patterns would show a heavier weighting of visual information when the reliable light was on, but a heavier weighting of path integration information when the unreliable light was on. This probe trial prediction is illustrated in Figure 5.1.

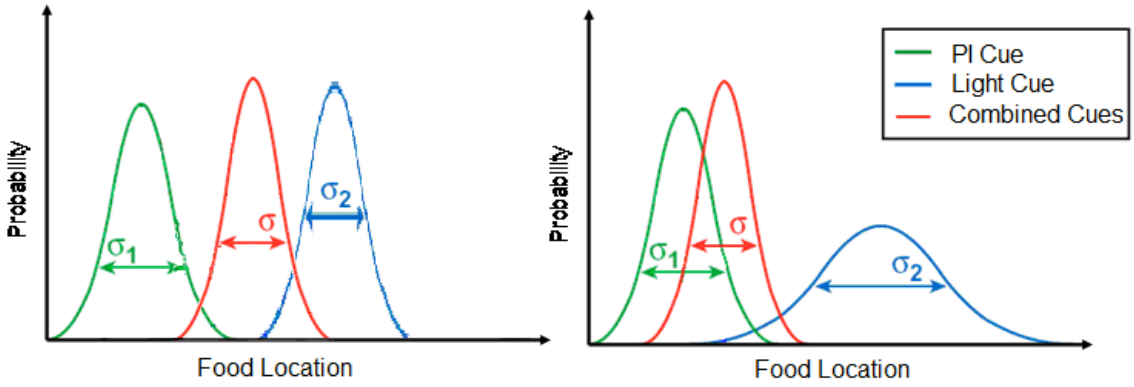

Figure 5.1: Prediction for optimal integration of visual and path integration during the probe trials. *Left* shows the integration of cues when an accurate, reliable torch light is on. *Right* shows the integration of cues when a diffuse torch light is on. When the diffuse light is on, it is predicted that the optimal integration of cues will result in a heavier weighting of path integration cues. Adapted from Knill and Saunders (2003)

The second part of this study included the behavioural task from the initial stage, while simultaneously recording HD cells. The advantages of coupling HD cell recordings with behaviour are three-fold. First, recording the cells that encode directional heading by utilising both path integration and landmark information should hopefully determine whether the uncertainty caused by an unreliable cue is expressed at a cellular level. Second, recording the firing direction of these cells should determine whether the cells integrate

these two cues based on cue reliability. Third, the simultaneous recording of cells and behaviour should establish whether these two measures correlate.

Based on observations by Gu et al (2008), the tuning width of the HD cells could be the parameter that reflects uncertainty. In particular, a prediction is that the more uncertain the system is, the greater the tuning width of the HD cells. Applied to the present study, it would be predicted that a diffuse light would increase the tuning width of HD cells in comparison to the accurate light. An increase in tuning widths across a trial can occur for two reasons; either the tuning width of a cell has actually increased, or the tuning curve itself drifts over a range of directions (Clark et al., 2009). When this drifting is summed together, the apparent result is that the tuning curve of the cell has increased. This drifting in preferred firing direction has previously been associated with uncertainty in HD cells. Goodridge et al (1998) found that after eight minutes, the HD cells of blindfolded rats significantly drifted. A drift in preferred direction would not only result in an increase in firing range, but also in a decrease in firing rate within a trial. Therefore, if a significant effect of tuning width or firing rate is found, a sliding time window analysis would need to be conducted to assess whether these parameter changes reflect a drift or if they reflect true changes to the cell's characteristics.

Both the firing rate and the directional firing range of an HD cell do vary from one region of the pathway to the next (Stackman and Taube, 1998; Sharp et al., 2001b). In particular, the firing rates of HD cells have shown to be relatively irregular across recording sessions (Taube et al., 2004). However, the determinants of tuning widths or firing ranges of HD cells are relatively unknown (Taube, 2007). To investigate whether one of these determinants is uncertainty, the firing rate and directional range of a cell that has been recorded in both the accurate and diffuse conditions will be compared.

The preferred firing direction of the HD cells can also be analysed to establish whether these cells demonstrate cue integration. As with the behaviour predictions, a Bayesian model would predict that HD cells will be influenced by reliable information more than unreliable information. Therefore, when the accurate light is on, the HD cells should rotate with that light more than when the diffuse light was on. The under-weighting of visual information in the diffuse light condition would consequently lead to a greater weighting of path integration information. This study was therefore measuring the tuning width, firing rate and mean firing direction of HD cells in order to see whether these cells reflect the weighting and integration of visual and vestibular cues.

As mentioned, the simultaneous recording of behaviour and HD cells can indicate the degree of correspondence between the two. Very few studies have examined this relationship, which is somewhat surprising as there is often a tendency to assume that these two measures correlate (Golob et al., 2001). However, a lack of studies may reflect the fact that simultaneously recording cells and behaviour can be difficult. Even though there have only been a relatively small number of studies, the limited data has produced seemingly mixed results. Initial observations by Mizumori and Williams (1993) suggested that HD cells do correspond to spatial behaviour. They recorded HD cells in the LDN of two animals performing a radial arm task. They found that an increase in performance of the task correlated with an increase in directionality of the HD cells. This finding suggests a strong coupling between HD cell firing and spatial behaviour. However, this finding should be approached with caution as previous findings have shown very little evidence of changes in directional selectivity of HD cells over time (Taube et al., 1990a; Taube et al., 1990b; Taube, 1995). The small data set (two HD cells), correlating averaged values and the confounding variable of background noise when calculating a signal-to-noise ratio have led some experimenters to question the findings from Mizumori and Williams (1993). For a review see Muir and Taube (2002).

Dudchenko and Taube (1997) provided a further insight into the link between HD cells and behaviour when they recorded HD cells from the ADN and the PoS. They trained rats to locate food in an arena that contained only one visual landmark, a cue card. The food location and the cue card location were not the same, but the spatial relationship between the two was kept constant. During the testing phase, the cue card was rotated by either 90° or 180°. They found that when the cue card rotated, the HD cells rotated by a similar amount. Moreover, they found that the rats searched for the food in a location predicted by the HD cells and the cue card. This finding suggests a strong coupling between the HD signal and spatial behaviour. However, unlike Mizumori and Williams (1993), they did not find an association between task acquisition and HD cell properties. Additionally, Dudchenko and Taube (1997) also found that when a rat was successfully trained to go to a completely novel arm after the initial cue rotation phase, the HD cell's firing direction did not change. Taken together these results suggest that there is an association between HD cell firing and spatial behaviour: however a causal link cannot be assumed. In other words, the current evidence has not shown that HD cell firing guides behaviour (Muir and Taube, 2002).

This idea of an associative but not necessarily causal relationship between behaviour and HD cell firing was reflected in the findings by Golob et al (2001) where rats were trained to locate a food reward in one corner of a square box relative to a cue card. At the testing phase, the rats were transferred to a rectangular box. The preferred firing direction for 90% of HD cells recorded in the ADN shifted by approximately 90° after entering the rectangular environment. This shift in preferred firing direction has been previously shown (Taube et al., 1990b), however the rats did not shift their search patterns. For example, if a rat was trained to locate a food reward in the corner located on the left side of the cue card in the square arena, they mostly searched for food in the corner located on the left side of the cue card in the rectangle. These findings indicate that the rat's behavioural response could not be predicted by HD cell firing.

It is therefore interesting to see whether the behavioural response of rats in this current experiment corresponds to the firing of HD cells in the PoS. Additionally, the response of both these cells and the rat's behaviour was assessed against a Bayesian model to see whether cue integration and cue uncertainty are expressed at both a behavioural and neuronal level.

This study was divided into two experiments. Experiment 1 examined the behavioural response of four trained rats during a Bayesian task. Two of these rats were then implanted in the PoS, but HD cells were only found in one rat. Experiment 2 recorded HD cells from this rat whilst the rat performed the task featured in Experiment 1.

5.2 Materials and methods - Experiment 1

5.2.1 Subjects

Four adult male Lister Hooded rats (weighing approximately 400 g) were housed in pairs [11:11 light:dark, with 1 hour (x2) simulated dawn/dusk] on a food-restricted diet (sufficient to maintain 90% of free-feeding weight) with *ad libitum* access to water. All animals were handled once a day in the week before training, in order to familiarise the rats with the experimenters. All procedures were licensed by the UK Home Office subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act 1986.

5.2.2 Apparatus

A circular wooden platform 1.2m in diameter was located in the centre of a curtained-off enclosure 2m in diameter (Fig 5.2). The platform was placed on a cardboard box measuring 50cm in height. Inside the box was a radio generating white noise, to mask any auditory cues.

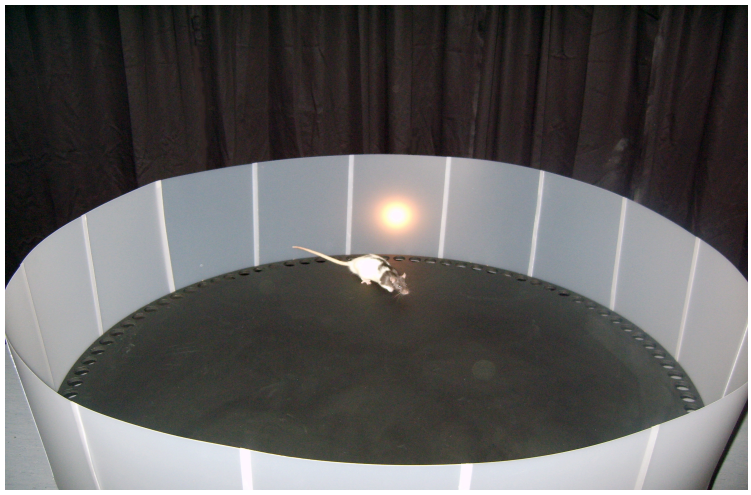

Figure 5.2: Wooden platform where the experimental protocol took place. A torch was located behind the platform walls in order to provide the salient light cue (in this case, the diffuse light).

The walls of the environment were made of translucent plastic which allowed the presentation of an accurate (reliable) or a diffuse (unreliable) torch light. The light was placed directly behind the translucent wall to create the accurate light and 30cm behind the wall to create the diffuse light. As the platform was raised off the floor, the torch was clamped onto a 25cm stand. When the torch was directly behind the wall, the light was sharply focussed, producing a 2cm diameter circle with clear boundaries. When the torch was placed 30cm behind the wall, the light was dispersed, producing a 5cm circle of light with blurred boundaries (Fig 5.3). As all main lights were switched off during the experiment, the torch was the only salient polarising landmark. During probe trials, two torch lights were used so the light could easily move location without having to physically move the torches. Only one torch was ever switched on at one time. The platform had 91 holes, with a diameter of 3 cm around the perimeter of the platform, with spaces of 1cm between each hole. This meant that there were 4° between each hole. Inserted into each hole was a small container which acted as a food well. A ball of cotton wall was placed inside each food well

to perform two functions. Firstly, it meant that in the baited wells, the food reward could be placed underneath the cotton wall to try and mask odour cues from the bait. Second, these cotton wall balls could be replaced regularly in all 91 wells to redistribute any odour cues. The platform was also rotated and cleaned with water between sessions in order to minimise local odour cues.

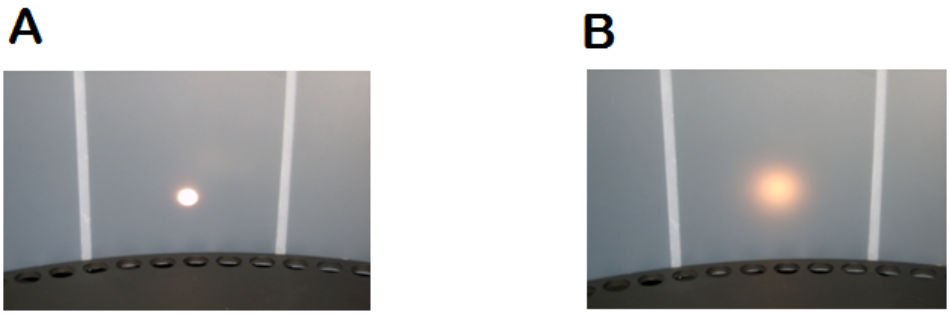

Figure 5.3: Presentation of the accurate (reliable) and diffuse (unreliable) torch light. **A:** The accurate light had sharp boundaries and had a span of 2cm. **B**: The diffuse light had blurred boundaries and had a span of 5cm.

5.2.3 Behavioural training

Training sessions took place in the experimental room described in the General Methods section and lasted for 20 minutes. Each of the four rats initially completed ten training sessions; each rat was trained separately. During a session, the rats were trained to shuttle back and forth on the maze between two baited food wells for twenty minutes. These baited wells were always located opposite each other and 90° either side from the light (Fig 5.4).

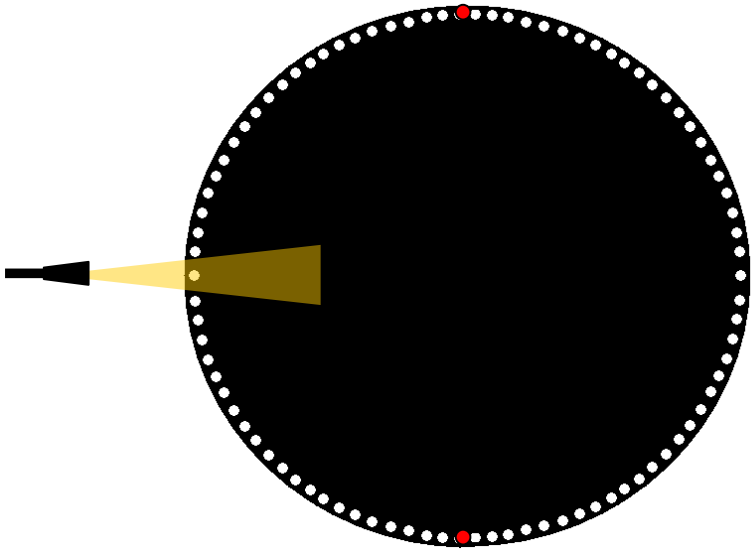

Figure 5.4: Schematic drawing of a bird's eye view of the platform. On this occasion, the torch is positioned to the west of the platform, producing a diffuse light. The two red dots indicate the location of the two baited food wells.

At the beginning of each session and before the rat entered the experimental room, the room was prepared by switching on the white noise and the torch light. The location of this light pseudo-randomly moved between each session in order to avoid the rats associating the light with any uncontrolled extramaze cues (e.g. a crease in the curtain). This torch either produced an accurate or diffuse light spot. The accuracy of the light was pseudo-randomly selected, ensuring that there were no more than three consecutive appearances of the same type of light. The main lights in the room were switched off and the curtains were drawn around the maze. One of the food wells (90° away from the torch light) was baited with a single Coco Pop (Kellogs). Another Coco Pop was also placed in a random location on the platform. This was done so that rats did not learn to shuttle back and forth between the baited wells using a simple body turn strategy. Instead, the rat had to explore the platform between each shuttle, encouraging the rats to use the light to locate the reward.

Once the room was prepared, one rat was brought into the experimental room in an opaque holding box. The box was taken through a join in the curtain and placed on the floor. These locations varied between sessions. The rat was then removed from the box and placed on to the maze so that the rat entered the maze from a different position for each session. Once the rat was on the platform the timing of the session commenced. Two experimenters were present in each session in order to allow efficient baiting of the two food wells. Both

experimenters would continually walk around the maze so that they could not be used as a reliable landmark by the rat. Whilst the rat was retrieving the reward in one food well, one of the experimenters would replace the Coco Pop in the opposite food well, out of the view of the rat. The Coco pop on the platform was also replaced at a new random location to discourage a simple body turn strategy.

After the ten initial sessions, all four rats were confident enough to explore the platform and retrieve the rewards from the food wells. At this point, each rat completed a further 25 sessions. These sessions were exactly the same as before, except this time the second experimenter recorded the choices of the rat for each shuttle run. A choice was defined as the food well that was first nose poked by the rat after the retrieval of the reward on the platform. The accuracy of these nose pokes or choices was then recorded. The recordings fell into four categories, indicating whether they chose the correct food well (0° error), the neighbouring food well (4° error), two food wells away (8° error) or more than two food wells away (12°+ error). The direction of these errors (clockwise or anti-clockwise) was also recorded. Each session was terminated after twenty minutes and the rat was returned to their home cage.

Towards the end of the training sessions, the food wells that should be baited were occasionally left empty. The torch light was also sporadically turned off for ten seconds. These two things were done in order to prepare the rats for the probe sessions.

5.2.4 Probe sessions

Probe sessions were performed to assess whether the rats were using the light as a directional cue or path integration information or an integration of these two cues to locate the food. The probe sessions were essentially the same as the training sessions, except that after four minutes of the rat shuttling on the platform, the torch light was turned off for ten seconds. An identical light was then switched on 20° away from the initial light (shining of a food well located five wells away from the original well). The initial nose poke was then recorded, whilst the Coco pops on the platform and opposite well were replaced. Initial nose pokes were not rewarded in order to ensure the rats were not using odour to locate the food well. This nose poke marked the end of the first probe trial within the session. The rats then shuttled back and forth between the two new baited wells (5 wells away from the original wells) for a further four minutes before the torch light was again switched off for ten

seconds. The initial light was then switched back on and the rat's nose poke was recorded. This was repeated so that each probe session contained four trials (Fig. 5.5).

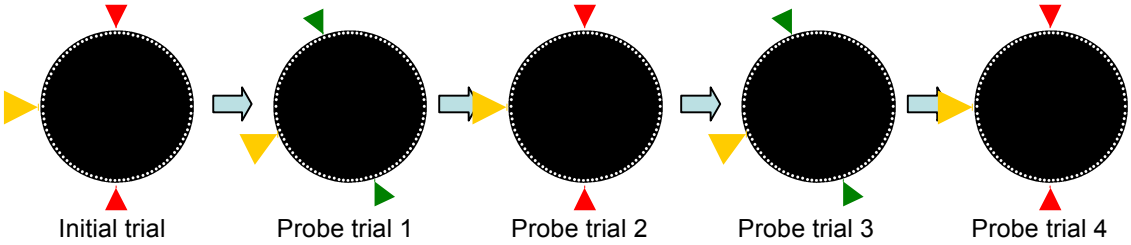

Figure 5.5: Schematic flow chart of the protocol in one probe session. In the initial trial for this probe session, the light was located to the west of the maze (yellow arrow) and rats shuttled back and forth between the two baited wells (red arrows) for four minutes. The light was then switched off for ten seconds, and a new light was switched on 20° away. This transition period is symbolised by the blue arrow. The rat was then probed by recording the rat's choice of well without the presence of a reward. Once this probe was recorded the new goal locations were baited (green arrows) for four minutes. The light was then switched off for ten seconds and the original light was switched on in order to carry out the second probe trial. This continued till the end of the fourth probe trial.

The probe sessions were performed using both the accurate and diffuse light. Each rat had two probe sessions using the accurate light and two sessions using the diffuse light. After these initial probe sessions, the distance which the light moved was revised to 8° (the shifted light thus shone of a food well located two wells away from the original well). This was done because it was felt that the 20° conflict might be too large to allow the Bayesian integration of cues (Jack and Thurlow, 1975). Each rat had one probe session using the accurate light and one session using the diffuse light in this revised set-up. Circular statistics were used to analyse the probe session data, including the circular mean and circular standard deviation. To compare the light conditions, a circular version on the one-way ANOVA (Watson-Williams) was used.

5.3 Materials and methods - Experiment 2

In the first experiment, four rats were used in the procedures described above. Two of these rats were then implanted in the PoS and HD cells were recorded from one of these rats in the second experiment.

5.3.1 Subjects

One adult male Lister Hooded rat from Experiment 1 (weighing approximately 400g at the time of surgery) was housed individually [11:11 light:dark, with 1 hour (x2) simulated dawn/dusk] on a food-restricted diet (sufficient to maintain 90% of free-feeding weight) with *ad libitum* access to water. All procedures were licensed by the UK Home Office subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act 1986.

5.3.2 Apparatus

The apparatus used in Experiment 1 was also used in this part of the study

5.3.3 Surgery and electrodes

The rat was implanted with moveable microelectrodes after the completion of Experiment 1. Description of these microelectrodes and surgical procedures can be found in the General Methods section. The electrodes were lowered into the PoS (Bregma coordinates: 6.7 AP, 2.8 ML, 1.6 DV). The animal was given one week to recover following the surgery.

5.3.4 Screening procedures

Recording commenced one week after surgery. Recording was done using multichannel recording equipment (DacqUSB, Axona Ltd). A description of the screening procedures can be found in the General Methods. Screening for HD cells took place in a room separate from the actual experimental room, to minimize the learning of extraneous cues in the recording environment by the rats.

5.3.5 Behavioural training

Training for this animal primarily occurred pre-surgery in Experiment 1. Two additional training sessions were performed one week after surgery.

5.3.6 Probe sessions

When an HD cell was found, the rat was taken into the experimental room in an opaque holding box. The rat was then placed on a raised holding box outside of the curtained enclosure in order to acclimatise to the room for five minutes. The rat was connected to the

recording device outside of the curtained enclosure. The exact same procedure used Experiment 1 was then conducted. The only modification made from Experiment 1 is that for the later two probe sessions the rat was briefly removed from the maze and placed in the holding box two minutes after the former probe trial and thus two minutes before the subsequent probe trial. The rat was removed so that the maze could be cleaned in order to reduce the presence of odour cues. Efforts were made to reduce odour cues because the rats performed very successfully in experiment 1 and it was thought that this success may have been due in part to odour cues. This confounding variable was tackled by moving the torches and therefore the goal wells to a new location. The cotton wool in the previously baited wells was also replaced with clean cotton wool. As the rat was removed halfway through a trial, it should be able to still use path integration to find the food reward. The implanted rat performed two probe sessions where the light shifted by 20° (1 accurate and 1 diffuse) and two probe sessions where the light shifted by 40° (1 accurate and 1 diffuse). The light shift was increased because HD cells tend to under-rotate with a landmark (Goodridge and Taube, 1995) and so a larger landmark shift would make it easier to detect a shift in the cells' preferred firing direction. The accuracy of behavioural choices in the probe trials was recorded in the same way as in Experiment 1.

5.3.7 Data analysis of HD cells

Cluster-cutting software (Tint, Axona Ltd) was used to analyse the data offline. A description of the cell isolation process can be found in the General Methods. Each cell's mean firing direction, peak firing rate, directional firing range and resultant vector length for each trial was calculated using the procedures detailed in the General Methods. All cells were required to meet a firing rate criterion whereby cells with a peak firing rate of less than 1.0 Hz across the session were excluded from further analysis.

Analysis of directional firing range and peak firing rate was performed on singe cell data using linear statistics. Comparing these two parameters across the diffuse and accurate conditions would address the issue of whether uncertainty is reflected at a neuronal level. As directional firing range and peak firing rate differ drastically between cells (Stackman and Taube, 1998; Sharp et al., 2001b; Taube et al., 2004), only those cells that were recorded in both conditions were analysed.

Analysis of HD cell mean firing direction was performed using circular statistics, examining mean values of cells that had been recorded simultaneously, i.e., as an ensemble (ranging

from 1 to 5 cells). Ensembles were analysed because previous studies have shown that HD cells from a single animal always act in concert and react together to environmental changes (Taube et al., 1990b). All circular analysis was also done using the CircStat Matlab toolbox (Berens, 2009). In order to analyse the rotation of HD cells from one trial to the next, the circular mean direction of cells in the first trial was subtracted from the circular mean direction of cells in the second trial to provide a measure of how far the ensemble had rotated. This was repeated for each subsequent trial of the session so that each session produced four rotation estimates. A mean of these four values were then taken to produce a mean ensemble shift for each session. In order to compare these actual shifts to the light shifts (i.e. the predicted amount of shift), the ensemble shifts were subtracted from the light shifts to produce absolute deviation values (deviation-from-expected).

These absolute deviation values could then be compared to zero. An absolute deviation value that was not significantly different from zero would suggest that the HD cells were rotating with the light. Similarly, absolute deviation values could be calculated by subtracting the cell ensemble shift from the shift in behavioural choice. Again, an absolute deviation value that was not significantly different from zero would suggest a strong degree of coupling between behaviour and cell firing.

5.3.8 Histological analysis

At the end of testing, the rats were then deeply anaesthetised with isoflurane before they were injected with sodium pentobarbital. They were then transcardially perfused using saline and then paraformaldehyde (4%). The brains were removed and stored in paraformaldehyde (4%) for at least one week before sectioning. The brains were sliced at 40 micrometers on a freezing microtome. The sections were then mounted and stained with Cresyl violet (Fig. 5.6). A full description of this process can be found in the General Methods section (Chapter 3).

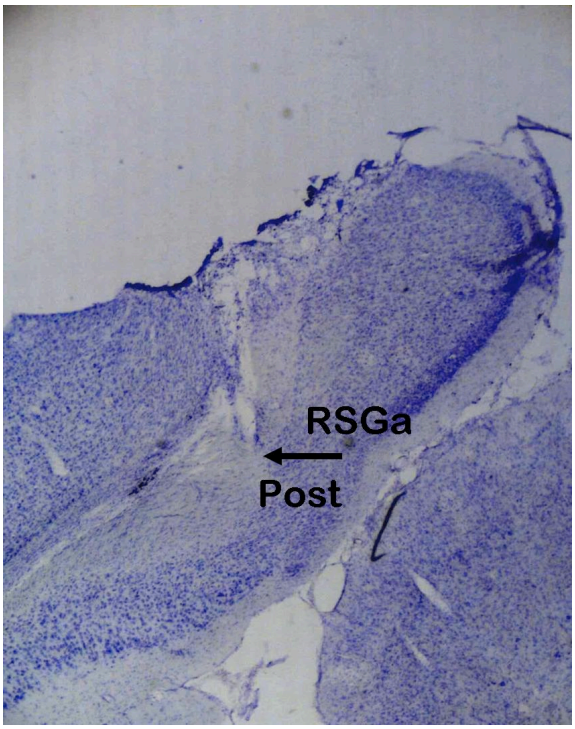

Figure 5.6: Histology slice from the rat implanted in the postsubiculum. The section has been labelled using Paxinos and Watson (2004) to indicate the location of the postsubiculum (Post) and the retrosplenial granular 'a' region (RSGa). From the section it appears that the electrodes were in the postsubiculum but it is important to note that the track is very near the Post/RSGa border and therefore the electrodes may have been in the retrosplenial granular 'a' region.

5.4 Results - Experiment 1

The data collected during the twenty-five training sessions are presented first, examining whether the rats' performance increased over time and whether the reliability of the light impacted on the accuracy of the rats' choices. The data from the probe trials are then presented, examining whether rats used a Bayesian strategy to integrate the landmark information with path integration.

5.4.1 Training data

Rats were trained over thirty-five sessions, with nose pokes being recorded in the last twenty-five sessions. A nose poke which was made in the baited food well was recorded as a correct choice, with a 0° error. The mean percent of correct choices for each session was then calculated. The ten 'pre-training' sessions meant that all four rats were reasonably good at the task (56.7% of choices were correct) at the beginning of the recorded training

session (Fig 5.7). A two factor repeated measures ANOVA analysing the mean percent of correct choices showed that that there was no significant main effect of light type ($F(1,3) = 2.099$, $p>0.05$) but there was a significant main effect of session ($F(11,33) = 3.063$, $p<0.01$). Figure 5.7 shows an improvement in performance over time and this was supported by a contrast test showing that there was a significant linear trend of mean percent of correct choices across sessions ($F(1,3) = 12.272$, $p<0.5$) . There was also a significant interaction between the type of light and session ($F(11,33) = 5.334$, $p<0.001$). It appears from Figure 5.7 that for the first few recorded sessions, the accurate light sessions produced more correct choices in comparison to the diffuse light sessions. However, after session 10 there appears to be little difference in the number of correct choices between the two lights.

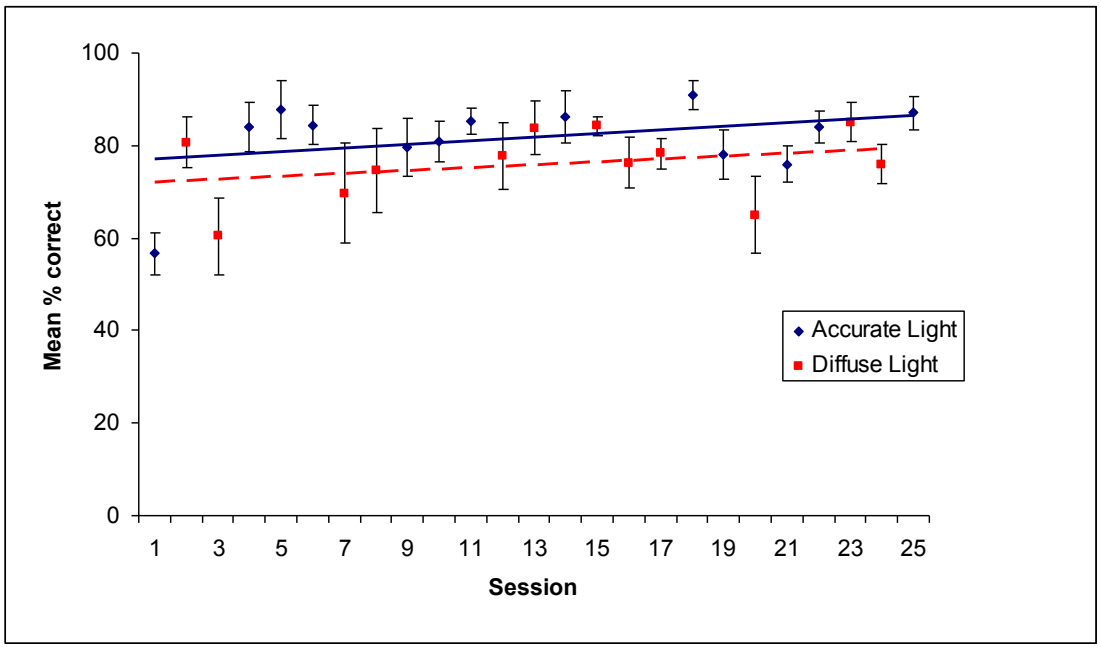

Figure 5.7: Mean percent of correct choices for the 4 rats in each of the twenty-five sessions. For half of these sessions the accurate light was on (blue data) and for the other half the diffuse light was on (red data). A linear line of best fit for both conditions demonstrates that the mean percent of correct choices increased over session and was generally higher when the accurate light was on, although there was no significant difference between scores when the accurate and diffuse light was on

5.4.2 Probe data

From the training data it appears that the rats improved over time. Additionally, the rats may have been slightly better when the accurate light was on, but this finding was not significant. In these training sessions, both the light and the rat's path integration signal could be used to locate the food. Additionally, rats may have also used static uncontrolled

cues such as odour cues to find the food. The impact of odour cues is raised later in the discussion. By placing the light and path integration signals in conflict, the probe sessions could assess the degree to which each cue influenced the rat's choice. The rats performed four probe sessions where the light was rotated 20° from its original position, two with the accurate light and two with the diffuse light. The rats then did a further accurate session and diffuse session where the light shifted by only 8°. During these sessions the light was moved four times to produce four probe trials within a session. As with the scoring of training sessions, a nose poke made at the well 90° away from the light was scored as correct (0° error). Unlike the training position, the location of the light moved between trials and thus the location of the correct well occupied two positions on the platform but they were always 90° away from the light. A correct score therefore indicated that the rats were using the light to find the food. A 20° error score (for the first four probe sessions) or an 8° error score (for the last two probe sessions) would indicate that the rat was using path integration to find the food. Alternatively, an error score of 20° in the first set of probe sessions or 8° in the second set of probe sessions could indicate that the rat was using an uncontrolled static cue to find the food. If the rat had integrated the light cue and path integration, an intermediate error score would be expected.

Figure 5.8 shows the frequency of choices made by the rats during the probe trials with both accurate lights (blue dots) and diffuse lights (red dots) when the lights were separated by 20° (plot A) and 8° (plot B). Both plots show that the majority of choices (92% for 20° light separation and 69% for 8° light separation) were made at either the correct location (0°) or at the previously correct location (20° or 8° away from the correct location). For the probe trials during a 20° light separation, there appears to be very little difference between choices made when the accurate light was used and when the diffuse light was used (Fig 5.8 Plot A). This is supported by a non-significant result from a circular ANOVA (Watson-Williams) examining the differences in choices (binned in 4°) between accurate and diffuse conditions ($F(1)=0.02$, n.s).

For the probe trials during an 8° light separation, there appears to be a small difference between choices made when the accurate light was used and when the diffuse light was used (Fig 5.8 Plot B). When the diffuse light was on, fewer correct choices were made in comparison to the accurate light and more intermediate choices were made. However, a circular ANOVA (Watson-Williams) examining the differences in choices between accurate and diffuse conditions was not significant ($F(1)=0.04$, n.s).

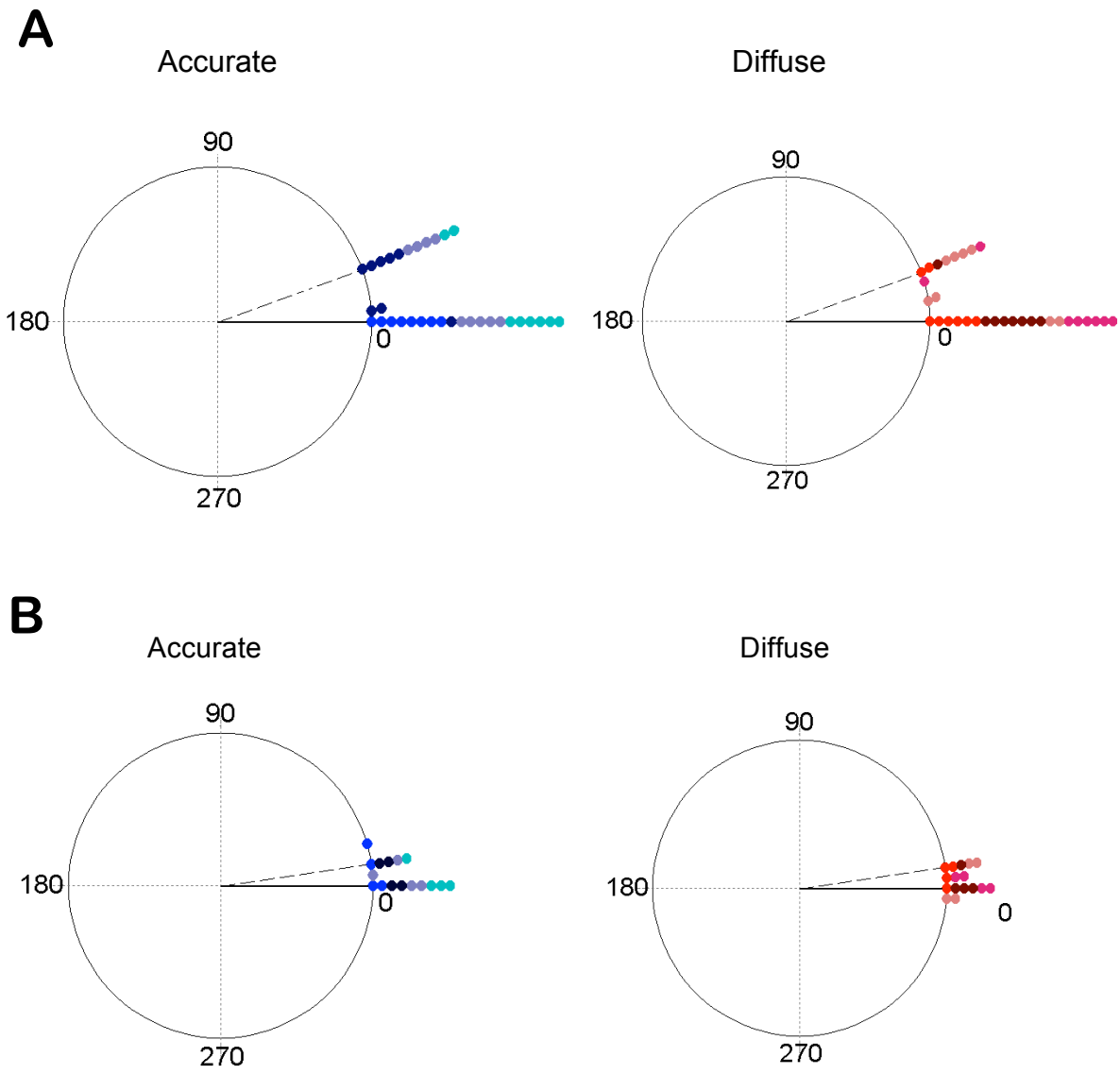

Figure 5.8: Circular plots in degrees showing the choices (nose pokes) of the four rats during a 20° light separation (plot A) and an 8° separation (plot B) when an accurate light (blue dots) and diffuse light (red dots) was on. The different shades of blue (accurate) and red (diffuse) dots represent data from different animals. The solid black lines indicate the location of correct choices (0° error) if the rat was using the light. The dashed black lines indicate the previously correct location (the well which was rewarded in the previous trial, 20° and 8° on plot A and B respectively). If the rat was using only path integration then searches should be clustered around this dashed line.

Taken together, the findings from the first experiment show that the choices in the probe phase were not influenced by the reliability of the light. There was no significant difference

in choice locations between the accurate and diffuse lights for both the 20° and 8° light separations.

5.5 Results - Experiment 2

HD cells were recorded from one rat (from the first experiment) whilst performing the probe trials described in the first experiment. The behavioural data collected during the four probe trials are presented first, followed by the electrophysiology data. This later analysis is separated into different sections based on the characteristics of the HD cell that were examined. The directional firing range and peak firing rate of the cells are presented first, followed by the mean firing directions.

5.5.1 Behavioural data

The implanted rat was recorded in two probe trials where the light was rotated by 20° (one accurate session and one diffuse) and two probe trials where the light was rotated by 40° (again, one accurate and one diffuse session). During these sessions the light was moved four times to produce four probe trials within a session. As with Experiment 1, a nose poke made at the well 90° away (alternating between clockwise and anti-clockwise) from the light was scored as correct (0° error) and suggested that the rat used the light to locate the food. If the rats were using path integration or an uncontrolled static cue to find the food, they would presumably search at the previously rewarded location and score an error of either 20° or 40° (depending on whether the light shifted by 20° or 40°). An intermediate error value would suggest that the rats were integrating the two cues in a manner predicted by a Bayesian model. Alternatively an intermediate value could suggest that the rat simply guessed the location of the food.

Figure 5.9 shows the frequency of choices made by the rat during the probe trials with both accurate lights (blue dots) and diffuse lights (red dots) when the lights were separated by 20° (plot A) and 40° (plot B). These plots show that the majority of choices (63%) were made at locations that suggest the rat was using a path integration cue or a stable extramaze cue to find the food and not the light (13%). This is supported by the findings that the circular mean choice for the 20° light separation (12.01° ± 8.7°) and 40° light separation (37.0° ± 3.9°) light separation were significantly different from a location predicted by the light (0°) (upper CI = 18.9°, lower CI = 5.2° and upper = 40.1°, lower = 34.4°, respectively). Analysis comparing behaviour between accurate and diffuse light conditions could not be conducted as there

are too few sessions. These data were however compared to the electrophysiology recordings to see if the behavioural choices were in agreement with the HD system.

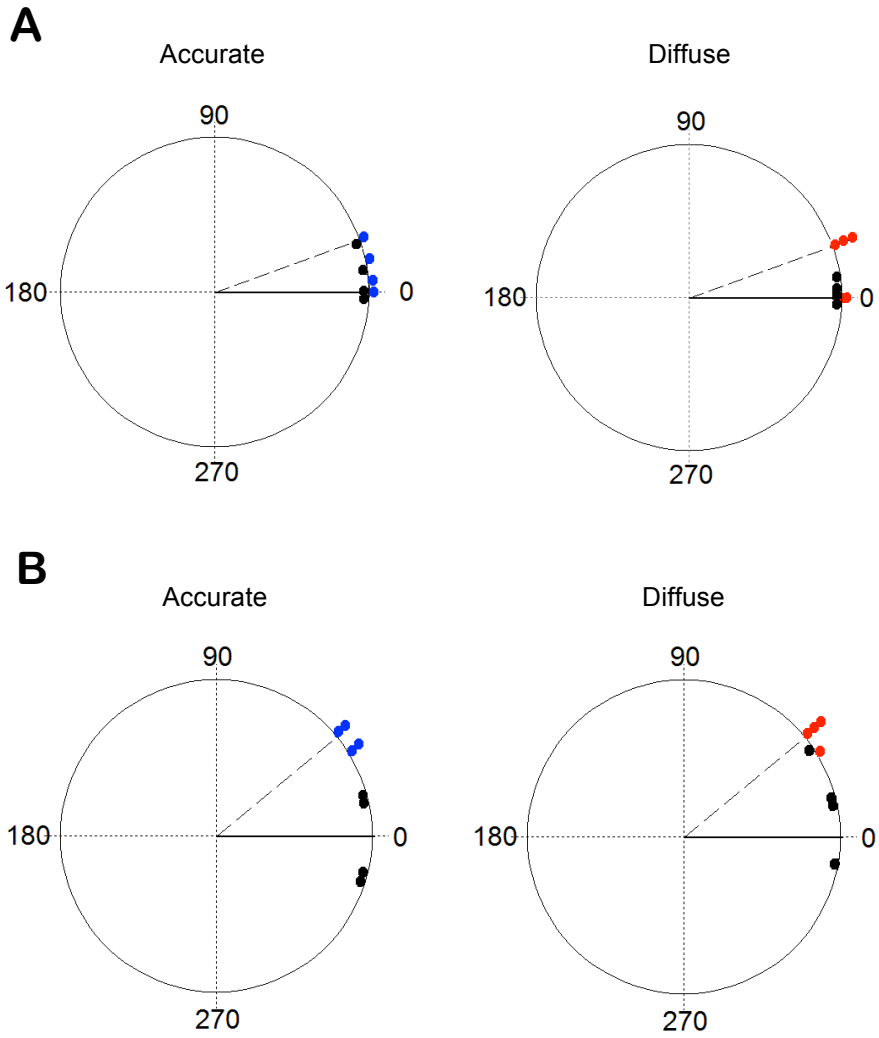

Figure 5.9: Circular plots in degrees showing the choices of the rat (coloured dots) during a 20° light separation (plot A) and a 40° separation (plot B) when an accurate light (left) and diffuse light (right) was on. The solid black lines indicate the location of correct choices (0° error) if the rat was using the light. The dashed black lines indicate the previously correct location (the pot which was rewarded in the previous trial, 20° and 8° on plot A and B respectively). Note that the coloured dots (blue-accurate and red -diffuse) typically cluster around the direction predicted by path integration (dashed line). Both the circular mean choice for the 20° light separation (12.01° ± 8.7°) and 40° light separation (37.0° ± 3.9°) light separation differed significantly from a location predicted by the light (0°) The black dots indicate the absolute deviations of the rat's HD cells (ensemble of 1-5 cells) during the accurate light conditions (left) and the diffuse light conditions (right). The black dots representing the absolute deviations in the accurate light plot significantly cluster around 0° (mean absolute deviation of 2.06°). The black dots in the diffuse light plot also significantly clustered but not around 0°. The mean absolute deviation in the diffuse condition shows a slight anti-clockwise shift (under rotation from the light) with an absolute mean deviation of 10.68°).

5.5.2 Directional firing range & peak firing rate

A total of six HD cells were recorded from the PoS of one rat. Five of these cells were recorded when the accurate light was shifted by 20°, four cells were recorded when the diffuse light was shifted by 20° and one cell was recorded when the accurate and diffuse lights were shifted by 40°. A Bayesian model would predict that the diffuse light would create more uncertainty when predicting the location of the food, in comparison to the accurate light. The directional firing ranges were measured for all cells to investigate whether the reliability of the light cue was reflected by the HD cells. More specifically, did the diffuse torch light lead to an increase in the firing range of the cells, in comparison to the accurate light? Only cells which fired in both the accurate and diffuse sessions were included in the analysis. Out of the six cells, four cells were recorded in both accurate and diffuse light conditions. The average directional firing range for the four cells during the accurate light sessions was 117.45° (± 15.9), in comparison to 120.11° (± 9.66) in the diffuse light sessions. Although the mean firing range was higher in the diffuse light sessions, a paired-samples t-test showed that this increase was not significant ($t(3) = -0.21$, NS).

The potential uncertainty created by the diffuse light was therefore not reflected in the firing range of the HD cells. However, this uncertainty could theoretically produce a reduced peak firing rate in the HD cells. The average peak firing rate for the four cells during the accurate light sessions was 22.4 Hz (± 17.1), in comparison to 16.04 Hz (± 15.0) in the diffuse light sessions. Although the peak firing rate was lower in the diffuse light sessions, a paired-samples t-test showed that this decrease was not significant ($t(3) = 0.56$, NS).

5.5.3 Mean firing direction of the HD cells

For each trial within a session, the mean firing direction of each HD cell was calculated. An example of one session is shown in Figure 5.10, where a diffuse light was rotated by 20°. Figure 5.10C shows the location of nose pokes (red dot) for each probe trial. For the initial probe trial, the rat searched at the location where the food was previously rewarded. This behaviour suggests that the rat was not using the light to locate the food. However, when examining the corresponding polar plots, there appears to be a small anti-clockwise rotation in the HD cell tuning curve from the first polar plot to the second polar plot. This shift in firing direction suggests that the rat was using the light to orient.

The choices made in the subsequent probe trials suggest that, like in the initial probe, the rat was not using the light to locate the food. It is difficult to determine whether or not the HD cell subsequently rotated by visually inspecting the data. Therefore the mean firing direction for the cell in each trial was calculated and subtracted from the former trial to formally assess whether or not the HD cells were coupled with behaviour. This analysis was performed for all four sessions.

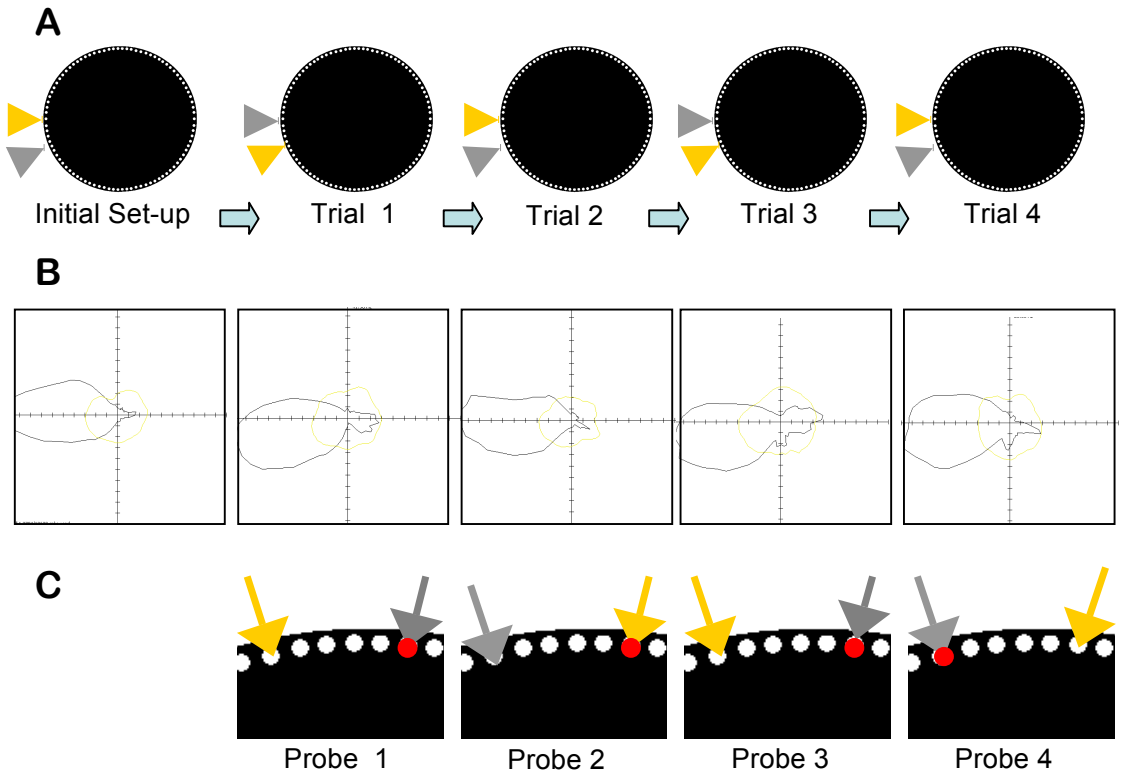

Figure 5.10: **A:** A schematic representation of the protocol used in the 20° of the diffuse light. The orange triangle represents the torch that was on and the grey triangle represents the torch that was off. Note that this session was conducted before the modifications of removing the rat from the maze whilst the platform and wells were cleaned, and the two torches were moved to a new location. **B:** The five polar plots of one HD cell recorded during this session. The first polar plot shows the firing of the HD cell before the probes were done. The subsequent polar plots show the firing direction of the cell during the probe trials Note that the HD cell shifts slightly back and forth between each probe in the direction of the light shift. **C:** A schematic representation of the choices made for each of the probes. A close-up section of the platform is drawn showing eight of the 91 wells. The yellow arrow indicates where the rat should search if it is using the light to locate the food. The grey arrow indicates where the rat should search if it is using path integration to locate the food. The red dot indicates which well the rat actually nose poked. Note that on only 1 out of the 4 occasions did the rat use the light cue to find the food

5.5.4 Comparison of behaviour and HD cells

All six cells were included in the mean firing direction analysis. As HD cells act in concert (Taube et al., 1990b), calculations were performed using the circular mean values of cells that had been recorded simultaneously, i.e., as an ensemble (ranging from 1 to 5 cells). To investigate the distribution of data the mean vector length was calculated to assess the degree to which these values clustered. Resultant vector length 'R' values which are closer to 1 indicate less variance in the data set. These data had an R value of 0.96 and a Rayleigh test showed that they were significantly clustered ($R = 15.31$, $p<0.001$).

To compare the extent of HD cell shift with the shift in behavioural searches, the latter was subtracted from the former to produce absolute deviations (deviation-from-expected). A small absolute deviation would suggest a stronger coupling between the cells and behaviour, compared to a larger absolute deviation. The mean absolute deviation for all cells was -19.04°, with a circular standard deviation of 16.87°. The absolute deviations were then compared to 0 to determine whether the extent of HD cell shift was similar to the shift in searches. The circular equivalent to a one-sample t-test showed that the absolute deviation values were significantly different from zero (upper CI = -0.73, lower CI = -0.49). This suggests that the shift in HD cells did not follow the shift in behavioural searches. The negative absolute deviation value (-19.04°) indicates that the HD cells were shifting to a greater extent in comparison to the behavioural searches. The next step in the analysis was therefore to determine whether these shifts in HD cell firing directions were similar to the shift in light location.

5.5.5 Comparison of landmark and HD cells

To compare the extent of HD cell shift with the shift in light, the latter was subtracted from the former to produce absolute deviations. The mean absolute deviation for all cells was 6.37°, with a circular standard deviation of 13.66°. These data had an R value of 0.97 and a Rayleigh test showed that they were significantly clustered ($R = 15.55$, $p<0.001$). The absolute deviations were not significantly different to zero (upper CI = 0.24, lower CI = -0.02). This suggests that the HD cells were following the light shifts.

In order to see whether the type of light affected the amount of shift in HD cell firing direction, absolute deviation values for the accurate light and the diffuse light were analysed separately. If it is assumed that the diffuse light is less reliable in comparison to the accurate

light, then in the diffuse light condition, a Bayesian model would predict the light cue would be given less weighting than it would in the accurate light condition. Thus, the HD cell shifts in the diffuse condition might consistently under rotate as path integration is given a greater weighting.

The circular standard deviations for the HD cell shifts in the accurate condition were very similar to those in diffuse condition (13.05° and 12.93° respectively) with identical R values of 0.97, suggesting that the reliability of the light was not reflected in the reliability of the cell shifts. The absolute mean deviation during the accurate light was, however, smaller than the absolute mean deviation during the diffuse light (2.06° and 10.68° respectively). Moreover, the absolute deviations in the accurate condition were not significantly different from zero (upper CI = 0.22, lower CI = -0.15), but the absolute deviations in the diffuse condition were significantly different from zero (upper CI = 0.38, lower CI = 0.004). This implies that when the accurate light was on, the HD cells reliably followed the light. However, when the diffuse light was on the HD cells showed significant under rotation (indicated by the positive absolute deviation value). This difference in absolute deviations between the two conditions can be seen in Figure 5.9 (black dots).

Taken together, the findings from the second experiment show that although the majority of choices (63%) were made at locations predicted by path integration or a static uncontrolled cue, the HD cells rotated by the same amount as the light cue. Additionally, HD cells rotated less when the diffuse light was on, suggesting that the cells were influenced less by the diffuse light in comparison to the accurate light. Finally, there was no significant difference in the directional firing range or peak firing rate of HD cells recorded when the accurate or diffuse light was on.

5.6 Discussion

This study consisted of two experiments, the first using a behavioural task and the second taking simultaneous recordings of behaviour and HD cell firing. These two experiments were conducted to assess whether rats/HD cells integrate path integration and landmark information, and if they do, whether this integration is consistent with Bayesian inferences. In the first experiment, rats performed slightly better with the accurate light during training but this was not significant and the probe trials indicate that the rats did not integrate information about the light cue and path integration to locate the food. Recordings of HD cells in the second experiment did however show evidence of cue integration. Moreover, the

findings suggest that the weighting of these cues was based on the perceived reliability of that cue.

5.6.1 Behavioural training

In the initial experiment, rats were trained over thirty-five sessions to retrieve food from a location 90° away from a light that was varied between accurate and diffuse. The rats chose the correct pot 81.4% (± 1.6) of the time in the accurate light condition and 76.0% (± 1.8) in the diffuse light condition. These values indicate a very good performance, considering the correct well was located 23 wells (90°) away from the light cue. To test whether a Bayesian model can account for these results, the data needs to be normally distributed (Fetsch et al., 2010). In other words, the majority of behaviour needs to be correct but crucially the behaviour must also show a degree of variability. To support this model specifically, this degree of variability needs to increase with a decrease in cue reliability. However, the rats' performance in this experiment showed very little variance, and the variance that was present did not increase when the reliability of the cue decreased.

A number of reasons could explain why the rats' performance on the task was so good. It could be that their path integration signal was highly accurate and reliable, or that the light cue was an excellent navigational beacon, even when the light was diffuse. In order to improve this behavioural task, the diffuse light could also be made more diffuse and less reliable, so that any behavioural differences between the two conditions would be more apparent. Another suspected reason for this impressive performance was that the rats were using a third cue to find the food – odour. To overcome this problem in the second experiment, the arena was cleaned between probe trials.

If these improvements were implemented and the reliability of choices between the two conditions was different, the next step in this revised experiment would be to examine behaviour when only one cue is available (Battaglia et al., 2003). Using vision-only trials (by disorienting the rat) and self-motion trials (by using a non-directional light source) the model can be tested to see if accuracy when given both cues is equal to that predicted by Bayesian integration of the individual cues.

5.6.2 Behaviour during probe trials

During probe trials, a conflict was produced by shifting the light by 20° but keeping the path integration signal stable. Like the behaviour during training, the confounding factor of odour

could also account for the probe data. These data indicated a bimodal search pattern, where the rats either searched at the location predicted by the light or the location predicted by path integration/uncontrolled static cue (Fig 5.8). Both of the wells at these two locations would smell of the food reward and so if the rat used their sense of smell to locate the reward, they would sometimes search at the well predicted by the light and sometimes at the well predicted by path integration. Alternatively, it may be that like the children in Nardini et al (2008) study, the rats were unable to integrate the two conflicting cues. Nardini et al (2008) found that unlike the adults which showed near optimal integration of visual and path integration cues, children under the age of eight produced optimal behaviour when only the visual information was available. They therefore concluded that this ability to integrate cues was not innate, but rather a developmental process. The bimodal choice patterns shown by the rats in this study may be indicative of an undeveloped process to integrate cues.

Another possible reason for a lack of cue integration in this study could be because the conflict between the light cue and path integration was too large. If the perception of the two cues is too large, an integration of these cues will not occur (Thurlow and Jack, 1973; Cheng et al., 2007). Efforts were therefore made to reduce the cue conflict and encourage cue integration in the latter probe trials. When the cue conflict was reduced from 20° to 8° the Bayesian model was moderately supported. When the accurate light was on, rats mostly searched at a location predicted by the light (56%) rather than using path integration/static uncontrolled cues (31%), or an integration of the two (0.06). However, when the diffuse light was on, the majority of searches were at locations predicted by path integration/static uncontrolled cues or a weighting of these cues and the light cue (50%). None of these differences were statistically significant however. This lack of significance may reflect the fact that the number of trials was relatively low in this part of the experiment (two accurate trials and two diffuse trials). Additional testing using this paradigm should reveal whether there is a true difference between choices made with an accurate light and a diffuse light.

5.6.3 HD cell responses

In the second experiment, recordings were taken of six HD cells recorded from the PoS of one rat which took part in the first experiment. Based on the observations of Gu et al (2008), comparisons of tuning widths from cells recorded in both the accurate and diffuse light conditions were compared. Gu et al (2008) found that the width of the tuning curves for MSTd neurons were narrower when two cues rather were available, in comparison to when

just one cue was available. However, in this present study, no significant differences in HD cell firing ranges were found between the accurate and diffuse light conditions. Neither were there any significant differences in the peak firing between these two conditions. Together these findings suggest that the uncertainty produced by unreliable visual cues is not expressed in the PoS HD cells. This lack of expression may not be too surprising given that many computational models assume that tuning curves of HD cells always maintain the same width (Zhang, 1996) and that within the same cell, firing rates of HD cells have shown to be relatively irregular within a trial (Taube et al., 2004).

Even though cue uncertainty due to cue unreliability was not witnessed in the HD cells, the cue weightings which result from this cue uncertainty were apparent. For example, when the accurate light was on, HD cell rotation was significantly similar to the degree of light rotation. However, when the diffuse light was on, HD cells no longer reliably followed the light. Instead, the cells showed an under-rotation where the mean firing direction of cells shifted to a location between that predicted by the light and that predicted by path integration/static uncontrolled cues (Fig 5.9). It therefore appears that when the reliability of the light decreased, the weighting of that cue also decreased and consequently the weighting of the path integration signal increased. This finding, together with the findings from the peak firing rate and directional firing range suggests that the HD system integrates visual and path integration information, but does not directly express the reliability of these cues. It may therefore be that uncertainty is coded at a neuronal level, but before the signal reaches the HD system.

In support of this possibility, the postsubiculum receives input from visual areas (Vogt and Miller, 1983; Clark et al., 2009) such as the primary visual cortex (V1). It is also know that there are neurons in V1 which have a bell-shaped tuning curve for visual orientation, shown by Hubel and Weisel (1962). It may be that the distributions expressed by these cells can code for the reliability of visual stimulus. Indeed, Vidyasagar and Siguenza (1985) presented sinusoidal gratings (pattern of black and white bands) to cats, whilst recording from V1, they found that a population of neurons in V1 demonstrated a decrease in the orientation tuning width when there was an increase in the spatial frequency of the stimulus. In other words, when the visual stimulus provided the visual system with fine detail, the tuning width of cells in V1 became tighter. Further recordings in visual areas and from other modalities such as vestibular neurons could establish whether these cells express cue reliability using Bayesian principles.

5.6.4 Coupling of head direction cells and behaviour

Interestingly, the cue integration observed in the HD cells in the second experiment was not reflected in the behaviour of the implanted rat. The rat's behaviour did not indicate a difference between the weighting of the light cue when it was accurate or diffuse. However, the choices did indicate a general bias towards using path integration information. This bias is similar to that found by Fetsch (2009) where they found that rhesus monkeys tended to overweigh vestibular information and under-weigh visual information. It is difficult to interpret exactly why the behaviour of the animal suggested a strong bias to path integration, when the HD firing suggested that the light cue was used to orientate. Having said this, a disagreement between HD cell firing and behaviour has been previously shown (Dudchenko and Taube, 1997; Golob et al., 2001). This disagreement particularly becomes apparent when multiple cues conflict. For example, Golob et al (2001) found that although a rat's search strategy remained constant between a square and rectangular environment, the firing direction of HD cells rotated by approximately 90°. The experimenters reasoned that the behavioural searches remained stable because the path integration signal remained stable. The HD cell firing however rotated by 90°, presumably because they were using the corners of the environment to orientate.

This idea was specifically tested by Frohardt et al (2002) using a modified Barnes (1979) maze. Essentially, this maze was circular and on the periphery of the maze there were multiple goal location (in this case doors separated by 45°) and was therefore not too dissimilar to the maze used in this current study. The experiment consisted of four conditions; a standard condition where rats had to locate the goal using either path integration or a large cue card, a blindfolded condition where the rat could only use path integration, a dark condition which was similar to the blindfolded condition, and finally a conflict condition where in a lit room, the goal location was rotated with respect to the cue card and room.

Frohardt et al (2002) found that generally rats performed very well on this task. However, when the rats made occasional errors, the findings were particularly interesting. The majority of errors occurred because there was an under-rotation of HD cells during a cue conflict. This under rotation guided behaviour but because the HD signal was not in correct reference to the goal location or in fact the location of any door , searches were made to a neighbouring door (45° away from the correct door), but in the same direction as the under-rotation. In addition to this, the realisation that the chosen door was incorrect led the

rat to select the correct door on subsequent trials, even though the HD cells maintained the same firing direction across trials. Together these findings, along with the findings from this current study indicate that a conflict of multiple cues can force dissociation between HD cell firing and behavioural responses. Further investigation taking simultaneous recordings will hopefully uncover the mechanisms behind this dissociation. Given the findings from this present study, it may be that dissociation occurs because HD cell firing is based on an integration of multiple cues but behavioural choices by the rat tend to be based on information exclusively from one cue.

5.6.5 Conclusion

The first behavioural experiment found that the performance of rats was at ceiling level, with very little variation in choices. The absence of a normal distribution was also apparent in the probe trials, where searches were bimodal. The bimodal searches implies that either the rats could not integrate landmark and path integration cues, or they were using an odour cue to find the food. A further study could establish whether rats cannot in fact integrate conflicting cues, or whether this study became the victim of a methodological problem such as an odour confound. Alternatively, the diffuse light may not have been diffuse enough or the conflict between the two cues may have been too great. The probe trials using a reduced 8° conflict supports this latter idea, where there was a small, albeit non-significant effect of cue integration using the diffuse light.

Although the behavioural findings in this study did not support a Bayesian model, recordings of HD cells in the PoS did show evidence of cue weighting based on cue reliability. When the accurate light was on HD cells rotated with the light, but they did not rotate with the diffuse light. Interestingly, the reliability of the cues was not expressed in the directional firing range or peak firing rate of the HD cells. Potentially therefore, this reliability coding could occur before the signal is sent to the HD system. Nevertheless, the HD firing showed that both landmark and path integration signals are utilised by the system, and when placed in conflict, the HD system can integrate these two cues. It is important to note however that uncontrolled static cues such as odour or auditory cues may also be used during orientation and therefore these results do not simply reflect a conflict between visual landmark cues and path integration.

The final experimental chapter examines cue conflict further, to establish how HD cells weigh visual and path integration information when they are placed in progressive conflict

with each other. As there was a lack of correspondence between the HD signal and behaviour in the present study, the subsequent study was designed so that the rats were not required to learn a behavioural task. Instead, the next study has focussed on the response of HD cells only, whilst the rats explored the same environment that was used in this present experiment.

Chapter 6 Exploring the conflict between allothetic and idiothetic cues

6.1 Introduction

Findings from the first two experiments along with a plethora of other studies have shown that HD cells use a combination of both visual (Taube et al., 1990b; Goodridge et al., 1998; Zugaro et al., 2004) and path integration cues (Taube et al., 1990b; Taube and Burton, 1995; Stackman et al., 2003) for orientation. The exact relationship between these two cues however is not known; some believe that the HD system favours landmark information over path integration (Mizumori and Williams, 1993) whereas some state the opposite to be true (Chen et al., 1994a). What can be agreed upon is that utilising these multisensory cues to create a single, reliable signal requires a degree of cue integration. The previous chapter contained preliminary findings from one rat, which illustrated that HD cells integrate these two cues based on their perceived reliability. The present chapter takes this initial finding and further explores the nature of cue integration by the HD system by manipulating the size of the conflict between the light cue and path integration.

6.1.1 Landmark dominance during cue conflict

Previous studies have examined the response of HD cells to a conflict between idiothetic and allothetic cues (Chen et al., 1994a; Goodridge and Taube, 1995; Knierim et al., 1995; Blair and Sharp, 1996) (for a more detailed description of the literature, see Chapter 2.6.2). The majority of these studies showed that HD cells were more likely to fire using landmark information compared to information from the path integration signal (Goodridge and Taube, 1995; Knierim et al., 1998). This landmark dominance has also been found in place cells (Jeffery and O'Keefe, 1999) where a cue card showed strong control over place fields. However, this control was soon lost if the rat had previously viewed the card as unstable. Knierim et al (1998) found that both place cell and HD cell firing was primarily influenced by landmark information as opposed to vestibular information. Interestingly however, this landmark dominance was only seen when the cues conflicted by 45°. At 180°, this dominance was not observed and the response from HD cells was mixed. These findings were also similar to a place cell study by Rotenberg and Muller (1997).

Knierim et al (1998) created only two degrees of conflict for most rats, thus the experimenters were unable to establish what size of conflict would cause the landmark to

lose dominance over the HD cells. In order to understand how the size of conflict between allothetic and idiothetic cues affects the integration of these two cues, the conflict situations need to be studied in finer steps. This present study therefore created a paradigm where conflicts ranged from 20° to 180°, with intervals of 20°. The cue conflict was created by moving the position of a light cue back and forth between two locations, around a circular arena. This landmark manipulation provided a conflict because the rat was not disorientated during the task and could therefore use path integration and also olfaction as a reliable cue.

The first aim of this study was to see if the cells' mean firing direction would rotate with the lights, and if so, to determine the degree to which they rotated. Given the findings from previous studies (Blair and Sharp, 1996; Knierim et al., 1998) it was predicted that for at least a 40° conflict, HD cell firing would be predominantly influenced by the light cue. Additionally, the stepwise paradigm was used to see if HD cells are predominantly influenced by the light cue beyond a 40° conflict.

6.1.2 Cue dominance or cue integration?

When the cue conflict was at its greatest (180°) Knierim et al (1998) found that the landmark dominance witnessed at a 45° conflict was no longer apparent and the response from HD cells was mixed. This current study aimed to quantify these variable results at greater conflicts, by using finer degrees of cue conflict. Based on previous findings (Blair and Sharp, 1996; Knierim et al., 1998) it was predicted that at small conflicts (<45°) HD cells will outweigh landmark information in comparison to path integration information. However, for greater cue conflicts this landmark dominance will be lost. Once the light cue loses dominance over the HD cells, the cells may switch to exclusively relying on the path integration signal (or an uncontrolled static cue such as odour) as the light cue may now be considered unreliable. In other words, the cells may switch from exclusively using landmark information to exclusively using path integration information. Alternatively, the landmark information may continue to influence the HD cells but the amount of influence that the landmark has may gradually reduce. In other words, the HD cells may use a compromise that shows the simultaneous influence of both cues. Given the findings from the previous chapter that demonstrate cue integration in HD cells, the latter was predicted.

6.1.3 Cue integration in different areas of the HD network

To fulfil a secondary aim of this study, recordings were taken from three different areas of the HD pathway; the postsubiculum (PoS), the reterosplenial cortex (RSP) and the anterior

dorsal thalamic nucleus (ADN). This was done to assess whether the findings from Knierim et al (1998) and Blair and Sharp (1996) from HD cells in the ADN could be generalised to other areas of the HD system. Goodridge and Taube (1995) did record from both the PoS and the ADN, but direct comparisons could not be made as only PoS HD cells were recorded in the second half of the experiment. The presence of conflicting findings in the field (Mizumori and Williams, 1993; Chen et al., 1994a) has led to the speculation that the influence of landmark manipulation has differential control over cells, with respect to different areas of the HD pathway (Knierim et al., 1998).

HD cells are known to have different properties depending on where they are located in the brain. For example, neurons in the RSP tend to have slightly narrower directional tuning widths in comparison to the ADN, and both are thought to have narrower tuning widths than cells recorded in the PoS (Wiener and Taube, 2005). It is generally thought that the peak firing rates of HD cells do not significantly differ from one region to another (Blair et al., 1998) but instead show large variability across the network (Taube et al., 2004). Yoganarasimha et al (2006) and Blair and Sharp (1995) however did observe that cells in the ADN had consistently larger peak firing rates in comparison to those in the PoS. Also, unlike the PoS, HD cells in the ADN also show anticipatory firing (Blair and Sharp, 1995). Moreover, cell activity in the ADN (Taube and Muller, 1998) increases with an increase in angular head velocity.

One potential reason why the majority of HD cells in the ADN but not the PoS show anticipatory firing and a modulation for angular head velocity is that ADN cells receive a strong path integration signal (Clark et al., 2010a) which makes them particularly sensitive to head movement. These cells can then provide advanced path integration information to the rest of the HD pathway (Blair and Sharp, 1995). Additionally it is thought that HD cells in the ADN receive this path integration signal from the lateral mammillary nuclei (LMN) and dorsal tegmental nuclei (DTN) (Hayakawa and Zyo, 1985). This is supported by findings that the peak firing rates of HD cells in the DTN (Bassett and Taube, 2001) and LMN (Stackman and Taube, 1998) are mediated by angular head velocity and turning direction. As well as the processing of path integration information, studies have also shown that the degree to which HD cells are influenced by visual cues may differ according to where the cells are in the HD pathway (Mizumori and Williams, 1993).

Figure 6.1: A schematic diagram taken from (Clark et al., 2009). The diagram shows the flow of allothetic and idiothetic information in the HD system. The diagram illustrates the idea that the PoS is the initial structure to receive visual information in the HD pathway.

It is generally considered that the HD cells in the PoS receive a stronger visual input in comparison to the ADN (Fig 6.1) , a structure thought to be located further downstream in the allothetic pathway (Clark et al., 2009). This has been shown experimentally by Goodridge and Taube (1997) and Clark et al (2010a) when they found that after lesioning the PoS and RSP respectively, HD cells in the ADN were no longer influenced by visual landmarks.

The retrosplenial cortex receives widespread cortical projections from visual cortices (Vogt and Miller, 1983) and it is also thought that visual information is received by the PoS via the RSP (van Groen and Wyss, 1992). Clark, Bassett, Wang and Taube, (2010a) found that RSP lesions caused a general decrease in firing for ADN HD cells and increased the time of anticipatory firing. More specifically, they found that when these rats experienced an environment where a landmark rotated, the HD cells failed to rotate. These findings led the authors to conclude that the RSP plays a crucial role in the firing of HD cells in the ADN. Moreover, this role that the RSP plays is thought to be particularly relevant to the integration of visual cues into the HD circuit.

These findings, that suggest the visual information is initially processed in the PoS and RSP (Goodridge and Taube, 1997; Clark et al., 2010a), may be reflected in the weighting of visual and path integration cues in these areas. In other words, HD cells in the PoS and RSP are thought to predominantly receive (Vogt and Miller, 1983; Wyss and van Groen, 1992) and send (Goodridge and Taube, 1997; Clark et al., 2010a) visual information as opposed to vestibular information. This bias for visual information may be apparent in the HD cell firing,

for example, HD cells in the PoS and RSP might show a greater response to visual cues in comparison to the ADN.

6.1.4 Attractor networks and transition dynamics

The idea that HD cells in different brain areas are differentially affected by idiothetic and allothetic cues would however be inconsistent with some versions of a dynamic attractor network (see Chapter 2.4). The main principle of a dynamic system is that the state of that system evolves over time. The mechanism by which this dynamic activity copes with noise involves the action of 'attractors'. Attractors are stable states which act as memories for the system (Touretzky, 2005). When noise enters the system, the main consequence of attractor dynamics is to bring the system back to these stable states. HD cells are typically modelled in the shape of a ring (Zhang, 1996) in order to represent the preferred firing directions of each cell.

The key characteristic of such a network is that cells are linked together through both excitatory and inhibitory connections (Boucheny et al., 2005). Essentially, it is thought that a cell will excite neighbouring cells but will simultaneously inhibit distant cells. In the HD system, this coupling of excitatory and inhibitory behaviour is thought to take on a Gaussian-like distribution, similar to that expressed by the firing of the HD cells themselves (Sharp et al., 2001a). Thus cells that represent similar directions will fire at the same time, but cells which represent opposing directions will never fire at the same time. When the rat's head moves, a vestibular signal is sent to the HD system, causing the activity to shift in the direction signalled by the vestibular input (Skaggs et al., 1995; Zhang, 1996; Touretzky, 2005). In conjunction, allothetic cues such as visual landmarks will influence the HD system. It is generally considered that landmark cues will initially set the cell's firing direction, whereas path integration updates this signal (Taube, 2007). Moreover, a path integration signal will tend to accumulate error over time and thus landmark information is also thought to act as a 'corrective' cue (Knierim et al., 1998). If all HD cells are part of the same attractor network then the relationship between all cells across all areas in the network would remain constant. Therefore cells in the RSP or PoS should respond to visual information in the exact same manner as cells in the ADN.

One way to reconcile the idea of differential cue weightings with an attractor network would be to suggest that there are local attractor networks. Redish et al (1996) proposed such a model termed a 'coupled attractor', where each area in the HD system contains its own

attractor network. This would predict that HD cells in the same area would have a constant relationship with one another but they would not necessarily have to have a constant relationship with cells from another brain region. One problem with this current 'coupled attractor' model is that it requires recurrent connections in the ADN, which are not thought to exist. Taking recordings of HD cells in different areas of the pathway should reveal whether or not these cells integrate conflicting cues in the same manner.

Further examination of the HD network and its attractor state was performed to fulfil the final aim of this study. By taking continuous neuronal recordings throughout each session, a direct observation of cell firing could be made shortly before and after change of the light location. Examining these time intervals should provide an insight into how activity in the HD circuit propagates from one preferred firing direction to another, after a sudden change in orientation. This investigation into the transition dynamics of HD cells has so far only received moderate attention.

Zugaro et al (2003) found that the establishment of a new preferred firing direction in HD cells was highly rapid (~80ms) and thus concluded that the cells' activity jumped from one preferred firing direction to another. However, this conclusion was only based on the short latency period of the updating of the HD cell signal. The animal's head was fixed in position during recordings and therefore HD cell firing over intermediate directions cannot be ruled out. Thus it may be that the cell's activity sweeps across intermediate directions in order to reach the new preferred firing direction (Redish et al., 1996). The present study therefore aimed to address this debate of whether a cell's activity jumps from one direction to another, or if the activity sweeps across intermediate directions.

Recently, Jezek et al (2011) examined the properties of attractor networks in the CA3 region of the hippocampus. Rats were trained in two boxes that had different light cues. During testing, the rats were placed in one of these environments and after minute, the cues were switched instantaneously to those in the other environment. They recorded place cells and theta activity in CA3 during these 'teleportation' trials. First, they found that place fields in the two environments rarely overlapped, demonstrating that CA3 had a different representation for each environment. During the teleportation or transition periods, the activity representing the initial environment briefly flickered to the activity representing the new environment. This flickering could occur several times within a trial and each flicker was extremely quick (approximately a tenth of a second). Interestingly, they found that theta cycles correlated strongly with either environment, but very rarely both environments. This

suggests that CA3 represented *either* the first *or* the second environment. An integration of these representations in CA3 firing was very rare. Given these findings (Zugaro et al., 2003; Jezek et al., 2011) it would be predicted that activity in the HD circuit would 'jump' rather than 'sweep' to a new direction.

In summary, this present study had three main aims. First, to determine the point at which HD cells switch from primarily using landmarks to relying heavily on path integration information. Second, to see whether HD cells in different brain regions were the same in this regard. Finally, to explore how activity in the HD circuit propagates from one preferred firing direction to another, after a sudden change in landmark orientation.

6.2 Materials and methods

6.2.1 Subjects

Six adult male Lister Hooded rats (weighing between 282 - 435 g at the time of surgery) were housed individually [11:11 light:dark, with 1 hour (x2) simulated dawn/dusk] on a food-restricted diet (sufficient to maintain 90% of free-feeding weight) with *ad libitum* access to water. All procedures were licensed by the UK Home Office subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act 1986.

6.2.2 Apparatus

The majority of the apparatus used in the experiment was taken from the previous experiment (Chapter 5). For a further description of the apparatus see section 5.2.2 and Figure 5.2. The two torches used in the previous experiment were also used in this experiment. Both torches were placed behind the translucent wall, producing dispersed 4-5cm circles of light. Only one torch was ever switched on at one time. Two torches were used so that the position of the light could easily move location without the experimenter having to physically move the torches. As all main lights were switched off during the experiment, the torch light was the only salient polarising landmark. The 91 holes around the perimeter of the platform were not baited in this experiment.

6.2.3 Surgery and electrodes

All rats were implanted at the start of the experiment with moveable microelectrodes. Description of these microelectrodes and surgical procedures can be found in the General Methods section. The electrodes were lowered into the brain in areas known to contain HD cells. Two rats (R321 & R344) were implanted in the PoS (Bregma coordinates: 6.7 AP, 2.8 ML, 1.6 DV), two rats (R409 & R410) were implanted in the ADN (Bregma coordinates: -1.8 AP, 1.4 ML, -2.1 DV), and two rats (R1696 & R1704) were implanted in the RSP (Bregma coordinates: -5.5/-6.3 AP, 1.3/1.5 ML, -1.2/-2.3 DV). All animals were given at least one week to recover following the surgery.

6.2.4 Screening procedures

Recording commenced one week after surgery. Recording was done using multichannel recording equipment (DacqUSB, Axona Ltd). A description of the screening procedures can be found in the General Methods. Screening for HD cells took place in a room separate from the actual experimental room, to minimize the learning of extraneous cues in the recording environment by the rats.

6.2.5 Recording procedures

When an HD cell was found, the rat was taken into the experimental room in an opaque holding box. The rats were then placed on a raised holding box outside of the curtained enclosure in order to acclimatise to the room for five minutes. The rat was connected to the recording device outside of the curtained enclosure and then returned to the holding box which was carried into the enclosure through one of three joins in the curtains and placed in a pseudorandom location on the floor. After a minute, the rat was taken out of the holding box and placed in the centre of the platform.

On the first session, the environment was set up so that one torch was on, whilst the second torch was switched off and placed 20° away from the first light (in either a clockwise or anti-clockwise direction). Recording began when the rat was placed in the environment. During the session, rats foraged for food. After 4 minutes of recording, the original light was switched off and the rat was left in darkness for 10 seconds. The initial switching off of the first light signalled the end of trial one. The second light was then switched on for 4 minutes and then switched off for 10 seconds. This second light is referred to as the ‘conflict’ light as

it conflicted with the original light position. This protocol was then repeated so that during each session there were five trials, where the original light was on for 3 x 4 minutes and the conflict light was on for 2 x 4 minutes. Thus, a single session was recorded for 20 minutes and 40 seconds. The position of the two lights never moved within a session, so the location of the light switched back and forth, five times during a session (Fig 6.2).

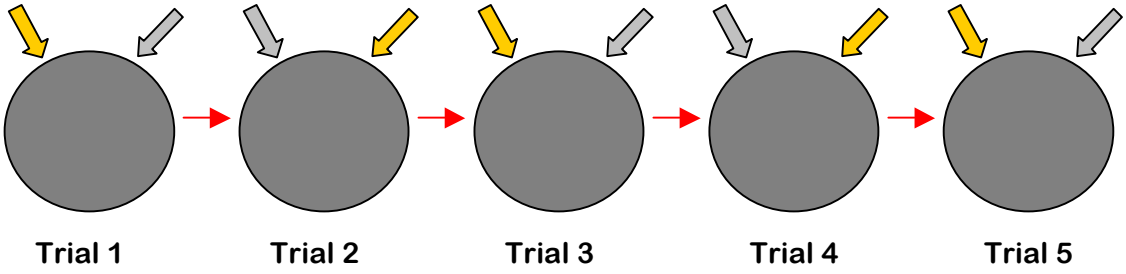

Figure 6.2: A schematic drawing of the experimental protocol for one session. The orange (light on) and grey (light off) arrows represent the two torches. The spacing between the pairs or arrows indicates the type of session, in this case a 40° session. The original light was switched on in trials 1, 3 and 5. The conflict light was switched on in trials 2 and 4. Each trial lasted for four minutes, in between each trial the rat foraged in darkness for ten seconds, represented by the red arrows.

For the subsequent session, both torches were moved to a new location around the circle and were now separated by 40°. This light separation continued to increase in 20° steps across each session. Each session was separated over a day, with the final day presenting a 180° light conflict (Table 6.1). The reason for increasing the light conflict sequentially was because the degree of experience that a rat has with a landmark can affect the degree to which they rely upon it (Knierim et al., 1995). It was therefore hypothesised that if the rats experienced a very large conflict on earlier trials, the HD cells would never use the light cue on subsequent trials. Although this design means that spatial and temporal effects are confounded, it does still mean that the integration of cues can be observed during conflict situations. Additionally, using a sequential method ensures that all rats have the same experience of the light at each stage of the experiment. This was particularly important for comparing HD cells recorded in different brain regions of different rats. Neuronal recordings were taken throughout the session in order to examine the cells' transition dynamics. At no point in the session was the rat disorientated or the platform cleaned. Therefore vestibular information and olfactory cues remained intact throughout the session.

Session Number (containing 5 x trials)	Degree of conflict
S1	20°
S2	40°
S3	60°
S4	80°
S5	100°
S6	120°
S7	140°
S8	160°
S9	180°

Table 6.1: The sequential presentation order for each light conflict. One session lasted 20 minutes 40 seconds (composed of 5 x 4 minute 10 second trials). The breakdown of a single session is presented in Figure 6.2

6.2.6 Data analysis

Cluster-cutting software (Tint, Axona Ltd) was used to analyse the data offline. A description of the cell isolation process can be found in the General Methods (Chapter 3). Each cell's mean firing direction, peak firing rate, directional firing range and resultant vector length for each trial was calculated using the procedures detailed in the General Methods. All cells were required to meet a firing rate criterion whereby cells with a peak firing rate of less than 1.0 Hz across the session were excluded from further analysis. HD cells which had an 'R' value of less than 0.3 within a given session were also excluded (van der Meer et al., 2010).

Analysis of directional firing range and peak firing rate was performed on singe cell data using linear statistics. Two-way ANOVAs were used to examine the effects of session and brain location of the HD cells on peak firing rate and directional firing range. Analysis of HD cell mean firing direction was performed using circular statistics, examining mean values of cells that had been recorded simultaneously, i.e., as an ensemble (ranging from 1 to 3 cells). All circular analysis was also done using the CircStat Matlab toolbox (Berens, 2009). In order to analyse the rotation of HD cells from one trial to the next, the circular mean direction of cells in the first trial was subtracted from the circular mean direction of cells in the second

trial to provide a measure of how far the ensemble had rotated. This was repeated for each subsequent trial of the session so that each session produced four rotation estimates. A mean of these four values were then taken to produce a mean ensemble shift for each session. These mean shifts were then subtracted from the predicted angle of shift (based on how much the light had rotated) to produce absolute deviations from expected rotation. The mean vector length of absolute deviations for each condition was calculated. The Rayleigh test (Berens, 2009) was then used to determine whether these absolute mean deviations clustered around a particular direction. Circular inferential statistics (Berens, 2009) were used to compare absolute mean deviation values against zero (the predicted deviation given perfect light-following), using a one sample test. The multi-sample test (circular analogue to the Kruskal-Wallis test) was used to calculate the main effects of brain region and session, as well as an interaction between the two.

To analyse the transition dynamics of each HD cell, each session was broken down into 0.1 second intervals. For each time point, the direction (in degrees) of the rat's heading direction was then extracted along with the information indicating whether or not that HD cell had fired. Plots were then created to illustrate when the cell had fired and what head direction that firing corresponded to.

6.2.7 Histological analysis

At the end of testing, the rats were deeply anaesthetised with isoflurane before they were injected with sodium pentobarbital. They were then transcardially perfused using saline and then paraformaldehyde (4%). The brains were removed and stored in paraformaldehyde (4%) for at least one week before sectioning. The brains were sliced at 40 micrometers on a freezing microtome. The sections were then mounted and stained with Cresyl violet (Fig. 6.3) as described in the General Methods section.

6.3 Results

6.3.1 Histology

Nine cells were recorded from rats implanted using PoS coordinates (four cells from Rat 321 and five cells from Rat 344), six cells were recorded from rats implanted using RSP

coordinates (four cells from Rat 1696 and two cells from Rat 1704) and two cells were recorded in rats implanted using ADN coordinates. Histology confirmed that the electrodes in Rat 344 were placed in the postsubiculum (PoS) (although the tracks are close to the PoS/RSGa border, see Fig 6.3), those in Rat 1704 were placed in the retrosplenial cortex (RSP), and those in Rat 409 and Rat 410 were placed in the anterodorsal thalamic nucleus (ADN) (Fig 6.3). Note that the electrodes in Rat 321 may have actually been in the subiculum rather than the postsubiculum and the electrodes in R1696 may have actually been in the secondary visual cortex rather than the retrosplenial cortex. Linear and circular statistics were later used (presented below) to examine whether the area in which the HD cells were recorded affected the peak firing rate, directional firing range and shift in the mean firing direction.

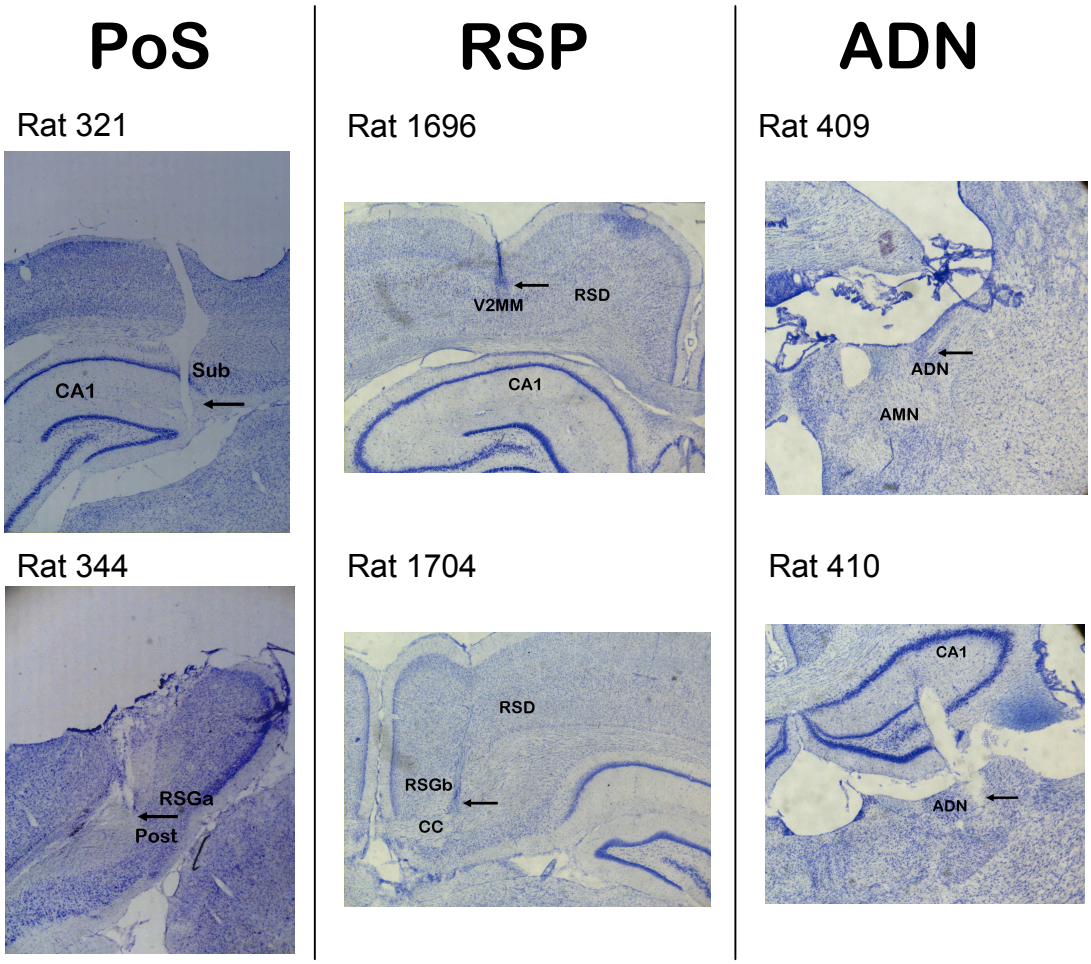

Figure 6.3: Histology of all six rats. Each section has been labelled using Paxinos and Watson (2004) to show the location of the CA1 region of the hippocampus, subiculum (Sub), postsubiculum (Post), retrosplenial granular 'a' region (RSGa), retrosplenial granular 'b' region (RSGb), retrosplenial dysgranular region (RSD), secondary visual cortex (V2MM), anterior dorsal thalamic nucleus (ADN), anterior medial thalamic nucleus (AMN) and the corpus callosum (CC) . Each arrow indicates the estimated recording site on the slide. Three brain regions were targeted: postsubiculum, retrosplenial cortex and the anterior dorsal thalamic nucleus. Note that the electrodes in Rat 321 may have actually been in the subiculum rather than the postsubiculum and the electrodes in R1696 may have actually

been in the secondary visual cortex rather than the retrosplenial cortex. Also note that the electrode tracks in Rat 344 are very close to the postsubiculum/retrosplenial border and therefore the electrodes in Rat 344 were possibly in the retrosplenial granular 'a' region.

6.3.2 Cell characteristics

A total of seventeen individual HD cells, in 8 ensembles of 1-3 cells in each, were recorded from six rats during 54 sessions of 270 trials. These seventeen HD cells met the inclusion criteria of having a peak firing rate of more than 1.0 Hz and a mean vector lengths ('R') of more than 0.3 (van der Meer et al., 2010). Across all seventeen cells, the average peak firing rate was 16.37 Hz (± 2.25), the average firing range was 120.8° (± 4.86) and the average 'R' value was 0.54. Data examining the peak firing rate and directional firing range from the individual neurons is presented first, followed by the analysis of mean firing directions performed on the ensemble data.

6.3.3 Peak firing rate and directional firing range

The mean peak firing rate in the PoS/subiculum appeared to be lower (3.64 Hz, S.D, 2.19) in comparison to the RSP (15.69 Hz, S.D, 13.58) and the ADN (29.78 Hz, S.D, 25.37). The mean firing range in each of the three areas appeared to be relatively similar. The mean firing range in the PoS/subiculum was 107.13° (S.D, 19.19), in the RSP was 132.65° (S.D, 30.6) and in the ADN was 122.67° (S.D, 17.18). As there was only a relatively small number of cells recorded in each brain region (particularly the ADN where only two HD cells were recorded), statistical analysis on peak firing rate and directional firing range were not performed on the data.

6.3.4 Shifts in firing direction

The mean firing direction in each trial was calculated for each cell's tuning curve. As each session contained five trials, five tuning curves were produced along with their respective mean firing directions (Fig 6.4).

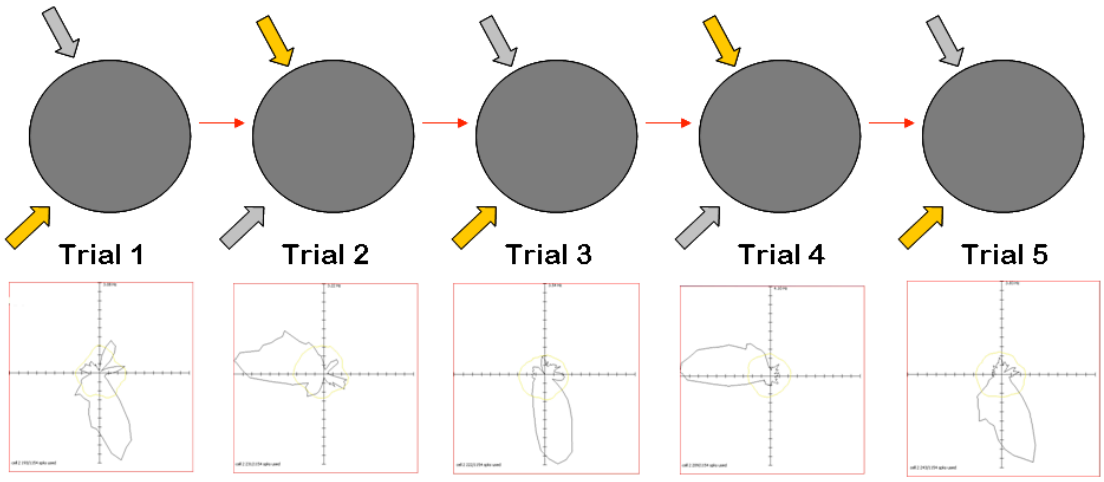

Figure 6.4: Five polar plots of one cell recorded from Rat 321 (PoS implant) during 120° light conflict session. The orange (light on) and grey (light off) arrows represent the two torches separated by 120°. Note that the HD cell in this example rotates by approximately 110° between trials, in the same direction as the light.

Using Matlab, HD tuning curves were plotted on a HD versus firing rate plot for all cells in all sessions. Figure 6.5 shows two examples of when the tuning curves rotated with the light during 80° and 120° conflicts. Figure 6.6 shows two examples during light conflicts beyond 120° when most cells failed to rotate their firing directions with the light.

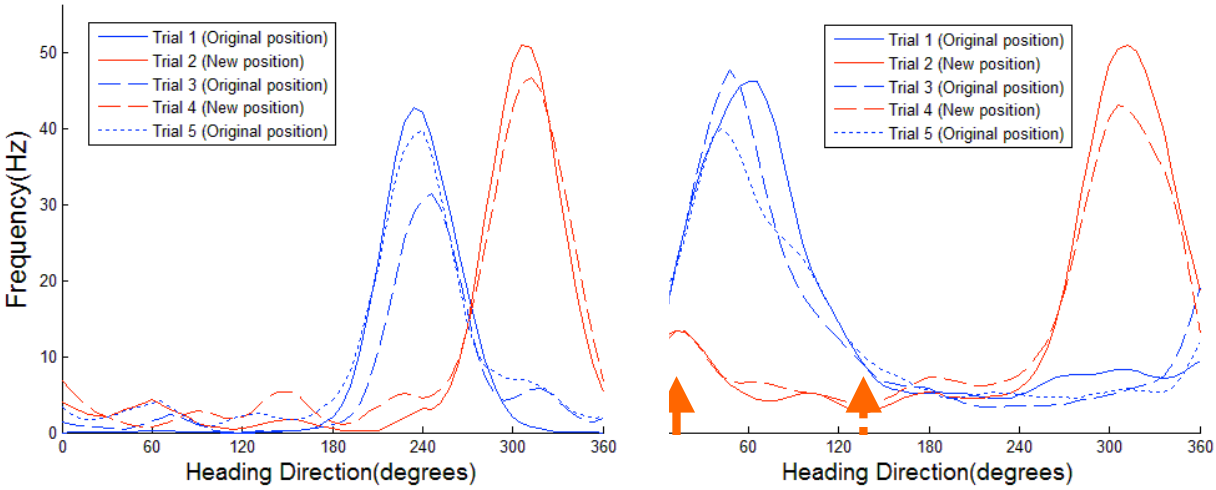

Figure 6.5: *Left* A frequency versus HD plot for Rat 409 illustrating the tuning curves for the 5 trials of an 80° light conflict session. The orange arrows represent to position of the two lights. The solid orange arrow indicates the position of the original light. The dashed orange arrow indicates the position of the conflict light. The tuning curves for the original light exposure (blue lines) had a mean firing direction of approximately 230°. When the light was moved to the new position 80° away, the tuning curves (red lines) had a mean firing direction of approximately 320°. Thus the approximate shift in mean direction was 90° (320°-230°) *Right:* A frequency versus HD plot for Rat 1696 illustrating the tuning curves for the 5 trials of a 120° light conflict session. The tuning curves for the original light exposure have a mean firing direction of approximately 50°, whilst the tuning curves for the conflict light exposure have a mean firing direction of approximately 310°. Thus the approximate shift in mean firing direction was 100° (Note that on this linear plot, the shortest distance between the red and blue tuning curves wraps around the plot)

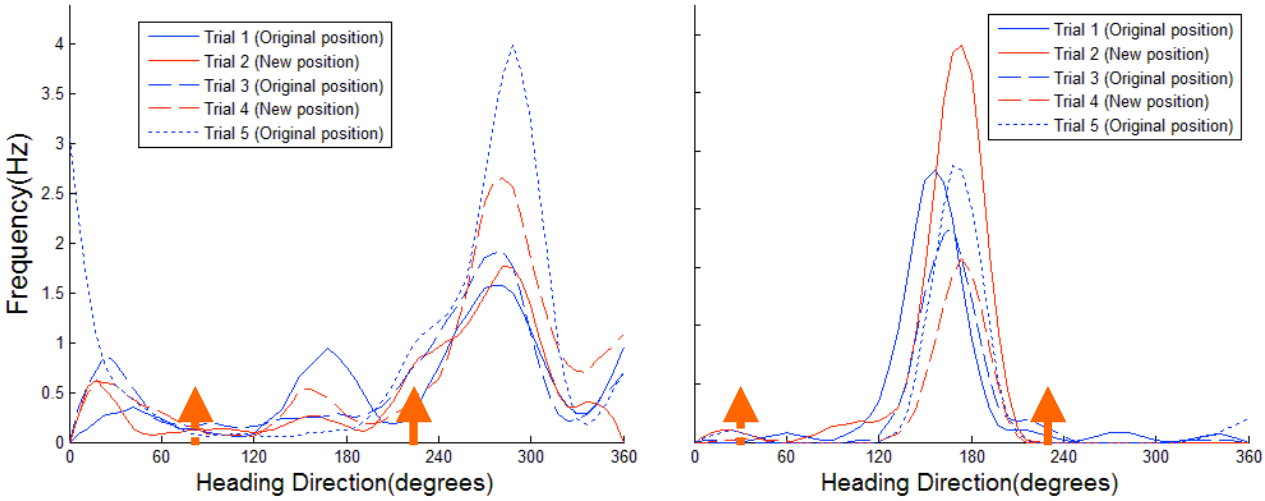

Figure 6.6: *Left* A frequency versus HD plot for Rat 321 illustrating the tuning curves for the 5 trial of a 140° light conflict session. The tuning curves for the original light exposure have mean firing direction of approximately 280°, as do the tuning curves for the conflict light exposure. *Right* A frequency versus HD plot for Rat 344 illustrating the tuning curves for the 5 trials of a 160° light conflict session. The tuning curves for the original light exposure have a mean firing direction of approximately 170°, as do the tuning curves for the conflict light exposure

Once all the mean firing directions were extracted for each trial, the mean firing shift was calculated for each cell by subtracting the mean firing direction from one trial from the subsequent trial. If multiple cells were recorded in a session, the average mean shift was calculated, because HD cells from a single animal always act in concert and react together to environmental changes (Taube et al., 1990b). All subsequent analysis was performed on ensemble data and analysed using the CircStat Matlab toolbox (Berens, 2009). Table 6.2 shows the descriptive statistics for the actual directional firing shifts of HD cells in comparison to the predicted shift (degree of light shift).

Light shift	Mean ensemble shift	Mean shift as % light shift	Abs. Mean Deviation (SE) (Undershoot)	Vector length	Rayleigh test
20°	13.7°	69	6.3 (± 2.4)	0.99	R=5.9***
40°	21.2°	53	18.8 (± 2.5)	0.99	R=5.9***
60°	41.2°	69	18.8 (± 2.4)	0.99	R=5.9***
80°	64.5°	81	15.5 (± 4.6)	0.98	R=5.9***
100°	66°	66	34 (± 6.0)	0.98	R=5.8***
120°	93.7°	78	26.3 (± 18.1)	0.7	R=4.2*
140°	69.8°	49	70.2 (± 15.8)	0.77	R = 4.5*
160°	50.9°	32	109.1 (± 12.4)	0.86	R=4.3*
180°	29.6°	16	150.4 (± 8.0)	0.94	R = 5.7***

Table 6.2: Calculations for each light rotation (i.e. each session). The table shows the mean shift for all cells per session shown in degrees and as a percentage of the actual light shift. Along with the absolute mean deviations (mean shift – light rotation) standard error, vector length and results from the Rayleigh test to show that they significantly cluster (*** = 0. 001, * = 0.05 alpha level)

There was a general trend for these absolute mean deviations or 'undershoots' to increase with an increase in light rotations (discussed in detail below). The variation within the data also increased with an increase in light rotations, which is reflected in the standard deviation values and vector length 'R' values (Table 6.2). However, column six of table 6.2 shows that all data points are significantly clustered (at an alpha level of 0.05* or 0.001***) within each of the light rotation sessions (Rayleigh test, see table 6.2). This suggests that all the recorded HD cells rotated their firing by similar amounts when exposed to the same light conflict. The comparison between the actual mean ensemble shift for each session and the expected shift (light rotation) is illustrated in Figure 6.7.

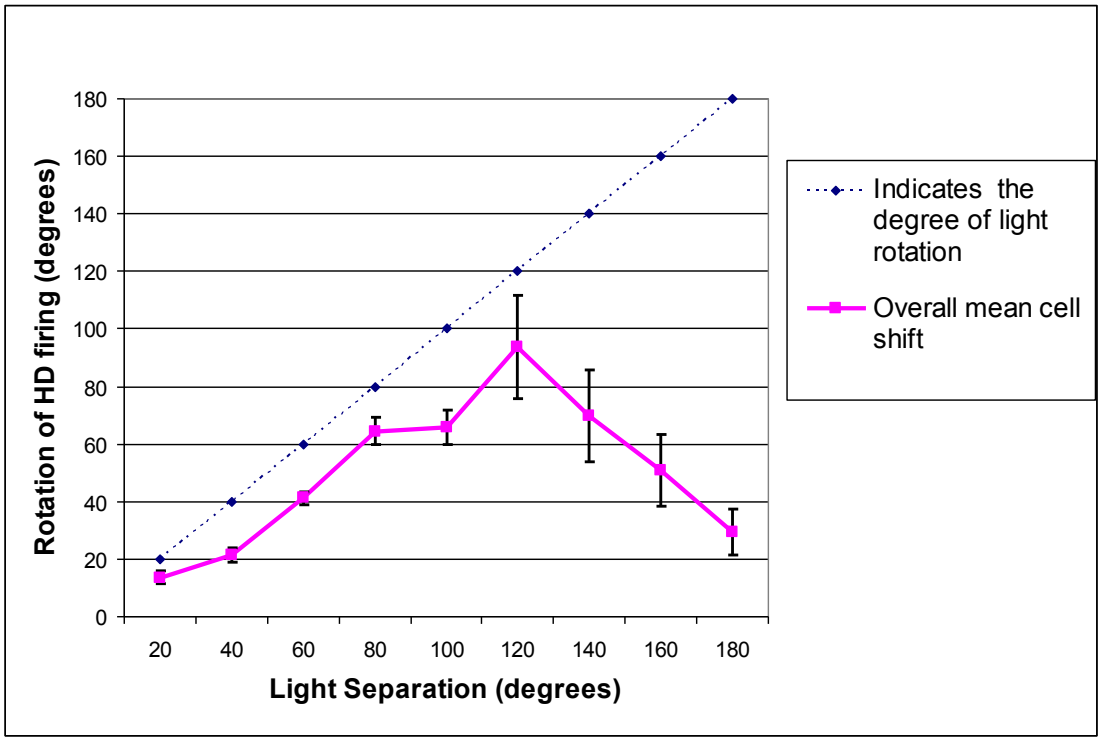

Figure 6.7: Plot showing the relationship between the expected shifts in degrees of the HD cells based on the light shift (blue line) and the actual mean ensemble cell shift (pink line) across each session.

The graph shows that the mean shift in cells steadily increases with the angular distance between the lights, up until the point at which the light shifted by more than 120°. This pattern in the data was quantified by calculating the amount of cell shift as a percentage of the actual light rotation (Table 6.2). The cells shifted their firing by more than 50% of the light shift up to 120° conflict, beyond which the firing shifted by less than half of the light shift. Beyond a 120° light conflict, the rotation of HD firing also shows a greater variation (larger standard error bars in Fig 6.7). However, it is important to note that this data is an average of all six rats and therefore it cannot be determined whether this variance reflects differences in HD firing rotations between rats or also within rats. An analysis looking at rats individually was performed to assess this, and is described later in this section.

To examine the shift of cell firing direction further, a multi-sample circular test was used. This test analysed the main effect of session, brain region and an interaction between the two, to see whether the mean shift of cells was affected by the extent to which the light rotated (session) and the brain region the cells were recorded from.

The multi-sample circular test revealed a main effect of Session ($\chi^2 = 21.7$, d f= 4, $p<0.05$), which can be seen in Figure 6.7. Post hoc tests were then used in order to further

investigate this effect. The mean cell shift for each session was compared to the degree of light rotation, by comparing the average undershoot (absolute deviation), measured for each light separation, to 0. An undershoot that was not significantly different from 0 would indicate that the cells rotated their firing directions by the same extent as the light. It was found that the undershoots for all light rotations except 120° were significantly different from zero and constantly under-rotated (Table 6.3), suggesting that the cells never rotated as much as the light (except 120°) Under-rotation of HD cells following landmarks are consistently reported in the literature and are typically around 15-20° (Taube et al, 1990b; Taube, 1995). Additional analysis was therefore performed where the actual mean shift of the cells was compared to zero. Mean shifts that were not significantly different to zero would indicate that the cells did not rotate and therefore were not influenced by the light. The mean cell shifts for all sessions were significantly different from zero (Table 6.3). This indicates that the on average, cells showed some rotation during all sessions.

Light shift	Mean undershoot (SE) in degrees	Undershoot different to 0? (Upper CI/Lower CI) in degrees	Mean ensemble shift	Mean shift different to 0? (Upper CI/Lower CI) in degrees
20°	6.3 (± 2.4)	Yes (11.46/5.73)	13.7°	Yes (17.19/5.73)
40°	18.8 (± 2.5)	Yes (22.92/11.46)	21.2°	Yes (28.65/17.19)
60°	18.8 (± 2.4)	Yes (22.92/11.46)	41.2°	Yes (45.84/34.38)
80°	15.5 (± 4.6)	Yes (28.65/5.73)	64.5°	Yes (74.48/51.57)
100°	34 (± 6.0)	Yes (51.57/11.46)	66°	Yes (80.21/51.57)
120°	26.3 (± 18.1)	No (74.84/-22.92)	93.7°	Yes (143.23/45.84)
140°	70.2 (± 15.8)	Yes (114.59/28.65)	69.8°	Yes (114.59/28.65)
160°	109.1 (± 12.4)	Yes (148.97/68.75)	50.9°	Yes (91.67/11.46)
180°	150.4 (± 8.0)	Yes (171.89/131.78)	29.6°	Yes (51.57/11.46)

Table 6.3: Calculations for each light rotation (i.e. each session), comparing the undershoot (absolute deviation) to 0° and the mean ensemble shift to 0°

Taken together these results suggest that the HD cells used a combination of both light and path integration information regardless of conflict size. As the platform was not cleaned during the session it is likely that the rats were also using olfactory cues to orient. The weighting of all these directional cues was however dependent on the session, where cells shifted their firing by at least 50% of the light shift during conflicts of 120° and less, but by less than 50% at greater conflicts. However, as mentioned earlier, Figure 6.7 shows data averaged across all rats and at a session level, where each session contains five trials. Therefore during light rotations, it cannot be determined from this graph whether cells rotated by the same extent as the light in some trials, and completely failed to rotate in the remaining trials, or whether the rotations in all trials within a session demonstrated an integration of light cues and path integration. In order to assess whether there were intra-session variations, the breakdown of ensemble shifts between each trial were plotted for each rat across all nine sessions (Fig 6.8).

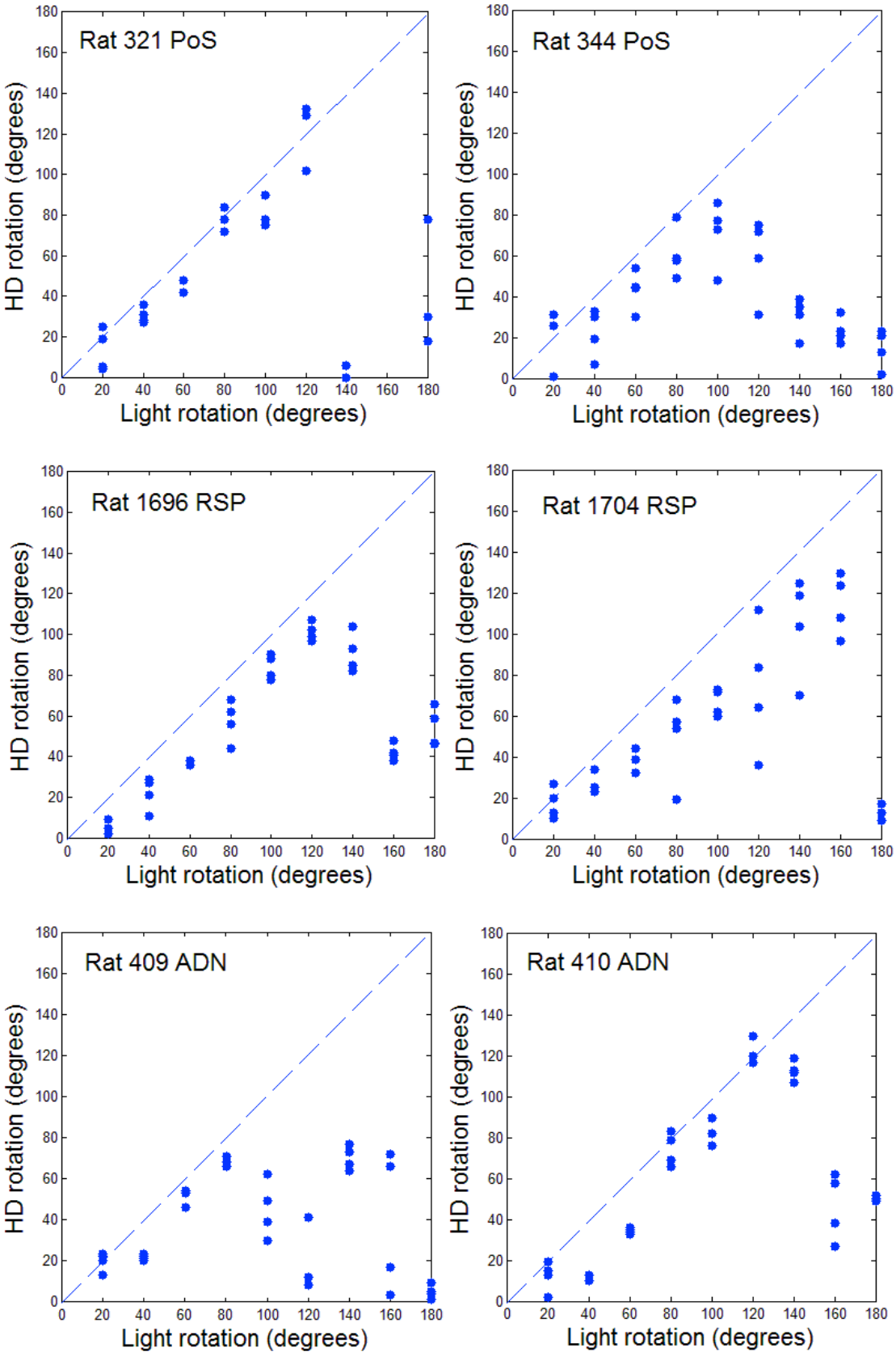

Figure 6.8: Plots showing the breakdown of mean ensemble shifts for each trial within a session. Separate plots indicate each of the six rats. Each blue dot represents the mean ensemble shift from one trial to the next. Thus, there are four dots for each light rotation (four light shifts per session). The dashed blue line indicates the amount the light shifted for each session.

Figure 6.8 shows that intra-session variation is relatively low. It appears that although intra-session variation generally increased across sessions, rotations in trials beyond a 120/140° cue conflict are markedly smaller than the light rotations. Most cells at this point tended to partially rotate between trials, with any intra-session variation due to some cells partially rotating and some cells not rotating (or drifting slightly). This suggests that for the majority of cells, the intermediate rotation values for the light rotations of 140° and 160° (Fig 6.8) are due to intermediate values expressed across all trials and not a combination of trials where cells rotated fully with the light and trials where they completely failed to rotate.

The multi-sample circular tests revealed that there was no significant main effect of brain area (χ^2 = 0.5, d f= 16, p>0.05). This can be seen in Figure 6.9 where each line depicts the mean ensemble shift for each brain across the nine sessions, along with the light rotation (black line). PoS HD cell firing appears to rotate slightly less in comparison to the other two regions but this is only really apparent when the light was rotated by 140°. For all the other light separations, the standard error bars show a great deal of overlap. There was also no significant interaction between session and brain area (χ^2 = 25.2, d f= 16, p>0.05).

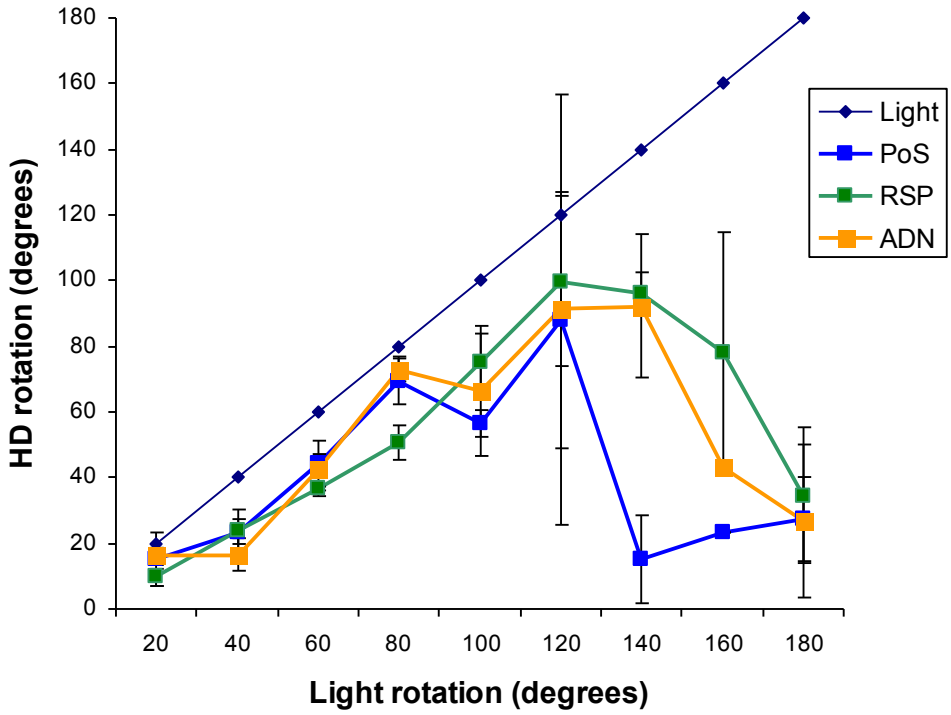

Figure 6.9: Plot showing the mean ensemble rotation for each brain area across all nine sessions. The blue line represents data from rats implanted in the PoS, the green line represents data from rats implanted in the RSP and the orange line represents data from rats implanted in the ADN. The black line represents the amount the light was rotated in each session.

In summary, the mean cell shifts for all sessions were significantly different from the light shift and from zero (amount predicted if HD cells used only path integration or uncontrolled static cues such as odour). These results suggest that the HD cell firing was based on a combination of directional cues. The weighting of this information was however dependent on the session, where cells shifted their firing by at least 50% of the light shift during conflicts of 120° and less, but by less than 50% at greater conflicts. The intermediate values expressed by the majority of cells during these greater conflicts were due to intermediate values expressed across all trials (cue integration) and not a combination of trials where cells rotated fully with the light and trials where they completely failed to rotate. There was no significant difference in mean cell shifts across session for each brain area.

6.3.5 Transition dynamics

The following set of analyses was conducted to determine at a fine temporal scale how activity in the HD circuit propagates from one preferred firing direction to another, after a sudden change in orientation. Previous work examining the speed of transition in the HD circuit (Zugaro et al., 2003) and the attractor dynamics of place cells in CA3 (Jezek et al., 2011) suggest that activity in the HD circuit might 'jump' rather than sweep over intermediate states to reach the new preferred firing direction.

The transition dynamics of each cell were observed by plotting the mean firing direction of the cell on a scatterplot as a function of time (binned in 0.1 seconds) for the interval of 0-1240 seconds. The majority of cell recordings produced plots that were highly difficult to interpret, for a number of reasons. First, there is a problem of sampling, as the rat may not have faced the preferred firing direction of the recorded cell at the time of the light transition. Second, rats were initially exposed to sessions with a relatively small conflict (20° then 40°). Coupled with a general under-rotation of the firing directions, this meant that the change in firing direction from one trial to the next was too small to observe a sweeping or jumping transition. Similarly, towards the end of the experiment, rats were exposed to very large conflicts which generally resulted in the firing directions rotating by only small amounts. This left a window in the middle of the experiment where the best observations could be made (for the light shifts of ~60° to 120°). From these remaining sessions, many cells showed a large variability, with a notable amount firing outside of two standard deviations from the mean firing direction. This was particularly true for cells with high firing rates (e.g. cells recorded in the ADN). Even with additional smoothing (using a smoothing kernel of 0.5, 1, 3 or 5 seconds) still produced plots which were very noisy because the rat's

head movements were very rapid. There were however, four interpretable examples (Fig 6.10) which are interesting to examine in detail.

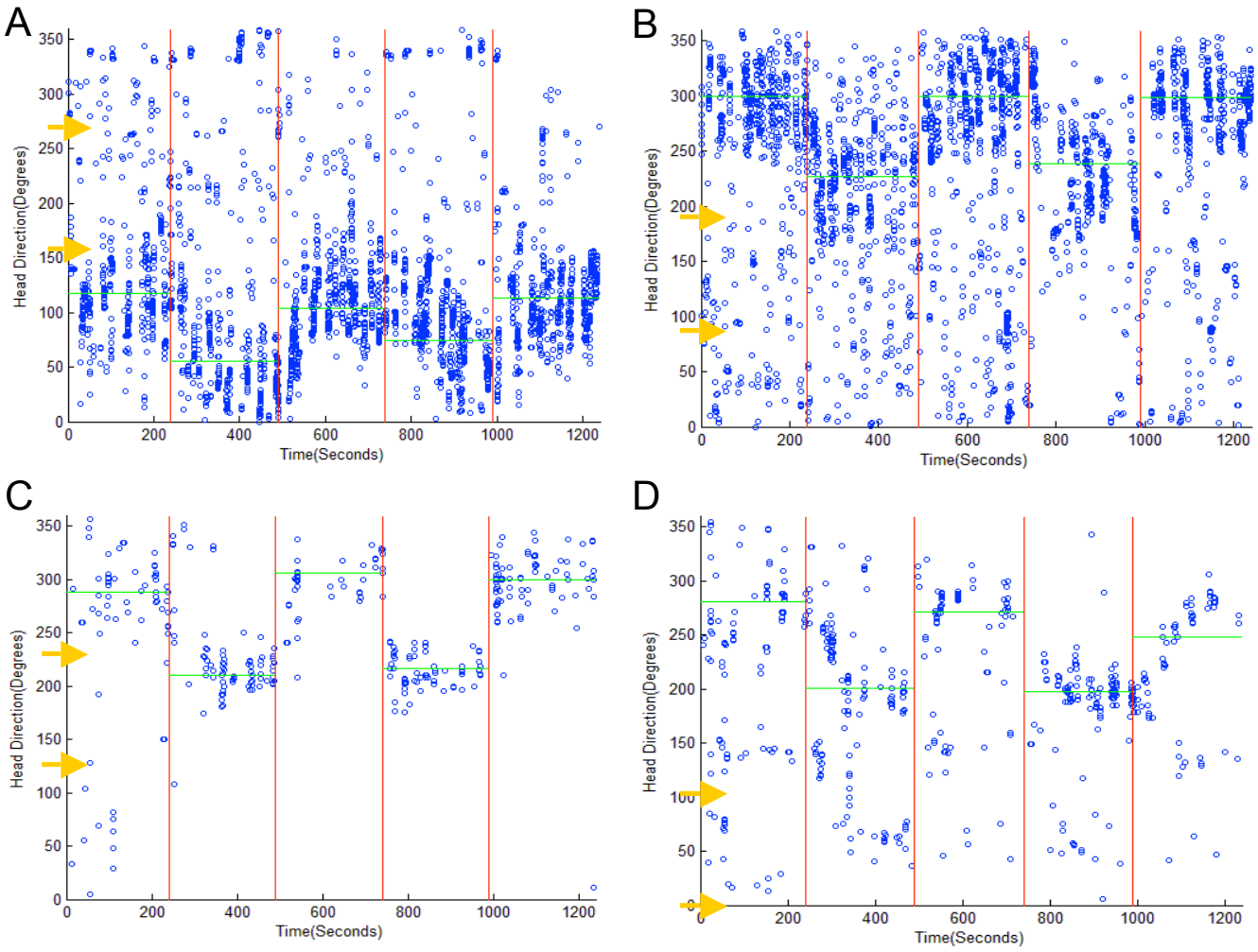

Figure 6.10: Time versus directional plots showing the firing of HD cells (blue dots) during four 120° light shift sessions. Plot A shows a cell from rat 409 (ADN) firing. Plot B shows a cell from rat 1704 (RSP). Plot C shows a cell from rat 321 (PoS) firing and plot D shows a cell from rat 344 (PoS) firing. The red lines indicate the time points where the light switches off for ten seconds, after which a light switches on in the new position. The green lines indicate the mean firing direction of the cell for that trial. The yellow arrows indicate the location of the two lights.

In order to look at the transition dynamics in these examples, the immediate points around the light change (red lines in the plots on Fig 6.10) were examined. There appear to be instances of cells firing at intermediate directions in all four plots with perhaps the clearest

examples of this in plot A (Fig 6.10). This intermediate firing is also apparent between the transition of trial 1 and 2 and trial 2 and 3 in plot B, between trial 1 and 2 and trial 2 and 3 in plot C and between trial 4 and 5 in plot D (Fig 6.10).

During the other trial transitions there does not appear to be this 'sweeping' of cell firing. However, a lack of intermediate firing does not necessarily mean that the firing of a cell 'jumped' from one direction to another. Due to natural sampling limitations (the animal could only sample one head direction at a time) the cells may not have fired at an intermediate direction because the rat's head was not facing that particular direction in time. This possibility was explored by taking an example where no intermediate cell firing was apparent (between trial 2 and 3 in Fig 6.11) and plotting the rat's actual heading position during this light transition (Fig 6.12). From this example it can be seen that this lack of firing could plausibly be due to the rat only sampling the intermediate space very briefly during this time (Fig 6.12). It may be predicted that this brief sampling should still cause the cell to fire, but it should be noted that this cell only fired when the rat was either stationary in that particular direction, or they sampled that particular direction multiple times in quick succession. Therefore a lack of intermediate firing between some of the trials may be because the rat did not sample the intermediate space enough for the cells to fire.

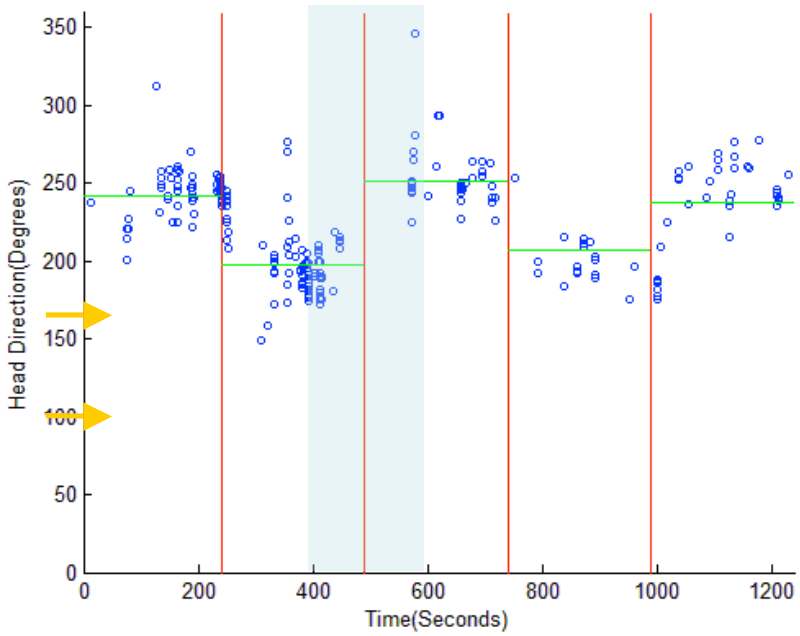

Figure 6.11: Time versus direction plot showing the firing of a HD cell from rat 344 during a 60° light shift session. Note that although there is indication of intermediate firing for the transition between trial 4 and 5, there is a distinct lack of intermediate firing between the other trials, most notably between trial 2 and 3 (highlighted in blue).

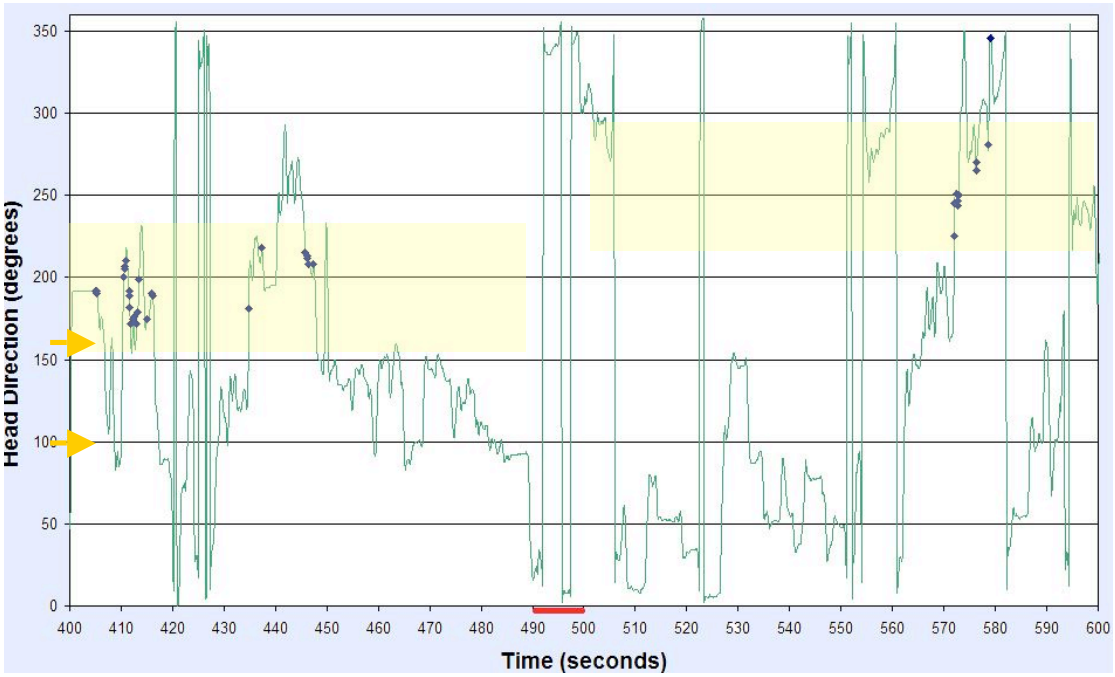

Figure 6.12: Time versus direction plot showing the firing of a HD cell from rat 344 (blue dots) during the blue highlighted section from Fig 6.13 (400-600 seconds). The green trace indicates the rat's heading direction. The red line indicates the ten seconds where the light switches off, after this a light switches on in the new position (490-500 seconds). The shaded yellow bands show the mean direction (with confidence intervals) for this cell during the two trials. Note that the rat's head did not dwell, but rapidly swept through these yellow bands during the light transition (490-500 seconds).

Taken together, these findings suggest that activity in the HD circuit may sweep continuously from one preferred firing direction to another, after a sudden change in landmark orientation. Figure 6.10 shows examples of HD cells firing at intermediate directions, supporting the idea that activity sweeps over intermediate directions before settling at the new preferred firing direction. However, not all transitions showed evidence of intermediate firing (Fig 6.11). This could either be because the activity jumped to the new preferred firing direction or because the rat did not sample the relevant heading direction during the light transition (Fig 6.12).

Finally, the sweeping of activity shown in Figure 6.10 may have important implications for the findings shown in Figure 6.7. Figure 6.10 shows that at the beginning of each trial, a cell will initially fire at a direction similar to the mean firing direction in the previous trial. The preferred firing direction of this cell will then gradually rotate and settle at a new preferred firing direction. This sweeping of activity at the beginning of each trial may explain why there is a clear degree of under rotation of the HD cells in Figure 6.7. To directly test this

idea, the previous analysis of the cells' mean firing shift and absolute deviation (undershoot) was repeated using only the last two minutes of each four minute trial. The new analysis is presented in Table 6.4 and in Figure 6.13.

Light shift	Overall mean ensemble shift	Mean ensemble shift for latter half	Abs. Mean Deviation (SE) for latter half	Vector length for latter half	Undershoot different to 0? (Upper CI/Lower CI) for latter half
20°	13.7°	23.3°	-3.3 (± 2.5)	0.99	No (2.6/-9.2)
40°	21.2°	22.5°	17.5 (± 2.8)	0.99	Yes (24.1/11)
60°	41.2°	45.6°	14.4 (± 3.7)	0.99	Yes (23.1/5.7)
80°	64.5°	68.7°	11.3 (± 4.4)	0.98	Yes (21.6/0.97)
100°	66°	80.2°	19.8 (± 3.8)	0.99	Yes (28.8/10.9)
120°	93.7°	104.3°	15.7 (± 12.1)	0.87	Yes (55.4/0.7)
140°	69.8°	71.7°	68.3 (± 14.6)	0.8	Yes (106.4/28.2)
160°	50.9°	66.7°	93.3 (± 14.6)	0.81	Yes (138.8/49.4)
180°	29.6°	27.6°	152.4 (± 6.8)	0.96	Yes (169/136)

Table 6.4: Calculations for each light rotation (i.e. each session). The table shows the mean shift for all cells during the whole session and the latter half of each session, along with the absolute mean deviations (mean shift – light rotation) standard error and vector length. Calculations are also shown for each light rotation (i.e. each session), comparing the undershoot (absolute deviation) to 0°

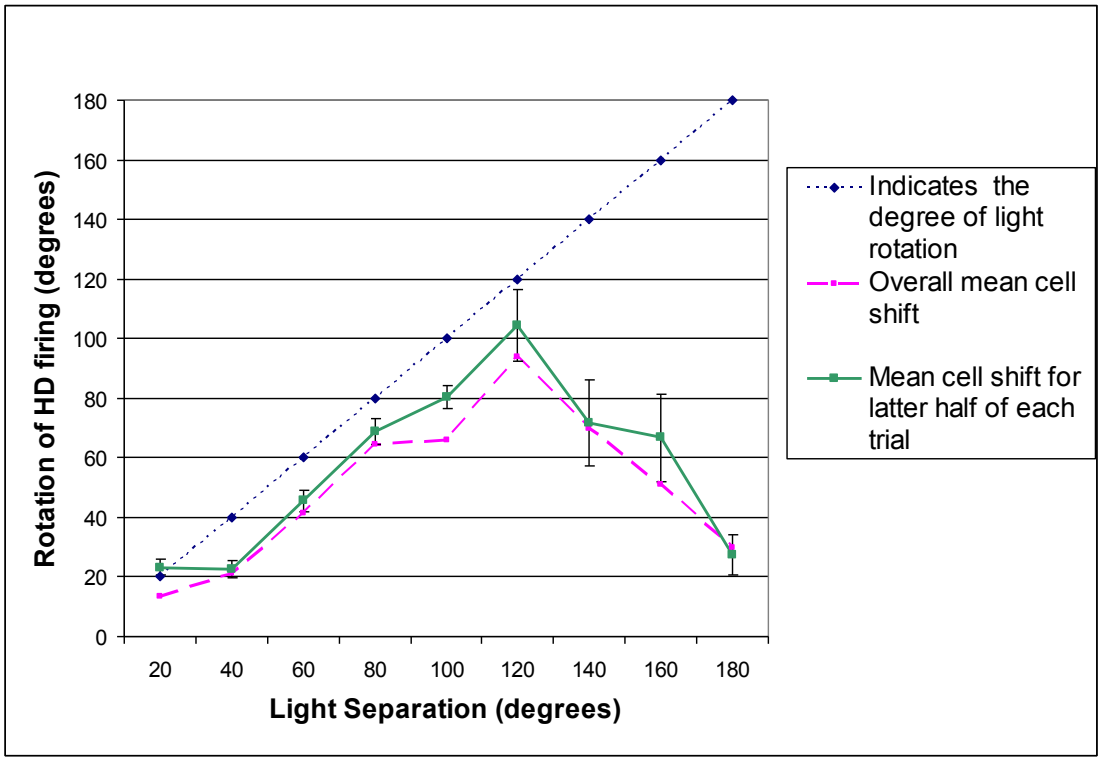

Figure 6.13: Plot showing the relationship between the expected shifts in degrees of the HD cells based on the light shift (blue line) the actual mean ensemble cell shift across each session (pink line) and the mean ensemble shift using data recorded in the latter half of each trial (green line) .

As can be seen from Figure 6.13, using only the data recorded in the latter two minutes of each trial rather than the whole trial resulted in a greater mean cell shift in all sessions except the last. However, the absolute mean deviations for each session (except the first) is still significantly greater then zero (Table 6.4). In conclusion, it appears that the sweeping of activity during the beginning of each trial can account for some of the under-rotation shown in the previous analysis using data from the whole trial. However, an under-rotation is still apparent when using only the data from the latter half of each trial, which suggests that the HD cells were using a combination of the light cue and other directional cues.

6.4 Discussion

One of the main findings from this study was that the shift in firing of HD cells during all cue conflicts demonstrated an integration of both light and path integration information. As the platform was not cleaned during the session it is likely that the rats were also using olfactory cues to orient. The weighting of these cues was dependent on the session, where cells shifted their firing by at least 50% of the light shift during conflicts of 120° and less, but by less than 50% at greater conflicts. This cue integration shows that during a conflict between

idiothetic and allothetic cues, the HD cells fired at an intermediate direction. This intermediate firing was also seen within the attractor network, where activity from a single cell appears to sweep over intermediate directions before settling at a new preferred firing direction. Finally, there were no apparent differences in cue integration between areas in the HD network.

The extent to which HD cells are influenced more by landmark than path integration cues or uncontrolled static cues will be discussed first. The presence of intermediate firing both spatially (HD cells firing at an intermediate direction during cue conflicts) and temporally (HD cell activity passing through intermediate states when the cell's preferred firing direction suddenly changes) will then be discussed. Comments will then be made about the apparent lack of differences in cue integration between areas in the HD network.

6.4.1 Threshold of visual landmark control

Within each session, the extent to which each ensemble shifted its mean firing direction across different rats was statistically similar (significant clustering of absolute mean deviation values for each session). This suggests that the conflict situation caused all cells to react in a very similar way, with little variation. The degree of variation between rats did however slightly increase with an increase in cue conflict, which is consistent with previous findings (Blair and Sharp, 1996; Knierim et al., 1998). This can be seen in the standard error bars from Figure 6.7.

The mean firing directions were also compared across sessions and a significant main effect of session was found. One explanation for this is that the degree of shift in mean firing direction was mediated by the degree of cue conflict. This interpretation would imply that these results are in agreement with previous studies that have examined changes in firing directions during cue conflicts, in both HD cells (Chen et al., 1994a; Goodridge and Taube, 1995; Blair and Sharp, 1996; Knierim et al., 1998) and place cells (Rotenberg and Muller, 1997). These studies showed that when the conflict between visual cues and path integration signals was relatively low (<45°) HD cells mostly relied upon visual information. Beyond a 45° conflict, cell responses were variable and demonstrated a much greater reliance on path integration information. The population of HD cells recorded in this present study showed very little variation in their responses during conflict situations, even when this conflict was as much as 120°. Typically, HD cells in sessions with a cue conflict of 120° or less shifted by around 70% of the total light shift, with all cells shifting by at least half. Cue

conflicts which were greater than 120° caused cells to shift by less than half of the total light shift

This 'threshold' value of 120° is considerably greater than that reported in previous studies (Chen et al., 1994a; Goodridge and Taube, 1995; Blair and Sharp, 1996; Knierim et al., 1998). One possible reason for this distinction is that the behavioural component in this present study led to the rats relying less on path integration. Knierim et al (1998) posed the idea that during foraging tasks rats can use different strategies to find the food (randomly search, systematically scan or follow the sound of the food being dropped) and that these different strategies may lead the rat to rely on one type of cue more than the other. Slight variations in the procedure used during this present study and Knierim et al (1998), such as the amount or frequency of food provided, may have led their rats to use different strategies. This idea can only remain speculative as neither study took formal recordings of the rats' behaviour. It should also be noted that the food itself and any other odour cues on the maze could also be used by the rat to orientate. This additional information would provide a reinforcing signal to a path integration cue.

An alternative reason for the difference in findings between this and previous studies is the type of strain of rat that was used. For example, Knierim et al (1998) used non-pigmented rats, whereas the present study used pigmented rats. Pigmented rats have superior vision in comparison (Prusky et al., 2002) and therefore the pigmented Lister-hooded rats used in this experiment may have relied more on visual cues simply because they had better vision than the rats used in Knierim et al (1998) . This interpretation would, however, fail to explain the differences between this study and Goodridge and Taube (1995) as they too used pigmented rats.

Arguably, the most likely reason why the present study found a greater dominance of visual cues is experience. It has been postulated that the degree of experience that a rat has with a landmark can affect the degree to which they rely upon it (Knierim et al., 1995). This idea has been incorporated into a model by McNaughton et al (1991). This model is essentially based upon the principle of an attractor network with the addition that there is also a learned association component in relation to landmark information. When a rat is placed into a novel environment, it is assumed that there is very little association between the rat's orientation and the position of the landmarks. The HD cells would therefore mostly rely upon a path integration signal in order to anchor. With a repeat exposure to the same environment, an association between the rat's orientation and the landmark configuration

develops. This association would then lead the HD cell to utilise landmark information to a greater degree than before.

In the present experiment, rats were initially placed on the platform and exposed to a very small cue conflict (20°). As this conflict was relatively small and the duration of the session was relatively long (twenty minutes) the rats may have established a relatively strong association with the light cue. Subsequent sessions of twenty minutes (of which there were nine sessions in total) coupled with a gradual increase in cue conflict may have caused the HD cells to weigh the light cue more heavily in greater cue conflicts (~100°) than they would have done if they didn't receive this prior experience. The process by which prior experience influences the weighting of information is described in Bayes' 1763 theorem. This theory takes into account top down processing based on expectations. When this is applied to cue integration, it suggests that previous knowledge about the reliability of a cue can influence the weighting of that cue (Yuille and Bulthoff, 1996). The applicability of a model based on Bayesian inference is discussed later in this section.

A behavioural study by van Wanrooji et al (2010) demonstrated the influence of prior knowledge on cue integration. Participants were presented with a combination of white noise and green LEDs that were located in different areas of an environment. The participants were then required to locate these audiovisual events and reaction times were recorded. Two conditions were used in this study, where participants were either exposed only to audiovisual stimuli that was always aligned in space, or they were also exposed to trials where the audiospatial stimuli was misaligned in space. They found that reaction times were consistently lower in blocks containing only aligned audiovisual stimuli than in blocks also containing pseudorandomly presented spatially disparate stimuli. The authors concluded that the participant's prior knowledge or expectation of the audiovisual congruence affected subsequent cue integration.

It would therefore be interesting to replicate this current experiment but randomise the order of conflict presentation to see if experience plays a crucial role in the weighting of allothetic and idiothetic cues. Indeed, Jeffery (1998) found that when rats were repeatedly exposed to a cue card that had previously been viewed as unreliable (the card was moved), the cue card progressively lost control over place fields. Additionally, it would be interesting to vary the time between the first light switching off and the second light switching on. In this current experiment, both lights were switched off for ten seconds. This period of darkness could be manipulated in a future experiment. Based on Bayesian principles;

increasing the duration of this period may cause the HD cells to follow the light at greater conflicts because error in the rat's path integration would accumulate.

6.4.2 Intermediate states

This current study provides two pieces of evidence that activity in the HD system occurs at intermediate states. First, during a conflict between idiothetic and allothetic cues, HD cells tended to fire in an intermediate direction between a direction predicted by the light cue and a direction predicted by path integration/uncontrolled static cues. Second, when a cue conflict occurred and the mean firing direction of a given HD cell changed, the cell's activity appeared to sweep over intermediate directions in order to reach the new preferred firing direction. These two pieces of evidence indicating intermediate states will be discussed in turn.

6.4.2.1 Cue compromise

The main aim of this current experiment was to build upon the findings from the previous chapter and further explore the nature of cue integration by the HD system. The discussion above highlighted that for conflicts of 120° or less, the HD cells shifted by around 70% of the total light shift. Interestingly, a conflict of around 140° caused most cell ensembles to rotate by only 49% of the total light shift in comparison to 78% for a 120° light conflict. This loss of landmark dominance can be seen in Figure 6.7 and arguably it was at this point in the experiment where the HD system detected a conflict within the environment.

When a sensory system detects a conflicting signal within their environment, that system needs to decide whether conflicting signals are a result of noise or because one cue is providing an unreliable signal (Nardini et al., 2008). Thus, during a 140° conflict or greater, the HD cells could do one of two things; either the light is perceived to be unreliable and so the cells use exclusively path integration information and/or other static cues such as odour. Alternatively, the directional cues are perceived to be 'noisy' and the HD cells use an integration of these signals. The findings from this current study support the latter view. The average ensemble shifts beyond 120° indicate that the HD cells started to rely more on path integration/uncontrolled static cues and less on landmark information. However, information from the landmark still influenced the cells during this conflict stage and cue integration was apparent. This landmark influence gradually decreased over time and with greater conflicts. In other words, the HD cells did not resolve cue conflict in a 'winner-takes-all' strategy, but rather they compromised.

6.4.2.2 Modelling of intermediate states

The previous chapter found that a Bayesian account of cue integration could successfully model the HD cells' mean firing direction during a cue conflict. In particular, it could model the finding that a more accurate light (that was presumably perceived to be more reliable) was more influential during cue integration in comparison to a diffuse light. It was therefore interesting to see if in this experiment, a cue integration model based on Bayesian inferences could predict how HD cells would integrate information, where presumably the perceived reliability of the visual landmark decreased over time at greater conflicts. Before a Bayesian model is discussed, it is important to note that although HD firing demonstrated cue integration, the findings in this study and the previous study show that the weighting of the directional cues are not expressed in the peak firing rate or directional firing range of HD cells. Therefore, reliability coding may not have occurred within the HD system but rather outside the system before the signal is sent to the HD cells. It is also important to reiterate that although landmark information was pitted against a path integration signal, it is likely that the HD cells were also using uncontrolled static cues such as odour in order to orient. Thus, findings that indicate HD cells were using path integration cues are also likely to reflect an influence of uncontrolled static cues, as these two cues would signal the same direction.

For small cue conflicts the HD cell firing indicated an integration of cues but fundamentally HD cells fired at a location nearer to that predicted by the light than predicted by path integration/uncontrolled static cues. For example, when the cue conflict was 20°, the firing direction of HD cells on average, shifted by 14° (i.e. 70% of the total light shift). A Bayesian model would suggest that landmark information was given a larger weighting (Fig 6.14) because the perceived variance of the light was smaller than that of path integration (Yuille and Bulthoff, 1996). Given that it was actually the light and not path integration that was manipulated, it may be that the HD system has an intrinsic bias towards landmark information. Some models based on Bayesian inferences have taken intrinsic biases into account (Scarfe and Hibbard, 2011). Indeed, it is thought that humans also have an innate bias towards giving a stronger weighting to visual information, above and beyond its actual reliability (Battaglia et al., 2003). Additionally, during the initial sessions where the conflict between the light and path integration were relatively small, a strong association between the rat's orientation and the landmark direction may have formed (McNaughton et al., 1991; Knierim et al., 1995) strengthening this landmark bias.

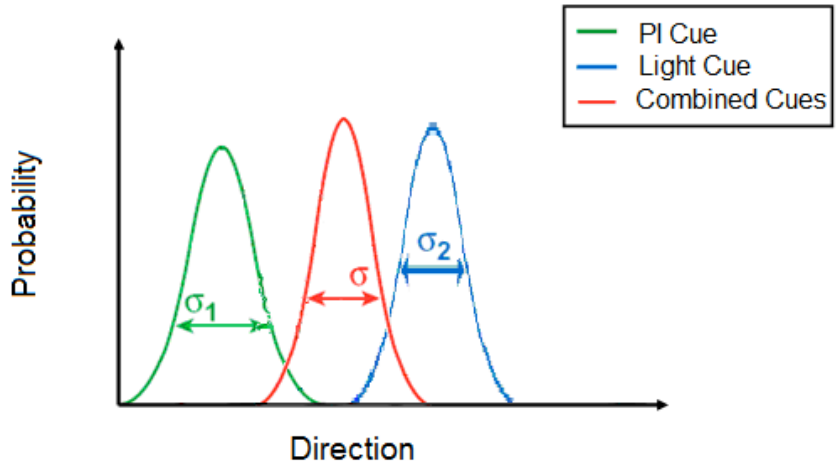

Figure 6.14 Prediction for optimal integration of visual and path integration during a 20° conflict session. The green normal distribution represents the direction signalled by path integration cues. The blue normal distribution represents the direction signalled by the light cue. Note that the standard deviation for this distribution is smaller in order to model the assumption that the HD system has an intrinsic bias towards visual landmarks. As a consequence, the optimal integration of cues results in a heavier weighting of landmarks cues (red distribution). This is shown in the data from the 20° conflict session, where HD cell firing shifted on average by 14°. The figure was adapted from Knill and Saunders (2003)

Interestingly, for most HD cells the ratio of landmark weighting and path integration weighting did not change for conflict situations until 140°. This may be rather surprising given that the conflict gradually increased and the amount of experience the animal had with the unreliable landmark increased. In fact, a Bayesian model should predict that an increase in both the level of conflict and amount of prior experience would cause the cells to reweigh the landmark and consequently path integration information. One way to possibly reconcile these findings with a Bayesian model is that during cue conflicts of 120° or less, the HD system could not detect a cue conflict, or at the very least could not detect that the conflict increased. Indeed, a Bayesian model bases its assumptions on perceived and not actual reliability. Thus if the HD system did not detect a conflict and instead perceived the light to be reliable (and given the intrinsic bias, perceived the light to be more reliable than path integration) this could explain the pattern seen in the data.

Beyond a 120° cue conflict, the cells appear to use a different strategy where landmark and path integration cues were now re-weighted (Fig 6.15). For example, when the cue conflict was 140° the firing direction of HD cells on average, shifted by 70° (i.e. 50% of the total light shift). This reweighting may suggest that the HD system detected a conflict between these

two cues. Given that the two cues were on average equally weighted, it suggests that the strategy of most cell ensembles was to reduce the level of noise in both cues, rather than assuming the landmark was unreliable and consequently ignoring it (Nardini et al., 2008). Furthermore, in the latter stages of the experiment, the reweighting of landmark and path integration cues by the HD system seemed to be modulated by the level of conflict between the two cues or the amount of experience they had with these cues, or both. For example, when the cue conflict was 180° the firing direction of HD cells on average, shifted by only 30° (i.e. 16% of the total light shift). This pattern in the data suggests that the HD cells were optimally integrating landmark and path integration cues. When the perceived variance of the light cue increased, the weighting of that cue decreased and the weighting of a path integration signal increased (Yuille and Bulthoff, 1996).

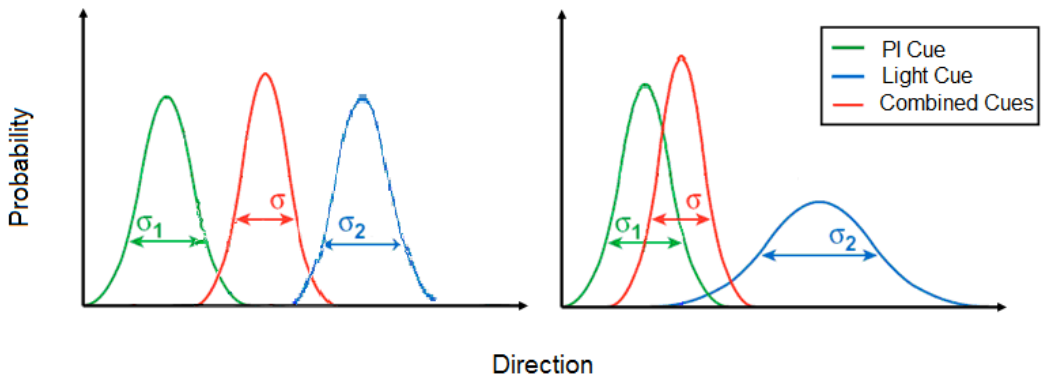

Figure 6.15 Prediction for optimal integration of visual and path integration during a 140° conflict session (Left) and a 180° conflict session (Right). *Left*: the standard deviation for the landmark distribution is no longer smaller than the standard deviation for the path integration cue. This is because the landmark and path integration information has been reweighted based on their perceived reliability. Consequently, the distributions are now equal and this is reflected in the data as during a 140° conflict, HD cells firing shifted on average by 70°. *Right:* the standard deviation for the landmark distribution has increased as the perceived variability of the landmark cue has increased. The standard deviation for the landmark distribution is now larger than the standard deviation for the path integration distribution. Consequently, the optimal integration of cues now results in a heavier weighting of path integration cues. This is shown in the data from the 180° conflict session, where HD cell firing shifted on average by 30°. The figure was adapted from Knill and Saunders (2003)

Thus it seems that a Bayesian account of cue integration can explain this data set if it is assumed that at 120° cue conflicts or less, the HD system is unable to detect a cue conflict.

When the HD system cannot detect a conflict or at least the size of the conflict, the weighting of the two cues is not based on their actual reliability and landmark information is preferentially weighted. When the cue conflict is detected, the HD system switches to using an optimal strategy where cues are weighted based on their perceived reliability. The interesting question is therefore; why can the HD system typically detect cue conflicts that are greater than 120° but not smaller conflicts?

First, it is important to note that this threshold of 120° cannot be taken as a definitive value. It is likely that this threshold value will be affected by a number of factors that have previously been discussed. Additionally, even in this study there were individual differences between rats. Nevertheless, it is worth speculating on what mechanisms might allow the HD system to detect a cue conflict that was previously undetectable at smaller conflict levels. This sensitivity in detecting cue conflicts of around 120° may be related to the size of the activity bump in the attractor network. Indeed, it may be that if the conflict size (or angle) is smaller than the angular span of the activity bump it may be that the conflict goes undetected. This is only speculation but it may explain why relatively large cue conflicts go undetected by the HD system.

6.4.2.3 Transition dynamics

The transition dynamics of individual cells were examined in order to assess how activity in the HD circuit propagates from one firing direction to another, after a sudden change in orientation. This examination was only possible when the cells rotated by a sufficient amount (~60° to 120°) that firing at the two directions showed little overlap. This data was somewhat surprisingly noisy, particularly for high firing rate cells. The high firing rate cells (typically ADN cells) produced large amounts of spiking across the twenty minute session. A percentage of these spikes occurred outside of the directional firing range and as a result, there was quite a substantial amount of this background firing for high spiking cells. This background firing also occurred in PoS cells but because the firing rate was much lower for these cells, the directional versus time plots were a lot less noisy and far easier to interpret.

From these interpretable plots, the cell's activity demonstrated clear occasions where the cell appeared to fire at intermediate angles when the preferred firing direction changed from one direction to another. These instances provide a fascinating insight into the mechanics of the HD network and suggest that the cell's activity sweeps across intermediate directions in order to reach the new preferred firing direction. As yet, no research has directly shown how activity in the HD system propagates from one firing direction to

another. Arguably, previous findings that have examined the latency period of HD cell firing and the transition dynamics of place cell activity (Zugaro et al., 2003; Jezek et al., 2011) would suggest the opposite; that HD cell activity would jump rather than sweep. Having said this, there were also notable instances in this current experiment where a sweeping of activity did not occur (Fig 6.11).

A lack of intermediate firing does not necessarily mean that the firing of a cell 'jumped' from one direction to another. As the rat could only sample one head direction at a time, the cells may not have fired at an intermediate direction because the rat's head was not facing that particular direction in time. In order to address this, the rat's actual heading position was plotted during a light transition where no intermediate cell firing was apparent. This example does indeed suggest that a lack of intermediate firing between some of the trials may be because the rat did not sample the intermediate space enough for the cells to fire. Interestingly, the HD cells only seemed to fire when the rat's head sampled the preferred firing direction of that cell multiple times, or remained stationary in that direction. One brief movement through the preferred firing direction did not appear to be enough to cause a cell to fire. At present, these data are only preliminary so it is difficult to confidently say whether a lack of sweeping activity is a consequence of low sampling, or an actual reflection of the cell's activity jumping from one preferred firing direction to another. If the latter is true, it suggests that the HD system incorporates both sweeping and jumping transitions, a stance which some researchers take (Zhang, 1996; Johnson et al., 2007).

6.4.3 Cue integration in different areas of the HD pathway

This current study found that there were no significant differences in the degree of shifts in cells recorded in the PoS, RSP and ADN. It was predicted that because visual information is initially received by the RSP and PoS (Vogt and Miller, 1983; Wyss and van Groen, 1992) and then sent to the ADN (Goodridge and Taube, 1997; Clark et al., 2010a) HD cells in the PoS and RSP would show a greater sensitivity to visual cues in comparison to the ADN. This difference was however not present in the current data and suggests that HD cells act in concert across different areas in the network and weigh cues in a similar manner. This idea lends support to the general principles of an attractor network (Boucheny et al., 2005). As mentioned before, more simultaneous recordings of HD cells from different brain regions within the same rat need to be performed in order to provide a definitive answer, as there is some compelling evidence that there are regional differences in cue integration (Mizumori and Williams, 1993; Blair et al., 1998).

6.4.5 Conclusion

To summarise, this present study showed that the shift in firing of HD cells during all cue conflicts demonstrated an integration of both light and path integration information. It is also likely that the HD cells were using additional cues such as odour as the platform was not cleaned during a trial. The weighting of these directional cues during cue integration was dependent on the session (the size of conflict and/or the amount of experience). During conflicts of 120° and less, the landmark cue appeared to have a strong exertion over HD cell firing. When the conflict was greater than 120°, this landmark dominance was lost suggesting that the HD system detected a conflict within the environment. Crucially, at this point in the experiment the cells integrated information rather than abruptly switching to using only path integration information or uncontrolled static cues. This data suggests that when the HD system detects a conflict between idiothetic and allothetic cues, HD cells use a compromise between these two cues, firing at an intermediate direction. This intermediate firing was also seen within the attractor network, where activity from a single cell appears to sweep over intermediate directions before settling at a new preferred firing direction. Finally, there were no apparent differences in cue integration between areas in the HD network.

Chapter 7 General Discussion

The main aim of this thesis was to determine how sensory information is weighted, integrated and expressed by the head direction system during situations where allothetic and idiothetic cues provided conflicting information. The main findings from this thesis are that during cue conflict, head direction cells integrate conflicting cues rather than a 'winner-takes-all' cue choice (Chapter 4, 5 and 6). In order to integrate sensory cues, each cue needs to be assigned a weighting (Yuille and Bulthoff, 1996). The work in this thesis suggests that the stability of the path integrator (Chapter 4), the perceived reliability of a visual landmark (Chapter 5), the degree of conflict between the path integrator and landmark cues and the prior knowledge of cue reliability (Chapter 6) can all affect the weighting of both landmark and path integration information. Interestingly, the integration of cues witnessed in all three experiments was expressed by the intermediate firing of HD cells, but not in the cells' peak firing rates or tuning widths. Finally, this intermediate firing was also seen within the attractor network, where activity from a single cell appears to sweep over intermediate directions before settling at a new preferred firing direction.

7.1 Background

A large body of literature has been dedicated to establishing what types of information is used by the HD system (Taube et al., 1990b; Chen et al., 1994a; Taube and Burton, 1995; Goodridge et al., 1998; Stackman and Taube, 1998; Zugaro et al., 2004; Shinder and Taube, 2011), but relatively little is known about how and when the system integrates these cues (Knierim et al., 1998). In the literature, it is currently unclear whether the HD system can integrates cues in an optimal fashion and if it can, does it always perform optimal integration? To address these questions, this thesis examined the response of head direction cell firing when the stability of a path integration signal was weakened and visual information varied (Chapter 4). These allothetic and idiothetic cues were placed in conflict, in order to assess the relative weighting that the HD system places on each cue (Chapter 5 and 6). It is important to note that in many cases, HD cells were probably able to use uncontrolled auditory and olfactory cues in addition to visual and path integration cues. Therefore these two cues are likely to have some influence on HD cell firing. Having said this, both olfactory and auditory directional cues have been shown to have little impact on HD cell firing (Goodridge et al., 1998).

A discussion of the findings from all three chapters is outlined below. A discussion of how these results complement each other will be given, along with suggestions for further work to address outstanding issues.

7.2 Stability of the path integrator

Findings from the first experiment illustrated that the type of environmental cue affects the influence of that cue on the HD system, but also the influence of these environmental cues is modulated by the strength of the path integration signal. Two types of allothetic cue were used; a cue card and geometric cues. Geometric cues were chosen because relatively few studies have looked at the influence of geometry on HD firing. Of those that have, many studies did not intentionally set out to examine how geometric cues influence HD firing (Golob et al., 2001; Dudchenko and Zinyuk, 2005). Consequently, it is difficult to say if their findings were due to the influence of geometry or to some other featural aspect of the environment. Further motivation to examine the influence of geometric cues on HD firing came from the extensive field of behavioural work looking at the influence of geometry on orientation and navigation (Garrad-Cole et al., 2001;Wall et al., 2004;Hermer and Spelke, 1996;McGregor et al., 2006;Esber et al., 2005;Pecchia and Vallortigara, 2010a;Margules and Gallistel, 1988;Reichert and Kelly, 2011), where findings were generally mixed.

One possible complicating factor in these behavioural studies is that goal localisation is used to infer the animal's heading calculation, even though these two things do not necessarily need to correspond (Golob et al., 2001; Frohardt et al., 2002). Behaviour is not, therefore, always an accurate determinant of spatial orientation. One way to avoid this problem was to replace behavioural observation with electrophysiological recordings of HD cells. Because the HD signal essentially stands as proxy for the animal's direction sense, it provides a useful means of assessing the animal's calculated heading. This signal was therefore used to explore the role of geometric cues in heading determination.

The results from the first experiment showed that geometric cues can influence HD firing but crucially, this influence was dependent upon the state of the path integration signal. The state of the path integrator was manipulated so that rats were sometimes deliberately disoriented, and sometimes they were not. It was found that when rats were deliberately disoriented, geometric cues did show an influence on HD cell firing. Conversely, when the

rats' path integration signal was relatively stable, geometric cues did not influence HD firing. Interestingly, in the same scenario, a visually salient cue card was highly influential.

Taken together, these findings suggest that geometry is only a relatively weak orienting cue for HD cells, since HD cell firing failed to rotate with geometric cues when a path integration signal was intact. This first conclusion may provide some insight into why there is a large discrepancy in the behavioural literature. Given the findings from the disorientation condition, it would be interesting to record HD cells in disoriented rats that were exposed to the environments used by Pearce et al. (2004). In their study, rats were initially trained in a rectangular pool with a submerged platform in one corner. They were then placed in a kite shaped pool, where one corner corresponded to the correct corner in the rectangular pool and one corresponded to the diametrically opposite corner of the rectangular pool. Their findings supported a local strategy account of geometric processing as rats showed a strong preference for swimming to the corner that was geometrically equivalent to the correct corner in the rectangular pool. Recording HD cells in a similar paradigm should reveal whether the cells of disoriented rats are sensitive to the local or global geometric features of an environment.

The findings from the first experimental chapter also suggest that geometric cues are processed in a different manner to visual landmark cues, since HD cell firing did rotate with a cue card when path integration was intact (Cheng, 1986; Zugaro et al., 2001a; Doeller et al., 2008). Finally, the findings from the first experiment suggest that changing the reliability of one cue, namely path integration, affects the degree to which that cue influences HD firing. This last finding has important implications for cue integration, as it suggests that the variability of a cue is inversely proportional to the influence or weighting of that cue and competing cues. This idea is one which is proposed by a Bayesian model of integration.

A Bayesian framework has been applied to models of cue integration (Yuille and Bulthoff, 1996) to predict how a system will integrate multiple multisensory cues to produce a single coherent signal. One of the main principles of a Bayesian model is that each cue has a degree of variability (perceived reliability) and this variability is inversely proportional to the weighting of that cue. Moreover, the perceived reliability of one cue will influence the weighting given to competing cues. Evidence of this reliability weighting was seen in the first experimental chapter. However, as both the landmark and path integration cues were manipulated (environments were rotated and rats were disoriented) it was difficult to directly test this Bayesian model. Thus, the subsequent two experimental chapters were

performed where the path integration signal remained intact, and a visual light cue was manipulated.

7.3 Intermediate firing

Evidence of cue integration as opposed to a 'winner-takes-all' choice between cues during a conflict between path integration and visual landmarks has been illustrated in all experiments. Before discussing the characteristics of HD cell firing, it is important to note that the second experiment demonstrated that HD cell firing was not necessarily congruent with behaviour. The majority of behavioural searches in the second experiment indicated that the rat was using path integration information to locate the food. However, the HD cells that were recorded during this task showed a shift in firing direction that was congruent with the shift in the light. This suggests that although the animal was using path integration to find the food, the rat's HD system was using the light or an integration of the light and path integration as a directional cue. Thus, the findings from the second experiment in this thesis suggest that when allothetic and idiothetic cues are placed in conflict, a dissociation between HD cell firing and behavioural responses can occur. This conclusion is supported by the work from Golob et al (2001), who showed that HD cells would use the corners of an environment as a directional cue, but the behavioural data indicated that rats were using path integration to locate the reward.

This lack of congruency also agrees with the other studies that have simultaneously examined behaviour and HD cell activity (Dudchenko and Taube, 1997; Frohardt et al., 2002). This small number of studies suggests that although there tends to be a correlation between behaviour and HD firing, there is currently no evidence to suggest that HD firing actually determines behaviour. This conclusion has important implications for the HD literature as there is often a tendency to assume that these two measures correlate (Golob et al., 2001). Consequently, it seems crucial to pursue these simultaneous recordings further, in order to greater our understanding of the link between behaviour and the HD system.

During the cue conflict in the second experiment, the mean firing direction of HD cells of an oriented rat rotated with an accurate light. However, when this light was diffuse, the HD cells demonstrated a clear under-rotation. These findings suggest two things; first, HD cells use both an integration of landmarks and path integration, and second, the integration of these cues appears to be based on the perceived reliability of the two cues. When the landmark produced a more reliable directional cue, the HD cells weighted the landmark cue

more. This idea is based on principles expressed in a Bayesian model of cue integration. Thus, optimal cue integration based on reliability weightings was apparent at an HD cell level. This type of integration has only previously been shown in behaviour (Nardini et al., 2008; Fetsch et al., 2009) and neurons located outside of the HD network (Gu et al., 2008).

Gu at al (2008) took recordings from neurons in the medial superior temporal (MSTd) area in primates. MSTd neurons are thought to be similar to HD cells in rodents as both have been shown to use visual and vestibular information to produce directional selective firing. Gu et al (2008) therefore recorded these cells during situations where the cells could use visual information, vestibular information or both types of information. They found that the width of the tuning curves for MSTd neurons were narrower when both cues were available in comparison to single cue conditions. Gu at al (2008) therefore concluded that the tuning width of the MSTd neurons reflected the reliability of the input (both cues produced a more reliable signal in comparison to a single cue).

Based on these findings, it was predicted that the tuning widths of HD cells would be larger when the diffuse light was on in the second experiment. However, this was not found to be the case. Similarly, there was no difference in the peak firing rate of the cells during the presentation of an accurate of diffuse light cue. As mentioned however, the HD cells did show a difference in firing between the accurate and diffuse light. The mean firing direction of HD cells significantly rotated with an accurate light but demonstrated a clear under rotation with the diffuse light. Taken together these findings suggest that although cue integration was apparent in the HD cell firing, the perceived reliability of the cues was not expressed by the HD cells. It may therefore be that uncertainty is coded at a neuronal level, but before the signal reaches the HD system. For example, Vidyasagar and Siguenza (1985) found that a population of neurons in V1 demonstrated a decrease in the orientation tuning width when there was an increase in the spatial frequency of the stimulus. It would therefore be interesting to take recordings from other modalities such as vestibular neurons to establish whether these cells express cue reliability using Bayesian principles.

7.3.1 Spatial intermediate firing

Findings from second experiment in this thesis showed that HD cells integrate landmark and path integration information. The third experiment examined this cue integration further by manipulating size of conflict between the light cue and path integration. The conflict between the light cue and path integration signal was gradually increased to establish the

degree of angular discrepancy between the two cues that would cause the HD system to detect a conflict and crucially, how this system then resolves that conflict.

With small conflicts, HD cells appeared to use landmark information more than a path integration signal. This landmark dominance has been previously shown in studies recording HD cells (Goodridge and Taube, 1995; Knierim et al., 1998) and place cells (Rotenberg and Muller, 1997; Jeffery and O'Keefe, 1999). Like previous studies (Taube et al., 1990a; Taube, 1995) HD cells at small conflict did show a marked under-rotation, suggesting that even though landmark information was the overriding directional cue, cells were still integrating path integration information. This landmark dominance in HD firing was apparent even when the cue conflict was 120°, suggesting that the HD system had yet to detect a cue conflict.

The 'threshold' value of 120° found in this present experiment was larger than expected and previously reported (Chen et al., 1994a; Blair and Sharp, 1996; Goodridge et al., 1998; Knierim et al., 1998). This may be due to the sequential order of presentation, allowing the rats to form a stable relationship between the landmark and their path integration signal (Knierim et al., 1998). This idea supports a Bayesian model and HD models which suggest that HD cell firing is influenced by prior, learnt associations (McNaughton et al., 1991). To establish the degree to which experience can account for the threshold of 120°, it would be interesting to repeat this final experiment where the degree of conflict in each session would be randomised. If the HD cells no longer rotated with the light when it was shifted 120° but instead weighted path integration information more heavily, it would suggest that previous experience of a cue (in particular the reliability of that cue) can affect the subsequent integration of that cue.

When the cue conflict was greater than 120°, the cells shifted on average by less than 50% of the total light shift. This loss of landmark dominance was arguably the point in the experiment where the HD system in most rats detected a conflict within the environment. Interestingly, at this point in the experiment HD cells integrated the two cues and the crucially for at least five out of the six rats there was no clear 'break point' at which the HD cells went from using the light cue to exclusively using path integration. This finding suggests that the HD system (or a related system) assumed that the conflicting signals was a result of noise rather than the landmark cue providing an unreliable signal (Nardini et al., 2008).

7.3.2 Temporal intermediate firing

All three experimental chapters showed that during a cue conflict, HD cells integrated information from both sources to fire at an intermediate angle. Findings from the third experiment also showed that this intermediate firing occurs when the preferred firing direction of a single cell abruptly changes. The transition dynamics of the HD system have so far yet be uncovered. One recent study by Jezek et al (2011) did examine the properties of attractor networks in the CA3 region of the hippocampus. Rats were trained in two boxes that had different light cues. During testing, the rats were placed in one of these environments and after minute, the cues were switched instantaneously to those in the other environment. During the transition periods, the activity representing the initial environment briefly flickered to the activity representing the new environment. Crucially, they found that CA3 represented *either* the first *or* the second environment and not an integration of these representations. Based on this work, it might be presumed that like activity in CA3, activity in the HD network 'teleports' or jumps between directions when a sudden change in orientation occurs. Additionally, Zugaro et al (2003) found that the establishment of a new preferred firing direction in HD cells was highly rapid (~80ms). Due to this short latency period, it was concluded that the cells' activity jumped from one preferred firing direction to another rather than sweeping over intermediate directions. However, this conclusion was only based on the short latency period of the updating of the HD cell signal.

The final experiment in this thesis therefore aimed to address this debate of whether a cell's activity jumps from one direction to another, or if the activity sweeps across intermediate directions. These mechanisms are not mutually exclusive and therefore the data may contain instances of both jumping and sweeping activity. The cell activity in the third experiment did demonstrate occasions where cells fired at intermediate angles when the preferred firing direction changed from one direction to another. However, there were also notable instances where this sweeping of activity did not occur. Preliminary findings suggest that a lack of intermediate firing between some of the trials may be because the rat did not sample the intermediate space enough for the cells to fire. Interestingly, the HD cells only seemed to fire when the rat's head sampled the preferred firing direction of that cell multiple times, or remained stationary in that direction.

At present, these data are only preliminary so it is difficult to confidently say whether a lack of sweeping activity is a consequence of low sampling, or an actual reflection of the cell's

activity jumping from one preferred firing direction to another. If the latter is true, it suggests that the HD system incorporates both sweeping and jumping transitions, a stance which some researchers take (Zhang, 1996; Johnson et al., 2007).

Based on this idea, it would be interesting to examine whether, like the mean firing directions, the transition dynamics are affected by the magnitude of cue conflict. According to the model proposed by Degris et al (2004) the degree of cell rotation may dictate whether the transition occurs in a sweeping or jumping fashion. The model predicts that when the cell's preferred firing direction rotates by a small amount, the neural activity sweeps over intermediate conditions before stabilising at the new firing direction. However, if the cell rotates by a larger degree, the cell's activity will jump from the old preferred firing direction to the new one.

7.4 Firing across the HD network

Finally, recordings of HD cells from multiple brain regions were taken to assess whether HD cells across the network respond differently to allothetic and idiothetic information. In the third experiment, recordings were taken from the PoS, the ADN and the RSP during cue conflict situations to see if HD cells in the PoS and RSP weighted visual information to a greater degree than cells in the ADN. This prediction was based on work that suggests that the PoS and the RSP receive visual information first and then send this information on to the ADN (Vogt and Miller, 1983; Goodridge and Taube, 1997; Clark et al., 2009; Clark et al., 2010a). In fact, the findings from the third experiment showed that there were no differences in the response of cue conflict between HD cells in the PoS, the ADN and the RSP. This finding suggests that HD cells in the network act in concert. It is however important to highlight the point that cells recorded in different brain regions were also recorded in different rats. Thus, to truly determine whether HD cells respond identically to directional inputs across the HD network, it would be necessary to take multiple recordings within the same rat.

7.5 Final conclusion

In summary, this thesis produced three novel findings. First, geometric cues appear to only influence HD cell firing when the rat is disoriented (Chapter 4). This finding may explain why behavioural studies have found conflicting effects of geometry on spatial orientation (Cheng,

2008). Second, HD cells can integrate cues optimally and crucially the weighting or reliability coding of these cues is not expressed in the firing rate or tuning width of the cells (Chapter 5 and 6). Nor is cue integration differentially expressed across different areas in the HD network (Chapter 6). Producing a cue conflict has thus illustrated that HD cells use a combination of both idiothetic and allothetic information to fire at intermediate directions. Intermediate firing was also evident in the third novel finding from this thesis. Examining the temporal characteristics of HD firing during a sudden change in orientation demonstrated clear occasions where cells fired at intermediate angles as the preferred firing direction changed from one direction to another (Chapter 6). These instances suggest that activity in an attractor network sweeps across intermediate directions in order to reach the new preferred firing direction.

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