Exploring the Ventral Hippocampal Formation

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

This thesis examines unit and field potential activity in the ventral hippocampal formation related to rewards, places, and changes in the environment. To examine this activity, a version of a biconditional discrimination task that is unlike previous versions was created. It is (1) compatible with recording from neurons during behaviour, and (2) elicits sufficient sampling of locations in the environment to estimate cell modulation by place. The thesis presents preliminary evidence for environment-dependent changes in activity in ventral hippocampal cells, baseline electrical profiles of the ventral subiculum, and evidence for ventral subicular cells modulating behaviour based on reward delivery and changes in the environment.

Evidence for Remapping in the Ventral Hippocampus

Dorsal hippocampal cells active at specific locations within environments are known to show different activity patterns in different environments. Some previously active cells will cease activity, while others will change the location within an environment for which they are active (known as remapping). While this phenomenon is known for the dorsal hippocampus, it is not well researched in the ventral hippocampus. This thesis presents preliminary evidence for ventral hippocampal cells changing activity between environments.

Preliminary Characterization of Ventral Subicular Activity

Ventral subiculum is an area intermediate between the ventral hippocampus and the ventral striatum. It is part of a circuit involved in the association of rewards and places. While studies have inactivated, stimulated, and generally recorded from areas including the ventral subiculum, very few studies of local electrical field activity there exist to date. In order to compare activity based on various conditions in a behavioural task, a baseline of electrical activity in a region is helpful to reference. This thesis presents a preliminary baseline profile of electrical activity in the ventral subiculum.

Additionally, very little is known about individual cell activity in the ventral subiculum. This thesis examines ventral subicular cells, and presents preliminary evidence that they can be modulated by both activities predictive of the delivery of reward, and the location of reward. It also shows evidence that these cells can have different activity for a single cell depending on which environment an animal is presently located in.

Abstract

The hippocampus (HC) is important for both memory and spatial navigation. These functions are thought to be supported by spatial firing patterns of "place cells" which are active in only certain locations within environments. Downstream from the hippocampus lies the ventral striatum (vStr), a structure involved in the motivational control of behaviour. While hippocampal cells have strong modulation by place, ventral striatal cells have strong modulation by rewards and cues that predict them. Together with the ventral striatum, the hippocampus forms a functional circuit in rats, thought to associate place representations with rewards in a context-, or situationally-, dependent manner. However, within the hippocampal formation there is an intermediate structure in the place-reward circuit between HC and vStr, the ventral subiculum. Due to its intermediate location between HC and vStr, the ventral subiculum is expected to combine aspects of hippocampal place related, and ventral striatal reward-related activity, in a context-dependent manner. However, while ventral subiculum has been implicated in context discrimination by studies performing lesions of that structure, little research exists on how ventral subicular cells are modulated by place, reward, or different contexts. Additionally, while the firing patterns of dorsal hippocampal cells reorganize between different environments, we currently have less evidence of this contextual discrimination in the ventral hippocampal cells that are more directly connected to the ventral subiculum. This project therefore explores ventral subicular cell modulation by place, context, and reward-related stimuli, while examining responses to changes in environmental context in the ventral hippocampus. To explore these structures, 5 male Long-Evans rats were implanted with dual-target implants, 4 of which performed a biconditional discrimination task (Context A: Cue 1 –> reward, Cue 2 –> no reward; Context B: Cue 1 –> no reward, Cue 2 –> reward) while recordings were made from ventral hippocampal formation targets. Local field potentials (LFP) were analyzed for spectral power and event-related signals, while neural firing activity was examined for event-related modulation of activity and evidence of context discrimination. Behavioural results show that as a group, rats learned to perform the task, although not all rats learned equally well. Preliminary evidence is presented for context-modulated event-related activity in ventral subiculum, and context-modulated neural firing rates in ventral hippocampus, as well as evidence for peaks in signal power in the theta (6-10Hz), delta (1-4Hz) and gamma (low gamma ~50Hz) frequency ranges in ventral subicular local field potentials. Thus, a preliminary examination of the ventral hippocampus and ventral subiculum shows functional intermediaries, combining context and reward-related signals.

Acknowledgments

I would like to thank the large number of people who have been responsible for me being where I am today.

My wife has been an unending source of support during the last couple of years. Resie, I truly could not have done this without you. Never stop being yourself. My family have supported me throughout, providing spaces to work and think, financial resources, and advice that have made the last several years vastly easier and more pleasurable.

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Finally, a number of members of present in van der Meer Lab have given me advice, help and friendship throughout these last years. Thank you to Sushant Malhotra, Eric Carmichael, Alyssa Carey, and Julien Catanese. You have done everything from helping with presentations to assisting me with looking after my rats. You have been completely invaluable on a number of levels. I'll miss you all.

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List of Abbreviations

AP	Anterior-Posterior
CA1	Cornu Ammonis subregion 1
CA3	Cornu Ammonis subregion 3
CC	Corpus Callosum
CS	Conditioned Stimulus (+: rewarded, -: not rewarded)
DG	Dentate Gyrus
dHC	Dorsal Hippocampus
dSUB	Dorsal Subiculum
EC	Entorhinal Cortex
HC	Hippocampus - also vHC (ventral subset), dHC (dorsal subset)
HCf	Hippocampal Formation
GA	Gauge
MEC	Medial Entorhinal Cortex
ML	Medial-Lateral
NAcc	Nucleus Accumbens
LEC	Lateral Entorhinal Cortex
LFP	Local Field Potential
LTD	Long Term Depression
SUB	Subiculum
vHC	Ventral Hippocampus
vStr	Ventral Striatum
vSUB	Ventral Subiculum
US	Unconditioned Stimulus
VTA	Ventral Tegmental Area

List of Symbols

Ω	Ohm - a unit of resistance to electrical current
μ	microns, or micrometres
$\chi 2$	A signifier representing a numerical distribution used to calculate statistical significance

Nomenclature

Rat Naming

Rats are named in this project according to standards used in the laboratory. This involves abbreviating rat names beginning with an R, and followed by a sequential number representing the rat's 'name' as well as the order of introduction to the colony room. A typical rat name would be R013 (the thirteenth rat introduced to the colony room).

Axis Abbreviation Conventions

As a way of abbreviating long structure names for different brain areas, positions along an axis such as the dorsal-ventral axis can occasionally be abbreviated. This typically results in dorsal positions being abbreviated with the letter 'd', and ventral positions being abbreviated with the letter 'v'. The result is that structures such as SUB (subiculum) and HC (hippocampus) can be additionally abbreviated as dHC (dorsal hippocampus), or dCA3 (dorsal hippocampal subregion CA3), and vSUB (ventral subiculum).

1 Introduction and Rationale

Rats, like humans, can learn to associate places with rewards. These place-reward associations are dependent on the context of their formation (Badiani and Robinson, 2004). For example, they allow for place preferences, where some environments, a type of context, are preferred over others because rewards are considered more likely (Bardo and Bevins, 2000; Tzschentke, 2007; Ito et al., 2008). Research has shown that the learning of place preferences is dependent on a circuit involving the flow of information from the hippocampus (HC), a part of the larger hippocampal formation (HCf), to the ventral striatum (vStr), part of a group of structures called the basal ganglia (Ito et al., 2008). However, before exiting the hippocampal formation, information destined for the ventral striatum typically passes through a second structure, the ventral part of the subiculum (vSUB), where it is routed to the ventral striatum, as well as other structures within the brain (Groenewegen et al., 1987; Amaral et al., 1991a; Amaral and Lavenex, 2007). While these three structures are known to be involved in the formation of place-reward associations, the exact mechanism of forming those associations remains nebulous. Two ways of examining the role of each structure are lesioning, and recording signals directly from each structure using electrodes.

Lesion studies remain a common way to study functionality of specific areas of the brain. They involve choosing a task that may require the functionality of a brain area, and then either surgically or chemically silencing its activity while observing the behavioural results. This allows for overall knowledge of the role that the area has, but typically shows less about the specific operations performed on information within the area. To examine specific representations of information within an area, direct recordings are usually required. These involve implanting electrodes within the area and measuring local voltages. These signals may be separated to obtain a record of both action potentials of individual neurons, and local field potentials that primarily represent coordinated synaptic currents. First, action potentials, seen as high frequency 'spikes' in voltage may be obtained and used to conduct analysis of individual neurons. This spiking activity may then be examined for modulation by specific features of an environment or task. Secondly, recorded local field potentials (LFPs) may be used to analyze increased coordination between brain areas, or specific cognitive events, such as the replaying of past memories. Knowing several methods of examining brain areas, we may then assess what they tell us about functionality in the HC, vSUB, and vStr.

The hippocampus, has been been extensively studied for more than a century (Ramón y Cajal, 1893). Examination of lesion studies of the hippocampus show it to be involved in several processes. Two of the most important are memory, and spatial navigation. With humans, the most obvious result of a hippocampal

lesion is the inability to form new memories (Scoville and Milner, 1957). With rats, which cannot be asked to describe symptoms orally, behavioural tasks such as water mazes are used to examine the results of hippocampal lesion (Morris, 1984). Such lesions in animals typically cause impaired performance in remembering which area of a water maze contains an escape platform, and as a result they spend more time searching for the escape platform (Morris, 1984; Clark et al., 2005; Broadbent et al., 2006). Recording studies have allowed for greater insight into hippocampal functionality. Studies of hippocampal cell modulation by the environment have repeatedly shown that a major correlate of HC cell spiking is physical location (O'Keefe and Dostrovsky, 1971). These 'place cells' fire within specific locations in an environment (O'Keefe and Dostrovsky, 1971; O'Keefe and Conway, 1978; O'Keefe and Nadel, 1978; O'Keefe and Recce, 1993; Royer et al., 2010). Notably, if the environment is changed, the physical location at which each 'place cell' is active will also change (Bostock et al., 1991), a result known as global remapping (See Section 2.2 - The Hippocampal Response to Differing Environments). This HC cell activity is therefore context, or environment, dependent; a necessary feature of place-reward associations. While context in this case refers to the environment, it is actually a much larger term encompassing all situational information used in cognition (Overton, 1964; Nadel and Willner, 1980; Hebben et al., 1985; Bouton and Moody, 2004). However, context-dependency has been studied more at the dorsal end of the hippocampus (Bostock et al., 1991; Leutgeb et al., 2005), and less ventrally, where cells are more often projecting to the vSUB and vStr. This evokes an interesting question of if ventral HC cells remap in the same manner as dorsal cells with respect to environmental contexts.

The hippocampus is connected to a second area, the ventral striatum, by a bundle of fibres (axons) known as the fornix (Groenewegen et al., 1987; Anderson et al., 2007; Humphries and Prescott, 2010). Lesion studies of the vStr have shown it to be involved in several processes, including learning (Joel et al., 2002; Khamassi, 2005; van der Meer and Redish, 2011b), motivation (Mogenson et al., 1980) and reward processing (Apicella et al., 1991; Schultz et al., 1993; Carelli and Deadwyler, 1994; van der Meer and Redish, 2009). As expected by its involvement in place-reward associations, this activity is also context-dependent (Koya et al., 2009). This context-dependency means that the associations are tied to a specific context, which in the case of this study is an environment that the association was created in. Studies of cells in the ventral striatum have shown that activity may be modulated by several features of behavioural tasks. These include the presentation of reward (Carelli and Deadwyler, 1994), cues predictive of reward (Schultz et al., 1992, 1993), and possible correlates of anticipation (Schultz et al., 1992; Khamassi et al., 2008; van der Meer and Redish, 2011a), where cells increase activity in expectation of receiving a reward. LFP studies of vStr have also shown that not only cellular activity changes with respect to task related events, with some frequencies changing in power with respect to events like delivery of reward (van der Meer and Redish, 2009). With hippocampal activity being modulated by place, and vStr activity modulated by reward, activity in the

intermediate structure, vSUB, should be modulated by elements of both.

The ventral subiculum lies immediately adjacent to the ventral hippocampus within the brain (Amaral et al., 1991b; Amaral and Lavenex, 2007). As such, it is often studied indiscriminately with the ventral hippocampus as part of the greater ventral hippocampal formation (Wiener and Arleo, 2003; Gruber et al., 2009). Specific studies do exist though, with lesions of ventral subiculum showing it to be involved in contextual discrimination, such that effects that would normally only happen in a specific environment will happen in all environments upon the loss of vSUB (Quintero et al., 2011). Unfortunately, cell and LFP studies that provided extensive information about hippocampal and striatal activity modulation by place and reward are sorely lacking. While evidence from the dorsal subiculum suggests that cells there are modulated in a non-context-dependent manner by place (Sharp, 2006; Lever et al., 2009), specific studies of ventral subicular cell or LFP activity relating to places and rewards do not exist.

Information lacking about ventral subicular functionality and the involvement of ventral hippocampus in context discrimination therefore create three main objectives for this work.

- Examine cellular activity from ventral hippocampus for evidence of remapping that would indicate context specific modulation by place, where a context will be defined as a distinct environment.
- Examine cellular signals from the ventral subiculum. These may be more modulated by place or reward, and so the experiment should look for evidence of both place cell related activity, and specific event-related modulation. It should examine if this modulation happens in a context-dependent manner as expected.
- Examine local field potentials (LFPs) from the ventral subiculum. Look for evidence of power increases in specific frequencies for use as a baseline for future experimentation. These LFP's may also by examined for evidence of event-related changes in frequencies reminiscent of those in the vStr.

These insights would add significantly to our understanding of place-reward associations, and how animals use them to learn from experience. To examine these aspects of the ventral hippocampal formation, a task is needed that can allow for examination of place, reward, and context related signals.

One way to examine context-dependency of an brain area is through use of an operant box. Operant boxes have been used to examine the context-dependency of behaviour in the past, including with respect to ventral subiculum (Honey and Good, 1993; Quintero et al., 2011). But to test for place related signals, an open area or track is also required to allow for cell activity in different locations to be apparent. Likewise,

examination of reward or other task event-related signals requires a way of differentiating signals relating to event modulation from other behaviour such as chewing or running. One way of differentiating those signals is to use a biconditional discrimination task (Trapold, 1970; Honey and Good, 1993), where the animal is forced to discriminate between two different conditions, such as differing cues, when deciding whether to perform an action such as approaching and nosepoking a receptacle to receive a reward. With these requirements in mind, it is possible to design an experiment.

The experiment chosen involves the construction of two open environments, differing in shape, colour and texture to examine cells for place related modulation. Rats are trained on a biconditional discrimination task in these environments, with either of two cues being randomly presented while the rat explores the environment in response to randomly dropped sugar pellets, and the rat being required to choose whether to nosepoke in a food receptacle or not to receive a reward. Nosepoking to the correct cue (CS+), which is alternated between environments, results in presentation of a sugar pellet reward, while nosepoking to the incorrect cue (CS-) results in a 5 second penalty that decreases the maximum possible food reward each session. Rats are implanted with an electrode array targeted to both the ventral hippocampal formation, and the dorsal hippocampus for use in comparing to known results regarding cell remapping. The rats then perform the biconditional discrimination task in the two environments while the electrodes are lowered through the ventral hippocampus and into the ventral subiculum. This allows for examination of place, reward and context in multiple ventral hippocampal formation locations, while simultaneously confirming that cells in the dorsal hippocampus can discriminate between the environments, even if ventral cells do not. This experiment builds upon an extensive research base, and as such, in-depth information on studies relating to each structure that formed the basis of this experiment will now be examined.

2 Background

2.1 Anatomy of the Place-Reward Circuit

General Anatomy of the Place-Reward Circuit

The connectivity of the structures involved in the place-reward circuit is not uniform. As a result, it is important to review the anatomy of the structures involved to inform discussion of their function in associating place and reward. The anatomy of the HC-vSUB-vStr place-reward circuit can generally be subdivided between the hippocampal formation (HCf), which contains the HC and vSUB, and the basal ganglia, which contains the ventral striatum (See Figure 3). Anatomy within the hippocampal formation can be thought of as defined along two primary axes, the transverse axis (See Figure 1), and the dorsal-ventral axis. The transverse axis refers to an order of structures within the formation roughly corresponding to the flow of information through the HCf. As defined by Amaral and Lavenex (2007), the transverse axis can be subdivided into the dentate gyrus (DG), the cornu ammonis (subregions CA3 and CA1 are most important for this experiment), which cumulatively form the hippocampus (HC) proper, the subiculum (SUB), the pre- and parasubiculum, and the entorhinal cortex (EC).

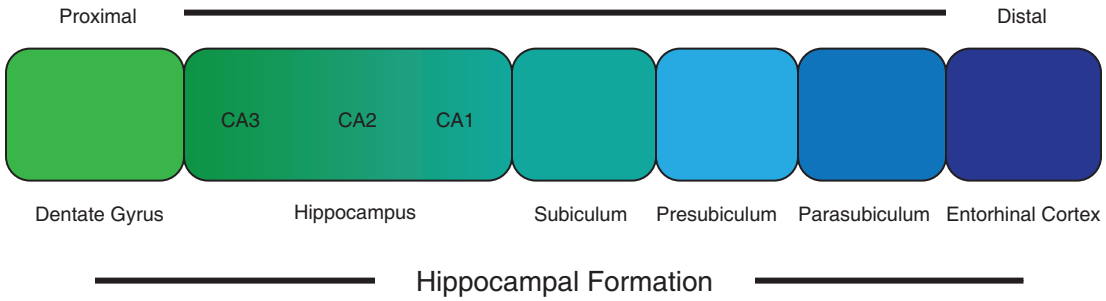

Figure 1: Transverse axis of the hippocampal formation
This diagram illustrates the concept of the transverse axis of the hippocampus, not to be confused with the dorsal-ventral axis. The hippocampus proper is shown as a gradient due to there being a lack of a firm dividing feature delineating the CA2 area from CA1 or CA3. A transverse axis likewise exists within each substructure of the formation.

The transverse axis terminology applies to all individual areas of the formation as well, such that CA3, CA1, subiculum, and other structures each have their own proximal and distal end. Information tends to flow along the transverse axis from proximal to distal structures, with the major exception of the EC. Connectivity with the EC completes a loop, providing input at the proximal end of the HCf, and receiving output at the distal end. (Amaral and Lavenex, 2007). While other axes exist to differentiate structure within the hippocampal formation, these two axes help define the great majority of the connectivity involved in the place-reward circuit.

The dorsal-ventral axis (formally referred to as the septo-temporal axis) exists roughly orthogonal to the

transverse axis. For the purpose of this project, I refer to the dorsal-ventral axis when examining either the brain as a whole (the depth of an electrode from the surface of cortex), or more commonly, the internal axis of a region, such as the hippocampus, where connectivity between structures differs. For hippocampal formation structures involved in the place-reward circuit, there are denser projections from ventral areas than from dorsal ones. This leads to predictions that will be developed in Sections 2.5 and 2.6.

Projections from the hippocampal formation to the ventral striatum are via a bundle of axons known as the fornix (Groenewegen et al., 1987; Amaral and Lavenex, 2007; Humphries and Prescott, 2010). This primarily ventrally originating bundle of axons carries both a strong subicular connection, specifically from the proximal end of subiculum, and a weaker connection from HC area CA1 to the striatum (See Figure 2;Amaral and Lavenex, 2007)[1]. The ventral striatum consists of a dense core of cells surrounding the anterior commissure, with a more sparse shell surrounding this (Humphries and Prescott, 2010). The ventral striatum principal cells are known as medium spiny neurons (MSNs), as opposed to pyramidal cells in the hippocampus. A gradient exists in the connectivity of HCf with the regions of the ventral striatum. The most dense connection is from the ventral HCf, and terminates in the caudomedial region of the vStr shell. More dorsal regions of the HCf have an increasingly sparse connection to more rostral and lateral regions of the vStr shell (Humphries and Prescott, 2010). Decreasing numbers of connections from all regions of hippocampus and subiculum terminate within the deeper regions of the vStr core. Ventral striatal areas to which the ventral subiculum is connected are in turn strongly connected with the ventral tegmental area (Humphries and Prescott, 2010). Humphries and Prescott (2010) provide an excellent overview of the region, including its many connections. Together, the hippocampal formation and ventral striatum are involved in a circuit that allows the association of reward with locations in space.

[1]Entorhinal cortex also projects to ventral striatum, although this connection studied in less detail than that from the hippocampus and subiculum.

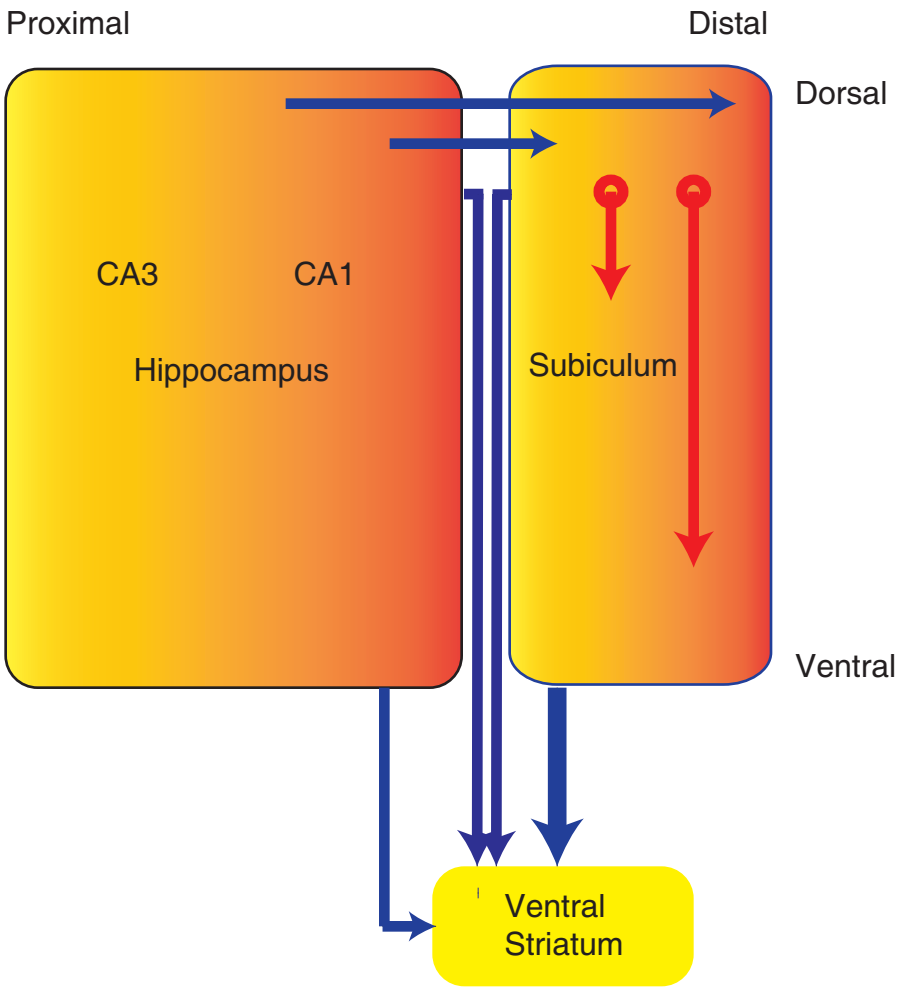

Figure 2: The place-reward circuit - simplified view
This diagram shows a simplified overview of the structure of the place-reward circuit. Blue lines are connectivity between structures, while red lines signify internal connectivity. Connectivity between hippocampus and subiculum occurs according to distance from the border between the areas. Distant areas project to distant areas, while close areas project to close areas. Dorsal areas of hippocampus project to dorsal areas of subiculum, while ventral areas of hippocampus project to ventral areas of subiculum. Inside the subiculum, information tends to flow only ventrally (Harris et al., 2001). While several regions of the hippocampal formation (areas with a colour gradient) are connected to the ventral striatum, the strongest projection is from the ventral proximal subiculum, as shown by the larger arrow

Functional Description of the vHC-vSUB-vStr Place-Reward Circuit

Functional descriptions of the hippocampus, ventral striatum, and the connectivity between them tend to respectively emphasize properties of places, rewards, and their association (O'Keefe and Dostrovsky, 1971; Moser et al., 1993; Carelli and Deadwyler, 1994; Pothuizen et al., 2004; Ito et al., 2008). As shown by Ito et al. (2008), unilateral inactivation of either hippocampus or ventral striatum alone was not sufficient to disrupt the association of place and reward. A disconnection between these regions was required to achieve this effect. Disconnection lesions occur by either directly cutting the axonal link between two areas, or by

lesioning one of two connected structures on opposite sides of the brain. In this manner, each side of the brain has a functional copy of one structure, so that both can contribute to behaviour without interacting. Ito et al. ablated the areas of hippocampus and ventral striatum on opposite sides of the brain, rather than the fibre bundles connecting them, and allowed for a simple examination of whether the lesions would separate place from reward. Ito et al. (2008) found that after disconnection lesions, rats had no preference for spatial locations where a pairing of sucrose with a flashing light was present, even though they remembered the pairing itself. It can therefore be said that place-reward associations are the result of a network, and are not simply the result of activity in one area.

With a general understanding of the hippocampus as having representations of places, and the ventral striatum as representing rewards, the place-reward circuit seems deceptively simple. However, the current functional descriptions of the structures in the place-reward circuit are somewhat at odds with their anatomy. Namely, although representations of place are found primarily in the hippocampus, most of those representations are found in the dorsal hippocampus (dHC), while the majority of the axonal connectivity to the ventral striatum originates in the ventral hippocampus (vHC) and ventral subiculum (vSUB). The next few sections expand on the functionality of each component of the circuit, while addressing the tension between their anatomical and functional descriptions. These sections therefore elaborate on the rationale for my experimental objectives introduced in Chapter 1.

2.2 Representations of Place - The Hippocampus

Early hippocampal *in vivo* electrophysiology established that hippocampal cells preferentially fire at specific locations in space (O'Keefe and Dostrovsky, 1971). Known as 'place cells', they have been found in both hippocampal areas CA1 and CA3, and collectively form representations of environments. Animals passing through environments with electrodes recording from their hippocampus will typically show a progression of place cells firing at successive locations on a track or on paths in an open environment.

The place-reward circuit requires animals to be able to discriminate between different environments so that they can learn where to expect rewards. Therefore, it is important to understand how place cells in the hippocampus respond to changes in the environment so that we can further understand place-reward associations. As mentioned, place cells fire at a given location in an environment (O'Keefe and Dostrovsky, 1971). However, due to limitations on the size of the brain, it is not possible to have a single cell represent a place in only one environment; there are simply not enough cells to do so. This leads to the concept of remapping, which occurs during changes in environment (spatial context) (Bostock et al., 1991). During remapping between environments, dorsal hippocampal cells may change either firing rate (the number of

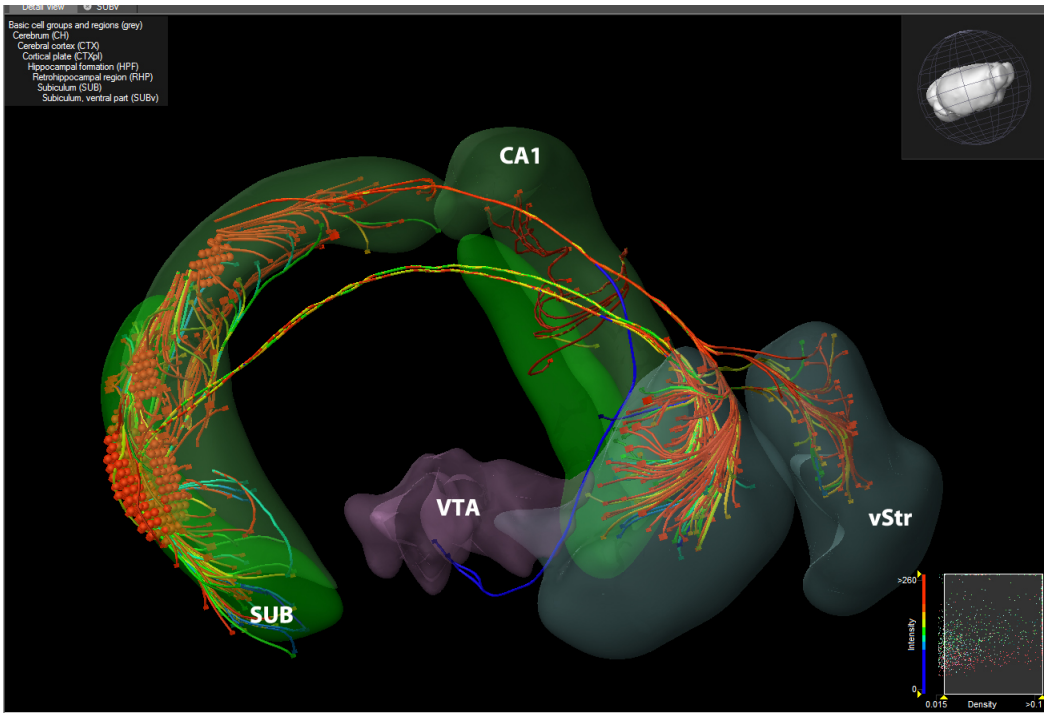

Figure 3: An overview of the location of vSub and subicular extrinsic connectivity
This volumetric figure created in the Allen Brain Atlas Brain Explorer (http://mouse.brain-map.org/static/brainexplorer) is from a mouse, similar in structure to a rat brain. Several experiments showing efferent external connections of the ventral subiculum (SUB; light green) and area CA1 of the hippocampus (dark green), as well as afferent connectivity to ventral subiculum. This figure shows the strong connectivity between the structures of the hippocampal formation (green) and the ventral striatum (blue). The connectivity from the subiculum to the ventral striatum is more dense than that originating from area CA1. Other external connectivity has been hidden. The axon bundles shown between the hippocampal formation and ventral striatum represent the fornix. Orientation of the brain is shown in the top right.

action potentials per second), or may become silent or change their active location in the environment (See Figure 4; Bostock et al., 1991; Leutgeb et al., 2005). Large changes in environment typically lead to dorsal hippocampal cells either showing no activity in the new environment, or being active in a different location of the environment, a result known as global remapping (Bostock et al., 1991) [2]. Since the cells involved in tiling (mapping) an environment and their location within different environments changes, we say a different ensemble (group) of neurons is active. These different ensembles are then the basis of 'cognitive maps' (O'Keefe and Nadel, 1978). It is extremely hard to record from an entire map due to the large number of cells involved, but global remapping shows us that single cells can be part of multiple environment maps. Global remapping therefore allows us to track when the hippocampus interprets environments as different.

[2] The alternative is changes in firing rates between moderately different environments, which is known as 'rate remapping'.

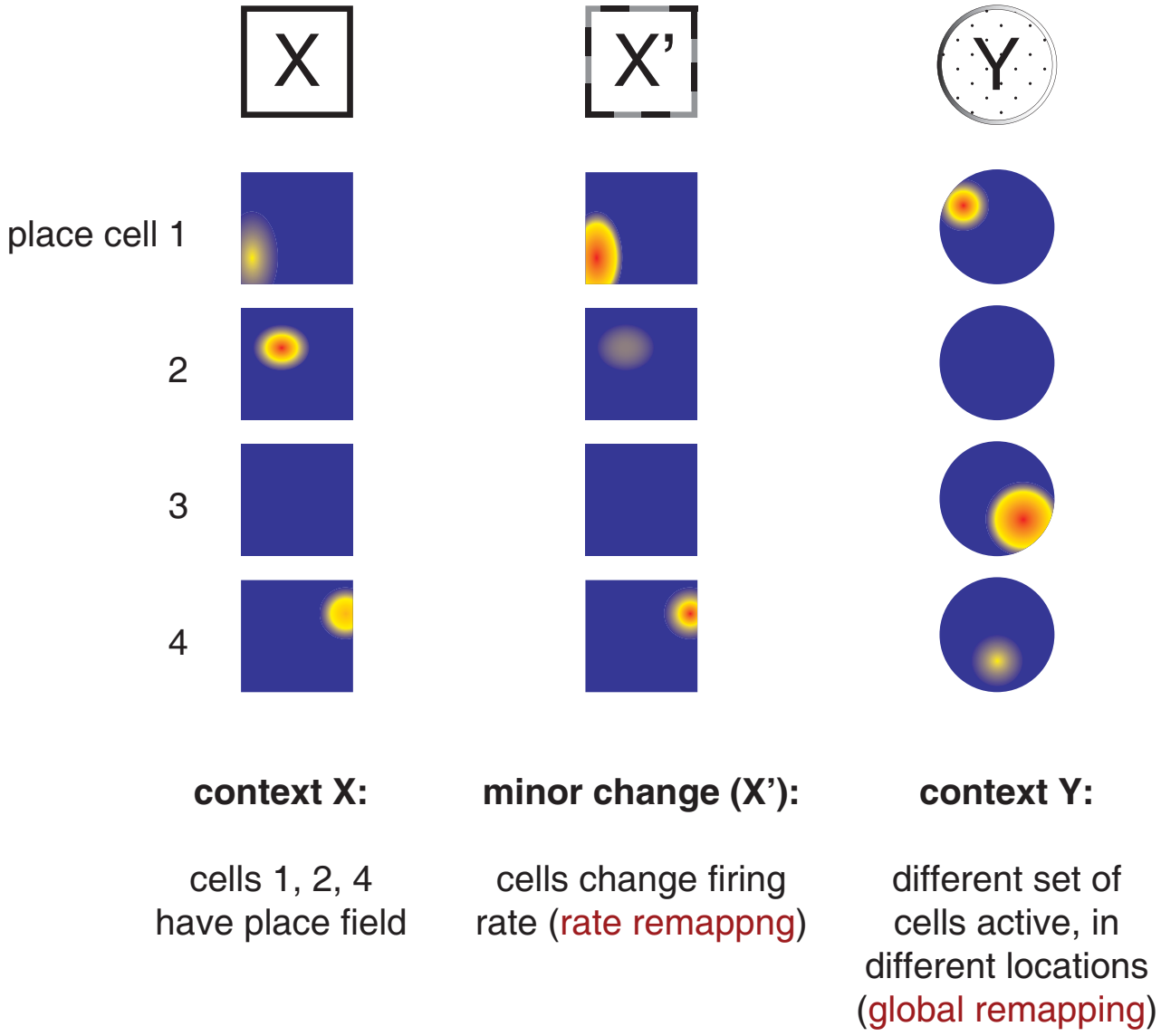

Figure 4: Hippocampal place cells and remapping
(Adapted from Malhotra, Cross, and van der Meer, 2012) This diagram shows how place cells behave within different environments. Two environments are shown, a square open environment (X), and a circular open environment (Y). It is possible to make minor changes to X to create an environment that an animal does not appear to perceive as completely different (X'). Place cells are represented as heat maps within a top-down view of each environment. When the environment changes in a minor way, cells may change their firing rates without changing the physical location at which they fire. This behaviour is known as rate remapping. When an environment changes drastically, such as between X and Y, place cells fire at different locations within the environments or may stop firing completely. This is known as global remapping. Remapping is considered to be representative of a subjective change in the environmental context for the animal.

A common way to think of remapping is in terms of context, where remapping is acting as a signal that the spatial context has changed. (Bostock et al., 1991; Leutgeb et al., 2005). Context itself is a complex term. One way of defining it would be as the set of information about a given setting or environment,

either spatial or nonspatial, which influences our understanding of, and responses to, the setting. This implies that remapping is not the only marker relevant to context, and that context is likely represented outside the hippocampus as well. This is indeed the case, as Koya et al. (2009) showed that it was possible to selectively lesion cells sensitive to a specific context of drug craving in the ventral striatum, preventing relapse. Nevertheless, although context has many components, spatial context, or knowledge of an environment changing, is a very useful marker for understanding how sensitive different structures are to context changes. The importance of context should not be understated, and although context likely requires the hippocampus, it does not solely rely on it. Therefore, as shown with the Koya et al. (2009) study of effects of context on drug relapse, understanding contextual representations outside the hippocampus can inform us about the brain's method of representing the environment, and structural involvement in the place-reward associations.

2.3 Representations of Reward - The Ventral Striatum

As mentioned above, one of the three main structures in the place-reward circuit is the ventral striatum, an area receiving hippocampal formation projections primarily from the ventral subiculum (and more specifically the proximal ventral subiculum) (Groenewegen et al., 1987; Amaral and Lavenex, 2007). Very important is connectivity to the ventral tegmental area (VTA), the source of the majority of dopamine signaling in the brain[3], and an area relevant to the functionality of the ventral subiculum (Amaral and Lavenex, 2007). VTA has dopaminergic synapses on to vStr and is strongly involved in its functionality (Taepavarapruk et al., 2000). The information gained from studies of vStr has given us significant insight into its involvement in place-reward associations.

The ventral striatum (vStr) has been postulated to have several functions, and is believed to be involved in multiple aspects of decision-making, including learning from feedback and in modulating ongoing behavior in response to reward-predictive cues, as explained by van der Meer and Redish (2011b). Early vStr work was focused on the path from motivation to action (Mogenson et al., 1980), based on yet earlier work which had implicated vStr in locomotion (Costall and Naylor, 1976; Kelly et al., 1975). More recently, evidence of vStr cell correlates with the vigor of conditioned responses has been found (McGinty et al., 2013). McGinty et al. (2013) found that the speed of approach to a reward was predicted by the firing rate of vStr neurons in response to a reward predictive cue. The latency between the presentation of the cue and initiation of movement was also predicted by this measure.

In another theory of ventral striatal function, often known as actor-critic models, the vStr serves as an

[3]Dopamine has been indicated in several processes, including reward processing.

evaluator, the critic (Khamassi, 2005). The critic uses differences between expected and received rewards that are represented in midbrain dopaminergic neurons (located in VTA) to evaluate the outcomes of previously taken actions[4]. These differences are known as reward prediction errors, and allow action outcomes to be evaluated either positively or negatively as a result. Results are then used to update the actor, typically the dorsal striatum, which then performs physical actions (Joel et al., 2002).

Calculation of a reward prediction error requires two components, the actual level of reward received, and the amount that was predicted. If actor-critic models are accurate characterizations of vStr involvement in decision making, we would expect vStr to respond to reward in some form, and indeed both reward responsive neurons (Apicella et al., 1991), and cells with a characteristic 'anticipatory ramping' (known as ramp cells) firing pattern up to reward sites (See Figure 5; Schultz et al., 1992; Carelli and Deadwyler, 1994) have been seen across many tasks and animal species.

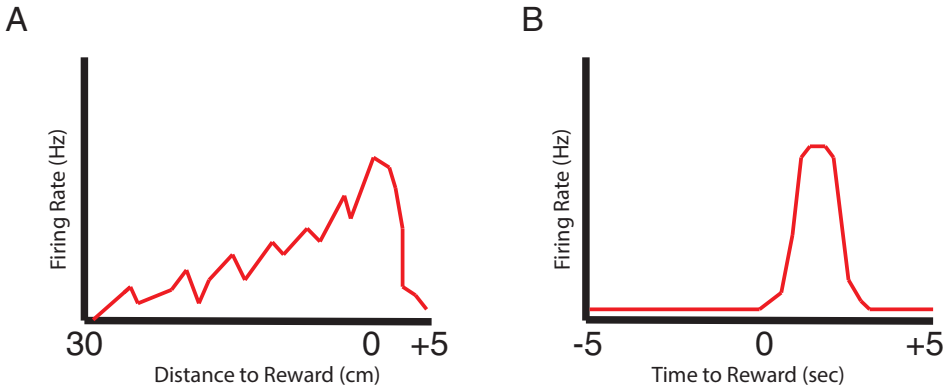

Figure 5: Simplified diagram of two ventral striatal cell types
This diagram shows a simplified diagram of two types of cell response patterns found in the ventral striatum. A) A 'ramp cell', which increases in activity as a reward location is approached. B) A reward excitatory responsive cell, where the firing rate increases after a reward is received, and then rapidly drops off.

This anticipatory signal is not restricted to only the reward, and can ramp up to reward predictive cues as well. One question is how these reward ramping signals came to exist. Upstream modulation from reward signals in hippocampus and the limbic system may be one possibility (See Figure 6 for an example of a possible interaction between hippocampus and ventral striatum; Malhotra et al., 2012), and indeed Royer et al. (2010) shows some evidence of this.

Given that hippocampal place cells remap in different environments, how do ventral striatal cells respond to a change in context? Current evidence suggests that these reward signals are dependent on spatial context as well. Koya et al. (2009) showed a marked decrease in the locomotion of cocaine sensitized rats when cocaine

[4]This information is presented to the critic by altered levels of dopamine released at synapses in the ventral striatum. Although dopamine likely has many functions, in this case we are concerned with bursts of dopamine acting as a signal of receiving a reward. This is similar to the way in which many drugs alter neural activity, as they can replicate the effects of dopamine release.

responsive vStr neurons were selectively inactivated. This selective inactivation had no effect in an alternate environment. This demonstrated that the cocaine reward signal was being selectively weighted depending on the environment, or context. Since reward-related signals are found in both the ventral hippocampus, and the ventral striatum, located both up and downstream of subiculum, we therefore may predict that they are present in the ventral subiculum as well.

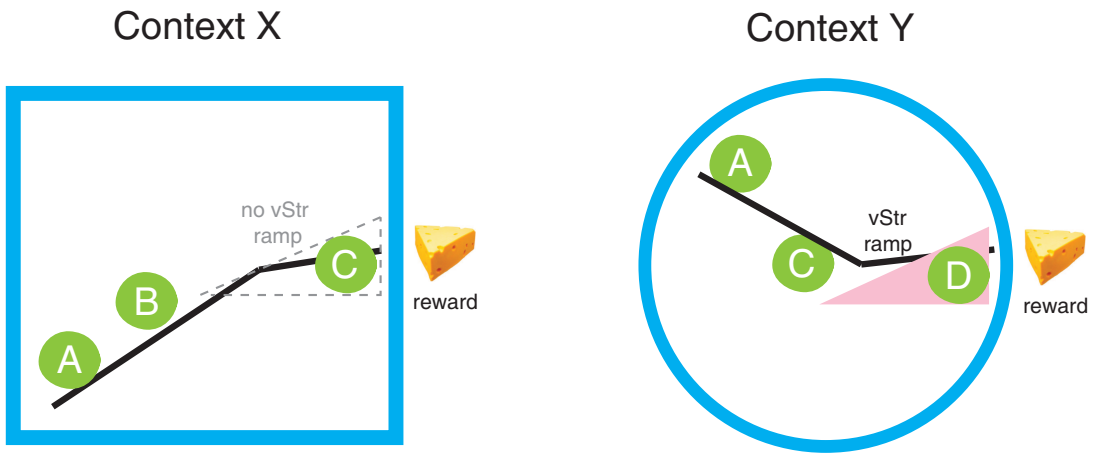

Figure 6: Context specific place-reward behaviour
This diagram shows a schematic of a possible interaction between reward associated ventral striatal ramp cells and hippocampal place cells in the two contexts used in Figure 4. Black lines represent paths taken towards the reward site during two potential trials. Green circles are hippocampal place cells, while the ramp represents increasing activity of a ventral striatal ramp cell. Note that during the switch from context X to context Y different sets of place cells are active in different locations, and an anticipatory increase in firing rate for the ventral striatal ramp cell is only seen in context Y as the reward is approached. The ramp cell does not increase in firing rate as the reward location is approached in context X.

2.4 Oscillations in the Place-Reward Circuit

The above section discussed neural activity at the spike level, but neural circuits also generate local field potentials (LFPs), which primarily reflect synaptic and dendritic currents. LFPs are then analyzed in terms of oscillations of varying power and frequency. Frequencies generated in specific structures tend to be characteristic for that structure. Oscillations reflect the coordinated activity of populations of neurons, and are thought to reflect organization of activity within and across brain areas (Gray et al., 1989; Singer, 1993; Stopfer et al., 1997).

Hippocampal place cell activity shows modulation by the 6-10Hz band of frequencies, known as the theta rhythm (Lubenov and Siapas, 2009). Theta in hippocampus is associated with many aspects of functionality and behaviour (Buzsáki, 2002; Klausberger et al., 2003; Sederberg et al., 2003; Colgin, 2013). Hippocampal theta is also coherent with theta in the ventral striatum during behaviour, and this coherence is particularly

strong when approaching reward (Berke et al., 2004; van der Meer and Redish, 2011a). Theta itself appears to be a locally generated feature of the hippocampus, although a connected area known as the medial septum appears to function as a pacemaker to maintain signal regularity (Brazhnik and Fox, 1999; Borhegyi et al., 2004; Colom et al., 2005). Since the subiculum receives information from hippocampus that already has a strong theta modulation, and projects to ventral striatum (which has, but does not likely produce strong theta signals), it is likely that subiculum is propagating those theta signals. This gives an experimental objective to examine theta and other frequency bands in the subiculum that may be involved in the place-reward circuit.

Theta rhythm modulation of neural spiking activity[5] originally found in the hippocampus has also recently been found in ventral striatal 'ramp cells' (van der Meer and Redish, 2011a), although as O'Donnell and Grace (1995) noted, it is unlikely that the ventral striatum is generating the theta signal internally because required local properties (membrane properties, specific excitatory-inhibitory circuitry) are not present. If the theta signal cannot be generated locally, and is required for specific theta phenomena found in ventral striatum, it is very likely an inherited feature from the hippocampus via the ventral subiculum. This is good evidence for the transmission of a reward signal, since while theta phenomena in hippocampus are often related to location in space, ventral striatal theta phenomena are related to both temporal and spatial distance from reward (van der Meer and Redish, 2011a).

In addition to theta, ventral striatum also shows prominent signals in the beta (~20Hz) and gamma (~50Hz) frequency bands (Berke et al., 2004). Ventral striatal gamma can be further subdivided into two separate bands of activity centred on the 50Hz and 80Hz regions. Ventral striatal Gamma-50 signal power increases after receiving a reward, and before initiating movements, while Gamma-80 increases slowly in power while approaching a reward (van der Meer and Redish, 2009). These two different bands of gamma may represent switching between processing information from prefrontal cortex.

While oscillations in the hippocampus and ventral striatum elaborate more on the functions of these structures, there is a disconnect between the anatomy of the hippocampus and the spatial signals that are expected to be sent through the connection from HCf to vStr. Theta signals, and place representations in general are more often found in the dorsal hippocampus, while the ventral hippocampus is more strongly involved in the place-reward circuit. We should therefore consider the gradient between the dorsal and ventral hippocampus before addressing the third place-reward circuit structure, the subiculum.

[5]This phenomenon is known as phase precession, and traditionally refers to the hippocampal type, where place cells fire at increasingly earlier phases of subsequent theta frequency waves as an animal passes through that cells place field (O'Keefe and Recce, 1993).

2.5 The Hippocampal Dorsal-Ventral Gradient - Unclear Changes in Place and Reward

Significant changes in connectivity occur along the hippocampal dorsal-ventral axis. These connectivity changes result in a gradual change in functionality along the dorsal-ventral axis (Bast, 2007; Fanselow and Dong, 2010). However, this change in functionality appears to contradict the view of the place-reward circuit with hippocampus representing place. The ventral hippocampus is more connected with the place-reward circuit than the dorsal hippocampus, but has more sparse representations of place than the dorsal hippocampus. Inactivation studies of regions along this gradient have given a general view of these differences. Lesions of ventral but not dorsal hippocampus have lead to altered stress and emotional responses in rats (Henke, 1990; Kjelstrup et al., 2002). Meanwhile, ventral, but not dorsal hippocampal lesions lead to deficits on spatial tasks, such as Morris water, and radial mazes (Moser et al., 1993; Ferbinteanu and McDonald, 2001; Pothuizen et al., 2004). These results together suggest that the more ventral the area of hippocampus, the less spatial in nature its functionality as compared to dorsal hippocampus. This is not surprising, as ventral hippocampus receives greater input than dHC from structures associated with arousal, stress, and reward responses (Pikkarainen et al., 1999; Pitkänen et al., 2000; Amaral and Lavenex, 2007). Though these results suggest vHC functionality is not exclusively spatial in nature, it must be noted that studies of vHC have demonstrated spatial representation, albeit at a different scale from that in dHC, as discussed below.

More in depth studies of cell responses along the dorsal-ventral gradient have elaborated on the behavioural view. As noted by Royer et al. (2010), a gradient exists in the likelihood of hippocampal cells being modulated by place, with far greater numbers of cells responding to place in the dorsal hippocampal regions, than those ventrally (Jung and McNaughton, 1993; Jung et al., 1994). Royer et al. (2010) also noted several other interesting features of spatially modulated cell activity in the ventral hippocampus. As shown by Kjelstrup et al. (2008) and Royer et al. (2010) among others, ventral CA3 pyramidal cells have larger 'place fields' (regions over which they are active) than do dorsal CA3 pyramidal cells. In fact, while dorsal cells typically have fields in the centimetre range, ventral cells can have fields up to at least metres wide. Royer et al. (2010) noted that these large fields were not necessarily continuous, with interruptions and peaks in activity over the larger area. This is more reminiscent of grid-like patterns of cell firing in the entorhinal cortex (Hafting et al., 2005) that also have dorsal-ventral gradients in activity. This leads to the possibility of spatial firing gradients being at least partially inherited from input structures (Witter, 1993), which then has implications for possible downstream representations of place in the subiculum. Royer et al. (2010) also noted behaviour of dorsal vs. ventral cells in a radial maze. Whereas the dorsal place cells only responded in one maze arm, and often in both directions of travel on that arm, ventral cells had place field

representation in multiple arms of the maze that differed in activity based on the direction of travel. This activity also differed strongly between the times when the rat was moving toward or away from reward in a separate test. They linked this behaviour partially to the context (direction relative to reward), offering support to the idea that like dorsal hippocampus, ventral hippocampal cells change firing activity based on the environment. This finding has a bearing on knowledge of both the subiculum and ventral striatum. In short, this gradient towards larger field size has a direct influence on what we might expect to find if we looked at activity in the ventral subiculum, since its ventral location would imply cells with representations of large sections of environment if place cells were present there. With reward signals present in both the ventral hippocampus and the ventral striatum, we would therefore expect reward representations in ventral subiculum as well. Stronger context-dependent neuronal activity in the ventral hippocampus combined with a sensitivity to position relative to reward would imply both of these effects being present in the ventral subiculum.

Of key interest is that while Royer et al. (2010) found vHC cell activity differed on inbound and outbound legs of a radial maze, those cells only differentiated between maze arm types on outbound legs, suggesting context-related effects. However, the context-dependent remapping studied in dorsal hippocampus (See Section 2.2) is not well studied in the ventral hippocampus. Based on known dorsal hippocampal results and the suggestive results from Royer et al. (2010), remapping of vHC place cells is a strong possibility. If the ways in which ventral hippocampal cells alter their activity based on changes in the environment could be discerned, it would give greater insight into the methods of integration of place with reward, and would inform studies of subicular function as an intermediary in the place-reward pathway. It is therefore important to examine remapping effects in the ventral hippocampus. This can be accomplished through methods similar to those used to study dorsal remapping, and is an experimental objective for my work.

2.6 Bridging the Gap from Hippocampus to Ventral Striatum - Finding the Role of the Subiculum

Insight from Lesion and Stimulation Studies - General Ideas

Several studies have also attempted to look at the place-reward representation that was suspected in this connection through lesions or stimulation of vSUB. One example is Laxmi et al. (1999), which showed that after vSub lesion there was a failure to learn to alternate directions of travel in a t-maze to receive a reward. While this result was compatible with the results of Ito et al. (2008), it also severed all other subicular output connections, and so can only serve as supporting evidence. Reversible lesions by Floresco et al. (1997) also showed an inability to remember spatial information related to past reward location in non-delayed recall,

while earlier results showed an inability to use spatial information to escape a Morris water maze (Floresco et al., 1996) [6]. These results consistently show evidence of a connection between spatial information, dopamine, and reward in ventral subiculum. My thesis examines evidence for this integration of place and reward at the cellular level.

Several other studies have examined subiculum as it relates to context. Since we know hippocampus and vStr have involvement in discriminating between contexts, it is probable that vSUB does as well. Vorel et al. (2001) noted that cocaine seeking behaviour was contextually dependent (in this study, a spatial context), and studied the effects of vSub theta frequency stimulation on the reinstatement of cocaine craving in specific contexts. They found that vSUB stimulation in the theta range of frequencies induced long lasting dopamine release in vStr, and caused reinstatement of cocaine craving. They speculated that the induced cocaine craving reflected a readout of a stored context in the hippocampus by vSub. Later, Biedenkapp and Rudy (2009) studied the effects of vSub lesions on contextual fear conditioning. It was found that contextual fear was impaired if the ventral subiculum was lesioned after fear conditioning had taken place, but not before, a result that implicates vSub as involved in at least one method of recall of contextual information. Quintero et al. (2011) studied the effects of vSUB lesions on responses to previously unremarkable stimuli when combined with a nausea inducing chemical. They found that for scenarios where no change in environment context occurred, responses to the chemical were as expected. However if the context was changed, responses generalized to all environments if vSUB was lesioned. This demonstrated that the ventral subiculum was not required for the correct behaviour, but was required to make associations based on the spatial context. Bossert and Stern (2012) recently revisited contextual addiction with heroin, based on non-contextual heroin studies performed earlier (Sun and Rebec, 2003). Their results largely match with those of Vorel et al., showing that if the vSub is reversibly inactivated, reinstatement of contextually induced heroin seeking is reduced. These results together seem to provide a fairly strong backing for the notion that vSub is at least involved in spatial context processing, and the context-dependence of drug cravings. We also know that vSUB influences the vStr by influencing dopamine levels that reach it, and that vSUB is involved with associating place and reward. The experiment should therefore examine responses to both reward and location in space. However, teasing apart the specifics of representations in vSUB requires both electrophysiological studies of vSub neurons, and studies of the ventral subicular LFP.

[6] The fact that the results concerned only non-delayed recall is difficult to interpret. One possibility is that while an initial memory trace was stopped from forming, hippocampal memory consolidation (see Carr et al., 2011 for information on memory consolidation through replaying experience) and repeated attempts of hippocampal led vStr memory formation (Lansink et al., 2009) by way of pathways bypassing vSUB may have compensated for immediate deficits.

Insight from Electrophysiology - Lacking in the Specifics

While ideally, ventral subicular electrophysiology would tell us how reward and place are represented by vSUB neurons, unfortunately, such studies are lacking. However, in addition to the projections to ventral subiculum from hippocampal region CA1, there is also a unidirectional intrinsic connectivity from the dorsal to ventral subiculum (Amaral and Lavenex, 2007). Therefore, we may learn more about the role of vSUB by examining dorsal subiculum (dSUB) neurons. Sharp (2006) found a substantial population of cells of dSUB had spatial firing fields that organized themselves around boundaries of the environment. Moreover, these boundary active cells seemed to not care about the particular shape or size of the environment, maintaining the same firing location. Therefore, the cells seemed context invariant. Many examples of these cells were later demonstrated, and matched computationally derived predictions of 'boundary vector cells' Lever et al. (2009). A more recent paper by Kim et al. (2012) examined the dorsal subicular firing patterns in terms of firing along the transverse axis. They found sparse, more canonical place type fields proximally (close to CA1), with more distributed grid-like firing patterns further from CA1. Interestingly, the cells also underwent remapping between two U-tracks that differed only based on familiarity and relative location in the environment. We can likely think of the transverse axis differences in terms of influences from the entorhinal cortex. Specifically, subiculum receives more non-spatial information from lateral entorhinal cortex proximally, but also a strong spatial input from CA1 (Amaral et al., 1991b; Amaral and Lavenex, 2007). Proximal subicular spatial modulation as a result is less like that in entorhinal cortex, and more strictly hippocampal. Distally, dSUB receives more spatial inputs from medial entorhinal cortex, leading to a greater influence of entorhinal grid-like spatial modulation (Amaral and Lavenex, 2007; Kim et al., 2012). Kim et al. (2012) also found theta signal phenomena in this structure, although they did not look ventrally, where the majority connection to vStr lies. These results on dorsal subiculum are important for several reasons. First, because they show ways in which hippocampal spatial signals are modified when in subiculum, moving from place fields in hippocampus to spatial fields modulated by relation to the boundary of the environment, scaling with the size of the environment itself. Second, because as mentioned earlier, all subicular intrinsic connections are unidirectional, always moving ventrally. That means there is a very good chance these spatial types of signals are being dispersed ventrally. These results gave some tantalizing hints as to what representations might be found in vSub neurons, although not addressing it directly.

Published reports of ventral subicular cell activity and LFPs are sparse. However, some indirect and basic electrophysiological studies have been performed. Petrulis et al. (2005) presents probably the only study with extracellular recordings of vSUB in behaving animals (Hamsters). Unfortunately, the study did not address place and reward representation. They found that for 32% of vSub cells, an analysis of cell firing

patterns showed a prominent theta modulation (around 8-9Hz), at least leading to the possibility of some theta related phenomena in rat ventral subiculum. However, beyond showing that theta (6-10Hz) would likely exist in rat vSUB, the study did not present any information on other frequency ranges. This is not to say that no other electrodes have been in the rat vSub before, as several studies have included recordings in ventral hippocampus without differentiating the subiculum as a different structure (Wiener and Arleo, 2003; Gruber et al., 2009). Gruber et al. (2009) had multiple recording locations in vStr, prefrontal cortex, and ventral hippocampus. They noted that vStr theta was coherent with the ventral hippocampus[7], and that this coherence could be disrupted by transient bursts of activity in prefrontal cortex cells projecting to vStr. Unfortunately, this signal was not differentiated between hippocampus and subiculum, and so there is averaging of the frequency spectra in the two areas. Jackson et al. (2011) recently measured the intermediate subicular LFP in slices of brain tissue, confirming the presence of theta, at least in vitro, as well as both a fast (~80Hz) and slow (~50Hz) gamma rhythm. Additionally, gamma was found to be locked to the rhythm of the theta band, while subicular principal cells were found to be entrained to gamma to variable degrees. The theta-gamma coupling was noted to be an important feature of hippocampal-subcortical communication (Tort et al., 2008) during learning and recall (Tort et al., 2009), and so we would perhaps expect this to be true of ventral subiculum-vStr connections as well. Of note was the fact that although such theta-gamma coupling was not consistent in the hippocampus (CA1), it was much more so in subiculum, suggesting that subicular rhythmicity is not just a copy of that in hippocampus. In either case, examination of the ventral subicular local field potentials in vivo will be an important approach, and establishing a baseline of subicular field activity is an objective of this experiment.

Hypothesized Ventral Subicular Place-Reward Representations and Designing an Experiment

Based on the results reviewed above, we may hypothesize the following:

- Given projections from dSUB and hippocampus area CA1 to vSUB, neurons are likely to have some modulation by place.
- Since dSUB projects to vSUB, and dSUB place modulation differs along the transverse axis, vSUB neurons may also have more distributed place activity distally, and more place field-like activity proximally (near HC)
- Since hippocampal inputs to vSUB are primarily from vHC, place modulation is likely to be sparse, as it is in vHC.
- Results of dSUB electrophysiology, vSUB lesion studies, and extensive hippocampal and striatal results, suggest activity is likely to be contextually dependent.

[7]Coherence in this case means that the signals were similar across one or more dimensions such as phase or amplitude.

- Due to vSUB connectivity with vStr, a reward centre, and involvement in dopamine modulation, vSUB neurons are likely modulated by rewards or expectation of rewards.
- Due to its intermediate location between HC and vStr, internal subicular connectivity, and vSUB's likely modulation by place and reward, there is significant probability that individual neurons can also be modulated by both place and reward.
- While the ventral subicular LFP is not well studied, it likely has a strong theta frequency band, and perhaps gamma rhythmicity as well.

These hypotheses suggest ideas for vSUB's involvement in the place-reward circuit. However, this subicular integration of reward and place in a contextually dependent manner has not been shown to date, and gives objectives for an experiment. Of particular interest is how both neurons and the local field potentials of subiculum respond to reward, with some activity expected around the reward points.

In order to test these characteristics, a possible task would require a component that would be expected to trigger remapping in dorsal hippocampus, as well as a large enough environment for some form of spatial representation to be present. Dorsal hippocampal activity should be assessed for evidence of remapping to show that any context-dependent signals in ventral subiculum can be measured. To measure reward and expectation of reward, the task should allow analysis of animal response to reward predictive cues, as well as reward reception. An implant that can record from two structures simultaneously would allow comparison of signals between hippocampus and subiculum, both in LFPs and neurons, while possibly demonstrating remapping in ventral hippocampus at the same time.

3 Materials and Methods

The motivation of this experiment is to achieve a preliminary examination of representations and signals found in the ventral subiculum, in order to further understanding of the involvement of the ventral subiculum in the place-reward circuit involving the ventral HCf and the vStr. To that end, the behavioural task should allow analysis of (1) reward-related signals, (2) place related signals, (3) context-dependent modulation of 1 and 2. These requirements are satisfied by a biconditional discrimination task [8], with an open environment to allow for analysis of place cells and other place modulation. I will first describe the experimental design and training protocols. I then describe the design of the recording electrode array and the surgery to implant it. Finally, I describe the procedure for analysis of recorded signals.

3.1 Experimental Design

All experimental procedures were performed in accordance with the University of Waterloo Animal Care Committee (AUPP #11-06). Since a specific aim is to test for signals of spatial representation for separate contexts and for signals of reward, a biconditional discrimination task (Honey & Good, 1993) was chosen where tone 1 (but not tone 2) is predictive of reward availability in environment A, and tone 2 (but not tone 1) is predictive of reward availability in environment B (Figure 7).

In this experiment, the two environments are two boxes of an approximately equal area, with features designed to be different enough to trigger hippocampal remapping. Box 1 / Environment A is a black rectangle with a single white wall, with the feeding apparatus recessed into one of the long sides of the rectangle. This box has a 'granular' texture, with a roughened floor, and originally a rough grit sandpaper on the surrounding wall (this was later replaced with a angled roughened aluminum brace around the inside perimeter of the environment [45 degree angle, 2.5 inch width]). Box 2 / Environment B has a single black wall, a smooth texture to its features with a painted white floor, and fine grit sandpaper on the walls (later replaced with a smooth brace similar to that in Box 1). Box 2 was shaped as an amphitheatre, with the feeder recessed into the 'stage' wall. The inside floor area of each box is somewhat less than $1m^2$. The surrounding wall is a fixed 50cm height, and a house light is present over the feeding receptacle in both boxes. The house light is turned on continuously at all times during a session during which the animals behaviour could alter events in the experiment (this excludes 5s penalty times from nosepoking to the incorrect tone, and times before or after the session). The feeder receptacle is embedded within sloped walls attached to the vertical surrounding wall to discourage sitting on the receptacle during recording.

[8] A biconditional discrimination task presents an animal with two different cues that may be responded to under two separate conditions, or contexts. Results of responding to one cue are different from responding to the other, dependent on the condition, or context. The animal must learn to respond differently under each condition.

In order to determine behavioural discrimination between the two putative contexts, two audio cues are presented while the animal was in one of the two boxes. One cue is active (CS+) and results in food delivery if the animal responds by nosepoking (insertion of the nose into a specified area, which triggers a sensor and delivers a reward) the feeding receptacle. The second cue is inactive (CS-), which, if nosepoked to, triggers a 5 second delay during which the house light is turned off, and behavioural activity can not start a new trial. The only requirement for beginning a new trial by triggering the sounding of a cue is significant movement in the box to ensure sufficient movement around the environment for the estimation of place fields.

To fulfill the movement requirements, the environment is first segmented into 10 zones by distance from the feeder receptacle. The animal must then traverse 7 unique zones each trial, and be in at least the 5th zone from the feeder receptacle to trigger cue presentation. The structure of a single trial is the presentation of a cue (either a solid tone or white noise) for 7.5 seconds, with the rat able to respond to the cue by nosepoking the feeder receptacle during this period if he chooses, and a consequence if the rat chooses to respond. Trials end when the animal either ignores a cue (no penalty beyond the lack of a reinforcing presentation of reward), or nosepokes to a given cue, receiving either a food reward if he nosepokes to the CS+ for at least 0.6 seconds, or the 5 second penalty upon nosepoking to the CS-.

Rewards are standard sugar pellets of a number sufficient to maintain 85% body weight during recording (5 pellets each trial, for 8-12g pellets per day). Reward delivery is followed by an inter-trial period that consists of a randomized short time (typically 5-11s), plus the previously mentioned movement requirement. Successful contextual discrimination is exhibited if a rat attempts to maximize reward intake by consistently responding to the CS+ in each box by nosepoking, regardless of the tone played, while selectively ignoring the CS-. Throughout each session, pellets are scattered at random intervals (20-30s apart with an 80% probability) to encourage increased exploration (approximately 6g pellets per day). Each day had two 25 minute sessions beginning with a randomly selected box, with successive rats having cue associations reversed. The order of cue presentation was pseudorandom, drawn from a distribution where the same cue occurs no more than 4 times in succession.

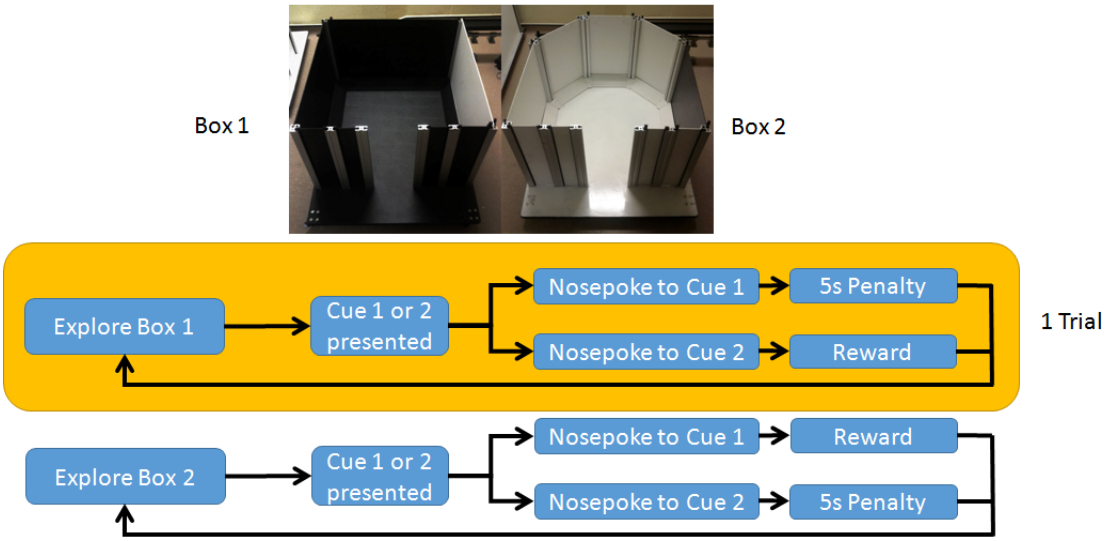

Figure 7: Experimental overview
This diagram shows the design of the experiment. The two boxes/contexts used in the experiment are shown as images at the top. A single trial consists of the time from when the rat is exploring, until either a timeout from a lack of response to a cue, or a nosepoke has been performed and the resulting condition has occurred. Cues are counterbalanced between rats so that subsequent rats have alternating cues signify reward in each box. Each recording day has a session in each box in a random order. Each session is 25 minutes in length.

3.2 Task Management

Task events were controlled and recorded by a custom-written MATLAB program, which communicated with a Neuralynx Digital Cheetah data acquisition system. This system was equipped with digital ports to communicate with automated food dispensers (Coulbourn H14-23R) and photobeams to detect nosepokes (Coulbourn H20-94). One food dispenser was connected to a custom designed delrin plastic feeder receptacle, with the other being used to pseudorandomly drop pellets within the enclosures. Recording cables were connected via a rotating commutator and tethered with elastic to reduce strain on implants from weight and rotational tension during recording. Environments were isolated from the researcher by a black curtain to minimize disruption.

3.3 Training and Behaviour

Prior to implantation, rats are trained each day for approximately 3 weeks to explore the environments and respond to cue presentation by holding a nosepoke at the reward receptacle for 0.6 seconds. A typical full session on a given day involves 25 minutes in each box, in a random order, with the active cue differing between boxes, but the same across all days for the same rat. Rats are initially handled for 10-15 minutes per day in the experimental room to familiarize them with both the experimenter and room. This period

usually concludes when an animal will eat pellets from the experimenter's hand. Rats are then trained to wear an LED tracking backpack while sitting on the experimenter's lap for 10 minutes per day until stress reactions subside. The backpack consists of battery powered LED's held in place around the animal's chest by a band of coban wrap.

Animals are put on a food restricted diet and given only 7-12g of food to maintain a slow decrease in body weight for several days. Once weight is slowly decreasing, animals are then placed in the environments for the normal 25 minute period each day, but with no trials performed and with the house light disabled. Pellets are typically scattered more frequently around the environment at this point to enable the rat to maintain body weight and encourage foraging behaviour. This phase typically lasts several days as well. Once the rat is foraging actively, trials are enabled, but without requiring a nosepoke. Rats are able to move about the environment, triggering cue presentation, with the feeder automatically dispensing pellets each time the rewarded cue is played. Once the rat has learned to associate the rewarded cue and/or feeder noise with the arrival of pellets at the feeder area, the rat begins to preemptively nosepoke the feeder receptacle in response to cue presentation. Once this has continued for a day or two, the task script is switched to require nosepokes to deliver reward, with no delay in the amount of time the rat must hold the nosepoke to be successfully registered. Rats typically understand this within the first day, although some rats do not understand the difference between rewarded and non-rewarded until considerable training is given. The time the animal is required to hold the nosepoke (delay time) is gradually increased over the next 1-2 weeks from 0s to 0.6s (the maximum time rats appear to nosepoke effectively with the current design of feeder receptacle).

Once the rat is actively nosepoking for 0.6s to the rewarded cue to receive the reward, and is maintaining body weight by completing enough trials across the two 25 minute periods, training is considered complete. The rat is returned to a resting period until surgery not more than 2 weeks later. The entire training period typically takes about 3 weeks per rat, although it varies based on the rat's temperament. Each training day is typically 1.5 hours in duration, includes the two 25 minute sessions, and is randomly placed before or after an equally long electrophysiology session in order to minimize scent cues in the environments. Environments are cleaned with a light detergent between sessions and between training and electrophysiology periods. Following habituation and training, surgery is performed to implant the electrode array. Rats undergo a one week recovery period, after which they have recording sessions while continuing to learn to discriminate the cues. Once recordings are taken from the estimated location of ventral subiculum, a session under extinction conditions[9] is performed and the brain is subsequently recovered.

[9]Extinction is a procedure in neuroscience wherein a previously known reinforcer of a behaviour, such as food presentation upon pressing a lever, is removed, and behaviour is assessed without the reinforcer. This may also be known as a probe trial.

3.4 Overview of Experimental Animals

The full experiment involved 7 animals (male Long-Evans rats), of which one served as a test for the implant procedure and design. Two animals failed to complete training due to anxiety on task. The remaining 4 animals underwent surgery and were recorded from over the course of the experiment, although quality of data between rats differed. Refer to Table 1 on the following page for an overview.

3.5 Implant Design

Implant designs involve creating an array that is cemented to the skull, with one or more bundles of electrodes that descend through the brain to the appropriate depth level to record from an area. To examine evidence for spatial representation and context discrimination, an implant with two target locations was chosen. Since ventral subicular representations are not well examined, this allows for comparison of novel signal data against expected results in a second area. The first target for recording is the ventral subiculum, with the second implanted target being the dorsal hippocampus (region CA1) where activity remapping due to differences in context is well known to occur. The ventral hippocampus and ventral subiculum are separated by around 0.5mm along the dorsal-ventral axis and are thus accessible by the same electrode at different depths. Electrodes are initially positioned on the dorsal side of the cortex, and are then required to traverse the dorsal-vental axis of the brain to reach ventral subiculum (at a depth of 9mm). To facilitate this traversal, the ventral subicular electrode bundle is implanted 5mm deep in cortex so that ventral electrodes have less distance to travel (4mm as opposed to 9mm).

To improve robustness of chronic implants, at most locations on an electrode array, electrodes are kept contained within steel cannulae to protect them from the environment. The dorsal hippocampus electrodes are located in a bundle of electrode holding cannulae (30 gauge, outer wall diameter $\sim$ 0.31mm) that rests on the surface of cortex, while the design of the ventral subicular bundle is a group of 4 cannulae organized in a parallelogram. Ventral subicular cannulae are separated by a small amount of space, to both maximize the area of the ventral subiculum that can be accessed, and minimize both tissue damage and likelihood of missing the entire structure. This bundle extends a full 5mm beyond the dorsal CA1 bundle and is gradually advanced into the brain during surgery to the 5mm depth, embedded in hippocampal tissue. Electrodes are then extended from the cannulae at the starting 5mm depth, until the target location of ventral subiculum is reached around 8.5-9.0mm.

One design constraint is the need to protect the implant from the environmental damage and electrical noise. This was accomplished by using metal and 3D printed cones that surrounded the implant while it was in use. Other methods of electrical noise control involve using both a grounding screw implanted in skull

Rat ID	Task	Training	Cells Isolated	Days Recorded	Notes
R001	LinearTrackTone	Complete	–	–	Testing rat for e-phys, targeting, and hardware. Ventral targets not hit, tetrodes went medial into midbrain
R010	ContextBoxTone	Complete*	8	07/08 - 08/01 (25)	Ventral targets not hit, tetrodes went medial into midbrain. *It was decided to not wait for rats to learn the task fully in order to save time. Rat was given food upon CS+, and never learned the task
R013	ContextBoxTone	Complete	11 (8 dHC, 1 vSub)	08/25 - 09/11 (18)	One vSub hit. Two ventral tetrodes ended in pre/para subiculum (unknown final location). Implant failed catastrophically after 2.5 weeks due to unknown reasons. Rat learned the task well
R015	ContextBoxTone	Halted	–	–	Training halted due to very slow learning and high anxiety. Rat was retired to the colony room.
R017	ContextBoxTone	Halted	–	–	Training halted due to slow learning after rat learned to make vertical 50cm jumps and escape the contexts. Rat retired to the colony room.
R021	ContextBoxTone	Complete	12 (11 dHC, 1 vSub)	12/04 - 12/16 (13)	One vSub hit. Experiment was eventually halted due to severe electrical noise in all recorded signals and recording hardware problems. Rat never appeared to learn the task.
R022	ContextBoxTone	Complete	47 (16 dHC, 24 vHC, 7 vSub)	01/20 - 02/14 (26)	1-2 vSub hits (1 confirmed). 1 tetrode in vHC at same time. Rat learned the task well.

Table 1: Experimental Animals

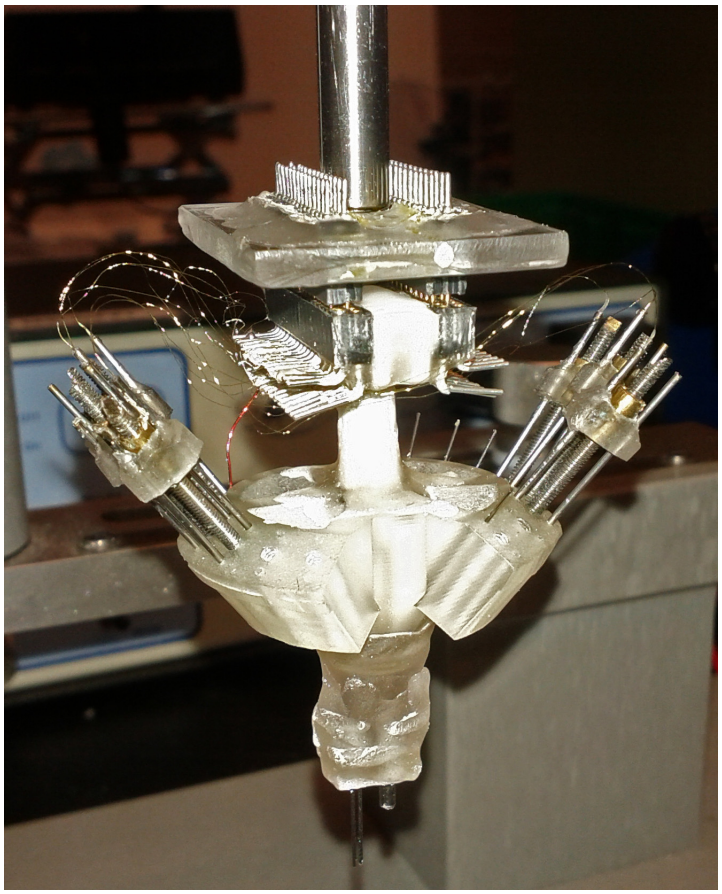

Figure 8: Implant design
Shown here is an image of a nearly complete implant, noting that the connection between headstage and pre-amplifier is via an MillMax connector. The two cannulae bundles can be seen protruding from the bottom of the implant. The subicular bundle is on the left side, and the shorter cannula to the right is the dorsal hippocampal bundle.

on the opposite side of the skull from the implant, and a noise reducing electrode in the dorsal CA1 bundle which is left in the corpus callosum, an area of relatively low signal strength compared to the dorsal CA1 or ventral subiculum. In practice, this is not yet sufficient to eliminate noise for very ventally located electrodes. For each rat, at least one of the vSub electrodes determined not to be on a correct descent trajectory to ventral subiculum (based on electrical signal profile) was repurposed for use as a reference electrode. This electrode was then moved to an area of low signal strength during the later periods of the experiment.

3.6 Surgery and Recovery

Surgery was performed on 5 male Long Evans rats (age 7-12 months at time of surgery). Animals were fasted for one day prior to surgery, and were placed under general anesthesia using gaseous isofluorane (5%). Animals were further anesthetized with i.p. sodium pentobarbitol (50 mg/kg at 240 mg/ml) and placed on a stereotactic holding device, remaining under light isofluorane administration (1-2%) for the duration of surgery. After visualizing markers on the skull, a small section of facial muscle (right side, approximately 2 mm) was separated from the right lateral skull ridge to allow vertical access to the ventral target. Craniotomies were drilled on the right side of the skull, with a grounding screw being attached to the opposite side of skull from the craniotomies. The dorsal hippocampal (dCA1) craniotomy was drilled using a 1.8mm trephine, while the vSub craniotomy was drilled freehand with a 0.5mm dental drill (Targets relative to Bregma (mm): dorsal CA1 - AP -3.8mm, ML +2.5mm, vSub - AP -5.8mm, ML +4.8mm). The implant was lowered into place until the 4 ventral subicular cannulae (30 gauge, outer wall diameter $\sim$ 0.31mm) touched the surface of cortex, and then was - using a small 3D printed targeting piece as a guide for alignment - lowered exactly 5mm into cortex until the end of the dorsal hippocampal bundle (14 gauge, outer wall diameter $\sim$ 2.1mm) touched the surface of cortex. The grounding screw was then attached to the implant, and a column of pink dental acrylic (brand here) was constructed around the implant for protection. Each tetrode was subsequently lowered into cortex by 1.28mm to assure each electrode was outside its holding cannula. The implant protective lid was secured with coban wrap and black electrical tape. Rats were given anafen (subcutaneous 3 mg/kg at 10 mg/ml) and ibuprofen (approximately 1 200mg liquid filled pill or 15 mg/kg at 100 mg/ml liquid Children's Advil per 250mL bottle of water) for analgesic effects, and the antibiotics Dualcillin (intramuscular in thigh, 0.1mL per side) initially and baytril (2.5 mg/kg at 50 mg/ml mixed in saline, injected subcutaneously during surgery, and daily for for the first two days of post-operative recovery) to prevent infection at the implant site. Animals were weighed and monitored daily for one week before running on task. Rats were typically connected to the recording system after 2 days to assist with advancing tetrodes to the desired depth and to check the quality of the tetrode recordings.

3.7 Electrophysiological Recording

Neural recordings were taken using tetrodes, spun compound electrodes consisting of 4 individual 17 μm platinum iridium (PtIr) wires. The purpose of using tetrodes is that use of 4 wires per electrode allows for triangulation of an electrical signal, which is the basis for isolating individual neurons. The wires are heated after spinning to achieve bonding of their insulation layer[10], and are plated with platinum solution (standard platinum black, mixed in lab) to have their electrical impedance lowered to between 200-350kΩ. All tetrodes were initially referenced against another tetrode positioned in corpus callosum (CC) above the hippocampal (dorsal CA1) cell layer. In some rats, the reference tetrode was noisy by the time of data collection, requiring that one planned dHC tetrode was returned to CC and used as a reference. While dHC tetrodes remained referenced against CC for the duration of recording, rat ventral tetrodes were switched to a dedicated ventral reference (One of the existing ventral tetrodes was moved to the lowest amplitude signal area seen during tetrode descent) by the time of data collection due to severe electrical noise issues. Signals are amplified using a pre-amplifier affixed to the implant, and transmitted by multi-channel wire to the Neuralynx recording system. The signals themselves are not only the action potentials of neurons, but are a combination of electrical changes caused by all ionic activity in the local grouping of cells around the electrode. The signals must be filtered in order to record specific types of neural activity. Electric field activity recorded by the Neuralynx hardware is filtered as follows: low frequency signals are filtered with a low-pass filter (1-425Hz) to record what are known as local field potentials (LFPs are a summation of cellular ensemble electrical activity that does not include high frequency events such as action potentials), while the putative action potentials are isolated using a high-pass filter (600-6000Hz), and are stored for later offline analysis.

3.8 Cell Isolation and Data Preparation

Stored putative action potentials (spikes) were sorted into clusters of data that represent putative neurons automatically using KlustaKwik (http://klustakwik.sourceforge.net), followed by manual neuron sorting using the mClust package (A. David Redish et al., University of Minnesota). Putative neurons were isolated based on standard criteria, and were checked for both isolation of the activity cluster from other clusters, and cluster cohesiveness[11] (L-ratio < 0.1, Isolation distance > 16). Obvious outliers in the neural waveforms were

[10] Wires are designed to have a conductive core surrounded by a non-conductive insulating layer. Causing the insulation layers of multiple wires to bond together makes the current wire arrangement permanent, and improves strength of the overall wire bundle.

[11] In short, cells are triangulated based on the voltages of neural action potentials recorded by each of the 4 electrode wires. Action potentials for a given neuron must have voltage patterns that are distinct and isolatable from those of other neurons. Thus, the term cluster cutting is used, which refers to isolating/cutting voltage spike clusters from the background of recorded activity.

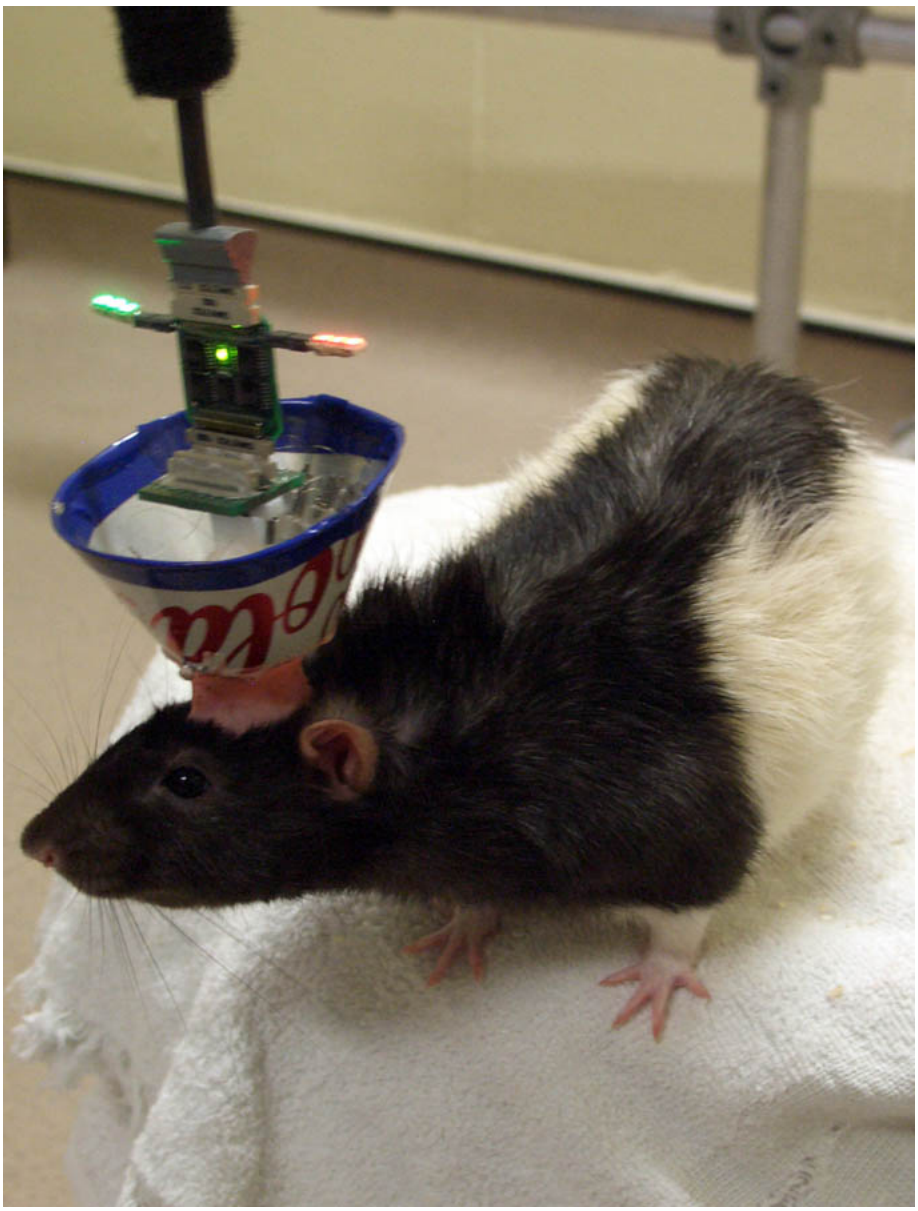

Figure 9: Rat during a rest period in the experiment after having undergone surgery several weeks previously Rat R001 can be seen here after surgery sitting on his resting pedestal (a modified flower pot covered with a towel). The coloured LED's seen at top are used for tracking the animal during the experiment. The cone for R001 and R010 were created from cola cans due to a lack of prototyped plastic parts. Since the cones were made of aluminum, they were grounded via a single wire, and served as a Faraday cage (This had no noticeable effect on noise levels).

removed. Autocorrelograms[12] were assessed for expected features, such as modulation by theta (6-10Hz) frequency signals in dHC. Isolated neurons were stored for later analysis.

3.9 Data Analysis and Statistics

To test whether contextual discrimination of the cues was taking place, behavioural data was analyzed based on several metrics. These included analysis of movement around the cue presentation time (2s window), per trial and daily averages of distances relative to the feeder receptacle around the time of cue presentation, percentage of trials where the rat nosepoked for rewarded and non-rewarded cues, and per rat tracking of cue responses during the electrophysiology stage of the experiment. Chi-squared tests (`chi2gof`) were used to assess the statistical significance of the nosepoke percentages to CS+ as compared to CS-. This was calculated both in individual contexts, and across contexts, and therefore assessed whether behavioural contextual discrimination was taking place. The observed values were the percent nosepokes of each condition, typically CS+ nosepokes and CS- nosepokes in either an individual or combined context. Expected values were calculated by assuming that a rat that has not learned to discriminate the cues by context would nosepoke equally to both CS+ and CS-. Since cue outcomes were reversed between boxes (nosepoking to white noise in one context would give a reward, but a penalty in the other context), a combined context rejection of the null hypothesis shows that cues were learned in a context-dependent manner.

Tetrode recordings were tagged with location based on histological localization and features of the local field potential for that tetrode. As current recordings from ventral subiculum are nonspecific, *in vitro*, or in other species of animal, exploratory analysis of LFP recordings was undertaken. Previously filtered LFP's were downsampled (4x) and spectrograms for each tetrode were computed using `mtspecgramc` (Chronux; params: moving window = 2s, step size = 0.05s, Fs = 1/median of timestep dt, pad = 2, fpass = 0 to 250Hz, tapers = 2,1,1). Windowed average spectrograms were isolated from the overall spectrogram (per-tetrode) for times around CS+/- presentation, Nosepokes to CS+/-, and reward delivery. LFP's were separated based on active (on task) and rest (on resting pedestal, plugged in, but not in the environments) periods per session, and frequency power spectra (PSD's) were calculated using `psd` (params: estimator = welch, Fs =1/median of timestep dt, opts: FreqPoints = 'All', NFFT = 'Nextpow2', SegmentLength = Fs*2, overlapPercentage = 25) for each recorded structure per day.

The project performs cellular analysis of ventral hippocampal formation neuron modulation by reward and place. To examine place and reward modulation, I divided the environment into a grid of individual bins, and calculated heat maps of neuron place-dependent firing using action potential locations convolved

[12] A measure of the difference in time between each neural spike and every other neural spike for one neuron

with a 2D gaussian curve, normalized and thresholded (> 0.05 second occupancy). The centre of activity of each cell's place field was taken for the first and last half of the session in each environment, and was used to visually compare activity between environments. This tested for the presence of remapping (it would be expected that remapping cells would have a much larger difference in centre of activity between contexts, than between halves of a recording session in a single context). To test for modulation of cell activity by task related events (cue presentation, reward delivery, nosepoking), peri-event histograms (PETHs) were calculated using a -2s to cue length window for CS+ and CS- presentation, nosepoke to CS+ and CS-, and reward delivery[13]. Statistical significance of task event-related modulation was assessed by using a larger histogram bin size (0.5s compared to 0.1s in the PETH) in order to capture larger scale significance of the activity. 100 time-randomized spike trains (records of neuron action potentials) were created for activity in a context. Observed bins in either the top or bottom 5% of activity for the randomized spike trains were considered to be significant. To account for variability, a new set of 100 randomized spike trains was generated and compared against for each 0.5s bin present in the statistical analysis.

3.10 Histology

After recording was completed for each rat, small electrolytic lesions were made by passing current through the recording tetrodes (two channels per tetrode, 10μA, for 10s). Lesions created in this manner are visible under microscopic examination, allowing for subsequent histological localization of the electrodes. After 24-48 hours, animals were deeply anesthetized using isofluorane, and were subsequently asphyxiated with carbon dioxide. Perfusion was immediately performed according to standard procedures using saline and formalin. Brains were extracted and stored temporarily in buffered formalin solution. After 48 hours, the formalin was mixed with 30% sucrose and vortexed, with the sucrose serving to prevent ice crystallization tissue damage during sectioning. After a minimum of two days, brains were sectioned using a freezing microtome in 50 micrometer slices, and were set aside on 4% gelatin slides (4 per slide) to dry for several days. Initial rats used a cresyl violet stain to visualize tissue, while later rats used a method involving counterstaining with glacial acetic acid to allow for a more accurate visualization of cell bodies. Cover slips were affixed with Permount (Fisher Scientific) mounting solution, and slides set aside to dry for several days before viewing. Slides were imaged under CCD camera microscope, and images showing tetrode tracks and electrolytic marks were saved. Sections of brain were compared against a rat brain atlas (Paxinos and Watson, 2008), and locations of each tetrode were recorded for later use.

[13] A peri-event histogram (PETH) looks at a window around each occurrence of an event. The time around the event is divided into bins, and activity is sorted into those bins. Activity in bins is then summed across all instances of the event to create a histogram of event-dependent activity

4 Results

The results consist first of behavioural data showing (1) that rats can discriminate between the two contexts, (2) that rats can discriminate between the reward predictive and non-predictive cues, and (3) that performance was not uniform across individual rats. Second, histological evidence is presented for electrodes located in both the ventral hippocampus and ventral subiculum. Third, individual cells are analyzed for evidence of place, context, reward, and cue modulated activity. Finally, LFPs are analyzed to determine general levels of electrical activity in the ventral subiculum, and to determine whether local fields are modulated by events during the behavioural task.

4.1 Context Discrimination Results from Behaviour

4.1.1 Global Average Rat Behaviour (Post Surgery) as Applies to Discrimination of Context

Do rats discriminate between reward predictive and reward non-predictive cues? To this this, averaged data was compiled across all days post-surgery for all animals running the standard version (task required nosepoking to trigger reward delivery) of the task (3 of 4 rats). One rat undergoing the task (R010) was excluded from the average due to behavioural modification of the task required for him to move[14]. Data was computed as a total of trials nosepoked out of total trials of each condition (CS+ or CS- presentation). It was expected that rats would learn to correctly nosepoke to presentation of the CS+ while ignoring the CS- (e.g. a day with fully accurate responses would show 100% of CS+ presentations were nosepoked to, with 0% of CS- nosepoked to in each context). Chi-squared tests were performed to check for statistical differences in responses to CS+ and CS- in a context (See section 3.10). The Chi-squared tests were computed for nosepoke percentages to CS+ vs. CS- presentations in each condition (black context, white context, combined contexts) with an expectation of equal amounts of nosepoking to both cues under the null hypothesis (that there is no difference in percent trials resulting in a nosepoke). Observed values were the percent nosepokes following CS+ and CS- presentation in each of the three conditions. All three conditions - black context ($\chi 2 = 139$, df $= 1$, $p < 0.01$), white context ($\chi 2 = 39$, df $= 1$, $p < 0.01$), combined contexts ($\chi 2 = 167$, df $= 1$, $p < 0.01$) - showed evidence that overall, rats learned to both discriminate between the two contexts, and to discriminate between reward predictive and reward non-predictive cues dependent on the context (Figure 10). Reaction to each specific cue also depended on its predictiveness of reward, with rats responding to a specific cue consistently more often when it served as a rewarded stimulus - tone cue ($\chi 2 = 126$, df $= 1$, $p < 0.01$), white noise cue ($\chi 2 = 41$, df $= 1$, $p < 0.01$). Thus, overall rats discriminated cues within the contexts,

[14]This rat did not move quickly upon presentation of the cue. Rewards were presented regardless of rat activity to encourage movement within the task, as this still allowed analysis of cell spatial modulation, as well as activity related to consumption of the reward.

and across contexts.

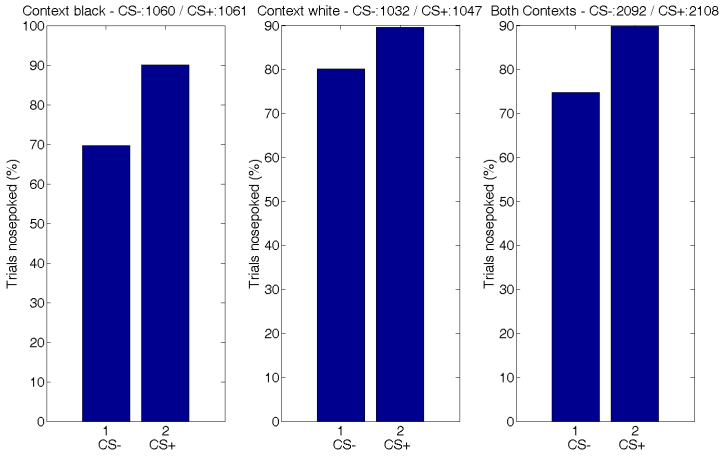

Figure 10: Rats discriminate between rewarded and unrewarded cues in both contexts
This data shows the percentage of trials nosepoked for each context across all trials for all days for rats R013, R021 and R022 (R010 excluded for task reasons). Rats not understanding the meaning of the contexts nosepoke the same amount to both CS+ and CS- presentations. Bars show the percent of each cue type nosepoked to in each context, and overall. The number of trials for each condition is shown at the top. This figure shows a clear increase in nosepoking behaviour to the rewarded stimulus in each context.

4.1.2 Comparative Rat Behavioural Learning Curves

Learning curves were created for each day post-surgery behaving on task for each rat, showing percent successful nosepokes and likelihood of ignoring of the CS- (Figure 11). Since percent totals do not give a full representation of the actual numbers involved, I additionally show the total number of trials under each condition. These data show that rats R013 and R022 had an increasing trend in the correct number of CS- responses by the end of recording (Figure 11), while CS+ responses tended to be consistently high (Typically around 90% by the end of recording, with the remaining ~10% split between ignored CS+ trials and early withdrawals from the nosepoke). Rat R010 has an unknown awareness of the task due to behavioural constraints (See footnote in section 4.1.1). Rat R021 did not appear to learn the task as expected, as the variables tracked do not show a successful increase in ignoring of the CS- while nosepoking to the CS+ (Figure 11). While rat R021 did learn to drastically increase the number of trials he performed per session, he responded similarly to both cues, leaving rats R013 and R022 as the only rats to show evidence of correctly learning the task.

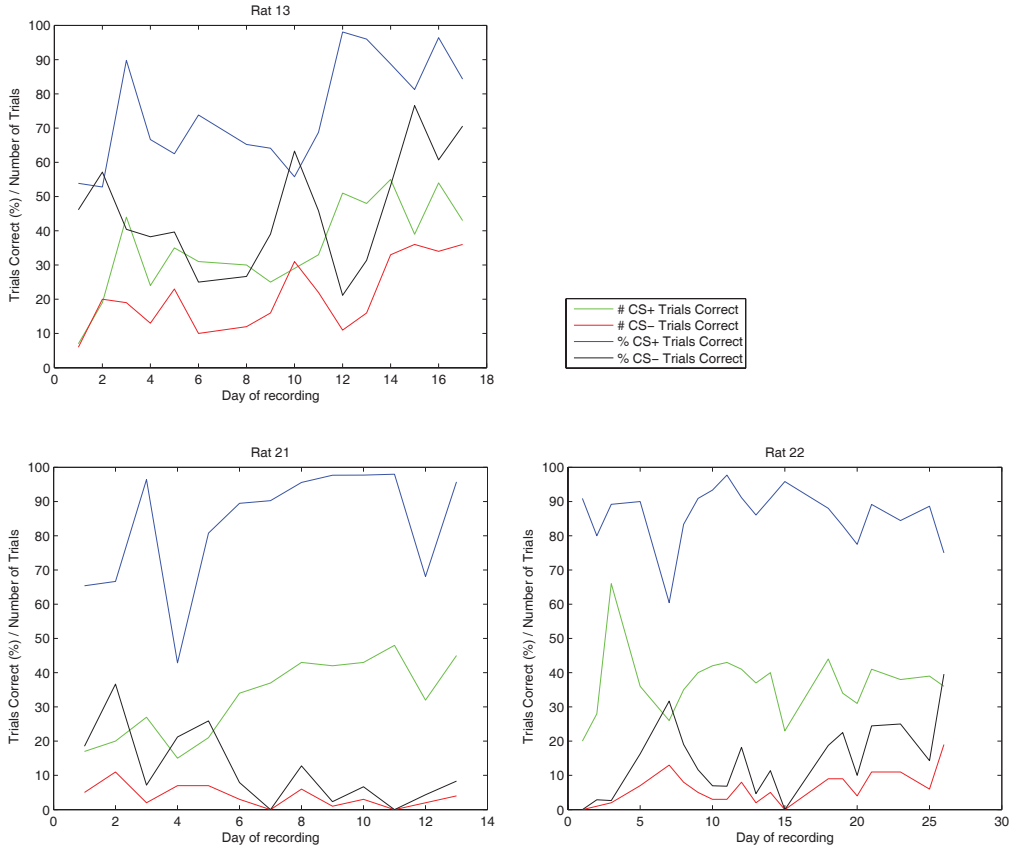

Figure 11: Rat learning curves - all days post-surgery on task

This figure shows the learning curve post-surgery for each rat that performed the task as intended. The blue line represents correct responses to CS+ as a percentage of total CS+ presentations. The black line represents the same for CS-. The green and red lines respectively represent actual numbers of correct CS+ and CS- response trials on each day rather than percentages. The Y axis doubles both as the percent correct or the actual number of trials the percent is based on for that day. While rats generally learned correct responses to CS+ (blue line), perfect performance would show CS- performance (black line) rising and becoming equivalent to the blue line in value.

4.1.3 Discrimination of Cues and Learning Results for Individual Rats

In additional to overall averages across rats, I assessed the average behaviour for each rat and single day behaviour to give a finer level view of learning progress. Results for individual rats are shown here as this allowed for a better examination of cue responses that often differed between rats in general. Using this format of showing individual rat performance, rat R013 (Figure 12) and rat R022 (Figure 14) learned the task during the recording period according to chi-squared analysis of responses to CS+ vs. CS- presentations in individual boxes and across boxes (See section 3.10 for more info on chi-squared analysis; Rat R013 - black ($\chi 2 = 99$, df $= 1$, p < 0.01), white ($\chi 2 = 55$, df $= 1$, p < 0.01), combined ($\chi 2 = 151$, df $= 1$, p < 0.01); Rat R022 - black ($\chi 2 = 11.9$, df $= 1$, p < 0.01), white ($\chi 2 = 3.1$, df $= 1$, p $= 0.08$), combined ($\chi 2 = 48$, df $= 1$, p < 0.01)), although rat R022 did not appear to learn to discriminate between the cues in the white context

by this measure. Rat R021 shows some evidence of learning (Figure 13) overall (black ($\chi 2 = 2.2$, df = 1, p = 0.14), white ($\chi 2 = 139$, df = 1, $p < 0.01$), combined ($\chi 2 = 1.59$, df = 1, p = 0.21)), but this was no longer present by the end of the recording period. Results for rat R010 clearly show a difference between response to CS- and CS+ presentations. Although the rat learned to move towards the feeder after CS+, but not CS- presentation, nothing can be said about the context-dependence of this movement since the nosepoke condition to trigger reward delivery was not required (the rat may have simply learned to run towards the noise of an activated feeder). Since the food would always be present, the rat also had no requirement to travel within the 5s cue period. Best case scenarios for a rat (R013 is shown) on a given day are presented in appendix 6.4 as an example of what these plots look like when learning is complete. While rat R013 and rat R022 clearly learned the task to a statistically significant level, rat R021 did not show evidence of learning, and rat R010 must rely on evidence from extinction conditions (See section 4.1.4 and accompanying footnote) to determine if any learning occurred.

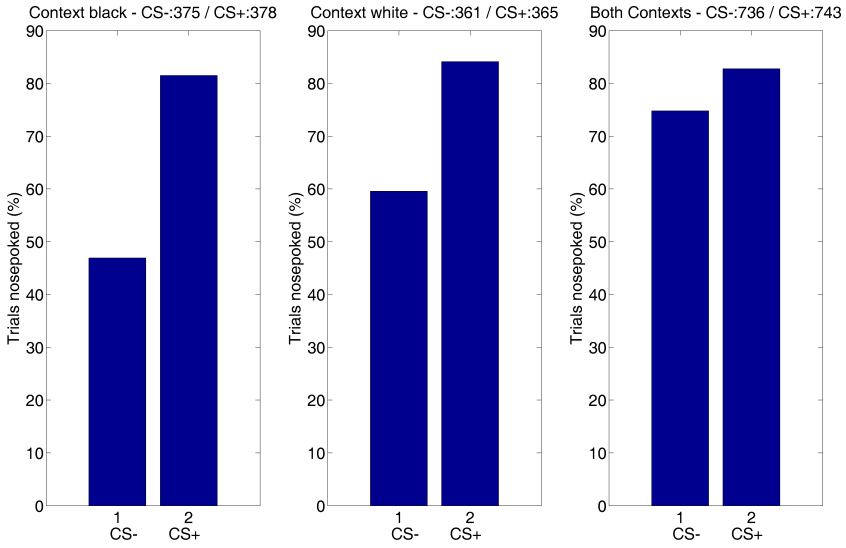

Figure 12: Nosepoke responses to cue presentation over all days for R013
Responses of rat R013 averages over all responses to cues for all days. Chi-squared tests were performed to determine statistical significance of differences between CS+ and CS- in each individual box, and across boxes in a context-dependent manner. Rat R013 is seen to respond much more often to CS+ presentations in each context, and overall (black ($\chi 2 = 99$, df = 1, $p < 0.01$), white ($\chi 2 = 55$, df = 1, $p < 0.01$), combined ($\chi 2 = 151$, df = 1, $p < 0.01$)), showing a context-dependent response to the cues.

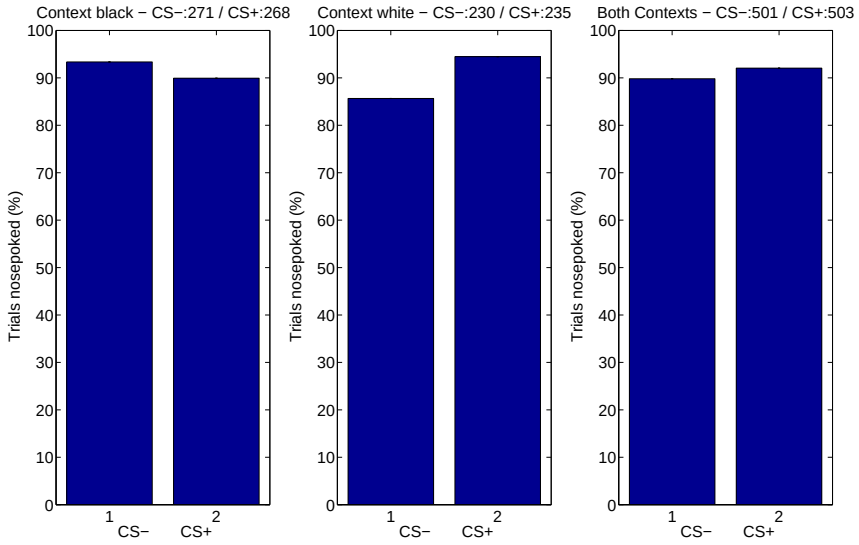

Figure 13: Nosepoke responses to cue presentation over all days for rat R021
Nosepoke responses are shown averaged over all days for rat R021. Only performance in the white context is significant in a chi-squared test ($\chi 2 = 139$, df $= 1$, $p < 0.01$).

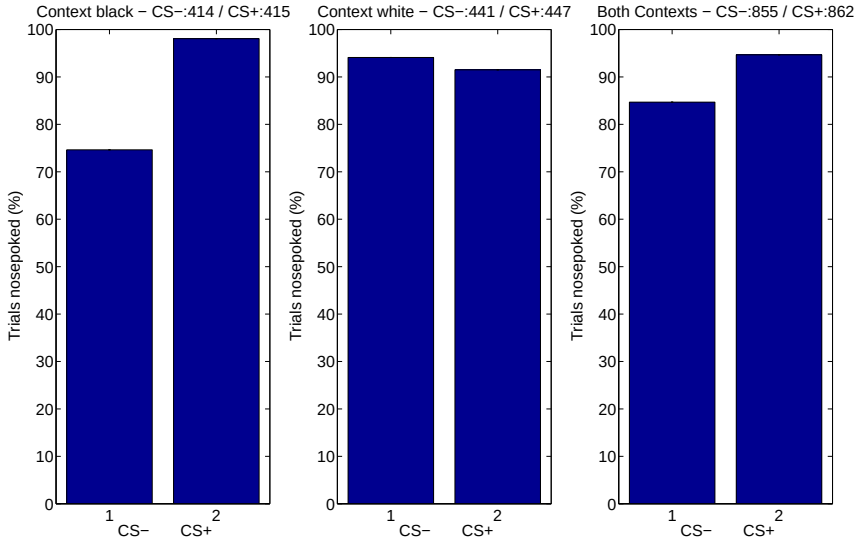

Figure 14: Nosepoke responses to cue presentation over all days for R022
Nosepoke responses are averaged over all days for rat R022. Black ($\chi 2 = 11.9$, df $= 1$, $p < 0.01$) and combined ($\chi 2 = 48$, df $= 1$, $p < 0.01$) contexts had chi-squared test results of $p < 0.01$. The white context was not statistically significant, but due to the combined statistics, we can say that the animal contextually differentiated between the cues.

4.1.4 Extinction Results (Cue Presentation Without Reward)

Testing for context-dependent signals in this experiment is based on the assumption that rat behaviour is influenced by memories of past rewarding behaviour. Since it is possible that animals could simply relearn the rewarded cues each day instead of relying on memory, a way of testing if cues are remembered is required

to discount this possibility. Two options were available, either examining the first trial of each day, or doing the final day of the experiment under extinction conditions. Since first trial examinations would have statistically required more trials than were available, extinction conditions were chosen. Extinction for the rats meant that they still received scattered pellets as before, but no pellets on correct cue response were presented. Extinction conditions were used on rats whenever possible (2 of 4 rats tested). Rat R010's extinction results were less useful for examination since he always received pellets for the correct cue, even without responding. However, it was possible that rat R010 would have remembered some of his training, and would have responded by nosepoking when no rewards were forthcoming.

In addition to the nosepoke percentages used before, distance peri-event averages (PEAs) were used to examine behaviour around cue presentation in finer detail. (PEAs)[15]. The distance peri-event averages were calculated to give per trial and average responses to cue presentation in a more readily understandable and analyzable format. In short, distance from the feeder receptacle is calculated at each point within a -2 second to cue length period for each trial. The distance is averaged across trials, and shown with standard deviation error bars. Distance from the feeder receptacle was used as a measure instead of speed because the rats had a tendency to respond to any cue by moving faster regardless of whether it was a CS- or CS+. However, only in the case of CS+ presentation for rats that learned the task, did the rat consistently approach the feeder receptacle.

Unfortunately the same methodology used to show rat learning also showed in this case that rat R010 likely did not learn anything about the differences between cues (Figure 15). In the case of rat R022, the rat appeared to become extremely frustrated during the second half of each extinction session, and stopped responding to cues in both contexts. As a result, the final 10 trials of CS+ and CS- presentation were removed from the analysis to make the results of the extinction conditions clear. Both distance peri-event averages (Figure 16; Shown for comparison with a similar figure from rat R010) and nosepoking percentages (Figure 17) show that rat R022 was consistently responding more to the CS+ presentation within each context and across both contexts combined (black and white chi-squared tests, and cross context cue tests were not done due to lack of sufficient trials, combined contexts - $\chi 2 = 8.03$, df = 1, p = 0.005). It is additionally interesting is that although rat R022 only showed knowledge of cues in one context during normal task days, during extinction, he demonstrated a full knowledge of cues in both contexts. In short, evidence from rat R022 under extinction shows that at least in the case of this rat, behaviour is not learned at the beginning of each session, but is rather retrieved from memory of the task conditions.

[15]In short, a distance PEA is a calculation of the distance of the animal from the feeder, performed in a window around the presentation of a cue for each trial. An average is calculated based on the individual trial behaviour, and individual trials are presented to show differences within a session.

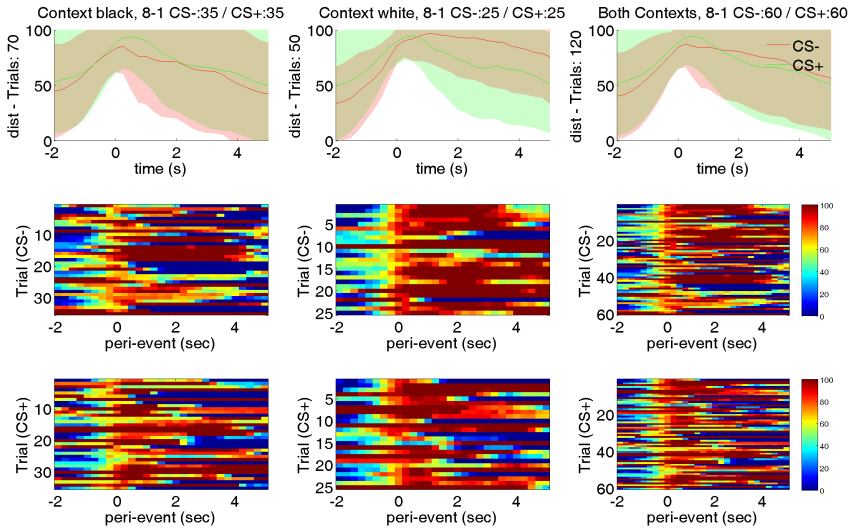

Figure 15: Distance peri-event averages and single trial behaviour for extinction for rat R010
Shown here is the distance in arbitrary units from the feeder as a function of time around the cue presentation (-2s to cue length window). The top row is a graphic of distance from feeder against time in 2D (green CS+, red CS-)with the shaded area being the standard deviation of the sample. The middle and bottom rows are a heatmap of distance from the feeder over individual trials, with individual trials being represented as rows. Dark blue represents that the rat was within the bounds of the feeder at that time point. Number of trials in each condition is shown at the top. Because rat R010 nosepokes were not tracked, a different method was required to assess response to cues. Distance to feeder was an appropriate measure in this case, although extinction results show rat R010 did not learn the task (gave equal responses to cue conditions), as expected.

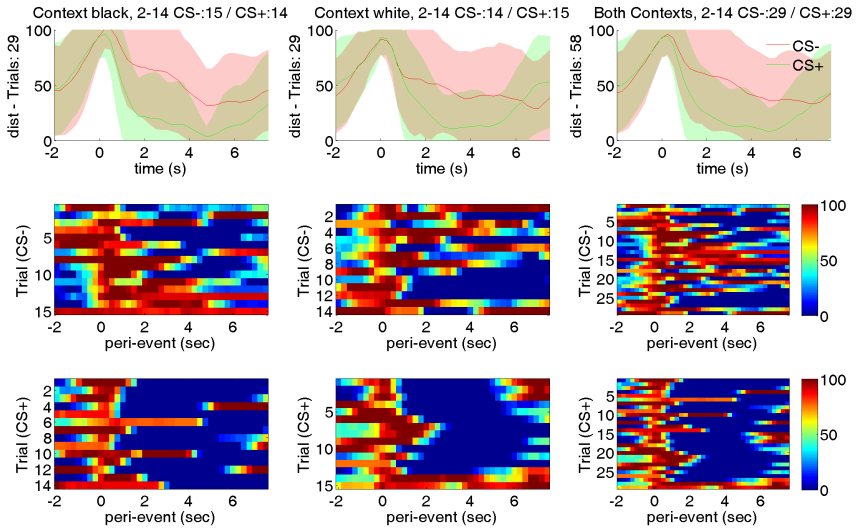

Figure 16: Extinction distance peri-event averages and single trial behaviour for R022 during the first half of the session in each context
The same distance measurement is shown for rat R022's extinction as was previously used for rat R010's extinction results, showing an accurate response to cues. Rat R022 is seen to be approaching the feeder in response to CS+ cues, while ignoring CS- cues most of the time in both contexts.

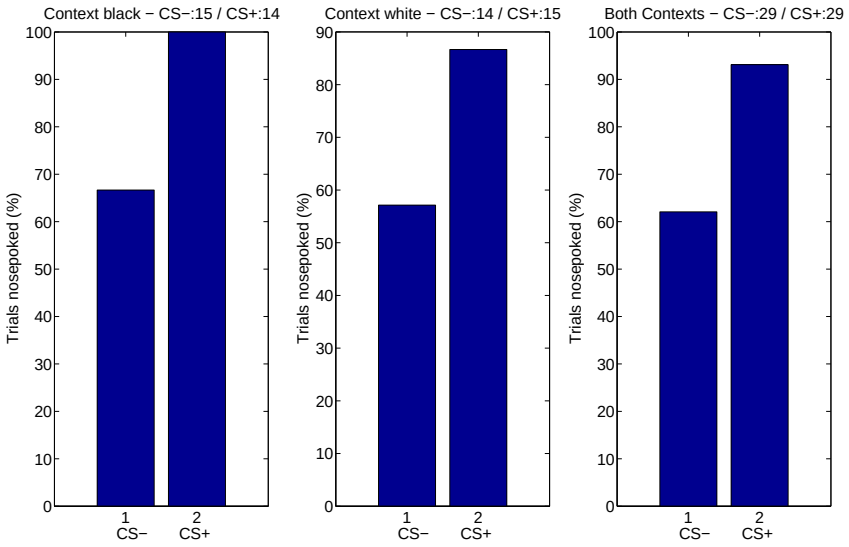

Figure 17: Extinction nosepoke percentages for rat R022 during the first half of the session in each context
Nosepoke responses for rat R022 show learning, with significantly more responses to CS+ as to CS- under extinction conditions.

4.2 Histology

Histology results are presented here for each structure targeted by electrodes for recording. Supplementary histological diagrams for individual tetrodes are present in appendix 6.5.

Although the implant of rat R013 suffered a catastrophic failure during routine testing, other methods described here were successfully used to ascertain the location of the R013 tetrodes. Both dorsal hippocampal tetrodes entered the CA1 cell layer. This was noted by an inversion of the direction of sharp waves in the hippocampal signal as noted for both tetrodes [16]. One ventral tetrode was known to penetrate vSub due to track location appearing to end roughly in the distal vSub, and cells being recorded in this location. An additional tetrode appears to have been present in either the presubiculum or parasubiculum. A high quality image is not available of rat R013's vSUB tetrode, although a low quality image is available in appendix 6.5. The other two ventral tetrodes were stopped further dorsal and are presumed to have been in the ventral hippocampus, although not the hippocampal CA3 cell layer, at the time of implant failure. Therefore, even with an implant failure, rat R013 tetrodes were localized to both dorsal hippocampus and ventral subiculum.

For all animals after rat R013 (Rats R021 and R022), tetrode locations were determined based on damage caused by small electrolytic lesions (see section 3.10 for a description of this procedure). Both rat R021 and rat R022 each had one tetrode in vSub, while at the same time having tetrodes in dHC. Rat R022 had an additional concurrent tetrode in vHC area CA3 at the time that vSub was finally reached. As a result, recordings for all three structures are available simultaneously. Rat R010 histology is not shown here. Due to the angle of the implant after surgery, tracks from rat R010's ventral tetrodes left intermediate hippocampus shortly after implantation and entered an unknown location of midbrain. As such, those tetrodes were not analyzed further. Overall, after recording was completed 6 useable (signal without excessive electrical noise or artifacts) tetrodes across 4 rats were present in dorsal hippocampus, 1 was present in ventral hippocampus, 1 was present in ventral dentate gyrus, and 3 were present in ventral subiculum across 3 rats. At least four useable tetrodes also passed through the ventral hippocampus during descent towards vSUB.

[16] Above a hippocampal cell layer, a sharp wave is a rapid downward deflection (drop in voltage) that quickly returns to a more normal level. When entering the layer, this deflection flattens out, and inverts into a rapid upward deflection below the layer.

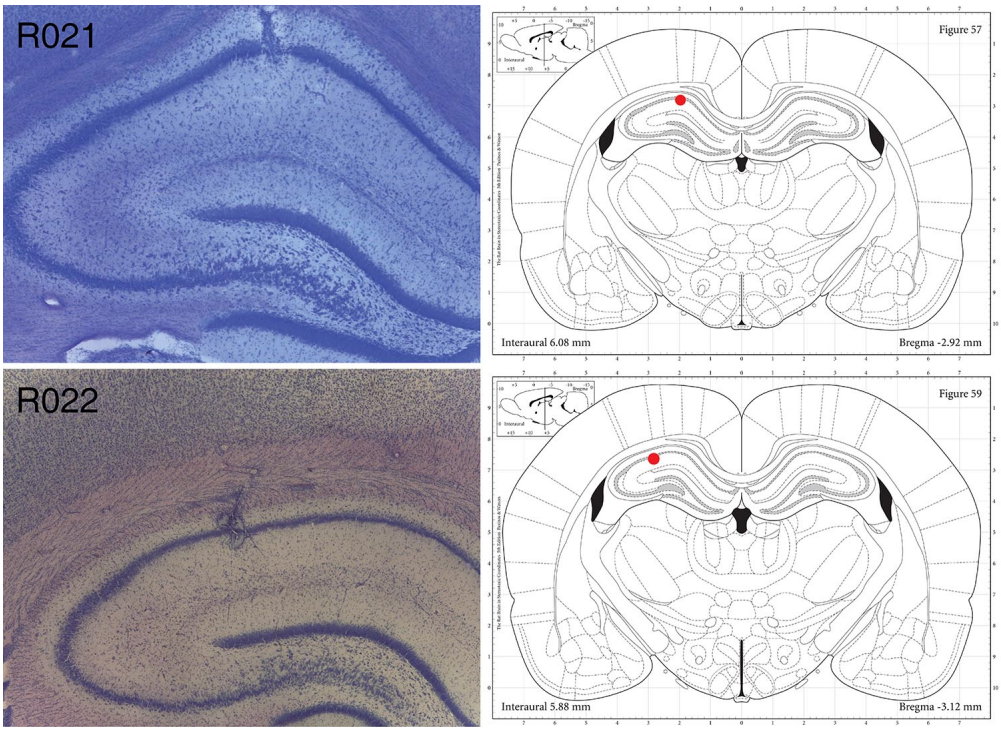

Figure 18: Dorsal hippocampal histology

Histological slices with glacial acetic acid counterstain are shown in the left column. Location of the tetrodes in dorsal CA1 are shown in the right column on coronal slices of brain. The top row and bottom row are images of tetrodes targeted to the dCA1 layer in both rat R021 and rat R022 respectively.

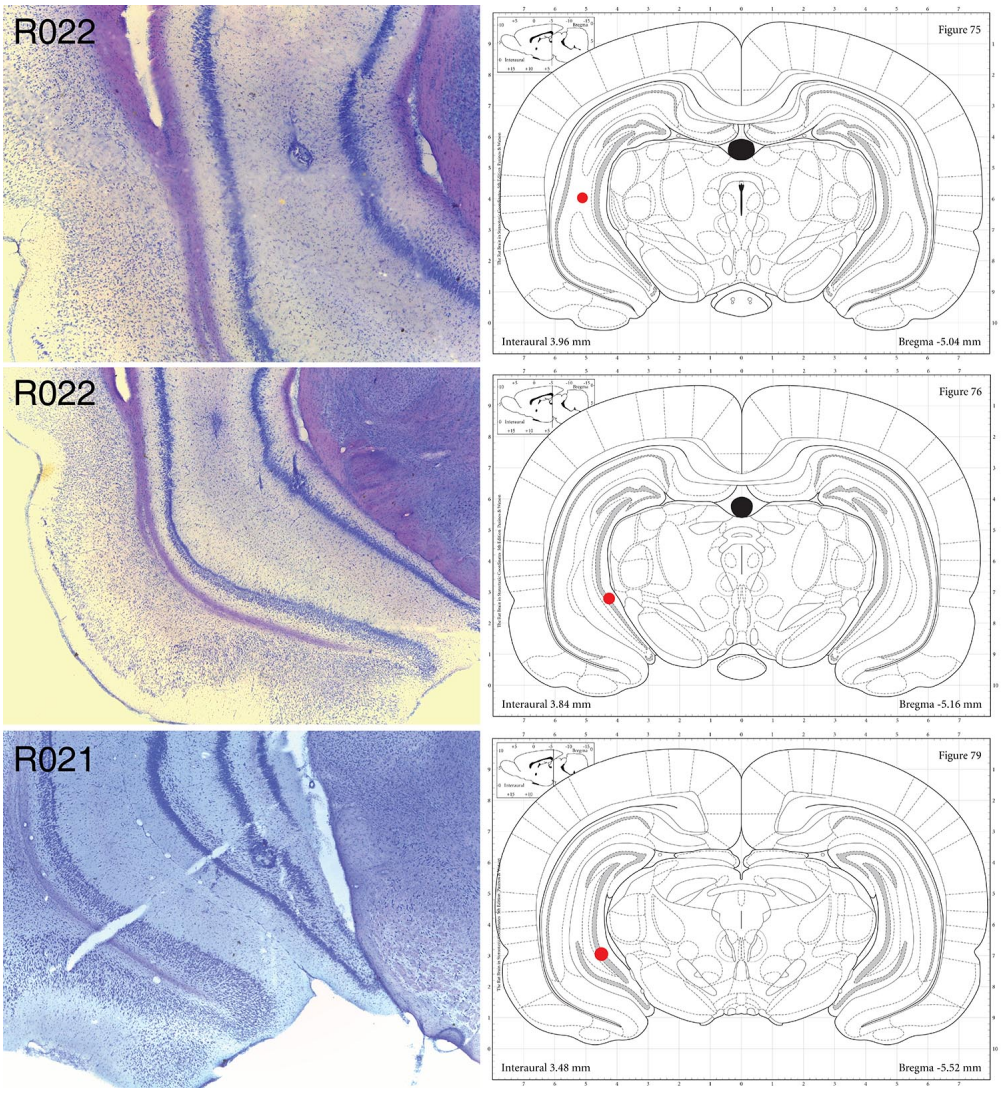

Figure 19: Ventral hippocampal formation histology

Histological slices with glacial acetic acid counterstain are shown in the left column. Location of the tetrode is shown in the right column on coronal slices of brain. The top row is a sample reference tetrode from rat R022 which was targeted to a quiet area on descent in order to cancel as much noise as possible on the vSub tetrodes. The middle row is a vCA3 tetrode from rat R022, while the bottom row is a ventral dentate gyrus tetrode.

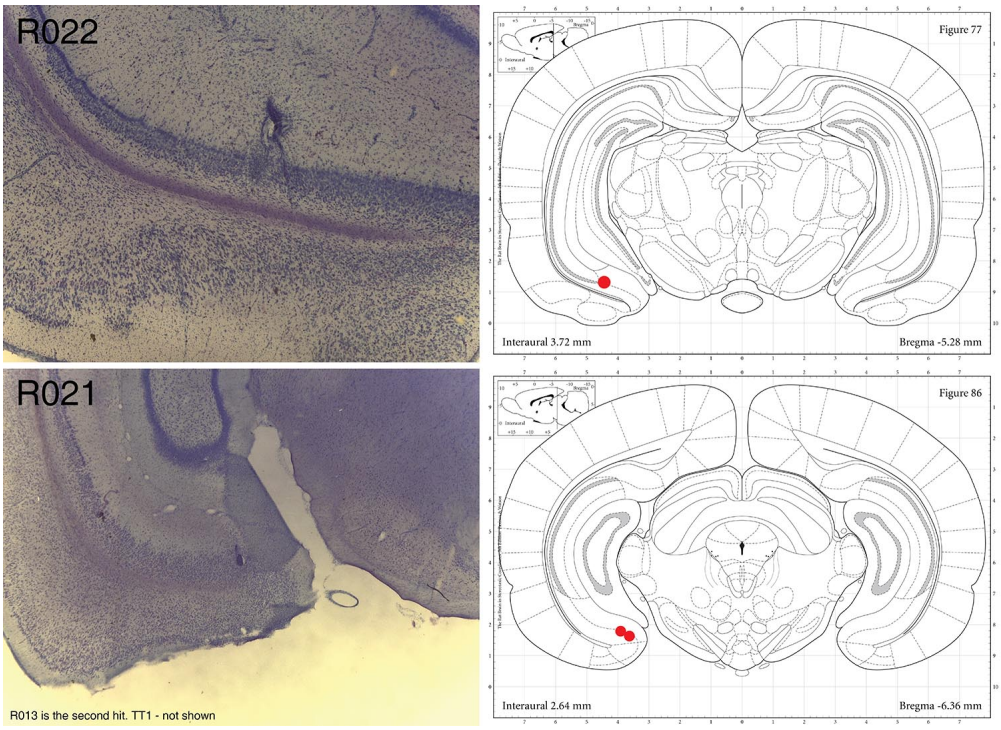

Figure 20: Ventral subicular histology
Histological slices with glacial acetic acid counterstain are shown in the left column. Location of the tetrode is shown in the right column on coronal slices of brain. The top row shows a proximal vSub tetrode from rat R022. From gliosis, the rat R022 vSUB tetrode is not obviously present in proximal ventral subiculum. However, cells were recorded from this location, so the tetrode was at least touching the cell layer. The last known location of the second tetrode attempting to hit ventral subiculum is seen faintly in the top centre of the image. This tetrode was several hundred microns behind the advance tetrode, but does appear to have recorded some spikes even though its final resting depth was not microscopically evident. It is possible, but not likely, that the lesion marker seen here is a combination of tracks from two tetrodes. The bottom row shows a distal vSUB tetrode from rat R021. Rat R013 had a tetrode in the same relative location, but is not shown here due to low quality of the image. The low quality rat R013 image is available in appendix section 4.6.

4.3 Results from Electrophysiology

4.3.1 General Results

Generally, there was an increasing trend in the quality of surgical targeting of structures and recordings from each successive rat. Rat R010 did not have a tetrode in the ventral subiculum, and did not have cells evident in dorsal hippocampus. Rat R013 through rat R022 all had tetrodes in both dorsal hippocampus and ventral subiculum at the same time, though they did not typically have neurons visible in both structures on the same day. Rat R013 had 11 cells isolated in total (10 in dHC, 1 in vSUB), and rat R021 had 12 cells isolated (11 in dHC, 1 in vSUB) before recording was terminated due to uncorrectable electrical noise issues. With improved implant assembly technique and electrical noise cancellation, rat R022 had 47 isolated cells (16 in dHC, 7 in vSUB, 24 in vHC) for a total of 70 cells recorded from rats R013,21,22. There were relatively

few active neurons present in vSUB on most days, though it's unknown how characteristic this simply is of the ventral subiculum in general, as it appears less dense than the hippocampal cell layer. Changes in LFP were tracked during the descent of the ventral targeted tetrodes towards vSUB. Information on the signal profile[17] of the tetrode descent from ventral hippocampus is available in the appendix (section 6.6).

4.3.2 Dorsal Hippocampal Confirmation of Remapping in Context

Before assessing if vSub or vHC cells changed activity based on the context or activities related to reward, it was first necessary to assess whether dorsal cells were undergoing global remapping as would be expected when the rat underwent a change in environment. Cell spiking activity was restricted to analysis of when the rat was moving, and was first plotted as a map of explored areas for each environment. Cells were then visually assessed for patterns of restricted spatial activity that were stable within an environment throughout a recording session. Of 30 dHC cells recorded, 10 showed visually assessed place related activity during the task. Of the 10 dHC cells with place fields during the task, 6 showed evidence of remapping between contexts, while one showed unclear evidence of remapping (60-70% showing remapping). Maps of spatial activity were generated for putative place cells, which were then smoothed through convolution with a 2D gaussian field[18]. Activity within each area of the environment was subdivided between the first and second half of the session in order to compare differences in activity within an environment against differences across environments. For cells that had different place fields between contexts, centre of mass of place fields were computed within environments and between environments, which allowed for a numerical estimation of the differences in place field location. Therefore, remapping was assessed visually both by comparing place fields and activity between environments (Figure 21), and also by comparing weighted centre of mass of half sessions against centre of mass between environments. Unfortunately, not enough cells were observed to perform a rigorous statistical analysis of the remapping that was found. However, since several cells were observed only being active in one context, this is a large difference in activity, even without accounting for cells that had only different relative place field locations. We can therefore say that global remapping is likely occurring in dorsal hippocampus, giving strong evidence the rats are distinguishing between the contexts.

[17] The recorded voltages in an area give a general idea of where a tetrode is due to characteristic signal features found in many areas. An example of this is characteristic 'sharp waves' found in the hippocampus. Notes of what features where seen on a certain day thus allows for creation of a general profile of the descent of an electrode through tissue.

[18] Roughly, this involves replacing a point location where a neural spike occurred with a circular gradient that rapidly attenuates in intensity.

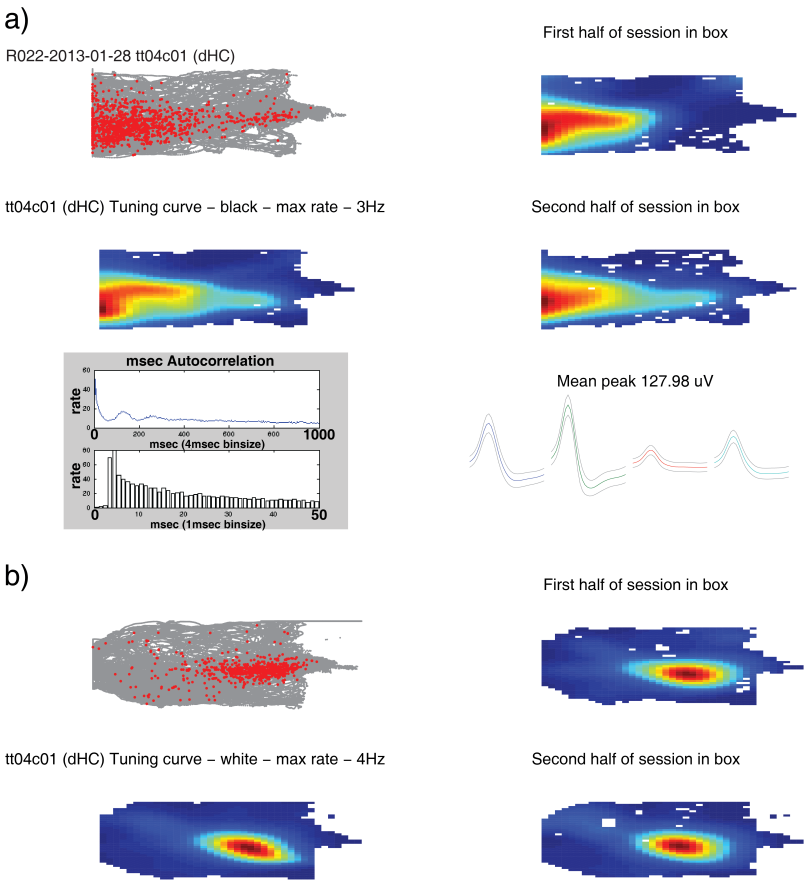

Figure 21: Sample dHC cell from R022 showing a difference in place fields between contexts
Cell R022-2013-01-28 tt04c01 (dHC) is presented here showing place fields in each of the two contexts. The top left box of each context is the coverage map in gray, with recorded action potentials shown as an overlay of red dots. A computed occupancy map for the entire session is shown below this as a heat map. The right most column contains the occupancy map subdivided by first and second half of the sessions (~12min each), and at bottom shows the waveform of the cell. The colour scale differs between the two contexts. The autocorrelation for the cell is presented in a gray box. The top section is a 4 msec bin-size showing the characteristic theta modulation pattern of place cells. The bottom is a 1 msec bin-size autocorrelation showing a lack of action potentials within the neural refractory period. a) The cell during the session in the black context. b) The cell during the session in the white context, showing different activity. The cell is seen to be remapping here, with the place field being in a different location of the environment in each context. The autocorrelogram of the cell showing theta modulation characteristic of place cells is shown next to the waveform.

4.3.3 Evidence for Cellular Remapping Where Behavioural Evidence Showed no Contextual Discrimination

As mentioned in the behavioural results, rat R022 did not appear to learn the task in the white context for at least 10 days after the beginning of recording. Analysis on the days prior to behavioural discrimination of cues was performed in order to look for any evidence of progress in learning the cues, and why the rat did not nosepoke accurately in each context. Both nosepoke behaviour, and cellular place field activity were compared on each day using the same methodology for cells as in section 4.3.2. Interestingly, although the

rat did not appear to understand the task as intended or differentiate between contexts behaviourally (no differences in attempted nosepokes), cells in the hippocampus did appear to at least partially remap (Figure 22). While evidence of differences in cellular response without behavioral differences do not represent a novel finding (Jeffery et al., 2003), it is interesting to note that they are happening here. This shows that HC cells are discriminating between environments without behavioural expression. The same phenomenon was not noted in the other tested rats as the relative cell counts were too low to estimate differences over days of recording. Section 4.3.3 has more samples of cellular remapping that occurred prior to obvious behavioural differentiation between the contexts. Therefore, we can say that even without behavioural evidence of cue discrimination based on reward delivery, learning of the contexts may be occurring. It is therefore still useful to examine evidence of remapping during these days in vHC.

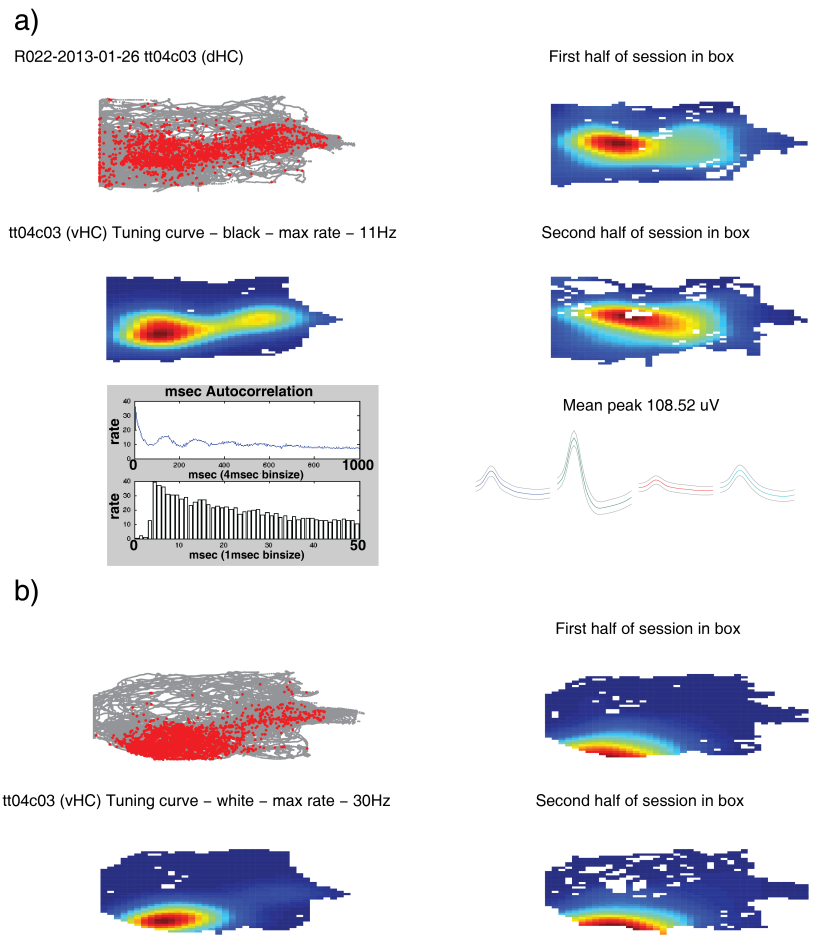

Figure 22: Activity of a dHC cell on a day before behavioural differentiation of the contexts
Cell R022-2013-01-26 tt04c03 (dHC) is presented here showing place fields in each of the two contexts. a) The cell during the session in the black context. b) The cell during the session in the white context, showing different activity. The cell is seen to be remapping here, even though this cell was recorded on a day when no behavioural differences between response to cues were evident. The autocorrelogram of the cell showing theta modulation characteristic of place cells is shown next to the waveform.

4.3.4 Ventral Hippocampal Cells Response in Contexts

Ventral hippocampal formation cells were examined, both for place field activity, and in response to cue presentation, nosepoking, and reward delivery across contexts. To examine place fields, the same methodology from section 4.3.2 was used. Response to events was then assessed by plotting time windowed (measured only for certain intervals) spike trains (a series of recorded spikes/action potentials) over all trials for a cell in each context, and calculating the peri-event histogram (PETH) of the spike trains (summing spiking activity sorted into a number of time bins subdividing the time window). To assess the statistical reliability of changes in firing pattern in response to the events, 100 randomized spike trains were generated, and used as a population sample to test the observed spike train against. Observed spike trains were ranked against these

randomized spike trains, and bins within the top or bottom 5% were considered significant (95% confidence interval). To account for signal variability between sessions, a new set of 100 randomized spike trains was generated and compared against for each 0.5s time bin present in the statistical analysis. Although observed vHC cells did not appear to globally remap in response to changes in the context, there may be a degree of rate remapping occurring (Figure 23), wherein the cellular firing rate changes between contexts without the location of the place field in the environment being altered (About half of vHC cells seen showed this trait - 4 of 7 with significant activity during the task). Ventral hippocampal cells also did not appear to have any specific place field within the environment and cells were not modulated by theta frequency signals, although most (5 of 7 with significant activity during the task) did show an increase in activity around the feeder location (Figure 23). Since cells with spatial modulation in the vHC are relatively sparse, the lack of observed place cells is likely due to that relative sparsity, as well as the lack of significant numbers of vHC cells examined.

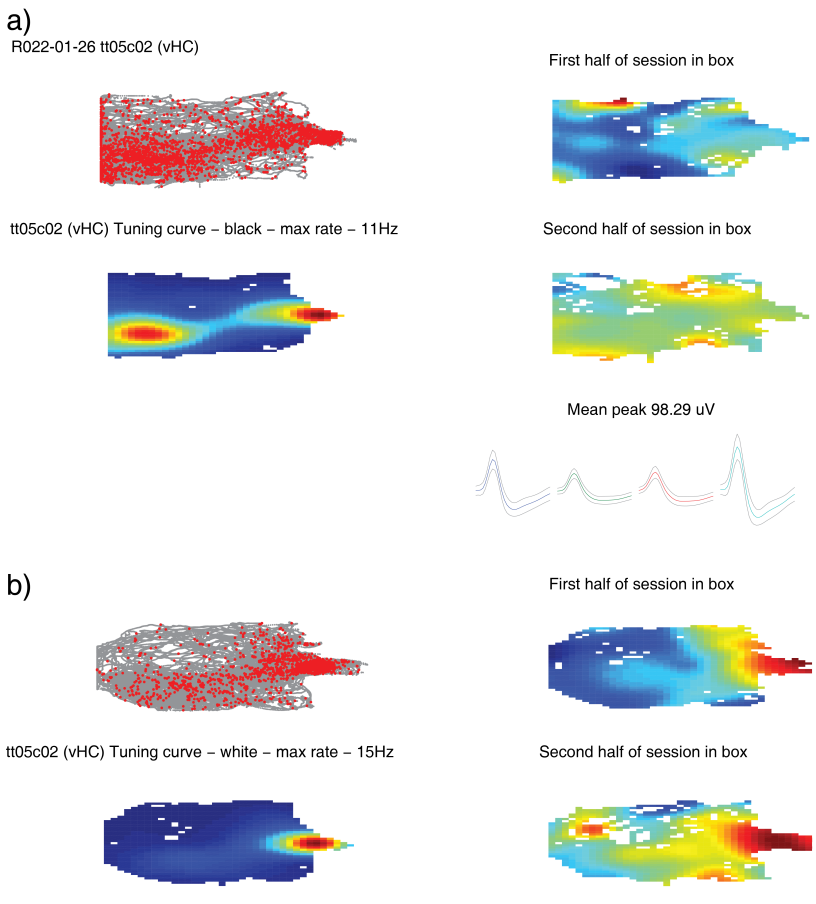

Figure 23: Ventral hippocampal CA3 cell response
Cell R022-2013-01-26 tt05c02 (vHC) is presented here showing place fields in each of the two contexts. a) The cell during the session in the black context. b) The cell during the session in the white context, showing a much lower maximum firing rate, and a stronger correlation of activity with the location of the feeder

Only two ventral subiculum cells were found which exhibited reliable spiking information for analysis. One of these was in the distal ventral subiculum (Figure 24), while the other was in proximal ventral subiculum (Figure 25). Neither cell exhibited a clear place field, with both appearing to fire in all locations within the environment. Interestingly though, there appears to have been modulation of the proximal vSub cell by the reward receptacle location, a feature not seen in the distal cell. This modulation by reward location is not particularly strong though when compared with other activity of that cell. The vSub cells also exhibited neither global or rate remapping, generally firing at the same maximum rate and in the same locations between contexts. Neither cell showed modulation by theta frequency signals.

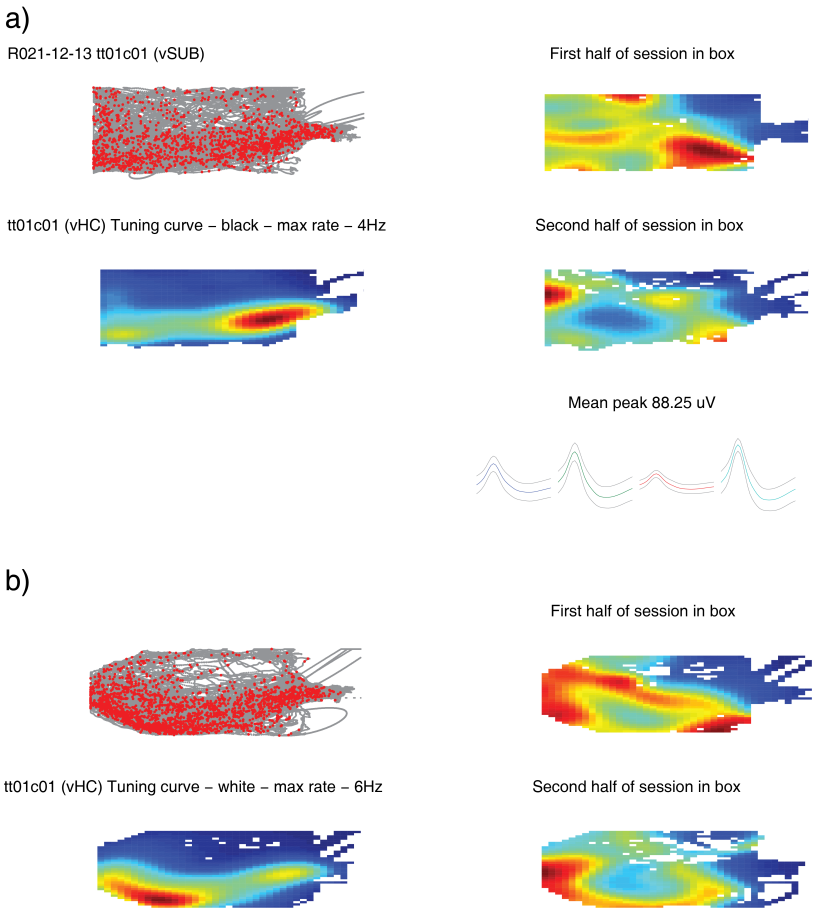

Figure 24: Distal ventral subiculum cell response
Cell R021-2012-12-13 tt01c01 (vSub) is presented here showing place fields in each of the two contexts. a) The cell during the session in the black context. b) The cell during the session in the white context. The cell does not appear to have a conventional place field, or remap during changes in context.

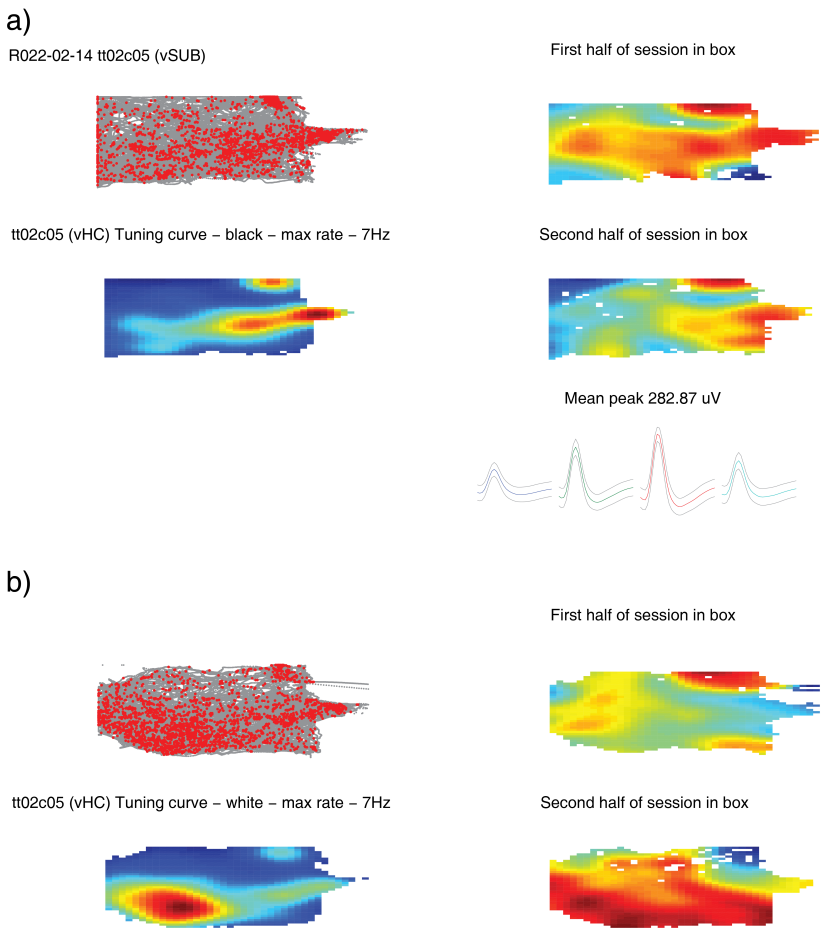

Figure 25: Proximal ventral subiculum cell response
Cell R021-2013-02-14 tt02c05 (vHC) is presented here showing place fields in each of the two contexts. a) The cell during the session in the black context. b) The cell during the session in the white context. The cell does not appear to have a conventional place field, or remap during changes in context.

However, the cells did have interesting firing patterns in response to events. In the case of the vSUB distally located cell, there was a clear modulation of firing rate in response to events, and that modulation was context specific. The distal cell had suppression of firing rate in response to nosepokes to CS+ presentation in the white context (and weakly to CS- presentation), but not in the black context (Figure 27). This could not be accounted for solely based on the speed of the animal. This was likely not due to behaviour in only one context, as during that day the rat treated both cues identically with respect to behaviour in approaching the feeder receptacle. The activity was not due to reward delivery because the animal must wait 0.6s after nosepoking before feeder begins triggering delivery of food pellets. The suppression of cell spiking activity took place within this time window. Although the cell responded in both contexts with increased firing rate after receiving reward, only in the white context prior to reward was activity suppressed during a period of similar movements by the animal (Figure 27). While there appears to be a increase in activity in response

to the CS+ cue presentation (Figure 26), this is actually closer to the average firing rate of the cell and is not likely related to the cue.

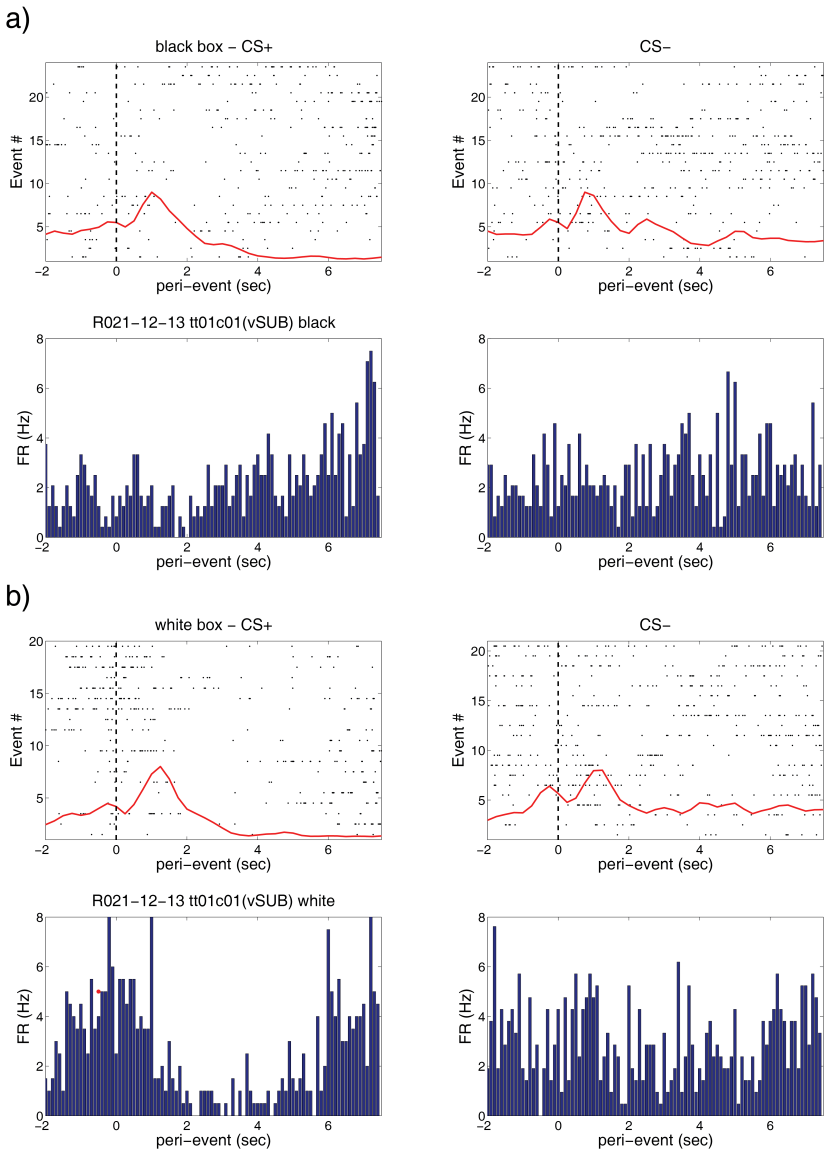

Figure 26: Distal ventral subiculum cell response to cues
This figure shows the responses of the distal ventral subiculum cell to cue presentation. The top of each diagram represents per trial windowed spike trains around an event (the dotted black line). Speed is overlayed over each spike PETH window in arbitrary units. Bottom diagrams are peri-event histograms of the data in the spike trains, represented as firing rate (Hz) around the event (sec). Red dots represent statistical significance of 0.5s bins of activity. A set of 100 randomized spike trains was generated for each context by randomizing the ISIs of the existing spikes. This was repeated once for each 0.5s bin, and the observed spike histograms were compared against the random spikes, with bins in the top or bottom 5% being significant. a) The responses of the cell in the black context. b) The responses of the cell in the white context. A suppression of activity is visible after CS+ presentation in the white, but not black context, which cannot be accounted for by speed.

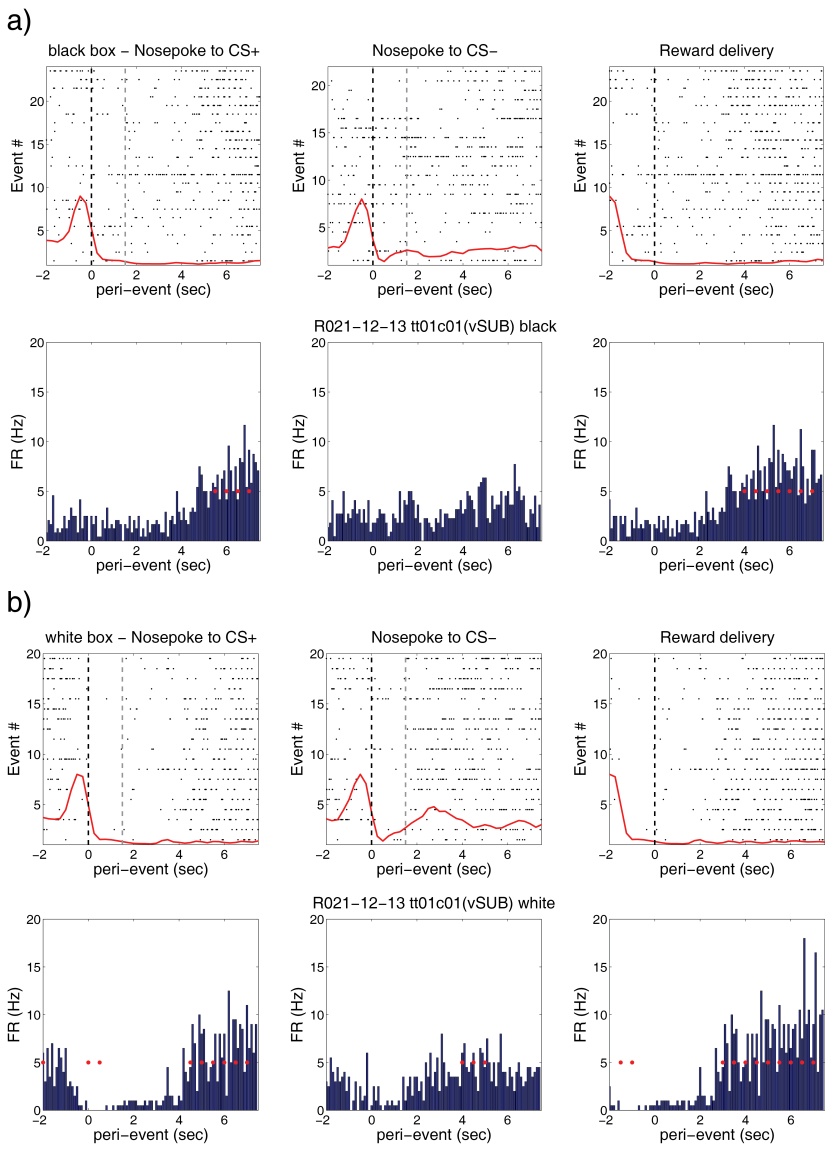

Figure 27: Distal ventral subiculum cell response to nosepoke and reward
This figure shows the responses of the distal ventral subiculum cell to nosepoking and receiving reward. The top of each diagram represents per trial windowed spike trains around an event (the dotted black line). The gray dotted line on the nosepoke spike trains represents estimated time of reward arrival at the feeder receptacle. Speed is overlayed over each spike PETH window in arbitrary units. Bottom diagrams are peri-event histograms of the data in the spike trains, represented as firing rate (Hz) around the event (sec). Red dots represent statistical significance of 0.5s bins of activity. A set of 100 randomized spike trains was generated for each context by randomizing the ISIs of the existing spikes. This was repeated once for each 0.5s bin, and the observed spike histograms were compared against the random spikes, with bins in the top or bottom 5% being significant. a) The responses of the cell in the black context. b) The responses of the cell in the white context, showing a strong suppression of cell spiking after nosepoke, and a strong increase in activity after receiving reward

Unlike the distal cell, the proximal cell did not appear to have any particular modulation of spiking activity by task related events such as cue presentation or reward delivery (Figure 28, 29). According to

the rank of observed spike trains against 100 randomized spike trains (repeated for each time bin in the histogram), spiking activity around cues, nosepoke and reward delivery for the proximal cell in the white environment was not elevated significantly compared to activity in the black environment.

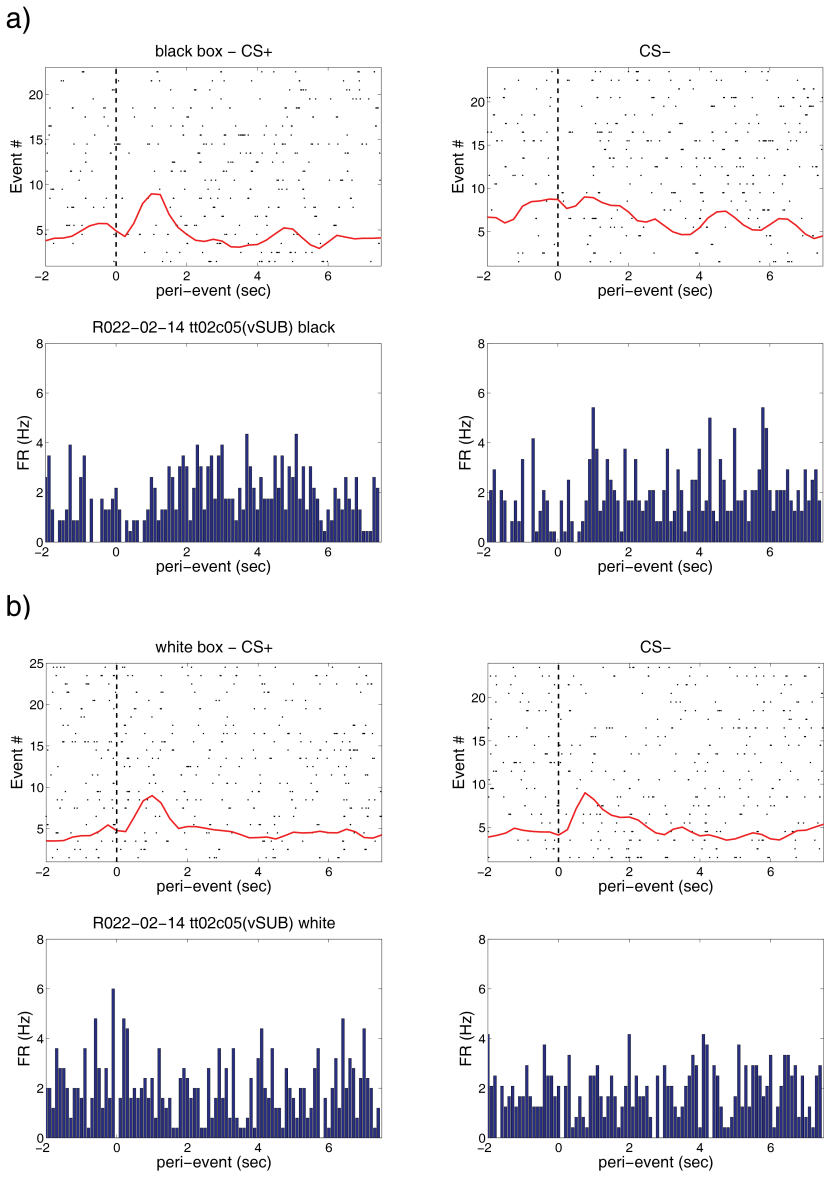

Figure 28: Proximal ventral subiculum cell response to cues
This figure shows the responses of the proximal ventral subiculum cell to cue presentation. a) The responses of the cell in the black context. b) The responses of the cell in the white context. No task related event modulation is present.

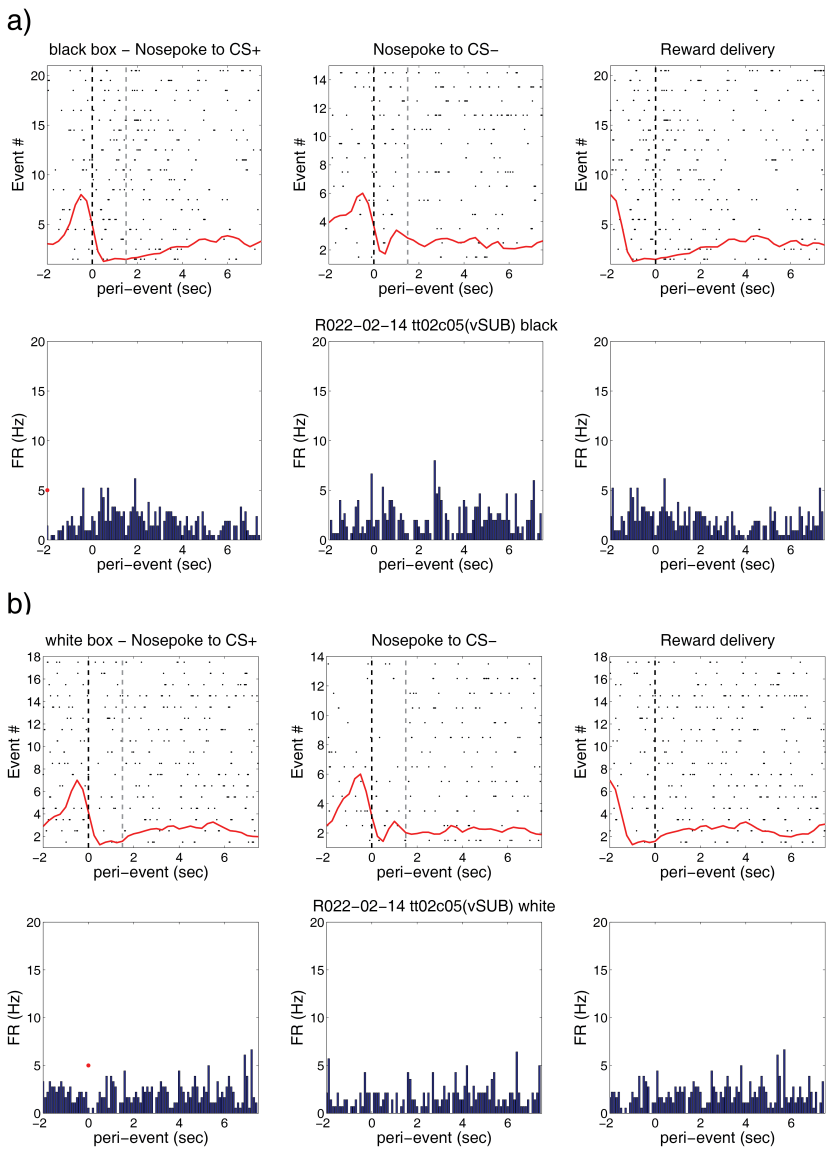

Figure 29: Proximal ventral subiculum cell response to nosepoke and reward
This figure shows the responses of the proximal ventral subiculum cell to nosepoking and receiving reward. a) The responses of the cell in the black context. b) The responses of the cell in the white context. No task related event modulation is present.

In summary, several interesting preliminary results were gained from vHC formation cells. First, there is evidence of possible rate remapping in ventral hippocampal CA3 cells. Second, firing rate increases near the reward receptacle in both ventral CA3 and proximal vSUB were noted. Finally, there is evidence for contextually dependent suppression of cell spiking in response to expected delivery of a reward in the distal vSUB cell, along with a context-independent increase in spiking activity after reward consumption.

4.3.5 Comparison of LFP's for Dorsal vs Ventral Hippocampal Formation: Resting and Active Patterns

After examining evidence for remapping, another target for the project was to examine local electrical field activity (LFP's) in the ventral hippocampal formation, and to be able to compare this with dorsal hippocampal LFP's. Signals were divided within sessions by whether the animal was on the waiting pedestal (before/after sessions) at the time (rest period), or not. For the rest period analysis, only times where the rat was on the pedestal before and after a session were considered. For active analysis, data was restricted to times when the animal was moving and not located at the feeder receptacle. Power spectra were computed using a `psd` function with a welch estimator (See section 3.10 for more details). Simultaneously recorded dHC and vHC formation data was compared for individual sessions. Electrical references for ventral tetrodes were located in electrically 'quiet' regions that did not show evidence of high frequency activity, and had minimal activity for lower frequencies (<10Hz). Evidence of multiple peaks in frequency power was present across signals. Dorsal hippocampus typically had delta (1-4Hz) and theta (6-10Hz) activity, while having power in the beta (13-20Hz) region on some days but not others. This beta signal appears to be a signal harmonic of the theta signal, as it is an exact double of the theta peak frequency. Ventral hippocampus (CA3) followed the same pattern as dorsal CA1 (Figure 31). Ventral subicular tetrodes recorded peaks in the delta (1-4Hz), theta (6-10Hz), beta (13-20Hz), and gamma ranges (30-80Hz), but these differed to a degree between rats (Figure 30, 31). In ventral subiculum, theta and delta frequency powers were both seen to drop in power (measured in decibels) during rest periods for locations on the distal vSub tetrode, while remaining similar in power between activity levels on the proximal tetrode. Thus, there may be decreased power in theta and delta in vSUB during periods of low activity. The ventral tetrodes were moving very quickly during recording days, and as such, are not shown compared as averages across days, but rather as snapshots of activity on a given day (during which tetrode location did not change). Overall, vSub LFP was noted to have obvious signal peaks in the delta and theta, and weaker signals in the beta (~20Hz) and gamma (~80Hz) range, while typical theta frequency signals from dHC and vHC were observed concurrently.

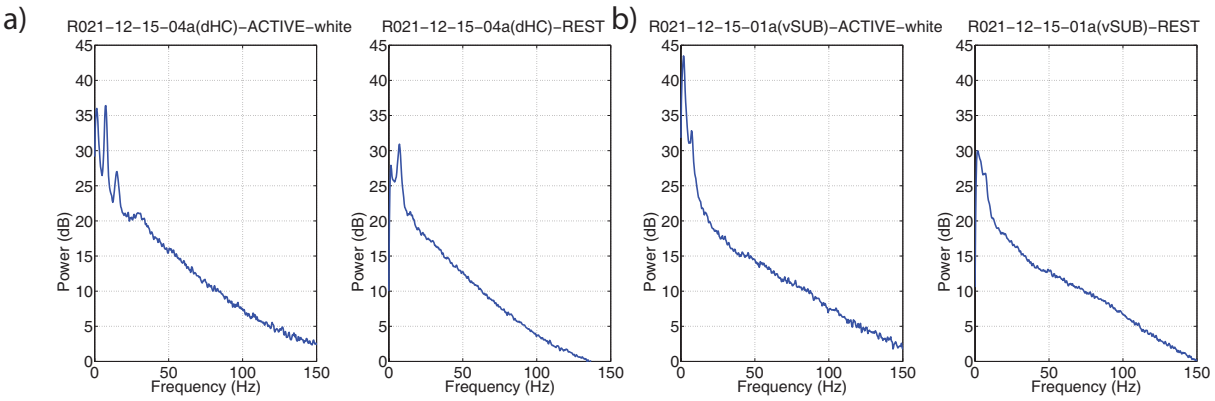

Figure 30: Power spectrum of R021 dHC and vSub

This figure shows power spectral density values (power in dB) for two separate structures recorded during the same session. Delta (1-4hz), theta (6-10hz), and gamma (~30Hz) are seen here. The signal at 14Hz for dHC appears to be a harmonic of the theta signal. a) Dorsal hippocampal power spectra, shown separated by active periods on task, and rest periods on the pedestal. The theta band has a lower power in the rest period. b) The same division of activity, but the power spectrum is shown for a vSub tetrode. A weak gamma band bump centred at the 80hz range is seen for the vSUB tetrode.

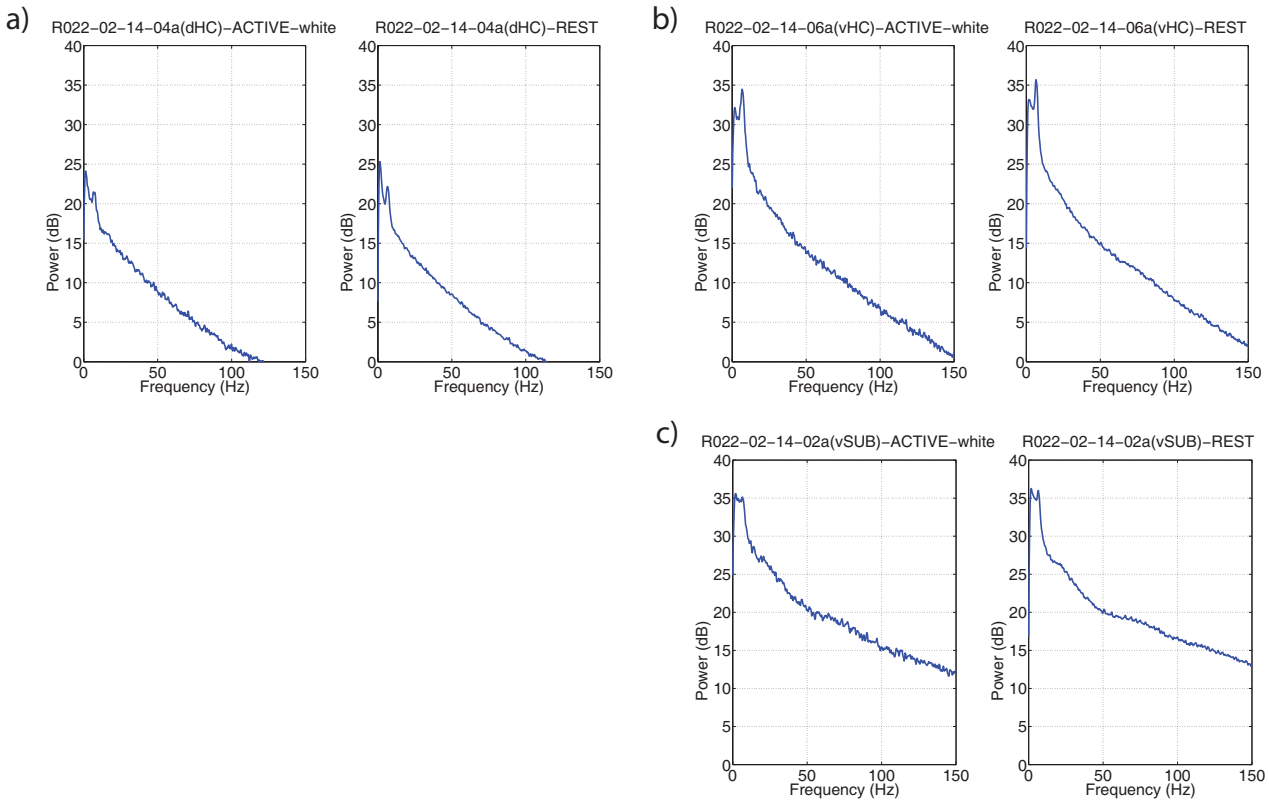

Figure 31: Power spectrum of R022 dHC, vHC, and vSub
This figure shows power spectral density values (power in dB) for three separate structures recorded during the same day. Delta (1-4hz), theta (6-10hz) and gamma (~50-80hz in this case) are all seen here. Beta signals are absent in this rat for this day, although some beta (~13-20Hz) can be seen on the vSUB tetrode. a) Dorsal hippocampal power spectra, shown separated by active periods on task, and rest periods on the pedestal. b) The same division of activity, but power spectra is shown for a vHC tetrode. Signals between dHC and vHC seen here are relatively similar, while differing in power. c) The same division of activity, but with the power spectra calculated for a vSUB tetrode. This tetrode is much more proximal than for R013 and R021. Theta and delta peaks appear blended, and a weak beta peak is seen that is not a harmonic of the theta signal. A gamma band in the 50-80hz range is also seen for the vSUB tetrode.

4.3.6 Ventral Hippocampal LFP response to Events

In addition to a general examination the HCf LFP's ventrally, one possibility is that ventral hippocampal formation LFP's would by modulated by task related events. To test this, spectrograms[19] were calculated over each session (`mtspecgramc` - Chronux), and individual spectrograms were extracted for small time windows around different event types: CS+/- presentation, nosepoke to CS+/-, reward delivery. These spectrograms were averaged to create the average spectrogram over all instances of an event type for a given day. Relative speed was displayed to allow for examining the possibility of locomotion modulated LFP signals. No obvious response of ventral hippocampal formation (either vHC or vSub) was noted to any of

[19] A spectrogram is used to display differences in the power of signals (dB) for each signal frequency over time. They can be thought of as a moving sequence of power spectra computed over a small window.

the task related events (Figure 32, 33, 34). Inspection of individual trials (not averaged) similarly did not reveal significant task related modulation. However, a ventral hippocampal increase in theta (6-10hz) noted around the time of cue presentation and presumed to be due to an increase in locomotion (Figure 32 - arrows) may not be present in proximal vSub. While theta power is not obviously increasing in vSub in response to speed, it is possible that delta (1-4hz) power is, as an average response was seen in some circumstances with increased speed (Figure 34 - arrows).

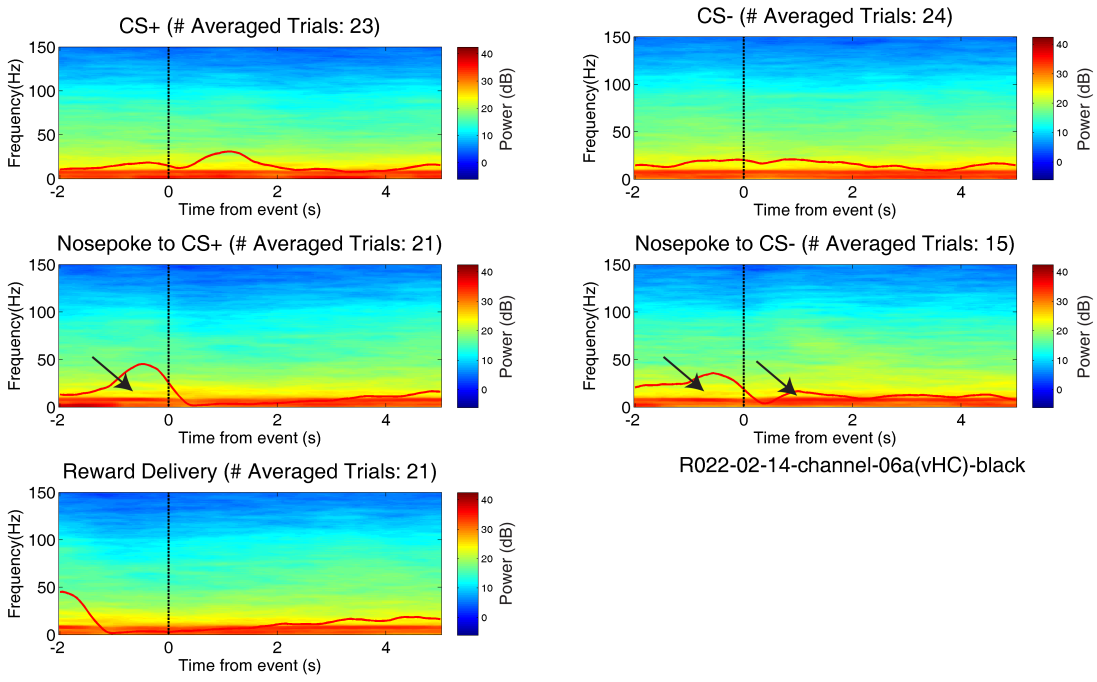

Figure 32: Ventral CA3 spectrogram of a single session windowed LFP response to events
Windowed average event spectrograms are shown here for CS+/CS- presentation, nosepoke to CS+, nosepoke to CS-, and reward delivery. Colour mapping is the same across all events for this figure. The black dotted line is the time of the event. The red line represents relative speed of the rat during windowed events. Note the increase of theta under the humps denoted by arrows. Of the two evident bands at the bottom of each spectrogram, the upper band is theta (around 8hz here), the bottom band is delta (around 2-4hz).

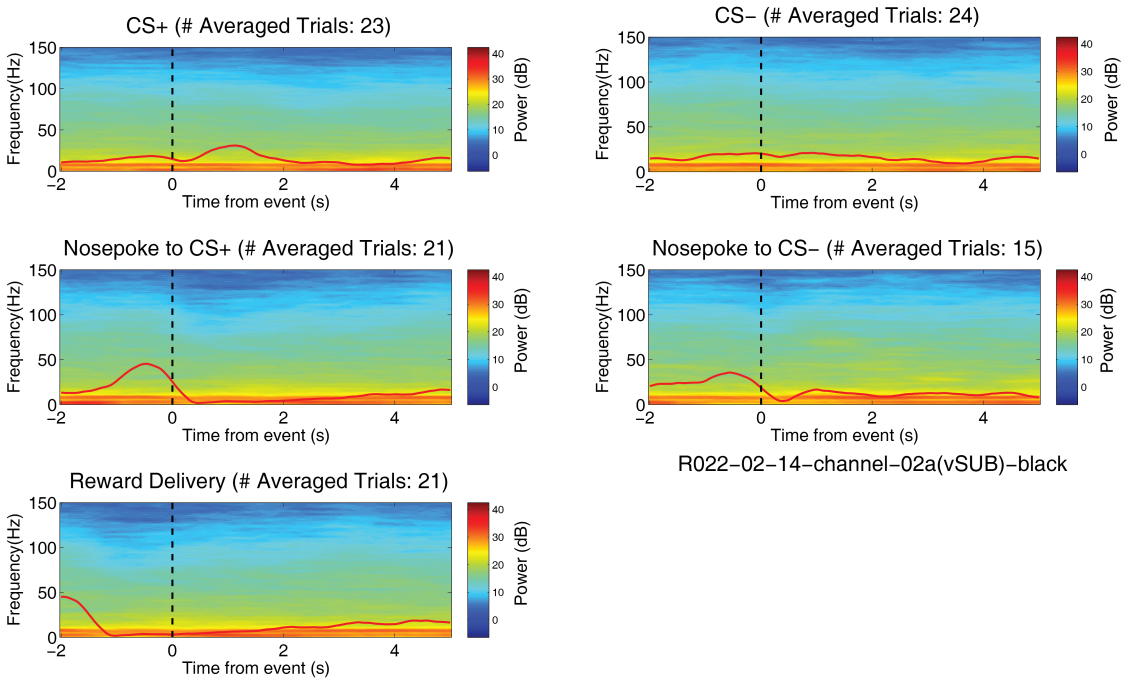

Figure 33: Proximal ventral subicular single session spectrogram of windowed LFP response to events This tetrode from proximal ventral subiculum shows no evidence of having LFP modulated by task related events, nor does it show evidence of increase in a frequency band in response to increase in speed (red line).

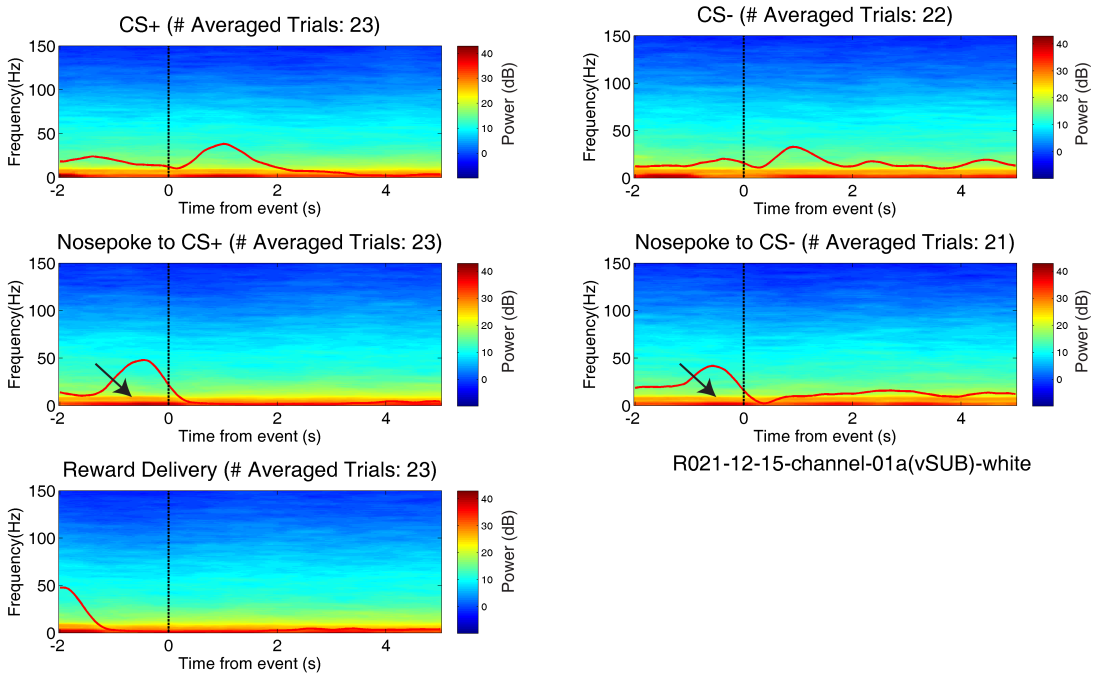

Figure 34: Distal ventral subicular single session spectrogram of windowed LFP response to events This tetrode from distal ventral subiculum shows no evidence of having LFP modulated by task related events. However, some evidence for increase in the power of the delta band is seen. Arrows highlight increases in delta signal power in the average spectrogram.

5 Conclusion and Discussion

5.1 Summary of Results

Seven male Long-Evans rats underwent training for this project, of which five completed training and were implanted with the two-target electrode array. The three most recent implanted rats had tetrodes that entered the ventral hippocampus and subiculum. The project presented a number of complex challenges such as specific targeting of two distant brain areas (the dorsal [dHC] and ventral [vSUB] poles of the hippocampal formation) concurrently, design of a novel implant, and creation of a specific behavioural task that. While formally similar to other previously performed tasks (Honey and Good, 1993), this project was asking novel questions that necessitated a modified design using open environments instead of enclosed boxes. Overall, the rats learned the task, although only 2 of the 3 rats with tetrodes in vSub clearly learned to contextually discriminate between the cues. Of the 3 successful vSub tetrodes, two entered the distal subiculum, while one entered proximal subiculum. Since electrodes must be slowly lowered towards a target location over days, patterns relating to vSUB were recorded. Specifically, electrode approach to vSUB was signaled by an electrical pattern visually noted with each rat: an extensive region of low power in high frequencies seen in LFP's prior to entering the cell layer during electrode descent. This allows for easier analysis of current electrode location in future experiments involving vSUB. A total of 70 cells in target locations were recorded across the 4 implanted rats performing the context task, with increasing numbers of cells isolated in later rats (47 isolated in rat R022). All vSub tetrodes passed through vHC previously during descent, allowing concurrent measuring of the dHC, vHC and vSub LFP for rat R022, and subsets of the three structures on other days and for other rats.

Of the 3 ventral subiculum tetrodes, all 3 had at least 1 isolatable cell, with only 2 vSub cells being usable in the cell spiking analysis due to thresholding and firing rate. Cells in vHC fared somewhat better, with increasing quality of vHC recordings in later rats. While no vHC cells were seen to undergo global remapping, or changes in context specific event-related firing, individual cellular firing rate did show some evidence of changing between contexts. Evidence of context discrimination was confirmed by both behavioural and observation of dorsal hippocampal results, although there were not enough dorsal cell recordings to show this conclusively though group statistics. Of the two vSub cells, modulation by task related events and context differed. Distal vSub had a cell which was modulated by both nosepoke to CS+ and reward, of which only the nosepoke to CS+ activity appeared to have contextual differences. Proximal vSub had a cell which was not modulated by CS presentation, did have increased activity around the reward receptacle relative to some other areas of the environment.

Concerning the LFP, vSub single session LFPs were noted to have obvious signals in the delta, theta, beta and gamma range. This may change based on location along the transverse axis as a strong beta signal was only noted on one of 3 tetrodes, but more tetrodes would be required to confirm. While a hippocampal tendency to respond to increases in speed with increased theta power was noted in the ventral HC LFP's, the same day responses of vSub LFP's did not show this behaviour. If anything, delta frequencies appeared more linked to speed than theta in vSub. No obvious responses in the beta and gamma range to events were noted, and vSub LFP theta and delta did not appear to change markedly in response to reward.

Overall, some, though not all rats learned to discriminate between cues in the behaviour task, and the implant was able to record from 3 separate structures concurrently. Although some evidence was seen for contextual representation in both vHC and vSub, it is very preliminary due to the paucity of cells isolated that were amenable to analysis. Exploration of vSub LFPs showed a lack of changes in signal power due to either reward-related events or cue presentation, although overall power spectra were broadly similar to hippocampal LFP's.

5.2 Behavioural Results and Learning

Due to the lack of previous testing using this behavioural apparatus, assessment of performance across rats was a priority. While a number of events may have altered the quality of learning by the rats, it remains true that only 2 of the 6 rats that trained on the task had an obviously discernible sense of the task (as demonstrated by a significant effect of context in a chi-started test). Although all 4 implanted rats performing the context task learned to respond to cues by nosepoking prior to implantation, results differed between rats. Two rats never appeared to learn to respond selectively to CS+ and not CS- presentation by nosepoking during post-surgery recording sessions, and two had training aborted due to erratic progress in learning the task. The method of increasing foraging by scattering free pellets on a random basis worked well, and as a result, rat coverage of the environment was sufficient for place field analysis. Rats were able to learn to nosepoke effectively to trigger food delivery for a maximum of 0.6s within the reasonable time frame for training (~20 days), which was sufficient to analyze responses to nosepoking as differing from reward delivery. Ways chosen to assess movement included peri-event averages (PEAs) of the speed of the subjects in response to each cue. It was quickly found that rats increased their speed any time an audio cue was played, and so the PEAs were modified to use distance to feeder in video-tracker (VT) units (rough approximation to a centimetre scale). A second measure for analysis was the amount of nosepokes to a cue condition as a percentage of possible nosepokes to cues. These were analyzed using a standard chi-squared

test to determine if responses to cue conditions were significantly different.

Two rats testing under the current task were not able to learn to nosepoke effectively in response to a cue while foraging. This appears to have largely been an anxiety effect, though the exact nature remains unknown. Even after careful cleaning of the environment, animals often had trouble being comfortable enough with the environment to forage, often preferring to simply sit on the feeder receptacle. This is likely due to the large open nature of the environment with relatively few potential safe areas for the animal except in the feeder receptacle area. While these rats were slowly learning to forage and respond to the cues by nosepoking, it was not possible to extend training to longer than a month to allow this to occur naturally. Additionally, one rat (R017) learned to escape the task environment by jumping over the 50cm surrounding wall, necessitating an immediate halt to training. Four rats learned to nosepoke in response to all cues, and due to time constraints, implantation was chosen to take place at this point. Rats either learned context specific cue conditions after implantation, or continued responding to all cues equally.

Overall results for the rats showed that as a group, they learned to discriminate between cues in a context-dependent manner, and individually, this is a result of strong learning from rats R022 and R013. For these two rats, after about 10 days, responses to cues began deviating from similar responses, and by 15 days, they were statistically different enough to show evidence of learning the cues between contexts. The distance peri-event averages (See 4.1.4) performed show a visual representation of how the animals respond to cues. Although the average differences in cue type response were not clearly statistically significant under that measure (95% confidence interval of average distances to feeder in the response window), they were certainly visually different on a per trial basis. Nosepoke responses on both a per day and overall average showed much clearer evidence for learning, with chi-squared analysis of differences in cue response being statistically significant for rats R013 and R022, while only being significant for rat R021 overall in the white context. Behavioural responses for rat R010 were generally not considered due to the experiment not requiring nosepokes for reward in his case, and the noise of the feeder activating with CS+ presentation itself being a cue to approach the feeder receptacle.

One possible confounding factor complicating this approach to analysis is the possibility the animal would simply learn the cues anew each session rather than remembering them from previous days. One possible way to address this is through analyzing the first trial during each session, before the rat would have a chance to learn anything. However, given that rats only appeared to learn the task during the final 10 days of recording, there was not enough information for statistical analysis. An additional approach is to test responses under extinction conditions, wherein animals must respond to cues in the absence of a reinforcing reward for correct responses. Unfortunately, rat R013, which had the most obvious differences in response to CS+ and CS- presentation, suffered a catastrophic failure of the implant, which resulted in an extinction

session not being possible. Rat R010 underwent extinction that did not produce any obvious results (R010 had food delivered irrelevant of responses to cues due to extreme fear of moving in the environment). Rat R021 did not learn the task (although chi square tests show overall statistical significance on the white context, this did not appear to be learning during each day, but rather random response differences), and suffered such significant hardware problems that recording was prematurely aborted. In hindsight, I should have performed a session under extinction conditions with rat R021, especially given results from rat R022's extinction results. Rat R022 underwent extinction with interesting results. During individual sessions of recording, R022 showed no evidence of learning to respond appropriately in the white task, though he quite obviously learned the cues on the black task. As noted in electrophysiological results for cells in R022's dHC CA1 area, global remapping appeared to occur, showing HC cells differentiated between the contexts. During the extinction, he refused to perform trials after the first half of the session, but during the first half, he showed appropriate responses of learning for both contexts, even after having shown no previous evidence of knowing cues in the white context. R022's extinction results are the most important for this project because R022 had by far the most number of cells recorded.

In summary, with some caveats, there is evidence of overall learning of the cues in each context, and there is strong evidence of learning for R022 due to results from extinction testing, but the task does not appear to be learnable, at least within the available time, for all rats. Rather, it appears that roughly half (3/6) of tested rats simply will not show a learning response for the differences between cues in the two contexts, even though the majority (4/6) are able to learn to nosepoke effectively in this task in response to a cue while foraging in the environment.

5.3 Confirmation of Existing Results

Since all laboratory equipment was being initially tested during this, and another concurrently running project, it was important to ensure equipment was operating properly by confirming the presence of existing phenomena before examining evidence for new discoveries. Since the implant design chosen involved electrodes in the extensively studied dorsal hippocampus, it allowed for analysis of the dorsal hippocampal remapping that is a common measure of context differentiation. The two targets also allowed for examining recording power spectra comparatively between structures. The several ways chosen to examine evidence of known phenomena included: Examining hippocampal LFP, evidence for dHC remapping, and checking vHC cells for known responses, e.g. large or non-evident place fields.

Ventral hippocampus, in this case CA3, is known to have smaller numbers of 'place cells' than are present

in dHC (Royer et al., 2010). Consistent with this observation, no vHC cells recorded showed any evidence for having a place field. Cells fired nonspecifically across areas in the environment. These cells may have included analogs of dHC 'place cells', but as the location was quite ventral in the vHC, it was not possible to tell if activity was related to place, as the cells relative place fields may have been larger than the environment (Kjelstrup et al., 2008).

In dorsal hippocampus, I examined evidence for global remapping of cellular place fields. Remapping is only expected to happen during the conditions of this task if the animal is using a different 'cognitive map' for the environment, treating it as a separate context (Bostock et al., 1991). While there were not enough cells to perform a proper statistical analysis of the evidence for remapping in dHC, cells were analyzed on the bases of place fields during the first and second half of each session, and weighted centre of mass for place fields was compared between contexts, and within the half sessions of each context. By that measure, and by visually examining place fields between contexts, remapping occurred regularly in cells in R022, as while other animals had cells in dHC recorded, these were not recorded after the task was behaviourally shown to be learned. Another interesting result is that cells in R022 also showed evidence of remapping even before the rat was obviously aware of the context differences. While occult learning of an experiment has been shown before, this is an interesting result in this case which may have bearing on future rats undergoing a similar task.

During recording, location of tetrodes within the dHC was assessed both via presence of cells, and polarity of the hippocampal sharp waves[20]. While this was good evidence for tetrode location during recording, another method of confirming that these were reliable hippocampal LFP signals was used as well, with LFP's being compared against known results for theta. Generally, hippocampus contains a strong theta band (6-10hz signal), modulated positively in power by physical activity. This theta band is directly tied to a number of phenomena like phase precession that may have direct bearing on how an animal interprets its location and the future. Both dorsal and ventral hippocampal signals showed strong evidence of power in the theta band. Furthermore this theta band was often stronger in power during periods of activity in the task, and appeared stronger during faster movement, while often significantly weaker when the animal was in a resting position on the pedestal before and after a session. Together, these three areas give some confirmation that the hardware was all functioning as intended, and that cells and LFP's being recorded were reliable, and not overly confounded by noise issues.

[20]Sharp waves are a feature of the hippocampal local field potential involving a concurrent period of powerful high frequency signals, and typically a rapid deflection from the current voltage with a slower recovery. A downward (negative) deflection signals the electrode is above the cell layer (dorsal), while an upward (positive) deflection signals a ventral electrode position. A sharp wave event not occurring with a rapid deflection signifies the presence of the electrode in the hippocampal cell layer.

5.4 Ventral Hippocampal Formation Results - Comparative Power Spectra, LFP Analysis, Contextual Representation and Response to Events

Since experimental results match known results for each area examined, we can assume the correct functionality of the implant, behavioural task, and recording equipment, and examine the evidence for place and reward modulation in the ventral hippocampal formation. Results for the ventral hippocampal formation were preliminary only due to the relatively small number of cells and tetrodes in the area. However even these preliminary results allow us to make observations, though more data would be required for statistical confirmation. To examine results, it is easiest to split them between those accomplished by examining regional power spectra, those looking at cellular correlates with behaviour and context representation, and in the response of the local field potentials to events. Additional observations would be possible given a larger number of cells and tetrodes across more rats.

Due to the nature of the two target implant designed for this experiment and the location of the targets within the brain, a relatively rare possibility to study three areas simultaneously was present. In addition to the intended targets of dHC and vSUB, which were recorded concurrently in all 3 rats with a vSUB tetrode, rat R022 additionally had one tetrode left in a relatively cell rich area of the ventral hippocampus that allowed for concurrent recording of dHC, vHC and vSUB. At present this is simply used to compare relative power spectra between the regions, but it is easy to imagine more powerful analyses being performed given additional rats.

Beginning with power spectra, as explained above, dHC and vHC generally agree with known results, showing a strong theta band. There is also delta (1-4hz) and gamma (30+Hz, several distinct frequency regions) visible. Peaks in the beta region (13-20Hz) were seen on some days, but these are exact doubles of the theta frequency peak and are likely harmonics. Evidence from existing studies, namely Petrulis et al. (2005) shows that we would expect theta signals in ventral subiculum, but given that other studies generally failed to distinguish separate ventral hippocampal formation structures, and the animals in the aforementioned study were hamsters, not rats, expected results were unknown. Generally, a theta band was strongly visible as expected in all vSub tetrodes. Additionally, although theta power differed between rats and on different days, results for dual structure recordings generally agreed with each other on frequency components. Additionally, while there were no strong gamma signals, gamma was seen in all vSub tetrodes at around 80Hz, while not being visible in the same frequency range in either dorsal or ventral hippocampal tetrodes. This matches with dorsal subiculum results from Jackson et al. (2011) which showed that gamma was generated independently by the subiculum. It is possible that the vSUB gamma signal is connected to ventral striatum's gamma signal. Gamma in ventral striatum is well established, exists in the form of

separate gamma bands in the 50hz and 80hz range, and has been connected to the presence of reward (van der Meer and Redish, 2009).

LFP's were also analyzed for responses to events, namely cue presentation, nosepoking and reward delivery. No obvious responses to the three categories of events were noticed. These results were computed as averaged windowed spectrograms for each day, so to confirm, trials were examined individually, although they suffer from a higher level of 'noise' frequencies that would be averaged out between trials normally. Although there is no evidence for ventral subiculum LFP's changing in response to events related to reward prediction or delivery, there was some evidence for changes in the frequency bands. As theta in both hippocampus and ventral striatum is modulated by speed, a higher average power can be seen in ventral hippocampal windowed spectrograms when examining against relative average speed of the rat at the time. But unlike hippocampus, it would appear that ventral subiculum lacks this feature of theta, at least for days examined. While there were a low number of days in vSUB recorded, this lack of theta modulation is suggestive of a difference in functionality, perhaps connected to increased limbic signal representation and less navigational representations. If anything, the slower delta region of frequencies appears more modulated by speed in ventral subiculum. None of these signal characteristics appears to be dependent on the context.

Next we may ask how cells in vHC and vSub responded to changes in the context. Since Royer et al. (2010) noted some differences in vHC activity between segments of a task that were suggestive of contextual representation, some form of change in vHC activity in response to a different environment would be expected. However, since the environment was still relatively small, and the tetrode locations in vHC were quite ventral, global remapping was difficult to assess. In fact, for four cells, what was seen was closer to a form of rate remapping, where the firing rate of a cell changes between contexts, but not the overall firing place correlates. A large number of additional ventral cells would be required to confirm rate remapping, but it seems to suggest that while ventral cells are sensitive to the context, they do not completely stop firing in response to changes in environment. Rather vHC cells only modulate their activity levels, even when the dorsal hippocampus is using a completely separate cognitive map of the environment. Although no vHC cells were seen to be modulated by theta activity, or showed evidence of place fields, several did show evidence of increased firing around the reward receptacle location, which is in agreement with the idea that vHC should have a degree of reward modulated activity that is passed on to vSub for further processing. The two ventral subicular cells studied also showed no evidence of a place field, much like those in vHC, although the proximal cell was more active immediately near the feeder receptacle in both contexts than a few centimeters away. However vSub cells also showed no evidence in the two recorded of changing rate in response to context either. The vHC cells studied were in an area of vHC that is connected to the subicular cells that project to ventral striatum, so if there was a simple copying of representations between structures,

we would not expect to see that difference in cellular responses to the environment. With the few results available, we can say that spatially, vSub showed no evidence for response to context changes, while vHC showed responses in the form of firing rate modulation, but not place field activity.

The final set of results to examine were the responses of ventral cells to events. Specifically, I examined the two ventral subiculum cells for evidence of changes in firing rate between contexts and between windows around different events (Spike PETHs). There were significant differences between the cell in proximal subiculum and the cell in distal subiculum. First, the distal cell responded with increased firing rate to delivery of a reward, but only in one context did it suppress activity shortly before the reward delivery in response to nosepoke (weakly correlated with CS+ presentation). However, the cell was modulated by nosepoke in one context, but not the other. This was not related to the apparent ignorance of rat R022 to cue differences in the white context at the time since the modulation by nosepoke was only seen in the white box, and is mostly present during the window before the feeder activates in response to the nosepoke. Like the vHC cells, both the proximal and distal cells had increasing activity immediately around the feeder receptacle, irrespective of the context. In short, the cells differed in response to context, but not with remapping. In fact, they showed changes in firing rate in response to events only in one context, while not in the other. The proximal cell, in the area directly connected to the ventral striatum also showed evidence of modulation by reward location. Although that signal is in agreement with the expected requirement of vSub representation of reward-related information, more cells would be required to confirm.

In conclusion, the project was limited by insufficient rats and cells recorded to make strong statistical statements about ventral hippocampal formation cells. However, there was still evidence for theta signals in subiculum, as well as gamma, possibly related to the downstream ventral striatum. Evidence was also shown for ventral hippocampus cells altering their firing rate in response to changes in context, even though they did not globally remap as dorsal cells did. No evidence of subicular LFP changing in response to task related events was noted, but the absence of theta modulated by speed of the rat was noted compared to ventral hippocampus. Finally, although ventral subicular cells lacked anything resembling a place field, they did alter their firing rates in response to events in the case of the distal cell, and reward location in the case of the proximal cell. In at least some of these cells, it appears those altered firing rates are contextually specific. These results may be relevant to studies of the ventral striatum since a contextually specific change in firing rate in response to reward allows information to be passed to ventral striatum that could partially account for its ability to trigger contextually dependent relapse to drug seeking, among other effects.

5.5 Suggested Future Experimental Targets for Ventral Subiculum and Ventral Hippocampus

Given the preliminary results shown here, it is possible to make several recommendations for future studies. First, it is critical to have a significantly larger number of rats when attempting to study these areas, unless the quality of recordings can be drastically increased. The low cell yield and signal noise issues were partially fixed by experience in how to effectively reference ventral tetrodes, as well as how to modify the recording setup, but these did not go beyond giving several cells in vHC and vSub to work with, and considerably more are needed. Altering the electrodes to have a lower electrical resistance may be required to record more subtle signals, although this is then likely to simply increase the noise. As this is a tradeoff, simply experimenting with the hardware parameters for the ventral hippocampal formation recording sites may be required. Cell yield has been further increased with later experiments in the lab, but these were too late to use in this project. Assuming a constantly increasing yield between rats, 3-4 extra rats should be sufficient to give a reasonable amount of cells to perform statistical calculations on.

Second, it is critical to improve the behavioural task to increase the likelihood of rats rapidly learning the differences between cue conditions. This would minimize time training uncooperative rats, as well as increase the likelihood of effective analyses during recording sessions. The best way to do this is likely to increase the penalty for responding with a nosepoke to a CS-. The easiest way to do that may be with a blast of air, which is at least worth testing if not too difficult to set up. Since a Masserman and Yum (1946) experiment's use of air blasts as punishment was sufficient to induce persistent neurotic behaviour in cats, it is likely that a smaller blast of air would be sufficient to alter rat behaviour. Failing this, eye-blink conditioning with a simple stimulating electrode is an alternative method to alter behaviour that may work. The expectation is that a higher penalty will significantly decrease both training time and rat anxiety by making failure and success conditions more obvious (Rats often appeared confused when they didn't receive pellets for CS- nosepoking).

Third, given the results in vHC and vSub, it may be prudent to drop the component attempting to find place fields in these areas in favour of looking only at event-related activities and firing rates. If it is desirable to keep looking for place fields on a larger scale, a larger environment will likely be required. Possibly roughly double in size to the current environments, or around 1.5m on a square edge. The modulation of activity in response to context and event could also be tested by using additional reward predictive cues, since CS+ modulated activity in one cell.

Finally, more stringent selection of rats may help significantly. All rats studied in this experiment were friendly towards the researcher, but all suffered from the effects of anxiety in the environments. It is

possible that with a larger pool of rats to choose from, selecting only very low anxiety animals could make a significant difference in how likely they are to learn the experiment. If rat selection remains impossible, it may be possible to alter the environments to reduce fearful behaviour, such as by temporarily having novel objects in the environments during the first few days of training to encourage exploration.

These suggestions therefore set out a possible future path to continue study in these areas as relates to context. Although difficult to study, an increase in cell yield and number of rats would likely make a large difference and allow for statistical proof of the effects seen here.

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6 Appendices

6.1 Challenges: Suggested Improvements

While both the behavioural apparatus and implant worked as expected, the small number of isolated cells, and the low number of electrodes in the ventral HCf are due to several factors. The 4 most important challenges I have encountered will be addressed here, along with possible solutions. They are: Rats not adequately learning the task, tetrodes not hitting the correct targets, electrical signal noise, and a low cell yield.

As previously mentioned, while 6 rats were trained in the two contexts for at least two thirds of a month each before implantation, only 2 knew the test parameters to a statistically significant level by the end of recording. Biconditional discrimination tasks are not new. Honey and Good (1993) studied the effects of hippocampal formation lesions on contextual behaviour on these types of tasks long before this project was undertaken. But the specific form of the task is new, and in its current iteration, it appears to simply be beyond some rats to learn within a reasonable timeframe. Length of training is a key point because while only two rats learned the task, it still took them at least a combined month of training and recording to do so. It is quite possible that some of the additional rats would have learned the task given additional time, or in the case of R010, perhaps the final form of the experiment would have proved sufficient to stimulate him to respond to cues after implantation. The fact remains though that R015, R017, and R021 did not learn the task, and while the environment was modified to be higher quality after R017's training, no other changes were made at this point. Additionally, training rats for longer than a month when a large number of subjects is required is not always feasible. In the experimenters opinion, the most likely reason why later rats did not learn to respond differently depending on the context is due to the insufficient differentiation between success and failure conditions. To explain, there was not sufficient punishment for responding incorrectly to a CS-, with the only response being an effective addition of 5 seconds till the next time a rat could trigger a cue presentation. Depending on the rat, this could be construed as a neutral result instead of negative. As such, the only reason a rat would ignore the cue is to optimize food delivery, which may not have been equally motivating to all animals. A small blast of air would be one possible way of altering unwanted behaviour without causing physical harm (Masserman and Yum, 1946), although a gentle stimulation of an eyelid to induce blinking would also be minimally invasive. It is likely that given increased motivation to respond more selectively to cues, more rats would do so within the initial training period, possibly cutting up to 10 days off the total training time per rat (~10 days extra time required to learn to discriminate cues after implantation).

Another major challenge was targeting of the tetrodes to the correct ventral location. Although targeting dHC CA1 was accurate in all animals implanted, only 3 of 5 implanted rats had the second vSub target accurately acquired as well. In later rats, this was assisted by the development of a small widget fitted over the dorsal bundle during surgery to make both targets reach skull level at the same time during insertion. The widget was then removed once the target bundles were assessed as being correctly oriented, and the implant was lowered into position 5mm into brain. While this did appear to ensure that the bundle was not incorrectly targeted, only 1 tetrode from each drive managed to reach subiculum due to a combination of noise canceling requirements and the overall size and variability of the bundles. While noise canceling will be discussed in the next paragraph, the bundle shape and variability is also quite important. The bundle itself was designed to have 4 tetrodes as the corners of a simple rhomboid due to a high chance of cutting across the width of the vSub because of its position in the brain. Unfortunately the rhomboid shape also meant that while at least one tetrode was likely to enter vSub, several others would likely be far off target as a result. In the experiment these off-target tetrodes were not always obvious, but in several cases were used for noise cancellation as reference channels. While one obvious solution is to simply increase the number of rats implanted, this is not likely to be an efficient way to study the area, unless isolated cell yield is increased greatly. Solutions involving changing bundle shape may not be a reasonable solution here due to possibility of missing the vSub altogether, although it may be possible to add an additional 1-2 vSub tetrodes to the bundle without drastically increasing tissue damage. Extra channels could be used for either targeting vSub or for dedicated noise cancellation, as discussed below.

Electrical noise in the electrophysiological recordings constituted probably the single greatest challenge in obtaining usable recording data. While a reference tetrode was in place in corpus callosum for each rat to minimize signal noise, an unknown quantity was how fast noise would increase with further distances from that tetrode. The result was that any tetrodes passing the level of intermediate hippocampus were crippled in their ability to have isolatable cells, as well as having numerous signal artifacts in the LFP. The only way to minimize this in the experiment was to dedicate at least one of the four existing ventral tetrodes to serve as a reference, and to hope it was not a tetrode on target for vSub. This was only possible for areas that did not pass through the hippocampal cell layer during descent, and could be safely moved to a relatively silent area of brain. Unfortunately, as this tetrode was still at least several mm dorsal of the ventral tetrodes, it still did not fully stop noise artifacts. The result was extra time requirements for cell post-processing, as well as difficulty in telling issues with the recording system apart from issues with the implant. In one case, an intermittent electrical noise problem remained unidentified for a week due to difficulty in locating a stable cause. The cause was narrowed to a faulty cable attached to the implant, which was promptly replaced. It remains possible that the only way to counter these noise issues is to either alter the structure

of the tetrodes to have a higher resistance and avoid picking up weak noise, or to have an extra dedicated ventral reference targeted lateral of the existing tetrodes towards the ventral hippocampal fissure, that would advance in tandem with the ventral tetrodes, stopping just dorsal to the vSub. A reasonable strategy would be to attempt both if possible.

Finally, a lack of isolated cells meant that even for tetrodes in vSub, located in what should be a relatively crowded location for cells, recorded too few to make any significant statistical conclusions about the cells in either vHC or vSub. There were three obvious reasons for this during recording. First, a large electrical noise signal made it very difficult to reliably isolate cells from each other that had similar electrical activity. Second, many cells recorded appeared to have very low firing rates, even though they were almost certainly not noise. Third, the great majority of cells recorded were so weak that the signal was 'cut-off', or too weak to reliably isolate from regular background noise. Aside from noise, which has been discussed previously, the last two reasons are most likely to do with the structure of the tetrodes, and the quality of the implant as a whole. With more implants made, the number of cells recorded was seen to increase significantly. Also, familiarity with the drive design allowed for more effort to be put into increasing precision of the work. The only obvious remaining possibilities are an acellular location, and weak transmission of signal. First, while the tetrodes were kept at a standard resistance level (See Materials and Methods - Section 3), it is possible this was too high, and that as a result, weaker signals (e.g. from cells in vSub) simply did not register well in the recording system. This could be fixed by lowering the resistance of level of the tetrodes during electroplating, but increases the possibility of a noisier signal, making this a tradeoff. Also, changing the wire composing the tetrodes may increase the quality of the signal. Although it is possible the tetrodes entered areas of vSub without many cells, it is unlikely due to the relative uniformity of the area. A effective solution to this problem remains elusive.

6.2 Evolution of the Experiment

Evolution of the environments

After initial test runs on a linear track in early 2012, the first environments for this experiment were constructed to use with rat R010 several months later. A metal feeder receptacle was designed to use with the environments and replace the previously existing plastic prototype. An overview of the initial test runs can be found in the Appendix under Timeline (Section 6.1). The initial environments consisted of a PVC base plate, with hardened 50cm tall 2mm thick walls of the appropriate colour, and the one opposing colour direction wall to the right of the feeder. The environments sat on wheels to allow for easy movement within the experiment room. The length of the longest walls in each environment was 60cm, giving an environment

large enough for small hippocampal place cells (See section 2.2), as well as some moderately large place cells to show variation in firing.

This version of the boxes had a metal feeder receptacle that was designed by engineering students working in the lab. These receptacles have house lights, a fan to remove uneaten pellets, and metal pellet collection basin. Due to the recessed location of the receptacle as compared to the open nature of the environments, rats quickly began to remain near the receptacle at all times. To encourage exploration, a scattered pellet feeder was hooked up and the dropping tube was affixed to the directional wall of each environment. This allowed for even scattering around the environment (the sugar pellets had a tendency to bounce and evenly distribute themselves). Feeder receptacles were then redesigned to a new delrin plastic model for rat R013 onward that consisted of a machined square basin connected to the plastic pellet delivery tube, and attached to the environment with velcro. A sloped incline was built around the basin to discourage rats resting in that area. This proved to be sufficient to prevent rats hiding there in most cases.

While this series of improvements increased the amount of exploration of the animals in the environments, the base that the environments sat on was not fully stable, and drooped in the middle slightly on each side. This allowed for the occasional pellet to escape the environment under the walls, as well as occasional pellets in the corner being impossible to reach for implanted rats. This was eventually fixed with another small redesign by Science Technical Services (STS) that involved putting textured aluminum slopes along the edge of each wall (slopes extended to 2.5' into the environment vertically and horizontally), while making the base more stable through reinforcement. The slopes were textured so that there was a very fine grain in the white amphitheatre box, while having a heavy grain in the black box, as this matched the textures of the environment floors. This design remains stable to date.

Evolution of the task

After the initial environments were completed, training began, althouth the task did not yet include scattered pellets to encourage exploration. Both rat R010 and rat R013 trained for over a month each, as training efficiency was not optimal at this point. During rat R010's behaviour and training, most of the components of the task were established. Scattered pellets were added to cause rat R010 to explore more of the environment during training sessions, and to complete trials more quickly. The initial zone requirement was also added (After rat R010 just sat in front of the feeder to trigger new trials), and required the rat to explore at least 2/3 of an environment to trigger a new trial. Several task-related software errors occurred during the training of rat R010 which caused temporary cessation in the training sessions. These only corrupted the training session beyond usefulness in one instance, but did cause interruptions in training that may have

been distressing to the rat. The number of recorded events and tracking points was slowly increased during training and electrophysiology for rats R010 and R013, with success and failure variables and nosepoke events being tracked in more efficient manners. This allowed analyses that previously had to be computed by hand to be performed automatically by the recording script. The task was also altered to prevent cues being presented while the rat was near the reward receptacle, as this allowed for more complicated analysis of rat response to cue presentation.

During the training of rats R015 and R017, realtime tracking of events was added to the task to allow analysis during training and recording sessions. Neither of these rats completed training. Rat R015 never achieved a high performance level, and R017 eventually learned to completely escape the environments by jumping over the 50cm walls. Exploration was improved during this period, with the number of environment zones being standardized to 10, and with the requirement that only unique zones during each trial would count towards exploration.

By the time of rat R021's training, the task was fully refined and finalized. Subsequently, training of rats R021 and R022 occurred without problems, although like earlier rats, they failed to learn to contextually discriminate the cues before implantation.

6.3 Overview of the Task Control Script

This experiment was controlled by a Matlab program designed to run as a state machine[21] The state machine (A copy of this used for rat R022 is present in the Appendix) is a single file with a large variable declaration section that defines the current parameters of the experiment including the current point in training. It is followed by a timed loop with four native states that control what is occuring in the task. The loop begins in state 4, which is the active state, and signals waiting for the rat to respond by foraging around the environment. State 1 is triggered by the completion of defined movement parameters, normally moving through half of ten zones defined in the environment, and being present in the furthest half of the environment to trigger the switch in states. State 1 responds to completion of events by playing a pseudorandom choice of either CS (a 1kHz tone, or white noise - both balanced to be the same loudness), and then switches to state 2. State 2 waits for the rat to complete a response to the cue, either by ignoring it (or not moving fast enough, the cue has a defined length, normally 7.5 seconds), responding appropriately, which allows for a consumption period and switches back to state 4, or by nosepoking to the CS- for the environment, which switches to state 3. State 3 is a penalty state, which disables the house light and holds other events in the

[21] When running, the program is always in one of several internal states that are continuously controlling parameters of the behaviour task. An example would be a state of 1, which both plays a cue, and tells the rest of the program that a cue is playing.

state machine from happening for 5 seconds, before reactivating the light and switching back to state 4. The script keeps track of success and failure variables, updates the Cheetah recording software with session events (e.g. EventID 39 records that the rat nosepoked to the correct CS, but did not hold the nosepoke long enough to receive a reward), and after the session period (currently defined as 25 minutes) is up, shuts down the state machine, saves current data, and awaits instructions.

6.4 Task Control Script

```
Define current box and rat
Define variables for training, ranges for Inter Trial Interval (ITI) and scatter feeding (SF)
Initialize success and tracking variables

Set the active tone for the experiment (EX. if box==1 && rat ==1; activeCS=1; end)
Make a block of CS types (block = [1 1 2 2];)
Make a randperm trial list of these blocks
    (trial_list = cat(2,trial_list,block(randperm(4)));)
Define the ITI times for this session
    (ITI_list = ITIrangeMin + (ITIrangeMax-ITIrangeMin).*rand(300,1);)
Create a scatter feeding time interval list
    (SF_list = fmin + (fmax-fmin).*rand(maxSFtrial,1);)
Determine whether a pellet will be deployed for each list item based on fprob probability variable
Create zones automatically based on variables for number of zones and allocentric position of the box
    (Currently 10 zones are created)
Initialize clocks
State is set to 0
Turn on the house light

  Begin main loop (while elapsed time is less than time to run)
    Update visual display (time remaining, success variables, total pellet intake, location/zone)
    Dispense any queued scattered pellets (if dispensory clock is elapsed)
    Iterate the scatter feed interval to next item in list
    If rat has just received a reward, check reward clock, and if expired, clear all audio
    State Loop
      State 0: (ITI)
        If script has just entered this state, reset ITI to next in list
        If ITI has elapsed, and zone requirements are fulfilled (greater than x zone currently)
          Set state to 1
      State 1: (Play CS)
        If script has just entered this state:
          Clear all sounds
          Play the correct tone (CS) from the trial list
          Set state to 2
      State 2: (Await Response)
        Check nosepoke sensor
        If script has just entered this state
          Reset CS response time clock
        If script has not just entered this state
          If current CS is CS+ (Active for this box)
            If rat was not nosepoking before now
              If nosepokes are required for CS delivery
                If rat is nosepoking
                  Initialize nosepoke clock
              Else
                Initialize nosepoke clock (for compatibility with later IF statements)
            If rat was nosepoking before now
              If rat is no longer nosepoking and nosepokes are required
                Record trial as failed due to early withdrawal from reward receptacle
                Increase failed trials by one
                Go to state 4
              If nosepokes are required
```

```
            If rat is currently nosepoking
              If required nosepoke time has elapsed
                Dispense 5 pellets to the reward receptacle
                Reset CS response time clock to give rat full time to eat pellets
                Reset dispensing pellet clock to avoid errors
                Increase successful trials and successful CS+ by one
                Go to state 4
          Else
            If nosepoke delay has elapsed (for compatibility as noted above)
              Dispense 5 pellets to the reward receptacle
              Reset CS response time clock to give rat full time to eat pellets
              Reset dispensing pellet clock to avoid errors
              Increase successful trials and successful CS+ by one
              Go to state 4
        If CS response time clock is elapsed
          Record trial as false negative
          Increase failed trials by one
          Clear all audio from speakers
          Go to state 4
      If current CS is CS- (Inactive for this box)
          If rat is nosepoking
            Record trial as false positive
            Increase failed trials by one
            Clear all audio from the speakers
            Go to state 3
          If rat is not nosepoking and the CS response timer has elapsed
            Record trial as successful (true) negative
            Increase successful trials and successful CS- by one
            Go to state 4
  State 3: (Penalty for false positive)
    If script has just entered this state
      Turn off the house light
      Reset the penalty clock
    If penalty clock timer has elapsed
      Turn on the house light
      Go to state 4
  State 4: (Zone movement requirements)
    If rat is not in foraging only period of training
      If script has just entered this state
        Reset the number of zones required to trigger a new trial
      If script did not just enter this state
        Compare the current zone to the list of zones the rat has visited
        If more zones are still required, the current zone is new (unique), and was just entered
          Subtract one zone from the number required
          Add the current zone to the list of zones visited
        If no more zones are required
          Reset the used zones list
          Go to state 0

  Pause 1/120 seconds and end loop

Turn off the house light
Make a noise to alert the experimenter
Save all variables and exit
```

6.5 Best Case Scenarios for Behaviour on a Given Day (Rat R013)

Not all rats learned the task equally. However, rat R013 appeared to learn the task most clearly overall. These two figures show a best case day for behaviour with each cue for rat R013 using the same methods explained in section 4.1.3.

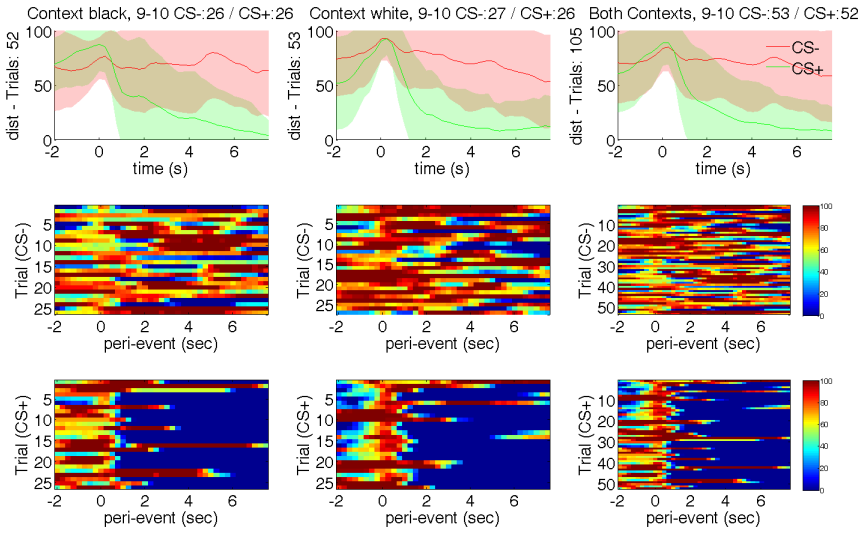

Figure 35: R013 best case individual day distance peri-event averages example of performance after learning Responses of R013 during a best case day where the rat was observed to be consistently having the correct approach to each cue, while occasionally testing the opposite condition. Shown here is the distance in arbitrary units from the feeder as a function of time around the cue presentation (-2s to cue length window). The top row is a graphic of distance from feeder against time in 2D (green CS+, red CS-)with the shaded area being the standard deviation of the sample. The middle and bottom rows are a heatmap of distance from the feeder over individual trials, with individual trials being represented as rows. Dark blue represents that the rat was within the bounds of the feeder at that time point. Number of trials in each condition is shown at the top.

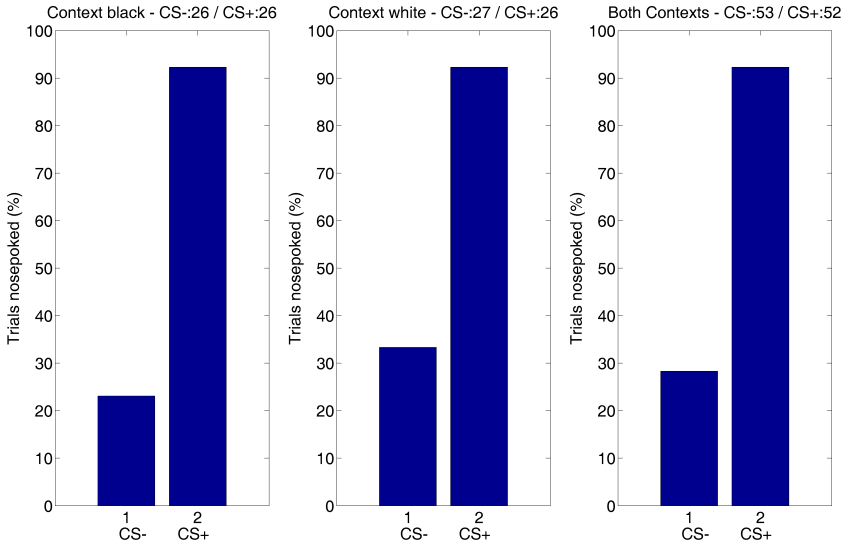

Figure 36: R013 best case nosepoke percentages for an individual day after learning
Responses of R013 during a best case day where the rat was observed to be consistently having the correct approach to each cue, while occasionally testing the opposite condition. Bars show the percent of each cue type nosepoked to in each context, and overall. The number of trials for each condition is shown at the top.

6.6 Individual Diagrams of Tetrode Histology for Each Rat

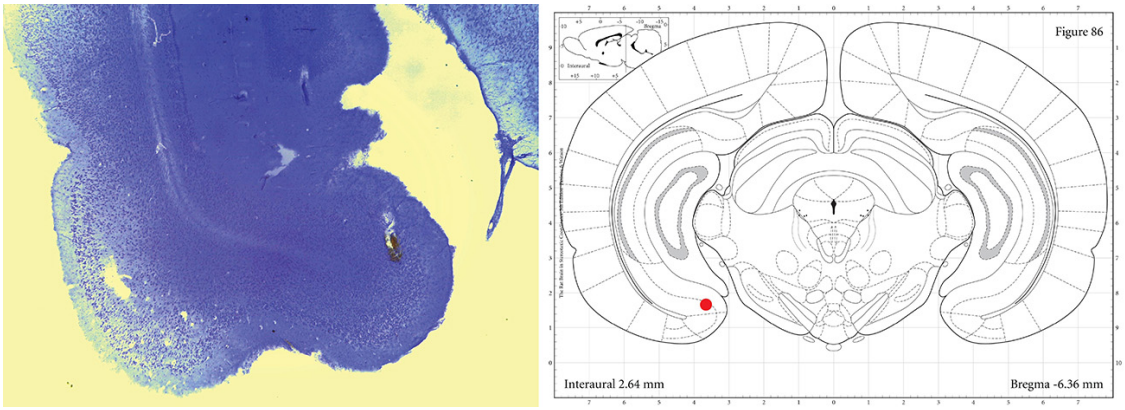

Figure 37: Rat R013 ventral subiculum tetrode

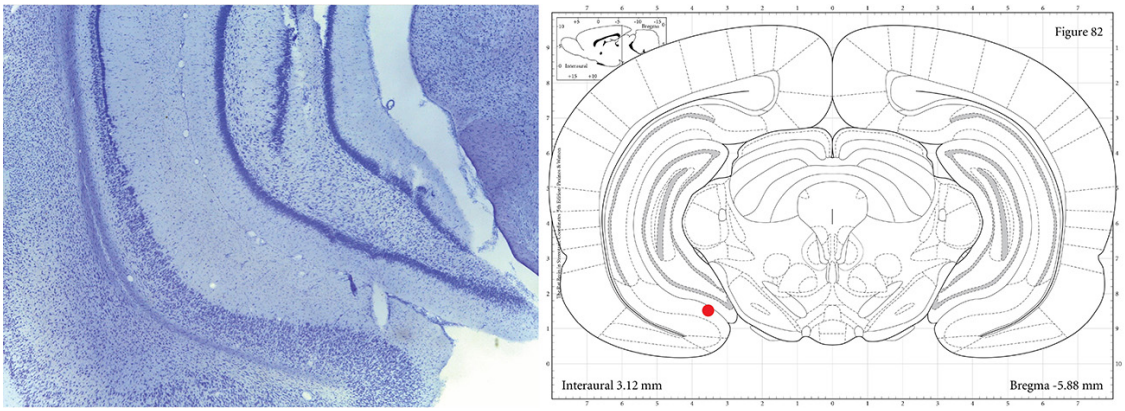

Figure 38: Rat R021 missed target on a tetrode targeting ventral subiculum

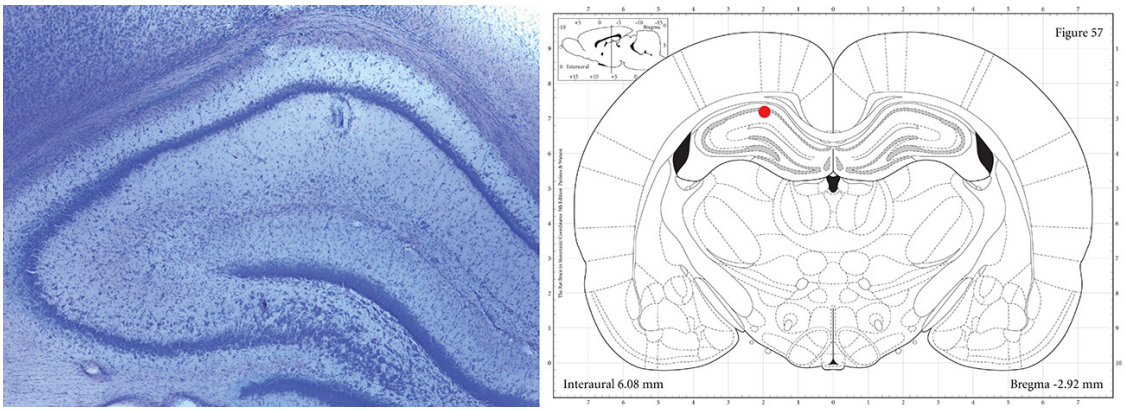

Figure 39: Rat R021 possible location of second dorsal hippocampal tetrode
Though the second dorsal tetrode final location was not obvious due to a lack of electrolytic damage visible on the slides, this may be an intermediate location shown by electrode track damage. Note the angle of the tetrode shown here. The previously shown dorsal tetrode may be visible in this image as a distortion slightly lateral and dorsal to the obvious track. The second dorsal tetrode did acquire the hippocampal cell layer during recording as determined by presence of hippocampal sharp waves (SWR).

Images for rat R021 reference tetrode could not be acquired due to tissue damage or missing location of induced electrolytic damage. Additionally one of the 3 ventral hippocampal tetrodes could not be found in histology, but was not present in a cell layer during the recovery of the brain. It was assumed to be in an intermediate quiet area and was being used as a signal reference.

6.7 Descent Epochs for Electrodes Targeting Ventral Subiculum

Descent of the electrode during electrophysiological recording towards vSUB tended to occur in 3 distinct epochs. Upon starting to record, tetrodes were typically in the intermediate hippocampus. This area tended to have both sharp waves and many frequency components (both high and low). As the tetrodes descended further, the high frequency components lowered in amplitude, and sharp waves disappeared at the ventral-most point of the hippocampus. This epoch tended to last for several mm of travel distance (until around +3.5 mm from the starting point in the cannulae). Finally, upon entering subiculum, high frequency components rapidly increased in intensity and spiking was once again evident. Since the experiment involved recording in multiple sites simultaneously, same day recordings are shown here (Figure 40) at different points of the descent.

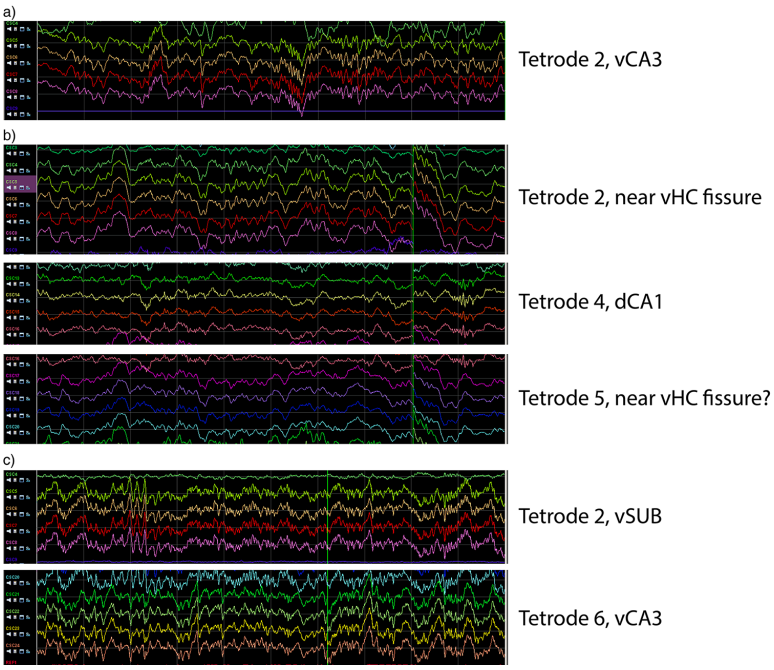

Figure 40: Descent epoch one - intermediate to ventral hippocampus
All descent data is from R022. CSC 5-8 represent tetrode 2, that eventually reached vSub. CSC 13-16 are tetrode 4 in dorsal hippocampus. CSC 16-20 are tetrode 5, which was targeted to vSUB but disappeared in the histology. It had a similar LFP to tetrode 2 at the time of ending recording. CSC 21-24 are tetrode 6, which was located in vHC CA3 at the end of recording. a) The eventual vSUB tetrode during descent while still in the ventral hippocampal cell layer. b) The vSUB tetrode after leaving the cell layer during the 'quiet' epoch of descent, with dHC and missing vSUB tetrode 5 shown for comparison. c) Tetrode 2 shown after reaching the vSUB, with Tetrode 6, currently located in ventral CA3 for comparison.

Glossary

actor-critic model	A model of striatal operation where the critic, theorized as the ventral striatum receives information on how successful an action was, and this influences future behaviour by the actor, theorized as the dorsal striatum.
cannula	A small metal tube. Cannulae are used to create the needle tips used for injections. They may also have flat ends instead of sharp ones to allow for protection of electrodes within the tube.
cell	By convention, neurons are often simply referred to as cells.
Chi-squared	A test commonly used to compare the observed results of an experiment to the expected results, and check how statistically significant they are.
cognitive map	In terms of the hippocampus, a cognitive map is the sum of all place cell activity in an environment that can be used for spatial perception and navigation.
confidence interval	The confidence level (ex 95 percent) at which results are considered to be statistically significant. This is generally presented as an interval around a population value
context	Context refers to the sum of information used to build a mental model of an event. In terms of this experiment, only environmental context is considered, which is the physical environment a subject is in.
context dependence	Many features of brain activity are dependent on the current context. For instance, some neurons in the hippocampus will only be active in certain environments, while being silent (no action potentials) in others.
dopaminergic neuron	A neuron which releases dopamine at synapses with other neurons. In the case of this experiment, dopaminergic neurons are typically present in the ventral tegmental area.
electrode	In this experiment, an insulated metal wire, used to measure changes in voltage in an area of brain. Electrodes may be bonded to other wires to allow for triangulation of electrical signals.
extinction	Extinction conditions occur when a previously reinforced behaviour (such as reward presentation when pressing a lever) is no longer reinforced. Behaviour is then assessed in absence of the reinforcer. When occuring on a single trial basis, extinction may be referred to as a probe trial.
firing rate	The number of spikes (action potentials) per second.
gauge	The cross-sectional size of a cannula. For a typical 30 gauge injection needle, the outer diameter of the cannula wall is 0.0125 inches, and the inner diameter of the hole is 0.0070 inches.
global remapping	In environments that differ significantly, hippocampal place cells may drastically change their activity between environments. This may include changing the location of a cells place field within the environment, becoming silent if previously active, or becoming active if previously silent.
hippocampal formation	A group of structures centred on the hippocampus that inclues: dentate gyrus, hippocampus, subiculum, presubiculum, parasubiculum and entorhinal cortex. For this experiment, I use the hippocampal formation to refer to the hippocampus and subiculum together, since they are often treated as such in experiments.

local field potential (LFP)	The sum of electrical field activity measured by electrodes when filtered, typically from 1-475Hz. The primary correlate of this signal is thought to be coordinated synaptic currents of local neurons.
nosepoke	A term that refers to a rodent breaking a directed infrared beam of light with its nose, typically by inserting the nose into a receptacle.
peri-event average	An average created from all instances of an event, within a given time window around that event. For instance, an average over all instances of the speed of a rat within a window around presentation of a cue.
peri-event histogram	A histogram calculated from all instances of a given event within a time window around that event. As this is a histogram, it shows the sum of activity within a time window around all instances of an event rather than an average.
place cell	A hippocampal neuron that has activity within an environment primarily correlated with a location in space.
place field	If a neuron has a strong correlation between its activity and a location in space, a place field is the area in space over which the neuron is active.
prediction error	The difference in value between an expected level of reward and an observed level of reward. The value may be positive or negative, and is used to inform future decision making processes.
ramp cell	A ventral striatal cell which increases its firing rate leading up to delivery of a reward. This increase in firing rate is dependent on both temporal and spatial factors relative to the reward delivery. It may represent a form of anticipation.
rate remapping	If an environment is not significantly different to an animal to trigger global remapping, rate remapping may occur instead. With rate remapping, instead of the place field of a cell changing location within an environment or becoming active/silent, the firing rate of the cell may change instead. In that case, a cell may begin having action potentials more or less often than it did in a previous environment.
remapping	Differences in activity of a neuron observed when animals are present in multiple environments. This is thought to represent ths switching of cognitive maps used to conceptualize the environment. Since the hippocampus does not have enough cells to represent each environment with completely unique ensembles, remapping allows the hippocampus to represent multiple environments using the same ensemble of cells. (See also: global remapping, rate remapping)
sharp wave ripple	As measured with an electrode, a rapid temporary increase in high frequency components of a signal (100+Hz; found commonly in the hippocampus) that is often associated with a slower temporary deflection from the average voltage. When an electrode is present within a hippocampal cell layer, the slower deflection disappears.
spike	A rapid spike in voltage as measured by an electrode. Typically, voltage spikes represent action potentials of neurons.
spike train	When a recorded signal is filtered to isolate only spikes, that series of spikes and associated timestamps is known as a spike train.
tetrode	A tetrode is a compound electrode with four wires. These wires are typically spun to eliminate space between them and are then heated gently to achieve binding of the insulation surrounding the wires. This creates a structure with both a higher resistance to bending than a single electrode, and the ability to

triangulate signals by calculating the differences in recorded voltage between each wire.