Systematic Mapping of the Electrical Activity in the Ventral Striatum during a Reinforcement Learning Task: An Analysis of the Micro and Macro Circuitry

Abstract

The ventral striatum (vStr), a structure involved in reward processing and goal-directed behaviour, receives input from multiple sources including the hippocampus, basolateral amygdala, and prefrontal cortex implying an integration of spatial/contextual, elemental/emotional and executive information in one region. The anatomical, morphological and electrophysiological differences within the vStr create a functionally heterogenous structure. The functional role of the electrical activity in the vStr has been studied at the unit, ensemble, and population levels (via local field potentials) and has revealed a variety of behaviour related changes. To date, the distribution of this functional electrical activity of the vStr has not been fully addressed in the literature. The firing patterns of some vStr neurons match the components of reinforcement learning models, however the central reward prediction error (RPE) component seems to be elusive in rodent unit and ensemble recordings. The modulatory properties of projections to the vStr from regions that explicitly encode RPEs in conjunction with recent discoveries in human patients suggests that RPE may be seen within the local field potentials. Here we propose a set of experiments that will address both the functional mapping of the vStr during a reinforcement learning task, as well as the influence of the hippocampal connections to the vStr. These experiments will use a silicon recording arrays implanted into the nucleus accumbens (NAc) to record the local field potentials and spiking activity across a two dimensional plane while the subjects engage in a spatial reward seeking task designed to generate reward prediction errors. By varying the reward location and magnitude we will be able to characterize the electrical activity across the NAc during a reward prediction error, something that has been reported in human fMRI but has so far eluded unit and LFP recordings in the rat. In addition, this study will also look to characterize changes in the NAc theta oscillations and modulation when the input from the hippocampus (thought to be the principal source of theta oscillations in the NAc) has been blocked.

The Ventral Striatum as a Complex Interface between Processed Sensory Information, Motivation and Action

The ventral striatum (vStr) has been considered to play general role in motivation and goal-directed behaviour (Mogenson et al., 1980), but more specifically it seems to be involved in Pavlovian/instrumental conditioning [1] (see debate with regards to Pavlovian/instrumental conditioning in the vStr in Corbit and Balleine 2003; Lex and Hauber 2008; Singh et al. 2011) Motivation itself is complex and contains many facets including action-selection, outcome perception, response vigor and anticipation (Niv et al., 2006, 2007). Motivation can be split into two general mechanism: goal-directed action selection which uses a response-outcome relationship, and habitual action selection which uses a stimulus-response relationship (Niv et al., 2006). In order to facilitate such complex behaviours the vStr must incorporate a great deal of information related to the internal states of the animal and the situations in which they find themselves, for example the value of a reward given a cost for obtaining the reward. The vStr is in an ideal position to integrate this information due not only to its anatomical connectivity but also due to some unique properties within the vStr itself. Some of these properties will be reviewed below to illustrate what is known about the layout of the vStr and where our current understanding falls short. Key elements of the vStr literature will also discussed which logically point to an alternative method for encoding certain task related neural correlates in the vStr.

The nucleus accumbens (NAc) is part of the vStr and has afferents from the hippocampus (HC), medial prefrontal cortex (mPFC), amygdala (Amy), and dopaminergic projections from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (Groenewegen et al., 1987; Humphries and Prescott, 2010), implying a convergence of multiple types of information related to context, emotion, higher cognition and reward detection into one region (Grace et al., 2007) (Figure 1). This multitude of information is congruent with the proposed role of the vStr in reward seeking and processing, motivational control and goal-directed behaviour (Nicola, 2007; Grace et al., 2007). The behaviourally relevant processing in the vStr is quite extensive. The degree of modulation via dopamine (DA) and its opponent serotonin (5-HT) via the raphe

[1] Though technically distinct instrumental conditioning contains Pavlovian elements which makes them sometimes difficult to distinguish

(Pazos and Palacios, 1985; Vertes et al., 1999; Daw et al., 2002) as well as local field potentials makes the vStr not only relatively complex but also very versatile.

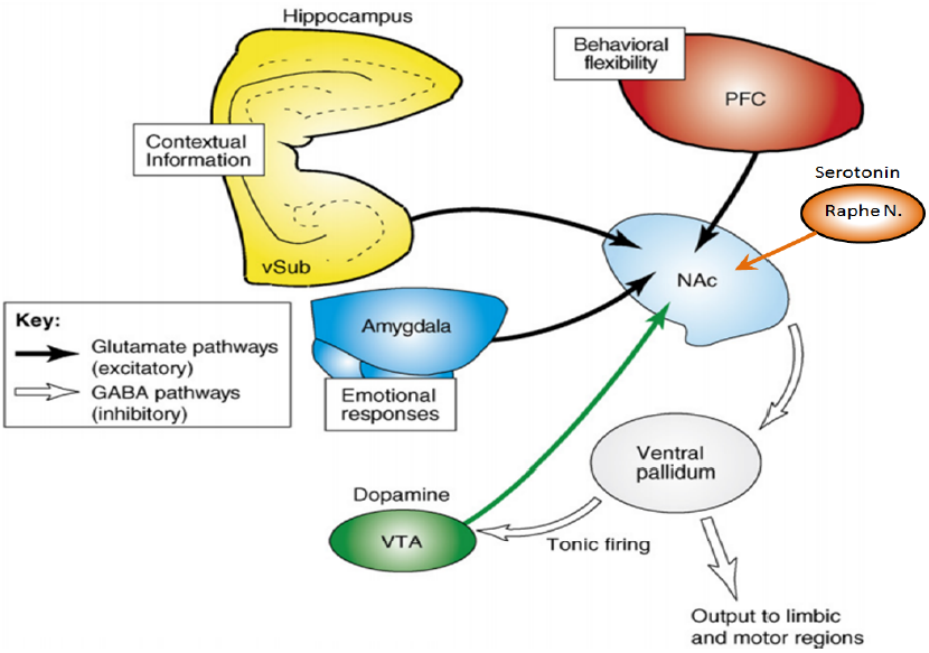

Figure 1: A schematic of the major projections to the nucleus accumbens (NAc). The major excitatory (glutamatergic) projections come from the hippocampus (via the ventral subiculum (vSub) having terminals with the highest density in the vStr), the prefrontal cortex (PFC), and the amygdala. The ventral tegmental area (VTA) which is a major source of DA activity related to reward perception and prediction projects directly onto the NAc (note the reciprocal connection via the ventral pallidum). The NAc receives a relatively moderate amount of serotonin from the medial raphe nucleus which has been shown to have an opposing role against DA (Daw et al., 2002). The major efferent fibers from the NAc pass to the ventral pallidum before moving on to other targets in the basal ganglia. Simplified and edited from the original image in Grace et al. (2007)

This proposal will address two key topics. The first being the heterogeneous nature of the vStr and how a better understanding of the distribution of electrical properties will help to fully understand the functional role of different subregions in the vStr during learning and reward prediction. The second topic will focus on the interactions between the HC and the vStr and will illuminate the modulatory effects of the HC on certain cells and oscillations in the vStr. A very brief overview of these properties and modulators will be discussed in order to address some key gaps in the literature regarding the local distributions of behaviourally relevant

activity across the vStr. Though this overview will show that a great deal is known about the vStr, it will also demonstrate how inhomogenous a structure it is and how a better understanding of its local properties is needed before its behavioural functions can fully be understood.

The Anatomy and Connectivity of the Ventral Striatum Display Regional Differences

The claim that the vStr is a heterogeneous structure stems from the differences in the gradient of afferents/efferents at the regional level down to the modulation of specific interneurons based on their receptor subtypes. These anatomical differences are given another level of complexity as lesions and inactivations of specific regions will lead to changes in specific behaviours therefore adding a functional distinction between regions. This diverse structure must be explored in some detail at the anatomical level before the evidence for any functional differences can be addressed.

The vStr consists of the NAc and the ventromedial portion of the caudate putamen (CPu) as well as the stria terminalis and the olfactory tubercle in the rat (Groenewegen et al., 1987). The remaining section of the CPu is considered to be the dorsal striatum (DS) and will not be covered, although it worth noting that the boundaries between the DS and vStr core are hard to define (Heimer et al., 1997). The vStr region contains a variety of afferents from the amygdala, HC, medial PFC, orbitofrontal cortex (OFC), thalamus, as well as projections from dopaminergic cells in the dorsal SNc and medial VTA (Groenewegen et al. (1987); Britt et al. (2012) for a review see Humphries and Prescott (2010) or van der Meer et al., *in press*). For the purposes of this brief overview only the NAc will be discussed in detail as it directly pertains to the proposed experiments.

Within the NAc are two principal types of neurons; medium spiny neurons (MSNs) which are not only the main GABAergic projection neurons and comprise more than 90% percent of the neurons in the NAc, but also form strong local connections to other vStr MSns (Plenz and Aertsen, 1996) and various interneurons, the most prominent of which are fast spiking interneurons (FSIs) [2]. These FSIs show strong local inhibition

[2]Large Aspiny interneurons (LAIs) and persistent and low-threshold spike cells (PLTS) also exist but are not discussed in detail

of MSNs within the vStr (Kawaguchi, 1993; Kawaguchi et al., 1995). *In vitro* and anaesthetized studies have shown that MSNs display alternating up and down states, which correspond to increased depolarization around the subthreshold level and a resting state respectively (Wilson and Kawaguchi, 1996). Transitions between these two states can be influenced by the timing and convergence of cortical excitation on theseMSNs (Wilson and Kawaguchi, 1996). This convergence has been shown in vStr afferents from the HC, PFC and BLA (O'Donnell and Grace, 1995; French and Totterdell, 2002, 2003). These convergent afferents display an interesting gating mechanism in which one input can put in the MSN in the up state and thereby facilitate a second input that can now cause the MSN to further depolerize and produce action potentials. Bursting activity in the PFC has also been shown to elicit depolarization patterns akin to the up state in the NAc core and may be facilitated at the level of local field potentials as well (Gruber and O'Donnell, 2009; Gruber et al., 2009). Blockage of HC projections to the NAc via lidocaine injection into the fornix can effectively prevent the MSNs from entering the up state (O'Donnell and Grace, 1995). This convergence and spike timing dependent gating of other inputs in the vStr suggests that the information being processed in a single MSN can be selective and therefore be adjusted in a task or state dependent manner.

At the macro level the NAc is subdivided into a core and shell component, with the core being morphologically difficult to distinguish from the dorsal striatum (Zahm and Brog, 1992; O'Donnell and Grace, 1993; Humphries and Prescott, 2010). With the exception of the rostral pole, which does not display a clear core/shell distinction, the core can be identified as the region encircling the anterior commissure while the shell portion comprises the ventral, medial, and lateral areas around the core (Zahm and Brog, 1992). The core and shell show an overlapping gradient of similar excitatory inputs that originate in the hippocampal region, prelimbic, dorsal agranular insular and dorsal anterior cingulate portions of the the mPFC, the basolateral amygdala complex, as well as the midline thalamic nucleus (Zahm and Brog, 1992; Humphries and Prescott, 2010; MacAskill et al., 2012). There are some subtle differences in the core and shell afferents that would suggest a functional distinction between the regions but at the same time there is a tendency for the shell efferents to eventually feed the core via the mediodorsal thalamus (see Zahm and Brog 1992). The MSNs in the core and shell have been shown to display different firing properties *in vitro* (O'Donnell and

here (see Kawaguchi (1993)

Grace 1993; *in relation to opioids* Brundege and Williams (2002)). The neuronal properties in the core and shell were compared *in vitro* and found only a few differences between these two regions with the exceptions being that core neurons displayed low-threshold spiking and that they were more receptive to afferent stimulation compared to shell neurons (O'Donnell and Grace, 1993). Local differences within the NAc core and shell can also be found related to DA. Excitation of the dorsal HC will trigger an increase in DA release in the core but not the shell, while stimulation of the ventral HC will cause shell DA levels to increase, but not the core (Peleg-Raibstein and Feldon, 2006) providing a possible functional segregation in DA signalling between these regions. This provides a degree of ambiguity at the purely cellular level that can better be addressed *in vivo* in order to detect behaviourally relevant differences in activity between these regions.

At the cellular and ensemble level there there is a clear difference between the core and shell. A unique property of the NAc core is the patch and matrix regions which bear resemblance to the patch and matrix in the dorsal striatum (Herkenham and Pert, 1981; Humphries and Prescott, 2010). Though the patch and matrix areas share major inputs with some subtle differences, they do have different projections with the patches going to the SNc and the matrix to the dorsal medial SNr (Groenewegen et al., 1999; Zahm and Brog, 1992). These unique properties in the core are difficult to study *in vivo*, but they do offer possible reason for the functional distinction between the core and the shell, that based on the subtle anatomical differences, would not be expected to be so sharp.

Based on these cases there are multiple sources of heterogeneity at the anatomical and electrophysiological levels in the vStr. The subtle differences between the efferents both within the core and between the core and the shell components of the NAc suggest that a potential difference in the hierarchical processing of information exists between these regions due to eventual looping of the shell projections back to the core afferents. The overlapping afferent gradients across the vStr combined with the convergence of multiple projections from the HC, PFC and amygdala on single MSNs suggests that these inputs can be modulated based on the timing of projections from these regions. This alone suggests a mosaic of highly processed sensory information across the vStr. The connectivity to the hippocampus which is one of the main afferent

projections to the vStr will be discussed in some detail to illustrate the complex relationship between the vStr and its limbic counterparts.

HC-vStr afferents originate principally in the ipsilateral ventral subiculum (vSub) (which itself receives inputs from the ventral CA1 subregion) with minor projections from the parahippocampal region (entorhinal and perirhinal cortices) (Groenewegen et al., 1987). The density of these subicular projections is the highest from the ventral region and decreases towards the dorsal region, while the NAc targets are the most dense in the caudomedial region and decrease in density as they go more rostral (Humphries and Prescott 2010 van der Meer et al., *in press*) (outlined in Figure 2). The HC projections to the NAc core seem to evoke larger responses in the direct MSNs than indirect MSNs (for a review of the indirect and direct pathways see Smith et al. 1998) suggesting a degree of hippocampal selectivity (MacAskill et al., 2012). The entorhinal projections target both the shell and core regions of the NAc while the HC/Sub projections terminate mostly in the shell with some minor overlap into the core. Projections from the hippocampal formation pass through the fimbria/fornix fibre bundle before reaching the vStr (Humphries and Prescott, 2010). This anatomical connection has been verified in anaesthetized rats by stimulating the fimbria/fornix which will invoke changes in the responses and LTP in the NAc (Boeijinga et al., 1990, 1993; Al'bertin et al., 2003). It is therefore likely that electrically induced disruptions to the fimbria/fornix would lead to a blockage of the hippocampal afferents to the vStr and would disrupt the transmission of hippocampal theta which has been shown to be influence the firing patterns of NAc cells through cross correlation analysis (Tabuchi et al., 2000). This possible disruption of the hippocampal influence in the vStr will be of particular interest in **Aim 2b**.

The complex convergence patterns of multiple afferents combined with the selective gating of their receptivity on NAc MSNs creates a heterogenous pattern of inputs from distal regions with strong behavioural correlates. In addition the regional differences between the core and shell and the patch and matrix mosaic would suggest a potential distinctions in information processing across these areas. Human fMRI studies have tried to isolated some behavioural correlates in the vStr but due to poor spatial resolution it becomes

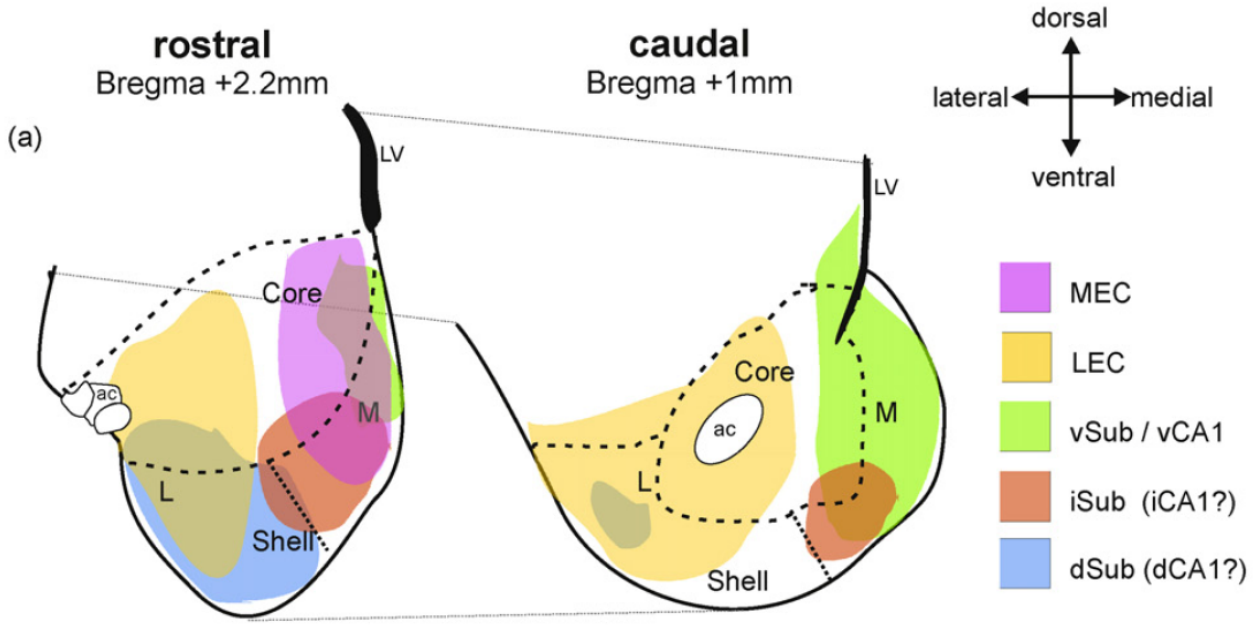

Figure 2: Schematic of the hippocampal formation and entorhinal cortex projections to the ventral striatum. The lateral entorhinal cortex (LEC) spans the lateral component of the rostral caudal axis, while the ventral subiculum/CA1 (vSub) covers the medial portions of the vStr. Both the medial entorhinal cortex (MEC) and dorsal subiculum (dSub) project mostly to the rostral region. The hippocampal inputs are more restricted to the shell portion while the entorhinal inputs span both the core and shell. This emphasises the overlapping distribution gradient of NAc inputs over across the ML, DV and RC axises. Figure cropped from Humphries and Prescott (2010)

difficult to claim any regional specificity. To elucidate the function distinctions between these regions researchers have typically adopted selective lesion/inactivation studies, or *in vivo* electrophysiology or some combination of both.

Lesions to the NAc Reveal a Behaviourally Relevant Distinction between the Core and Shell

Early work in vStr lesions focused mainly on locomotion, with damage to the vStr leading to increased movement (Kelly and Roberts 1983; see note in Pennartz C et al. 1994). However this relationship is in itself does not say much about the role of the vStr. Later work expanded the notion of the vStr being a key component of motivation by showing that lesions to the NAc would reduce motivational excitement (Balleine and Killcross, 1994) and that DA depletion in the NAc would lead to a reduction in responses that

were conditioned to provide a reward (Ranaldi and Beninger, 1993; Robbins and Everitt, 1996). This change in conditioned response lead to further lesion studies in an attempt to dissociate exactly what role the vStr could be playing in conditioned learning.

Since the vStr receives input from areas such as the HC and amygdala, it is not surprising that selective lesions to the vStr will impair behaviours requiring explicit or discrete contextual cues and Pavlovian learning (Kim et al., 1993; Cardinal et al., 2002). Pharmacological lesions to the NAc shell and core produce impairments but did not abolish acquisition in the Morris water maze (Morris, 1981) and spatial win-shift tasks [3] (Annett et al., 1989; Seamans and Phillips, 1994). Transient disconnection between the vSub and the NAc via lidocaine injections into both regions produces increased errors on a non-delayed random foraging but not on on delayed spatial win shift tasks suggesting that this connection could be important in non-delayed procedural tasks (Floresco et al., 1997). These previous studies did not specifically target the core and shell components of the NAc which show differences in their afferent and efferent connections as previously mentioned. A clear example of the distinct core/shell functionality came from Ito et al. (2008) who found that lesions to the core will cause a deficit in the number of correct trials during cue conditioning, while lesions to the medial shell region instead showed an impairment in the use of spatial cues and conditioned place preference. This further demonstrates a functional differentiation between the core and shell and the need for specific recording across both areas simultaneously.

This highlights the regional distinctions that are expected based on the anatomical gradients and subtle differences in the connectivity of the shell and core as mentioned earlier. The distinction between the behavioural correlates of the core and shell would suggest an separation in the activity of cells within these regions based on spatial or nonspatial cue components of a task. Cells encoding components such as reward-paired cues would thus be expected to be localized within the core rather than the shell. However, this distinction is less clear *in vivo*.

[3] The local specificity of these lesions was low and both the shell and core regions experienced cell loss

Multiple Goal Related Signals are Ambiguous in Location Across the NAc

Another approach to understanding the distribution of functionally relevant loci in the vStr is to record directly from a subject engaged in a specific component of a task. A multitude of studies have found specific behavioural correlates in the firing patterns of cells in the NAc (for an overview see Pennartz et al. (2011). These include changes in phasic NAc activity during reward prediction/anticipation (Khamassi et al., 2008; van der Meer and Redish, 2009a) (Figure 3), responses to cues paired with rewards (Roitman et al., 2005; Day et al., 2011; Goldstein et al., 2012), aversive stimuli (Roitman et al., 2005), task related actions (Roesch et al., 2009; Ito and Doya, 2009; Day et al., 2011), the type of reward (*food/water versus cocaine* Carelli et al. 2000, textittype of food Kalenscher et al. 2010), the magnitude of the reward received (Giertler et al., 2003; Goldstein et al., 2012), reward location (van der Meer and Redish, 2009a), and reward delivery (Day et al., 2011; Ito and Doya, 2009; Khamassi et al., 2008). Some of these reward anticipation related changes in NAc activity are best exemplified by the reward ramping seen in certain NAc neurons as the animal either waits for a reward or approaches a reward location (Khamassi et al., 2008; van der Meer and Redish, 2009a) (Figure 3). Some of these anticipatory cells have been shown to increase their activity until the animal has reached the reward site and then promptly reduce their firing to around zero. These ramping cells show theta modulation (discussed in a later section) (van der Meer and Redish, 2009a). These theta modulated reward ramp cells offer an opportunity to investigate the hippocampal dependence of some vStr cells (**Aim 2**). It is also of interest to address the effects of hippocampal inactivation on these spatially modulated reward related cells in the vStr (**Aim 2**).

These neural correlates of behaviour have thus far only been loosely defined as being in the vStr or NAc with some studies showing specificity to either the core or shell region. The behaviourally relevant activity in the vStr is further complicated given the anatomical projections from the VTA and the SNc to the NAc, two areas which show a strong change in their phasic firing based on predicted reward outcome. It is expected that these prediction signals would also appear to some degree within the reward encoding regions of the NAc and possibly in a regionally distinct manner given the variation in DA projections in the NAc. The DA signals from the VTA and SNc are tightly linked to reinforcement learning (RL) models which rely on this

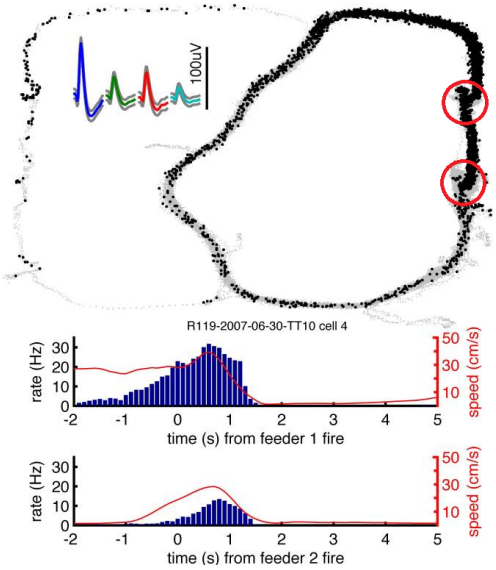

Figure 3: An example of a ramping anticipatory reward cell. The upper panel shows the locations of spikes around a multiple T-maze with two reward locations on the right side (red circles). As can be seen in the maze plot, the density of the cell firing increases as the rat gets closer to the reward sites. The lower two plots show the peri-event time histograms around the time at the first reward site (above) and the second reward site (below). The pattern before feeder one shows an increase as the subject approaches the feeder location but then persists until the reward reaches the track (1.5-2s after feeder arrival). The pattern at the second feeder location is similar but with noticeably lower activity. Taken from van der Meer and Redish (2009a).

outcome prediction value to determine their future action selection. Therefore it would be expected that the vStr would play a key role in this prediction error system or show some unit activity related to some of the RL components (discussed below), however this is still elusive at the level of unit recording and local field potentials (LFP) in rats.

Striatal Correlates of Reinforcement Learning

A naive reinforcement learning agent must learn to achieve the highest possible value from the actions it takes in an environment (Sutton and Barto, 1998). In the simplest sense the agent must have a representation of a set of states and a set of available actions in each of those states. The agent will select the action that yields the highest value. This is a process that must be learned through experience. The agent must balance

exploration of the possible action options with exploitation of the best available actions in order to optimize its performance. At the heart of reinforcement learning is the reward prediction error (RPE), by which an agent will make a prediction about the outcome of an action and then based on the difference between the actual outcome and the expected outcome will lead to a shift in the weights regarding the action values. This RPE has been a characteristic property of DA neurons in the VTA and SNc which display changes in the phasic DA projection activity when the subject experiences unexpected result based on previously known contingencies (Schultz et al., 1997). The DA RPE uses stimulus representations through time to predict the discounted value (sum) of future rewards (Schultz et al., 1997). This lead to a proposed relationship to temporal difference reinforcement learning (TDRL) in which the immediate action need not be rewarded provided there is some future reward (Sutton and Barto, 1998; Schultz et al., 1997). Due to the nature of the limbic and DA projections to the NAc as well as the DA dependent morphological specificity within the MSNs and FSIs it would appear that the vStr would be an ideal location for encoding some correlates of the prediction error or other TDRL components, however the evidence for a clear prediction error signal in the vStr has not been very convincing. However prior to discussing error prediction in the vStr it becomes important to address the role of DA within the vStr.

At the cellular level there is some evidence of the modulatory effect of DA on specific cell types within the vStr. *In vitro* applications of DA have been shown to inhibit striatal neuron firing, but did not alter their membrane potentials thereby limiting the excitability of striatal cells (Calabresi et al., 1987)[4]. Recordings of FSIs and large aspiny cholinergic interneurons (LAIs) have shown that the addition of dopamine will produce membrane depolarization (*FSIs in* Bracci et al. 2002, *LAIs in* Aosaki et al. 1998). Whole cell patch clamping revealed that these FSIs were showing a subthreshold membrane oscillation between 20-100 Hz (contains the beta and gamma bands) and that the first spike of the FSIs characteristic burst pattern was triggered by these oscillations (Bracci et al., 2003) . Berke (2009) expanded these findings to the freely moving rat finding that when given a DA agonist, that there was a clear shift from the gamma-50 (50Hz oscillation in the electrical potential within the region) to the 70-100Hz gamma-80, a switch that is also seen in undrugged rats immediately after receiving a reward *in vivo* (van der Meer and Redish, 2009a). Taken together these

[4]These applications were systematic and could have indirectly altered the activity of the recorded MSNs

data suggest that the higher frequency oscillations in the vStr are influenced by DA levels, and thus it would be expected that there would be a degree of change in the cells inhibited by these FSIs within the vStr during DA release related events such as RPE which should be detectable in the LFP and firing patterns of NAc cells.

It is important to stress the heterogeneity in the locations of VTA DA release across the NAc (Wightman et al., 2007) but also the variability DA receptor types across cells in the vStr, as these cells could react in different manners leading to specific relationships to the RPE properties of nigrostriatal DA. D1-like receptor knockouts in NAc interneurons have been shown to depolarize in a similar way to wild types in both LAIs and FSIs but D2 receptors were shown to modulate the degree of inhibitory GABAergic input to the interneurons, suggesting that there is a degree of DA specificity based on the type of receptor being expressed in the interneurons of the striatum (Bracci et al., 2002; Centonze et al., 2003). Pawlak and Kerr (2008) found that the application of DA to striatal neurons with specific DA receptor subtypes could have different effects on long term potentiation (LTP) and long term depression (LTD). D1/D5 receptor blockage could prevent both LTP and LTD while D2 blockage would only delay LTD and expedite LTP. These studies on the effects of DA on certain cell types suggests that DA can mediate synaptic changes, cell specific inhibition/excitation, and local field potentials in the striatum with a high degree of cellular specificity. In addition the DA modulation of local LTP/LTD suggests that there should a change in the NAc activity while learning via RPE (**Aim 1c/d**) as well as a heterogenous response to DA activity in the VTA such as RPE (**Aim 1a/c**). Taken together DA is thought to be capable of influencing the cells in the NAc and thus it would be expected that the RPE activity in the VTA/SNc would lead to some visible changes in the vStr in relations to RPE as well. This holds true when the brain is viewed at the macro level as in fMRI recordings.

Several human fMRI experiments have been able to identify a clear change in the local blood oxygen level depletion (BOLD) signal within the vStr when a subject experiences a RPE (Berns et al., 2001). These RPE induced BOLD changes in the vStr have been related to appetitive or operant conditioning (Pagnoni et al., 2002; O'Doherty et al., 2003), aversive stimuli (Jensen et al., 2003), reward magnitude (*NAc specific* Knutson

et al. 2005), as well as an anticipatory ramping of activity (*NAc specific* Knutson et al. 2001, textitfor a review see Montague et al. 2004). Specifically, in the O'Doherty et al. (2003) experiment, the authors were able to match the learning rate parameter of a TDRL model to the change in the BOLD signal during a RPE within the vStr, OFC, and cerebellum, suggesting that these regions closely fit the DA RPE response. A fast-scan cyclic voltammetry measurement of DA levels in the NAc of rats performing a classical conditioning task (Day et al., 2007) found that the DA level would initially signal the reward but not the predictive cue, while after some time this increase in DA would shift to the predictive cue instead, exactly as would be expected from the Schultz et al. (1997) data. This, in conjunction with reward-response related changes in the NAc LFP during cue presentation and reward outcomes recorded from human patients with deep brain stimulation implants (Cohen et al., 2009) would suggest that an RPE is detectable in the NAc but not in the units per se but rather at a population level. It is important to note that some of these experiments have pointed to the vStr being involved in prediction errors as well as the evaluation of a goal. But as noted by Hare et al. (2008), there is an inherent issue in applying a general linear model (as is often the case in fMRI analysis) since this form of analysis is susceptible to issues arising from variables that are themselves correlated. Through a more thorough analysis and experimental design Hare et al. (2008) were able to dissociate the PE related changes in the vStr from the goal value signal in the medial orbital frontal cortex. Together these results would suggest that there is a strong relationship between the RPE changes in dopamine projections to the vStr and the degree of BOLD or chemical activity in the vStr under multiple RPE related conditions which to date has not been as clear at the cellular level *in vivo* (**Aim 1c**).

Despite this line of evidence from non-invasive human studies relating the striatum to RPE, this response has not been evident *in vivo* in the freely behaving rat. Roesch et al. (2009) found that when rats engaged in movement towards a reward site following an odor cue, the majority of the recorded vStr neurons seemed to show phasic activity only when the rat activity moved towards the reward site, thereby suggesting that that encoded the value of the current action. They also found a very small percentage of vStr neurons that seemed to encode anything related to reward prediction. This is in contrast with what would be expected from the human and DA based PE responses as shown in Roesch et al. (2007) where the DA neurons fired

strongly based on bidirectional prediction error encoding regardless of the action taken. Despite the clear phasic change in the VTA/SNc in response to a RPE, no such effect or residual change seems to exist in the activity of neurons in the NAc. This does not exclude the influence of an RPE which may still be present within the local field potentials (LFP) as has been seen in humans (Cohen et al., 2009). Cohen et al. (2009) found that not only is there a change in the NAc gamma band power upon winning or losing money, but that these gamma band events (40-80Hz in this case) were slightly offset to the lower frequency alpha oscillations (8-12Hz). Depending on the trial outcome the timing of the increase gamma activity with rewarded trials eliciting more gamma activity following the alpha wave and loss trials having more gamma activity preceding the alpha wave (Cohen et al., 2009). Thus it is possible that these RPEs may manifest in other ways at the population level. Though the evidence for cellular encoding of RPEs in the NAc is almost non-existent, there is a great deal of unit activity related to other motivational and RL components.

Ito and Doya (2009) identified a percentage of neurons in the NAc and ventral pallidum (VP) which showed firing patterns correlated to action choice, reward outcome, reward probability, differential action coding, and state values. They employed a comparison of the mutual information to assess the mutual dependance of any two of the variables [5]. The Ito and Doya (2009) study did not explicitly test for prediction errors in the NAc/VP. NAc neurons have also been shown to display increased activity related to cue value and response movement (Roesch et al., 2009). These RL related cells point to an interesting connection between the DA activity in the VTA/SNc and suggest that the information being processed within the NAc is in fact much more complex than a pure reward prediction signal from the DA afferents. The Ito and Doya (2009) and Roesch et al. (2009) studies have looked at spiking activity but lack an analysis of subthreshold membrane potentials and the LFP in the vStr, implying an area of investigation. Cohen et al. (2009) have shown that there are changes in the gamma band that coincide with different phases of the alpha oscillations in humans during a RPE which also suggest that cross-frequency modulation could be playing a part in the RPE process. With studies so far dismissing the RPE signal in the vStr being based on unit recordings, and the general agreement that changes at the population level the vStr through human experiments, it is worth investigating

[5]This supplement to their regression analysis offers a way to circumvent the issues of correlated variables in general linear models from earlier

the changes across the vStr in the LFP signals across multiple frequency bands (gamma seems to be the most promising).

The idea of state space is a key issue in RL. An agent needs to have some information about the environment even if this is as simple as determining what state it is currently occupying and what state it will transition to when a particular action is taken. The hippocampus has long been associated with a neural representation of space and context and due to its strong projections to the vStr seems like a good candidate for providing at least some of the information needed to represent or determine the animals current and future states. The importance of the hippocampal-ventral striatum interactions will be discussed below.

Hippocampal-Ventral Striatum Interactions

The hippocampal region consists of the hippocampal formation (CA1, CA3, dentate gyrus and subiculum) and the parahippocampal region consisting of the presubiculum, parasubiculum, paraentorhinal, perirhinal and entorhinal cortices (Witter and Amaral, 2004). The hippocampal region contains cells with space encoding properties: place cells (O'Keefe and Dostrovsky, 1971; Wilson and McNaughton, 1993), head direction cells (Taube et al., 1990; Whitlock and Derdikman, 2012), grid cells in the MEC (Hafting et al., 2005), border cells (Solstad et al., 2008), as well as conjunctive cells with more than one of the aforementioned properties (Sargolini et al., 2006) (*some of these are reviewed in* Moser et al. 2008. In addition to these spatial cells, the hippocampus also contains a great deal of extra-spatial firing correlates. An example would be the pre-play and replay seen in place cells (Johnson and Redish, 2007; Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996; Gupta et al., 2010) which represents a behavioural sequence of previously experienced locations.

The majority of the HC projections that innervate the vStr travel via the ventral portion of the Sub which itself receives the majority of its input from the the ventral hippocampus (Groenewegen et al., 1987). The vHC has not been studied in as much detail as the dorsal portion, and it becomes important to acknowledge some of the differences in the HC along the septotemporal axis (roughly corresponds to the dorsal (septo) and ventral

(temporal) portions). The ventral HC which contains afferents from the hypothalamus and amygdala, seems to play a larger role in contextual and anxiety/fear related memories and the main connections between the PFC and the HC are via the ventral HC (Moser and Moser, 1998; Bannerman et al., 2004; Verwer et al., 1997; Hoover and Vertes, 2007). The ventral HC also has larger place fields (Jung et al., 1994; Kjelstrup et al., 2008), as well as decreased theta power and decreased theta modulation (Royer et al., 2010). In addition to the decreased presence of stereotypical dHC spatial signals, the vHC also seems to involve emotional processes (Henke 1990[6] Bannerman2004, Fanselow2010) and lesions to parts of the vHC will abolish cued tone fear and will have a less profound effect on contextual fear compared to dHC lesions (*ventral CA3* Hunsaker and Kesner 2008, *ventral CA1* Rogers et al. 2006. With the majority of the vStr input from the HC originating in the ventral portion of the Sub which in turn receives a biased amount of information from the vHC this suggests that the vStr receives a complex representation of context and emotional [7].

The hippocampal region displays a variety of oscillatory dynamics in the theta (7-10Hz), gamma (30-120Hz), and beta2 (23-30Hz) frequencies as well as sharp-wave ripples (SPW). Each of these oscillations shows some behavioural relevance. SPWs in the HC are large amplitude LFPs that occur when the animal is immobile, during consumption of food, or during slow wave sleep (Buzsáki and da Silva, 2012). Beta2 oscillations in the mouse HC have been shown to increase in power when the subject is in a novel environment (Berke et al., 2008). Hippocampal gamma, which is prominent during awake behaviour seems to have two sources, one is intrinsic to the HC with frequency of roughly 40Hz, and one in the entorhinal cortex (˜90Hz) (Colgin et al., 2009). CA1 shows an interesting synchronization with the 90Hz gamma in the MEC and the 40Hz gamma in the CA3 and since these 40Hz and 90Hz oscillations show a switching in their relative power, this would suggest a mechanism for information transfer between CA3 and MEC inputs to CA1. This becomes of particular interest to the HC-vStr interactions as the CA1 gamma signals phase locked to different parts of the theta cycle suggesting a possible cross frequency interaction (Colgin et al., 2009; Colgin and Moser, 2010). This HC theta oscillation requires a more detailed examination.

[6]Though the damage to the HC was quite extensive in this study

[7]This suggests that the representation of the hippocampus in figure one is a gross oversimplification

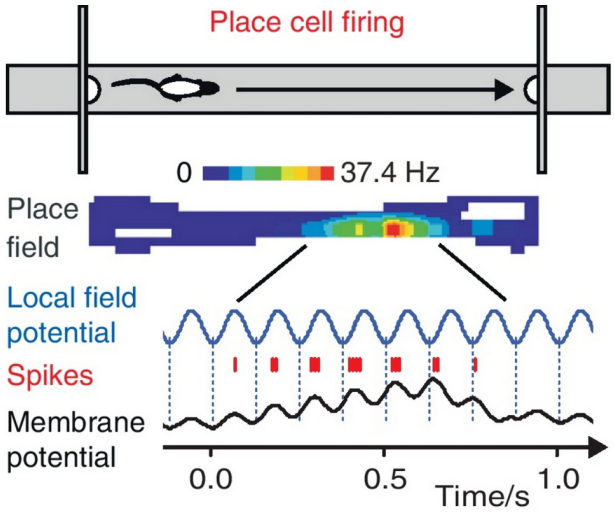

Figure 4: A visual representation of theta phase precession in a hippocampal place cells as a rat moves through the place field. As the rat enters the place field the membrane potential is low, the firing rate is low and the spikes occur at the peak of the theta LFP. As the rat moves towards the center of the place field the membrane potential increases, the firing frequency increases and the timing of the spikes precesses behind the peak towards the trough of the LFP. As the rat moves out of the place field, the membrane potential decreases back to baseline, the firing rate decrease (more abruptly) and the spikes now occur at the trough of the theta LFP. Taken from Burgess and O'Keefe (2011).

The most prominent oscillation is within the theta band which displays strong sources in the hippocampus (Vanderwolf, 1969)[8]. Place and grid cells display theta modulation, showing very clear theta phase precession (O'Keefe and Recce, 1993; Hafting et al., 2008). This phase precession occurs when the firing of a place cell has a slightly higher frequency relative to the local theta oscillations (Burgess and O'Keefe, 2011; Malhotra et al., 2012) (Figure 4). The functional significance of the theta phase precession is currently debated but could pertain to spatial reconstruction and positioning (Jensen and Lisman, 2000), lookahead and planning (Jensen and Lisman, 1996), and rapid learning via cross correlation between sequentially activated place cells and spike-timing dependent plasticity (Skaggs and McNaughton, 1996) (*see review in* Malhotra et al. (2012). These spatial properties and theta oscillations are of particular interest when linking places to rewards in the vStr which has also been shown to display theta modulation/phase precession relative to the hippocampal theta van der Meer and Redish 2011). Phase precession offers a unique connection to the HC

[8]Though the actual source of hippocampal theta seems to be the medial septum diagonal band of broca (*for a discussion see* Buzsáki 2002)

and can be used as a tool to investigate HC interactions with other regions displaying this phenomenon (**Aim 2**).

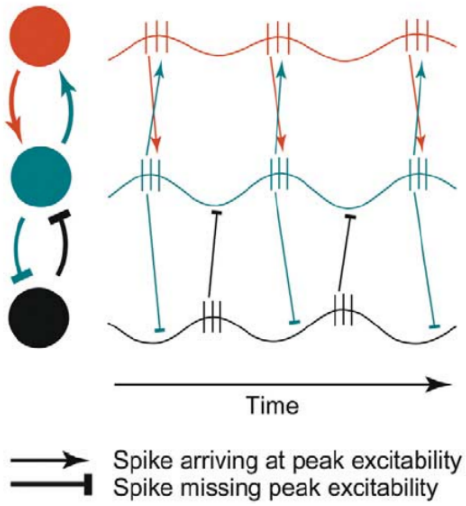

Figure 5: A schematic of three cells that show a similar periodicity but have different phases of firing. The top cell (orange) and the middle cell (teal) provide excitatory projections to one another during the peak of each wavelength taking into account synaptic delays. This ensures that they are able to receive signals from one another at an optimal rate. The bottom cell (black) and middle cells are not in phase and thus fail to elicit a change in postsynaptic firing rates since the cells are less receptive to excitatory input when they are in a trough. The distal component is not evident in this drawing, but this process has been proposed to be effective across large distances. Taken from Fries (2005).

Neurons with coherent phases and periods of input can convey information more efficiently (Buzsáki, 2004; Fries, 2005; Womelsdorf et al., 2007) (Figure 5). This would suggest that two distal regions in the brain whose phases were coherent during periods of input could increase the effectiveness of any communications. The convergent inputs to the vStr could be modulated by coherent frequencies in the vStr and the afferent origin and would offer an effective method of selective gating in MSNs with multiple limbic inputs as discussed earlier. This idea of distal LFP coherence can seen in the vStr and HC. van der Meer and Redish (2011) recorded from CA1 and the vStr simultaneously on a rewarded multiple T-maze. This task looked at the correlation between the HC and vStr theta oscillations, which seemed have the same average frequency in both regions but different theta power (Figure 7). They found that the reward ramping firing patterns of some cells of the vStr were theta modulated in that they displayed theta phase precession (Figure 6). The theta phase precession was evident in the cells that displayed the ramping firing pattern but not in cells with

other firing patterns, thus suggesting that the hippocampal theta signal could be selective for certain neurons in the vStr and only at certain times (van der Meer and Redish, 2011). The lack of evidence for strong theta sources in the ventral striatum (O'Donnell and Grace 1995, *reviewed in* Malhotra et al. 2012) and strong coherent firing between HC and vStr neurons (Tabuchi et al., 2000) suggests that the vStr theta is a product of the HC influence rather than being an independent theta source (van der Meer and Redish, 2011). Tabuchi et al. (2000) measured the degree of spike coherence between the HC and NAc and though they did not find a strong theta oscillation within the vStr they did notice that the cross-correlograms of several HC-vStr cell pairs displayed a peak in the theta range. These paired HC-vStr neurons carried a similar theta modulation during the 1s period immediately prior to receiving a reward but not 1s following the reward acquisition similar to the pattern of high gamma power prior to but not after reward sites (van der Meer and Redish, 2009b). The notion of HC driven vStr theta signals can be explored by an inactivation of the HC fornix/fimbria projections to the vStr coupled with a large recording array (this will form the basis for **Aim 2**).

Several experiments have disrupted the connections between the HC and the vStr. Lesions to the HC and NAc shell in opposing hemispheres lead to impairments in the acquisition of appetitive contextual conditioning (Ito et al., 2008). This suggests that rats engaging in a spatial learning task would also suffer performance impairments following HC-vStr disruptions. Pharmacological disconnection of the vSub and NAc via lidocaine led to impairments in the foraging behaviour in a non-delayed task but not during a delayed task, the opposite of what had been seen with similar vSub-(contralateral) PFC inactivation by Floresco et al. (1997). It is possible that the HC-vStr interactions are involved in exploratory goal directed behaviour, this could be tested by transient inactivation of the HC-vStr projection neurons during a task that requires this type of behaviour.

One hypothesis would be that an inactivation or blockage of the HC-vStr connections would lead to altered activity in theta modulated reward ramp cells in the vStr and could lead to further changes across other LFP bands which have been shown to display similar patterns of activity to the HC-vStr cell pairs in terms of

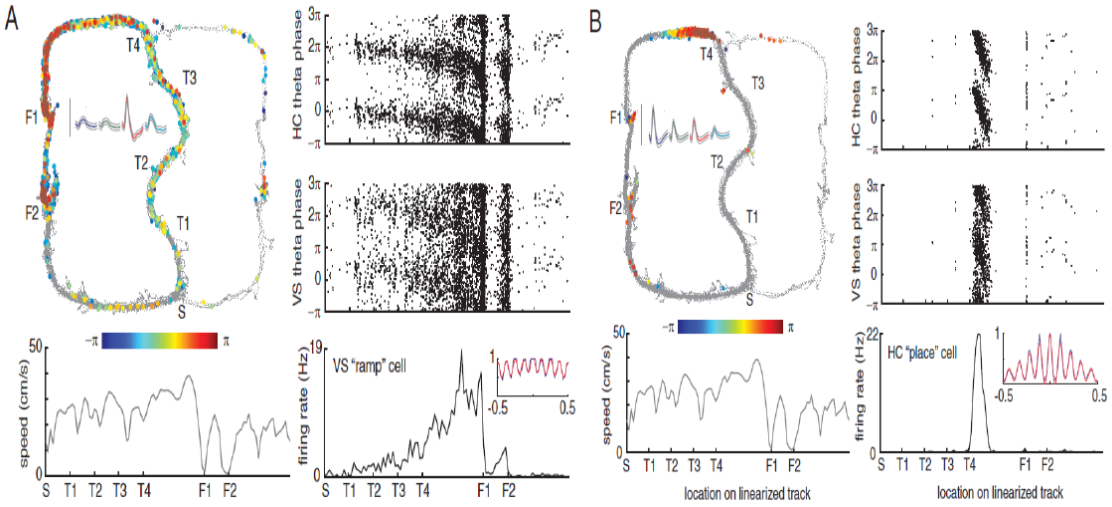

Figure 6: (**A**) An example of a vStr ramp cell that displays theta phase precession. The phase of the ramp cell can be seen to change as the rat approaches the first reward site (F1) and then resets and precesses again when approaching the second reward site (F2). The scatter plots of the linearized position versus the theta phase in right boxes in (**A**) show the characteristic "banana" pattern in the cell firing as they precess towards π as they approach F1. A similar precession can also be seen prior to F2 but with a much lower firing rate. (**B**) A HC place cell shows a specific location of elevated activity on the track and a very clear phase precession (black phase firing plot) within that place field only (compare the upper right box to the firing rate within the place field in the lower right box). Taken from van der Meer and Redish (2011).

reward approach. If the HC-vStr connection does in fact govern the association of reward contingencies with locations in the environment, then interruptions in these connections should impair place learning. **Aim 2** seeks to perform these inactivations and record any changes in the LFP and activity in theta modulated reward ramp cells on a spatial reward learning task, something that to the best of our knowledge has not been done using large scale unit and LFP recording in the vStr. The complex and distinct behavioural correlates of other vStr LFP bands become of interest as they will also encode spatial components or show some cross-frequency coherence or correlation. The LFp oscillations within the vStr are reviewed here.

Rhythms within the Nucleus Accumbens

Oscillations in the local field potential are the result of the average fluctuations in extracellular voltage across a 100-300μm area and have been proposed to play a role in local and distal synchronization/coherence, cellular modulation, and possibly the transfer of information[9] (Berens et al., 2008; Buzsáki, 2004). These LFPs can be the result of either local intrinsic generation, non-local inheritance, or passive volume conduction. The intrinsic generation of an oscillation is dependent factors like subthreshold synaptic currents, average membrane potentials, and the limits of spiking frequency. Intrinsic generation implies that changes in the LFP are the result of changes in the membrane properties of the cells in that region and are likely be the source of the signal. Inheritance implies that the oscillation is not generated locally but instead it is transferred through connectivity to another region that can oscillate at a given frequency. Inherited signals become of particular interest when addressing the influence of the region that generates the signal to region that is not capable of intrinsic generation. Finally volume conduction is a passive process by which a strong oscillatory source will spread to nearby regions even though this may influence processing in the receiving areas. In this respect volume conductance can be viewed as an artifact. The proposed origin and nature of the different LFP frequencies in the vStr will be discussed briefly.

The vStr delta (1.5-3Hz) and both gamma-50 (45-50Hz) and gamma-80 (70-100) seem to arise from the intrinsic neuronal properties. This seems to be due to the nature of FSIs in the striatum which show a interburst frequency of 2.5Hz ($\pm$ 0.9) *in vitro* which falls into the delta range, and an intra-burst firing rate of 52 $\pm$ 13Hz which puts it in the lower end of the gamma range (Taverna et al., 2007). This suggests that the delta and gamma rhythms are a product of the striatal FSIs and could be intrinsic to the vStr. It is worth noting the small fraction of FSIs compared to MSNs ($< 1\%$ of striatal neurons (Luk and Sadikot, 2001)) and the distribution of FSIs favouring the lateral striatum (Gerfen, 1985) suggesting a possible gradient in delta/gamma power across the lateral to medial axis in the striatum. The beta band (15-25Hz) has recently been of interest in the vStr however the source of the signal is unclear (Howe et al., 2011; Leventhal et al.,

[9]It is always worth noting that these LFPs may actually be an epiphenomenon and they themselves do not contain information, however mounting evidence would suggest that this is not the case

2012) but due to the beta modulation and oscillatory coordination selectivity for the beta band within the basal ganglia, it would appear that this is a good place to look for possible origins (Leventhal et al., 2012). The theta oscillation (7-10Hz) seems to be primarily driven by the HC input. Though theta oscillations have been reported in the NAc MSNs (*during head movement and rearing* Leung and Yim (1993)), they do not show any sustained intrinsic theta generation (5-8Hz in this case) nor could they be driven to sustained theta oscillations by stimulation of the fornix (O'Donnell and Grace, 1995). A low density recording across the striatum has revealed an increasing gradient distribution of beta, theta and gamma (only gamma-50 was analyzed) power from the dorsolateral to ventromedial part of the striatum (Berke et al., 2004). The behavioural roles of these oscillations will be addressed here and will demonstrate a need for a systematic mapping of each of these oscillations across the vStr, and a need for an investigation into the potential role of these oscillations in the elusive reward prediction error signal.

The theta oscillation has multiple behavioural correlates in the vStr and suggests that the interaction between the vStr and HC can have a profound effect on not only the theta LFP but also theta modulated cells in the vStr. Nearly all of the inputs to the NAc show some degree of theta rhythmicity (*reviewed in* Malhotra et al. 2012). A dual recording experiments by van der Meer and Redish (2011) and Berke et al. (2004) within the HC and vStr have found that not only is there a clear theta signal within the vStr but the frequency is quite similar to the HC (Figure 7B). Though the NAc does not seem to generate theta oscillations, it does show some strong behaviour related changes in the theta signal. Tabuchi et al. (2000) found that the degree of theta modulation vStr cells that were paired with HC cells would only be present immediately prior to receiving a reward but not after. Gruber et al. (2009) found that the vHC and NAc core displayed theta coherence during exploratory behaviour but not during operant behaviour while the vHC-NAc shell displayed a consistent coherence regardless of the behaviour. NAc reward ramping cells have also been shown to display theta phase precession (van der Meer and Redish, 2011)). Though vStr cells that are entrained to the hippocampal theta would be expected to encode some form location signal, this turns out to not be the case (Berke, 2009) and begs the question of what other function could be played by hippocampal theta entrainment. Together these findings suggest a rich coherence between HC theta signal relative to other possible theta sources. It

becomes of interest to interrupt these HC and vStr theta oscillations during a task that would require some form of place learning in order to address the persistence or even existence of theta modulated NAc cells such as phase precessing reward ramp cells (**Aim 2a**).

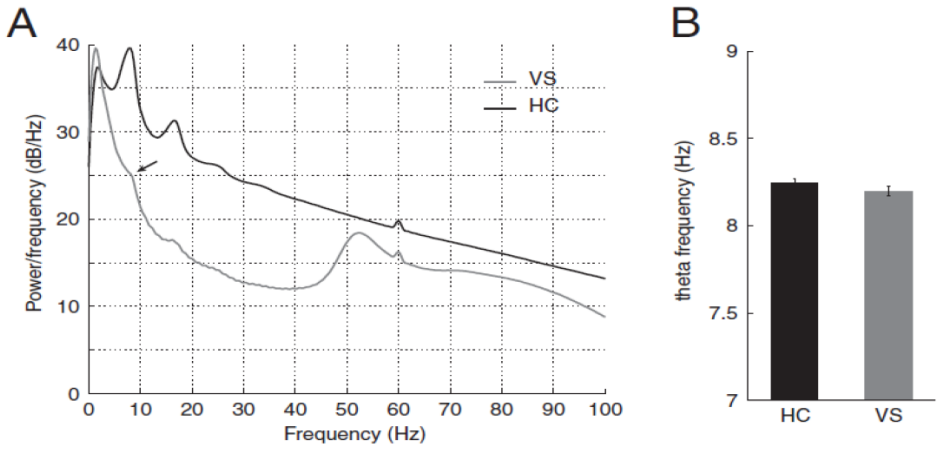

Figure 7: (**A**) A power spectrum of the LFP signals in the HC and vStr. Note the strong theta (7-10Hz) peak and its 16-20Hz harmonic in the HC compared to the relatively small power of the theta signal in the vStr. Also of interest are the prominent gamma-50 and gamma-80 peaks in the vStr but not the HC. The notch peak at 60Hz can be ignored. (**B**) Outlines the average theta frequency in the HC and vStr which does not seem to be different between the two. Taken from van der Meer and Redish (2011).

Compared to other vStr oscillations, relatively little attention has been paid to the delta rhythms in the vStr. Awake rats have their highest delta amplitude peaks during periods of immobility and face washing and low amplitudes during mobility and rapid eye movements (Leung and Yim, 1993). The NAc delta oscillation is suppressed in the presence of DA from VTA stimulation (Leung and Yim, 1993), which would imply an interesting relationship to the RPE signal encoded within the projection neurons to the vStr. A recent paper by Gruber et al. (2009) identified a relationship between the PFC and the NAc core in the delta band LFP. During operant lever pressing there is a strong coherence between these regions within the delta band, but not during random exploration. Taken with the aforementioned HC-NAc core theta coherence during exploration but not operant behaviour and the ability of both the HC and the PFC to influence the up states in striatal MSNs, this would imply that there is a behaviour dependent switch between these to input regions that carries a strong oscillatory marker (Gruber et al., 2009). There is an interesting relationship between

the PFC and VTA which display coherence at 4Hz and a phase coherence to the HC theta oscillations at 8Hz (2:1 cycles) (Fujisawa and Buzsaki, 2011). These three regions are main inputs to the vStr and suggest that an investigation into the delta band in the vStr would be merited and may provide some insight into the possibility of further NAc afferent coherence switching during specific behaviours.

Only recently has the beta band in the vStr become of interest *in vivo*. Howe et al. (2011) found that an increase in beta power was influenced by the delay time between reaching a goal and receiving the reward. Recent experiments by Berke et al. (2008)[10] and Howe et al. (2011) have found that beta oscillations within the vStr show increased power with experience. Though not explicit, this combination of learning and reward delay sensitivity would suggest that there are some prediction encoding properties in the beta signal that show some similarities to the prediction errors in RL. A recent study by Leventhal and colleagues (2012) proposed that increases in the striatal beta band power were reflecting a state change in the basal ganglia when a cue signaled a new action to be taken. Recording from the striatum as well as other nuclei in the basal ganglia they found that the beta signal was simultaneous but not necessarily identical across the basal ganglia, suggesting a possibility for large scale coordination (Leventhal et al., 2012). One would predict that this large scale synchronous activity would be altered during a reinforcement learning task especially if the value of the actions at decision points were to abruptly change. Though Leventhal et al. (2012) found a strong relationship to cued action, it would be of interest to investigate these beta signals without a cued action decision, but rather an internal representation of action selection as a result of a gradually learned relationship. The time difference between a cued and learned action-outcome values would also become apparent based on an unexpected outcome which could alter any planned reaction in the beta signal. This interruption in the predicted action should evoke a change in the working memory which would require some degree of hippocampal influence as well (see **Aim 1c** and **Aim 2a**). This makes the beta band a potential candidate for RPE signaling within the the vStr due to its relationship to action values and the vStr gamma oscillations (Howe et al., 2011) that have been shown to correlate with RPEs in humans (Cohen et al., 2009).

[10] Beta2 (23-30Hz)

The distribution of gamma oscillations across the vStr is expected to change in a task dependent manner due to the heterogenous distribution of limbic afferents, FSIs and DA receptor specificity. Striatal FSIs show phase-locking to local gamma oscillations (*in vitro*: Sciamanna and Wilson 2011, *in vivo*: van der Meer and Redish 2009b; Berke 2009). Recordings of behaving rats revealed that there were two populations of FSIs that had a preference to either fast or slow gamma (van der Meer and Redish, 2009b). This selectivity amongst the FSIs becomes of interest when we also consider the dorsolateral-ventromedial increase in the gamma power gradient (Berke et al., 2004) since the concentration of FSIs is greater in the dorsal/lateral regions of the striatum, thus suggesting a preference within the ventral population to fire bursts within the gamma range. This raises some questions related to the gamma induced changes across the gradient and how they may be influenced by DA which as discussed earlier has selective modulatory properties in FSIs. It is also of interest to identify the pattern of gamma oscillations across the vStr. The nearby piriform cortex has been shown to display gamma oscillations (Neville and Haberly, 2003) and could be acting through volume conductance and merely appear to originate in the vStr (Berke, 2009). This seems unlikely since the ventromedial portion of the vStr shows the highest gamma power compared to the more ventrolateral vStr which is closer to the piriform cortex. Morra et al. (2012) also compared the gamma-80 power relative to the distance from the piriform and concluded that gamma was local and not the result of volume conductance. The possibility of gamma volume conductance can easily be addressed by measuring the distributions in the gamma power across a large regularly spaced recording array in the vStr that to date has only been attempted with irregularly spaced electrode bundles (Berke et al., 2004; Kalenscher et al., 2010; Morra et al., 2012) (see **Aim 1a**). These previous attempts to map the gamma distributions has also failed to fully address both gamma bands which have been shown to display differences in power as a result of behaviour as discussed below. This gamma distribution is also of behavioural significance as the RPE encoding DA projection neurons in the VTA have selective modulatory effects across the FSIs in the NAc and would suggest a local variation in any changes in the gamma band as a result of an RPE.

Within the gamma band there are two frequency ranges seem to show a functional distinction. Both the gamma-50 and gamma-80 bands will be addressed here to illustrate the the potential role of these oscillations

to encode some elements of learning, reward perception and anticipation and potentially a RPE. The first clear evidence for a behavioural distinction between both gamma-50 and gamma-80 oscillations came from joint recordings of vStr LFPs and FSIs (van der Meer and Redish, 2009b). On a multiple T-maze, rats would display an increase in the gamma-50 at and while leaving the reward sites (an effect that seemed to increase in power with experience although this could be confounded by time spent at the reward site) while gamma-80 would increase prior to arrival at the reward site but did not linger upon leaving the site (van der Meer and Redish, 2009b). The gamma-50 and gamma-80 signals will gradually develop a more stable degree of task related modulation with experience implying that learning has a modulatory effect on these bands, with gamma-50 increasing in power over time while gamma-80 seems to decrease in power with experience (van der Meer and Redish, 2009b). The same study also noted a tendency for gamma-50 to increase in power when a correct choice was made while an error trial would result in an decrease. The onset of a reward delivery at the feeder site has a greater effect on the power of the gamma-80 oscillations than on the gamma-50 (van der Meer and Redish, 2009b). This raises an interesting question regarding any potential reset or depreciation of these experience dependent gamma changes in power when the learned paradigm is altered. It is not known if a change in the learned reward contingencies would lead to reset in the changes in gamma power or if there would be a slow progression which would be expected from traditional Q-learning RL models as the agent unlearns previous action values. **Aim 1c** offers the opportunity to study this change in gamma when the reward value are abruptly reset between recording session.

Kalenscher et al. (2010) have reported changes in the gamma rhythm related to specific elements of the reward seeking task. The first being the increased gamma-50 (with some gamma-80) power upon entering and exiting a reward site (consistent van der Meer and Redish (2009b) and that the type of reward could also be discriminated[11]. Secondly they found a weak correlation between the gamma signal the velocity/acceleration of the animal (Kalenscher et al., 2010). They also found gamma phase locking in both MSNs and FSIs and that some of these gamma bursts were task related suggesting a task induced modulatory effect within the vStr. This phase locking relationship suggests that the MSNs and FSIs are preferentially modulated within

[11]It is worth noting that this discrimination between the reward types would be associated with the time it takes to consume the reward, certain olfactory cues given in proximity to the reward site, and the time spent at the reward site.

the gamma band, which would imply that the gamma signal in the vStr is having a profound effect on the cells rather than a superfluous artifact of volume conduction. Due to the inhomogenous distribution of tetrode recordings the authors then looked for regional differences between the core and shell recording sites. In one subject they noted that none of the task related gamma oscillations were found in the core, thus suggesting that the shell would be the likely source, but this was not expanded within this study, and suggests that a regional distribution of task related gamma changes needs to be fully characterized across the NAc. They found that the differences in gamma power across the different tetrode sites seemed to be the result of locally differentiated sources as they saw some tetrodes with highly correlated power time series while others showed poor correlation suggesting that the gamma signal was heterogenous in strength across the NAc (Kalenscher et al., 2010).

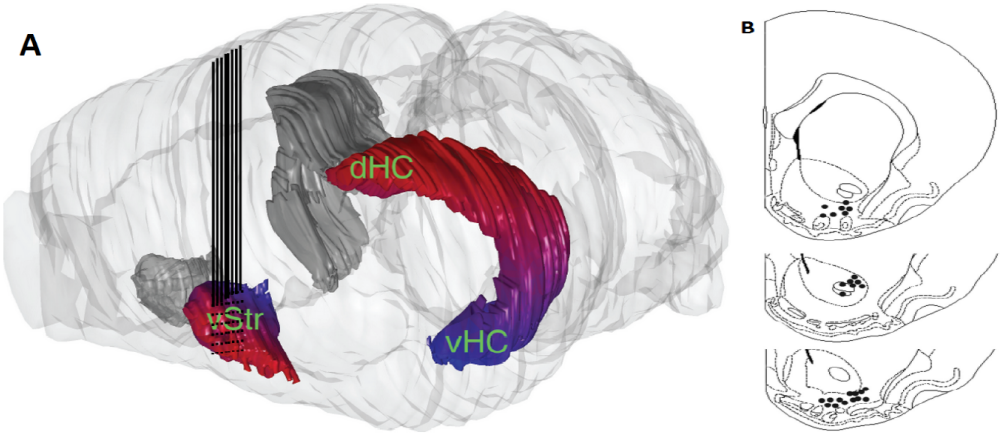

Figure 8: Comparison between a representation of location of a silicon probe array (black lines) in the vStr from my current experiment (**Aim 1**) which has been verified through histology (**A**) and the location of the tetrode bundles used in the Kalenscher et al. (2010) paper (**B**). (**A**) The probe was implanted at an angle with the lateral section extending slightly to the anterior. Note the more consistent coverage compared to the tetrode bundles in the Kalenscher et al. (2010) experiment.

The Kalenscher et al. (2010) study attempts to address key questions in the vStr literature related to task dependent gamma changes and regional distributions. Though they and others (Berke et al., 2009; Morra et al, 2012) have shown a differences in the power of gamma-50 and gamma-80 they failed to address the true regional distributions of these separate gamma band signals, by using irregularly spaced and somewhat clustered (see Figure 8B) tetrode bundles that only ever record from the core or the shell in a given subject.

A solution would be to use a rigid recording array that spans the NAc in two dimensions as per Csicsvari et al. (2003) in the hippocampus (Figure 9) and would thus allow for the identification of not only the localization and possible distinct sources of the gamma-50 and gamma-80 oscillations but other vStr rhythms that show some event related changes as well (beta and possibly delta) (**Aim 1**). Previous experiments that have made claims about regional distributions in the LFP within the vStr (Berke et al., 2004; Berke et al., 2009; Kalenscher et al., 2010; Morra et al., 2012) have never addressed the idea of prediction errors. It has also been shown that there is an inverse relationship between the beta and gamma band oscillations in the vStr with respect to learning (Howe et al., 2011). Beta appears to increase in power with learning while gamma-80 (gamma-50 was not addressed) decreases. This would also allow for the localization and relationship of task dependent changes in phasic activity in the NAc (as mentioned before) to be addressed. In addition, lesion data has shown a behaviorally significant distinction between the shell and core component yet the majority of electrophysiological studies in the area have recorded ambiguously from both regions suggesting that the current understanding of the role of the vStr may not be fully realized.

Pick Either This

In summary, we know that the theta, beta, delta and gamma oscillations show distinct behavioural correlates in the vStr. The heterogenous nature of the vStr and evidence for specific differences in afferent densities and connectivity would suggest a local distribution of oscillations that pertain to certain behaviours. Some studies have shown that gradients exist in the theta, beta, and gamma oscillations in the vStr but thus far these experiements have either not fully addressed all the distinct bands in regards to behaviour, or they have not recorded with a high enough density to see any local changes in the regions that would be expected to elicit a behaviour dependent change in the regions that seem to require

OR

The oscillations across the delta, theta, beta, and both gamma bands seem to show some clear behavioural correlates and add a further dimension of task dependent functional coding in the the vStr. The direct inter-

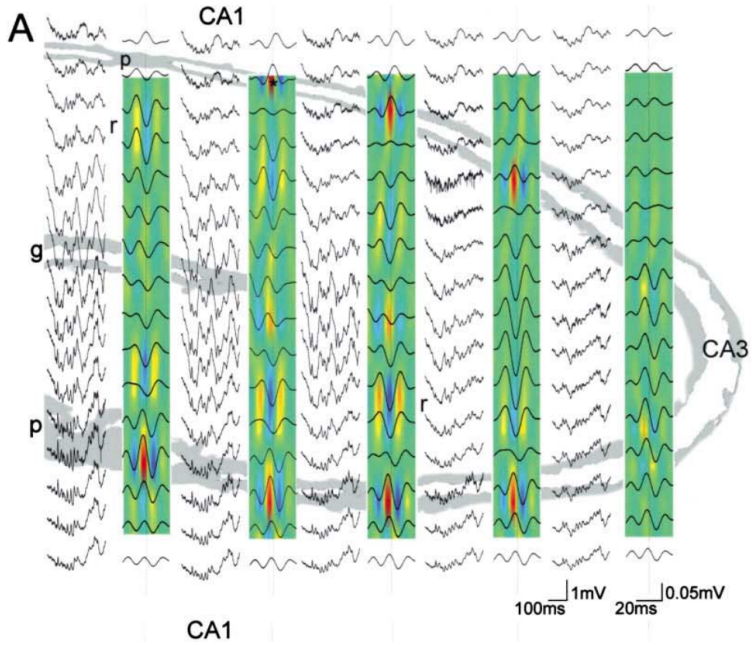

Figure 9: An example of a silicon probe recording array that spans a large portion of the hippocampus overlaid onto the actual position of the probe in the CA1/CA3. This example shows the local changes in gamma signals. The power of the gamma signals seem to follow the laminar curvature of the HC. This same technique has not been applied to the vStr with a large recording array. Since the vStr lacks this laminar organization and neuron orientation, it is unlikely that such as pattern would emerge and makes the localization of sources and sinks as has been found in hippocampal theta oscillations very unlikely. Taken from Csicsvari et al. (2003).

action between these oscillations and the firing activity of NAc MSNs and FSIs as shown by phase coupling and phase precession implies that not only could subpopulations of cells with similar frequencies and phases be in an optimal position to transfer information, but this also leads to interesting relationships with vStr afferents displaying region specific phase coherence within specific bands depending on the behaviour of the subject. Taken together, LFP analysis during active behaviour provides the opportunity to determine LFP function correlates but also how these changes in oscillatory power and frequency may add to the function distinction within certain regions of the NAc.

Attempts to determine the behavioural relationship between changes in LFP frequencies and power in the rat have so far only addressed spatial or goal directed actions but thus far have not looked directly at the

prediction errors or the acquisition of an uncued task from an experimentally naive state. Based on the RPE induced changes in the gamma band in human vStr recordings (Cohen et al., 2009), it is possible that the vStr LFP could contain valuable information related to reward prediction as the VTA DA projections have been known to alter the firing properties of FSIs in the NAc which are prime candidates for generating intrinsic gamma oscillations. Using a large regularly spaced recording array across the core and shell regions of the NAc while a rat learns to associate rewards with particular locations and then has those reward contingencies change unexpectedly, should be able to determine any changes in the local NAc LFPs as a result of a RPE.

Aim 1: Determine the distribution of local field potentials and spiking activity within the ventral striatum during a spatial reinforcement learning task

Aim 1a) - Map the power and coherence of local field potentials in the delta, beta, theta, gamma-50 and gamma-80 bands across the NAc.

Aim 1b) - Map and analyze behavior related changes in the spiking activity of NAc MSNs, FSIs and other striatal interneurons and their relation to these LFPs.

Aim 1c) - Determine if any changes exist across the local field potentials and spiking activity while the rat experiences a prediction error.

Aim 1d) - Determine if any changes exist across the local field potentials and spiking activity while the rat learns about reward locations reward magnitudes.

Optional Aim 1e) - Asses the cue induced changes in the LFP and map them to the anatomical regions that have been proposed to play key roles in their the shell and core of the vStr.

Aim 1: Experimental Design, Data Acquisition and Analysis

In order to address **Aim 1**, the task must be able to elicit a variety of different changes across multiple frequency ranges. Previous experiments have shown that approaching a reward site will elicit a change in the gamma and beta bands and that voluntary movement will cause hippocampal theta oscillations which also manifest in the vStr LFP. Though this task does not contain an operant mechanism which has been shown to cause changes in the delta band, other subtle behaviours such as passive grooming and head movements will also cause these to oscillations to manifest. Therefore the task should include voluntary movement and reward sites that can be sampled or skipped in order to map all four of the bands of interest. To promote the occurrence of a reward prediction error the task needs a consistent set of action-outcome contingencies that can be manipulated by the experimenter. Having the subject start from a naive state also allows for any experience/learning based changes across the vStr to be recorded. These learning changes would also be seen when the reward contingencies are changed across session which would require the subject to relearn the task to varying degrees. The experimental setup described here has been used on three subjects and has successfully elicited the behaviours needed for RPE and reward contingency learning.

An apparatus consisting of a 10cm wide elevated square track (1m x 1m) with a reward receptacle at each corner is used (Figure 10). Each reward receptacle has a photobeam emitter and detector pair placed at the reward delivery end in order to sense the animals nose entering the receptacle and avoid accidental tripping due to other bodily movements. A second set of photobeams will signal what arm of the track the rat is currently occupying. The rat must trigger two arm photobeams in a counterclockwise sequence in order to activate the next receptacle. Once the rat nosepokes at an activated receptacle for more than 500ms a computer controlled script delivers a specified number of dietary pellets via an automated pellet dispenser. The number of pellets is set by the experimenter for each reward site.

This testing apparatus allows for the magnitude of the reward to be manipulated. For this experiment the number of pellets at each sites is (2, 0, 2, 4; for sites 1-4 respectively) and they are delivered probabilistically (90%). The probabilistic reward system was chosen to isolate signals from reward reception and consumption

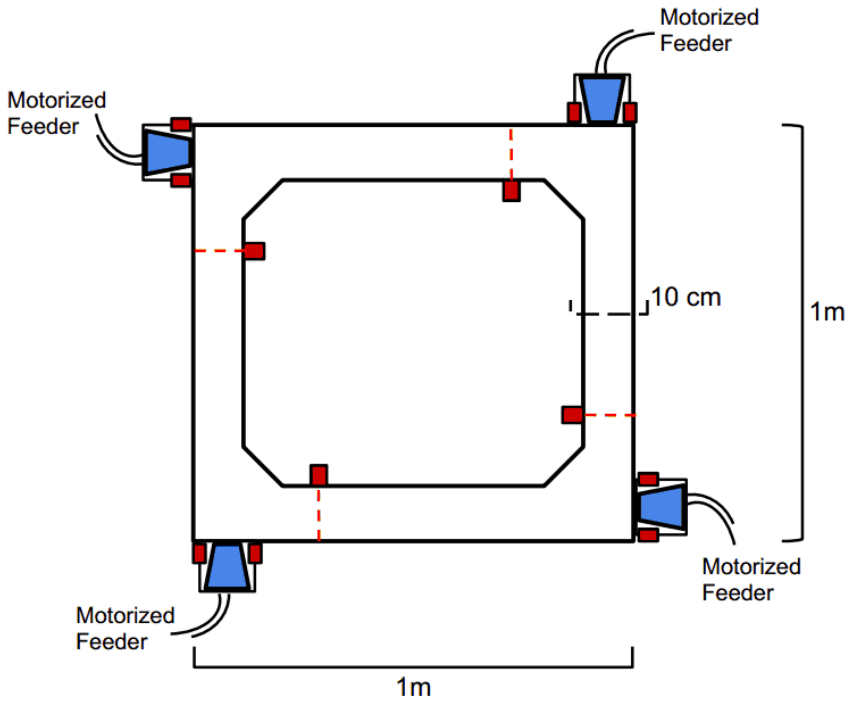

Figure 10: The testing apparatus (1m x 1m in size, 10cm wide). The rat must run counter-clockwise in order to activate the feeders. The square track has a reward receptacle at each corner (blue) with a photobeam emitter and detector (red) to detect when the rat pokes its nose into the receptacle. Additional photobeams are located on the track close to the receptacle (red dotted). These second photobeams are used to determine if the rat is running in the correct direction as they must trip two of them in sequence in order to have pellets dispense at the next feeder.

and not from the location alone. This chance reward delivery also allows for some negative prediction errors[12]. This non-uniform reward pattern allows for an assessment of reward magnitude related changes in the LFP/unit activity and reward ramping cells. This reward distribution pattern provides an indication that the subject understands the task when they consistently skip the non-reward site. Since these values remain consistent for the initial phase of testing it will allow for the observation of any learning related changes across session (**Aim 1d**). Once the subject has reached a high performance score ($> 90\%$ of non-rewarded sites skipped during three consecutive sessions), then the second phase begins where we test for any changes in the LFP and spiking activity in response to a reset of the known reward contingencies by switching the

[12]Rats do show an understanding of probabilistic reward delivery (Roesch et al., 2009) yet with such a high probability this should still elicit a RPE since the animal is expecting a reward, but this RPE may be more subtle if the rat fully understands the contingencies

non-reward site with the 4 pellet site (**Aim 1c**). This reversal should elicit a negative prediction error at the previously rewarded site and a positive prediction error at the previously non-reward site. This would be carried out until the subject regained the $> 90\%$ performance level for three sessions, then the sites would revert back to their original values. This second reversal would also determine if the animal retains the same degree of understanding from the initial setup, and thus addresses an issue in the RL literature related to extinction (Redish et al., 2007), which though not explicitly analyzed here would be of some value if some RL component related neural activity or LFP signals are found.

The subjects consist of 3-5 adult male Long-Evans rats. Each subject is implanted with either a single B-stock Neuronexus Buz64L or A8 silicon probe (Figure 11) in the left vStr (coordinates: AP = -0.5 to -2.0, ML = -1.0 to -2.5, DV = 5mm) and can be moved down further using a movable drive with a vertical range of ˜6mm (modified from Vandecasteele et al. 2012). A moveable wire reference electrode is also implanted in the same craniotomy but at a more dorsal location within the corpus callosum (DV: -2.5mm). The probe is moved on a daily basis in small increments, while the reference need only be moved to optimize the signal to noise ratio. The movement of the probe allows for a larger number of cells to be recorded within a specific DV range on the Buz64L due to its high density of recording sites in the DV axis. Though this does not provide an ideal mapping scenario for the entire range of the NAc, it does offer a greater understanding of the LFP distribution within a wide (1.4mm ML) area on a given recording session. Due to session to session changes in the reference signal, an important caveat is presented, that being that comparisons become difficult across sessions and thus a larger picture of the DV axis becomes less clear using the Buz64L. Therefore moving the probe daily increases the number of different cells that can be recorded at the cost of large area NAc mapping. The A8 probe need not be moved in this manner since the DV range of the recording sites is much more spread out and thus covers the majority of the NAc. The wide vertical recording range would allow for within session comparisons of the LFP distributions and regional changes. Referencing is key to a) removing large movement and chewing artifacts which can manifest as irregular oscillations, b) ensuring that the LFP is actually being extracted from the vStr and not from external sources that can convey signals across larger portions of the brain (eg: slow wave volume conductance). To our knowledge this is the first time that high

density silicon probes have been used in recordings of the ventral striatum *in vivo* (lower density probes were used to validate telemetry recordings *in vivo* but this did not address the properties of the signals in the vStr, rather only the identification of the gamma-50 signal as a proof of reaching the NAc targets Fan et al. 2011).

The electrical signals from the probe are multiplexed using a small headstage. The data is then collected using a 256 channel Amplipex KJE-1001 recording system. Continuous wide-band data (0.1-9kHz at 20kHz) will be acquired for offline analysis. This data is coupled with position tracking information gathered from a headstage mounted pair of LEDs and an overhead camera for use in the analysis of approach and exit velocity/acceleration. Signals from the feeder releases and the receptacle photobeams is also stored in the Amplipex system via a series of analog inputs. These analog signals will form the basis of the event identification during analysis (Figure 12 top).

Data analysis will employ the FieldTrip toolbox (http://fieldtrip.fcdonders.nl/; some of these analysis methods are outlined in Vinck et al., 2012), a broad open source neural analysis toolbox for Matlab. This toolbox offers some advantages for both **Aim 1** and **Aim 2** by combining strong LFP and spiking analysis with some artifact correction (the accuracy of which has yet to be tested with the Amplipex data sets recorded so far) and graphical mapping to histology images of the target regions. The "UltraMegaSort2000" (http://physics.ucsd.edu/neurophysics/links.html) scripts will be used to detect spikes in continuous data and MClust (http://redishlab.neuroscience.umn.edu/MClust/MClust.html) will be used to sort spikes into putative units for analysis. These tools will be supplemented by custom scripts to extract unit, ensemble, and LFP data. A basic outline of the analysis components is presented in Figure 12 (bottom).

Comparisons will be made between the different feeders to assess any changes related to the magnitude of the reward. The rewarded trials (90%) compared to the pellet withheld trials will help to identify any reward related changes or RPEs. Comparisons between arly and late (within session) rewarded and unrewarded trials to address any short-term experience or learning induced changes, whereas a comparison between the naive early sessions and later sessions at optimal performance for the initial reward location/magnitude can

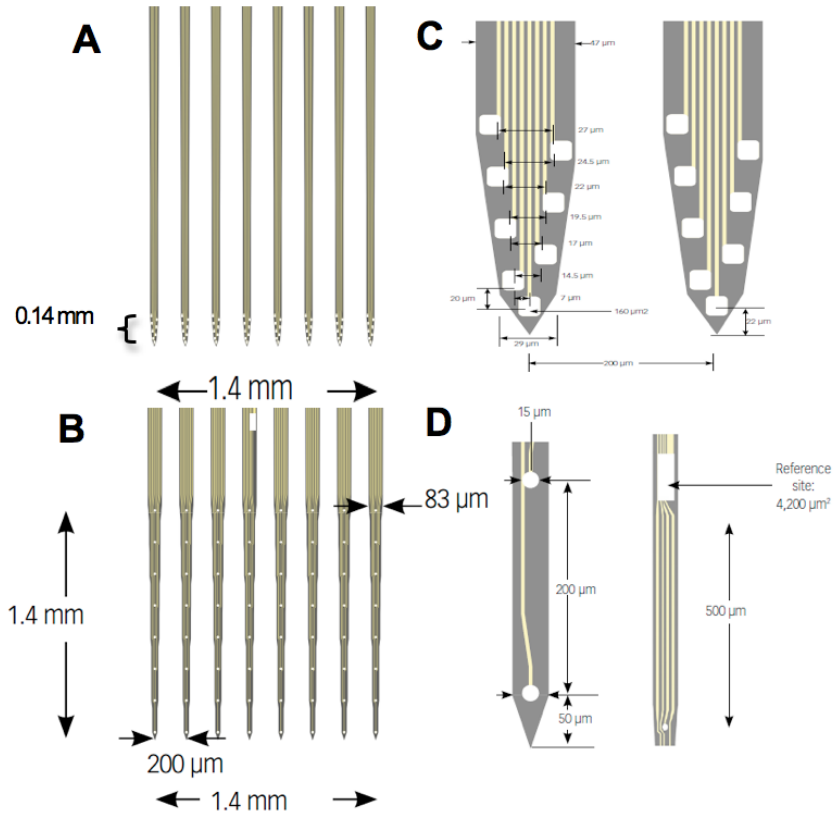

Figure 11: Detailed site maps for the Buzsaki 64L probes (A/C) and Neuronexus A8 (B/D). The two probes have 10mm long shanks but offer different degrees of spatial resolution in their recording array. The Buzsaki 64L offers the same ML span but with recordings sites only spanning roughly 400 microns in the DV axis which would provide a much higher spatial resolution (C) which becomes beneficial when looking at the specific neural activity in a smaller region of interest. Recording sites on the A8 are tenfold further apart (200 microns) compared to the Buzsaki 64L (20 microns) (compare C and D).The A8 probe has a recording array that spans 1.4mm (ML) and 1.4mm (DV) with an additional reference recoding site located on the fourth shank 500 microns above the most dorsal electrode. This allows for sampling of a larger area and would be more beneficial in LFP analysis. Images taken from the Neuronexus catalog (http://www.neuronexus.com/images/NeuroNexusCatalog.pdf).

test for any long term changes in LFP or spiking activity. These same comparisons will be made after the reward values have been switched which should promote the occurrence of RPE on the first few trials in the altered paradigm. For LFP analysis spectrograms of the peri event epoch averaged over multiple trials across sessions will be used for comparisons between the four reward sites, while within trials averages will be used for the rewarded and unrewarded trials. Testing for learning related changes becomes more difficult

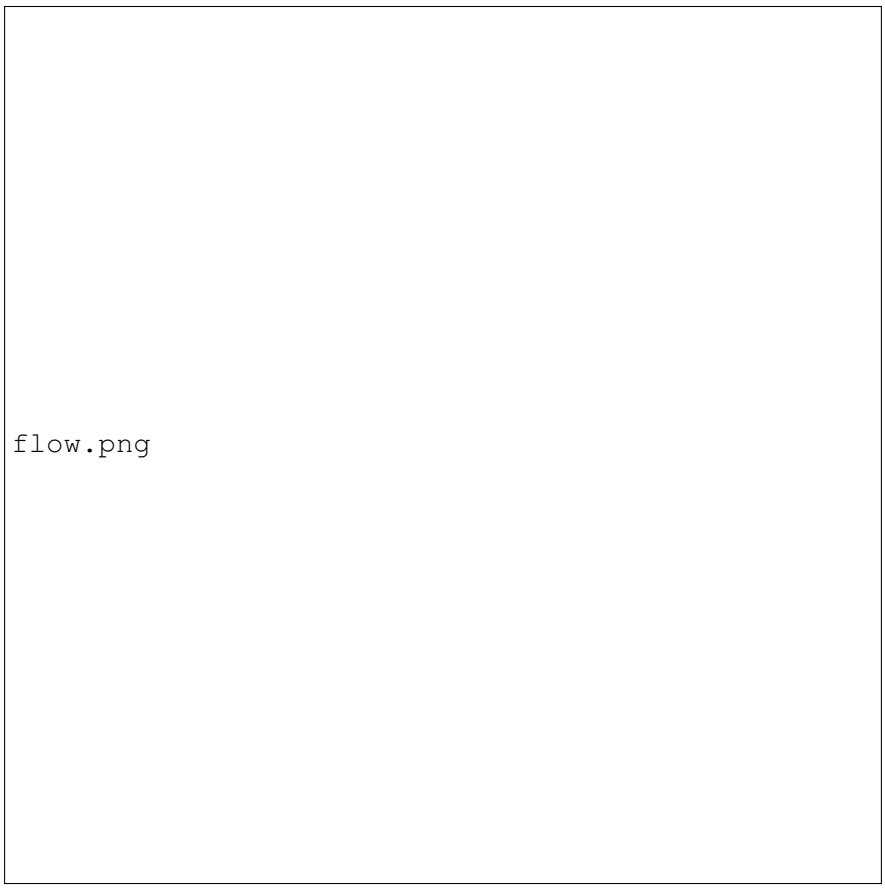

Figure 12: Flowchart of the data acquisition process (above) as well as the proposed analysis components (bottom). Note the electrical/optical stimulation (red) is only involved in **Aim 2a**.

so the trials must be binned in some way that will distinguish early trials from those later in the session. This binning approach would also be used to compare the early session to the optimal sessions. Analysis of the firing patterns of cells during these peri event epochs would be conducted in a similar manner using raster plots of the average firing rates. Further analysis of specific phase preferences/precession, modulation and coherence will be done in addition to the rasters and spectrograms.

To address Aim 1 a power spectrogram of the different bands will be overlaid onto the anatomical location of the probe (as per Figure 13) during specific rewarded and RPE epochs. This same method will be applied to other behaviours such as approach and exit from reward sites, resting behaviour, different running velocities and accelerations, grooming and chewing in order to isolate any confounding behaviours. This should be

able to identify local differences in the power across the NAc during events of interest. It is expected that since the FSIs in the NAc have the ability to generate gamma oscillations and that these intrinsic oscillations can be modulated by VTA DA projections, that there should be some local changes in the NAc during an RPE event or during reward acquisition. In addition, it would provide a better perspective into the local distribution of other oscillations such as the theta signal that pertains to the different vStr afferents which would be expected to produce regional changes based on the behaviour of the rat.

Early analysis of one of the subjects implanted with a Buz64L has yielded some promising data. Clear gamma oscillations can be seen across 56 channels on the probe with varying levels of intensity (Figure 13). This would suggest that there is some spatial distribution regarding the gamma oscillations across the medial lateral axis in the more dorsal region of the NAc (The lower rows in Figure 13 correspond the more medial shanks while the upper rows correspond to the lateral shanks) and already begins to provide some, albeit not definitive, answers to **Aim 1a**.

Aim 1b looks to identify any changes in the properties of NAc neurons that show modulatory changes based on certain LFP bands such as phase precessing reward ramp cells (van der Meer and Redish, 2009a), and how these modulations change with behaviour. This analysis will require the isolation of spikes within the continuous data via an automated program such as "UltraMegaSort 2000" which can then be used with MClust to isolate neurons whose activity is modulated by an LFP band or neurons that show change in firing activity that correlates with a particular component of the task. The activity of these behaviour or LFP modulated cells can then be assessed during the events mentioned in the previous section. The two probes types offer the opportunity to record across a large area and detect if any of the cells showing modulation within a band have a regional distribution that coincides with the local inputs or any gradients of the modulating oscillator. Even if this fails to identify any new neural correlates of goal directed behaviour, it would still be of interest to investigate any of the known cells such as anticipatory ramp cells and their regional modulation by theta and other bands.

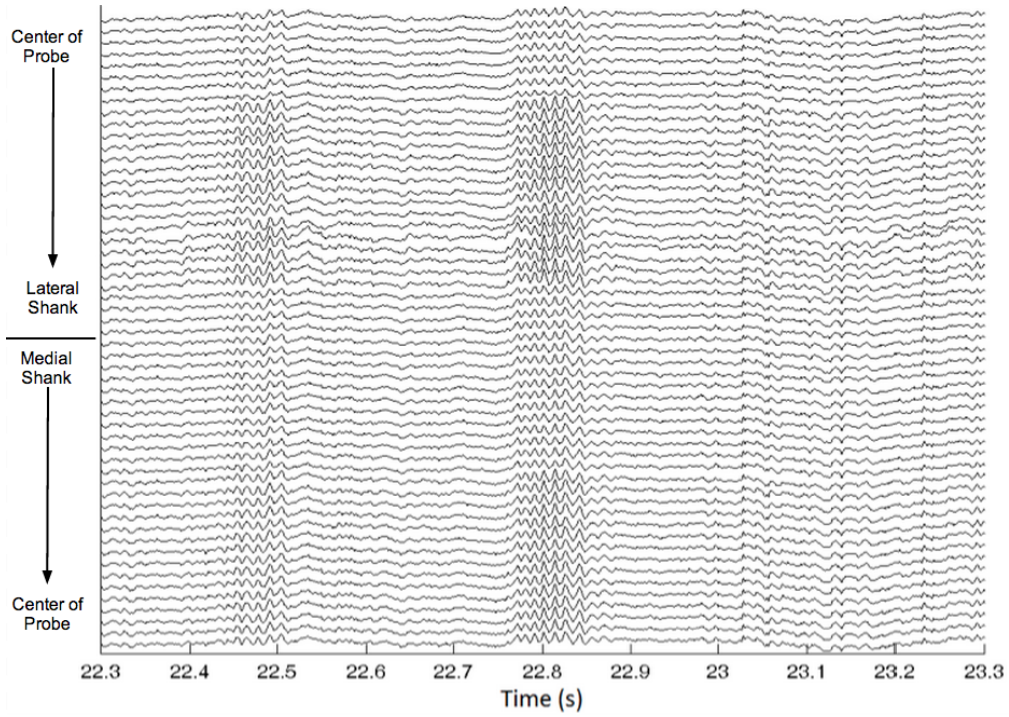

Figure 13: A sample (taken from session R036-2013-05-29) of the gamma oscillations seen in a one second window during a recording session with a Buzsaki 64L probe. Note the difference in the amplitude of the gamma burst across the channels. This suggests a local difference in the power of the gamma oscillations. This signal distribution offers some initial insight into **Aim 1a** with respect to the gamma band and demonstrates the ability of the silicon probe to detect changes in the LFP signal across a large array. At a quick glance 15 putative spiking units were identified in addition to the above LFP signal, and we believe that more can be identified upon closer inspection.

The identification of RPE induced changes in the LFP (**Aim 1c**) should be the most profound during the reversal phase of the experiment. The feeder that would yield the most pellets is switched with the unrewarded site which should produce a negative RPE at the old 4 pellet site and a positive prediction error in the old no reward site. This distinction between a positive and negative prediction error becomes of interest since certain VTA/SNc DA terminals in the dorsal striatum have been identified as RPE encoding but the direction is not clear (Oyama et al., 2010). Should an RPE exist in the LFP, as has been seen in humans in the gamma band (Cohen et al., 2009), it is of interest to test these RPEs over time to determine if any learning or experience based changes exist (**Aim 1c/d**). Though it seems likely that there would be some change in the gamma band as a result of a RPE, even if this turns out to not be the case, it is still of interest to address any

regional differences in the LFP signals which have been shown to increase or decrease in power as a result of learning. The RPE task offers the opportunity to assess initial task learning as well as adaptation. The changes in the delta band which have been shown to pertain to operant tasks in the core (Gruber et al., 2009) can also be further localized as well.

A optional modification to the current experimental design would allow for the identification of cue related changes in the LFP and neuronal activity as well (**Optional Aim 1e**). Visual cues have been shown to modulate oscillations in the beta (*phase reset in response to a known cue in* Leventhal et al. 2012) and possibly gamma bands (*noted observation in* Berke 2011) as well as certain populations of cells in the NAc show phasic firing related to the onset of these reward paired cues (Berke, 2011; Roitman et al., 2005; Day et al., 2011; Goldstein et al., 2012). In addition, lesion data suggests a regional difference in cue guided behaviour between the NAc core and shell (Ito et al., 2008). We thus hypothesize that a) the local distribution of changes in the gamma and beta bands would correspond to the regional differences in cue-guided behavior, and b) that the activity of action value encoding neurons should be altered based on whether a cued paradigm or spatial task is being employed. A simple modification to the current apparatus could distinguish between spatial-reward pairs and non-spatial cues in order to test these hypotheses. The same track would be used with the addition of LEDs at each receptacle (currently in place but not in use). The subject would then be conditioned to respond to the lights rather than the learned 0-2-4-2 sampling pattern. If used during the same session as the normal protocol, a tone could be used that would inform the rat of the switch from the spatial sampling to the cue sampling pattern, then direct comparisons can be made between the cued and place related changes in the LFP and spiking to determine if there is a regional preference for the type task or a shift in neural firing rates or LFP power when going from the cued task to the spatial (or vice versa).

Data from two early probe implants have shown that noise reduction is an issue with these probes. The first implanted subject used a skull screw above the cerebellum as the reference. Through the first probe coupled with the skull reference screw we were able to discern certain oscillations (mostly theta with some gamma) but they were highly susceptible to movement and chewing artifacts and spike detection during recording

was difficult, if not impossible. The second subject (used in Figure 13) was implanted with a similar Buzsaki 64L in the same location but this time the recording probe was moveable and one of the two references was connected to single reference wire 3 mm above the probe tip that allowed for variation in the location of the reference while the other reference wire was attached to the skull mounted Faraday cage and skull screw. This implanted reference offered a much better signal to noise ratio and made visualization of the LFP much more clear and allowed for spikes to be seen during offline analysis. The implanted reference was also more resistant to movement artifacts but chewing was still an issue. A possible solution for the chewing artifacts which is currently being tested in our lab is the use of liquid rewards, though peristaltic muscle movement in the throat and tongue lapping could still produce some noise it should be much less than the vibrations in skull from the teeth or from the temporalis muscle during chewing. The most recent implant used a moveable wire reference electrode and thus far has had an even better noise reduction. The A8 probe offers a reference electrode on the central shank which though not independently moveable, should also offer a good method for reducing noise, however, it is still relatively close to the recording site and could subtract some valuable signals. A second option for dealing with this noise issue is to use artifact removal software during the analysis phase. FieldTrip offers such a tool but it has not yet been tested on continuously recorded data from the Amplipex system.

The data acquired during **Aim 1** will provide a high resolution snapshot of the changes in the LFP and spiking activity across a large two-dimensional grid in the NAc during place-reward learning and during prediction errors. A logical step would be to expand this picture to the afferents of the NAc, specifically the HC. The relationship between the HC and vStr has been well defined with regards to the theta band and in relation to exploration and operant behaviours, but *in vivo* inactivation has not addressed any potential changes in the theta modulated reward ramp cells. It is also of interest to address other LFP bands such as gamma, beta and delta. Dual site recordings in the vStr and HC would help to better understand the properties of vStr cells that show HC theta modulation in relation to place-reward or cued learning as well as any changes in working memory when the reward contingencies are altered (as per the above experiment).

Aim 2: Investigate the relationship between the ventral striatum LFPs/spiking activity and the hippocampus and how disruptions to the hippocampal projections to the ventral striatum will alter the activity within the NAc.

Aim 2a) - Use optical inactivation, electrical stimulation or pharmacological agents to disrupt the hippocampal projections to the ventral striatum to determine the response of theta modulated reward cells, HC-vStr LFPs, and reward acquisition strategies.

Aim 2b) - Determine the degree of hippocampal influence on the NAc in the gamma band oscillations during a spatial reward learning task using high density recordings to identify pairs of neurons and the degree of synchrony/coherence in different LFP bands.

Aim 2: Experimental Design, Data Acquisition and Analysis

The testing protocol and apparatus will be identical to those described in Aim 1. The implant surgery will be the same for the ventral striatum silicon probe as well. In addition to the recording devices in the vStr, an additional pair of bipolar electrodes will be placed roughly 60 microns apart in the ipsilateral fimbria/fornix bundle as per Al'bertin et al. (2003) which contains the main projections from the vSub to the vStr (coordinates AP=-1.3mm, ML= 0.9-1.9mm, DV= 3.7mm) (Figure 14). This will allow for transient stimulation of the projection bundle to induce disruptions in the signals being sent from the HC to the vStr. A second approach would be to block the glutamatergic projections from the vSub pharmacologically. This alternative would involve the implantation of a cannula in the vSub which could apply micro injections of the GABA agonist muscimol which has been popular in hippocampal and subicular inactivation (Bonnevie et al., 2013; Biedenkapp and Rudy, 2009), or the sodium channel blocker lidocaine (Floresco et al., 1997). However subicular neurons have been shown to have variable reactions to GABA (Taube, 1993) making this the less viable option.

The clear alternative to electrical stimulation and pharmacological inactivation would be the use of optical activation/inactivation which not only offers greater selectivity based on the viral expression but also much

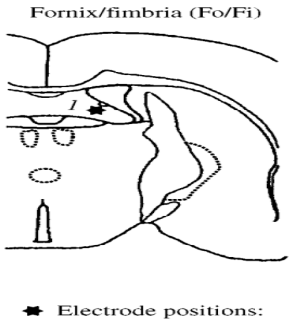

Figure 14: The location of the proposed fimbria/fornix stimulating electrode site. The electrodes will be on the ipsilateral side to the HC and vStr recording sites. Taken from Al'bertin et al. (2003).

better temporal resolution. Britt et al. (2012) have shown that bidirectional manipulations of glutamatergic projection neurons in the vHC, BLA and PFC via optical stimulation can elicit excitatory postsynaptic currents (EPSCs) in the NAc, with the vHC showing the greatest increase. They employed an adeno-associated virus, channelrhodopsin-2[13] (ChR2-EYFP) for stimulation or Natronomonas halorhodopsin (NpHR)-EYFP for inhibition, that was associated with the alpha-CaMKII promoter which would allow for the targeting of the glutamatergic projection neurons. Ultimately this Britt et al. (2012) study found that regardless of the source, the presence of increased glutamate in the NAc was sufficient to reinforce instrumental behaviours. This would allow for **Aim 2a** to be carried out. We are in communications with Sylvain Williams at the Douglas Institute who has found that good expression levels of the proton pump Arch in vHC pyramidal cells can be achieved by infusion of an adeno-associated virus that also contains a yellow fluorescent protein tag. To ensure the optical stimulation is having the correct effect in the targeted cells, this would require additional recording sites either via silicon probes or tetrode bundles in the vHC/vSub.

The exact timing of the electrical stimulation within the task would be determined based on any epochs of HC-vStr synchrony found in pre-testing recordings using the same subjects using the two recording sites. Of particular interest would be the periods of noticeable theta phase precession in the vStr as the rat approaches the reward site as described by citetVanderMeer2009. Muscimol would have a much longer period of inacti-

[13] As noted in Britt et al. (2012), channelrhodopsin-2 can take weeks to months to travel down an axon. This is something that needs to be considered in the timeline

vation and would require a series of test days which would either be under muscimol inactivation or normal conditions, making it more difficult to extract the specific changes related to RPE and learning.

Should these vHc/vSub disruptions yield a promising change then the next logical step would be to characterize this relationship using either a second silicon probe or multiple tetrode bundles into the vSub (AP=-5.8mm, ML= 4.8mm, DV= 8-9mm) which has been shown to have the strongest connections to the vStr. This alone will allow **Aim 2b** to be accomplished and would provide one of the few examples of recordings from the vSub. There is a lack of electrophysiological recordings in the vSub in the first place is due to the distance from the skull which makes tetrode recording difficult as the tetrode wires would either bend and be inaccurate or would require a cannulae to get them to the target and thereby damage a large portion of the HC on the way down. Another issue is the increased noise when recording in the deep ventral region since this is so close to the base of the skull and will suffer from chewing artifacts. The silicon probes offer a solution to the problem of electrode rigidity and have been used successfully in very deep HC recordings (Royer et al., 2010). Sebastian Royer has been able to show good recording signals in the vSub using the same Buz64L probes (unpublished data, learned through correspondence).

Analysis would be conducted in the same manner as for **Aim 1**. Particular attention would be paid to the LFP synchrony of HC and vStr cells. This could be accomplished using cross-correlation analysis surrounding salient task epochs such as feeder nose pokes and reward delivery cues (feeder motor sounds), pellet arrival, as well as approach and exit periods as per Tabuchi et al. (2000) would be useful for identifying any correlates of changes in HC-vStr pair activity related to these events.

Conclusion

The proposed series of experiments would address four unclear themes in the literature. The first being a systematic mapping of the LFPs and spiking activity across the NAc which to date has only had recordings done in irregular locations and has not covered the wide spectrum of behaviourally relevant LFP bands. The second question addressed here is the possibility of a neural correlate of a RPE in either the unit activity

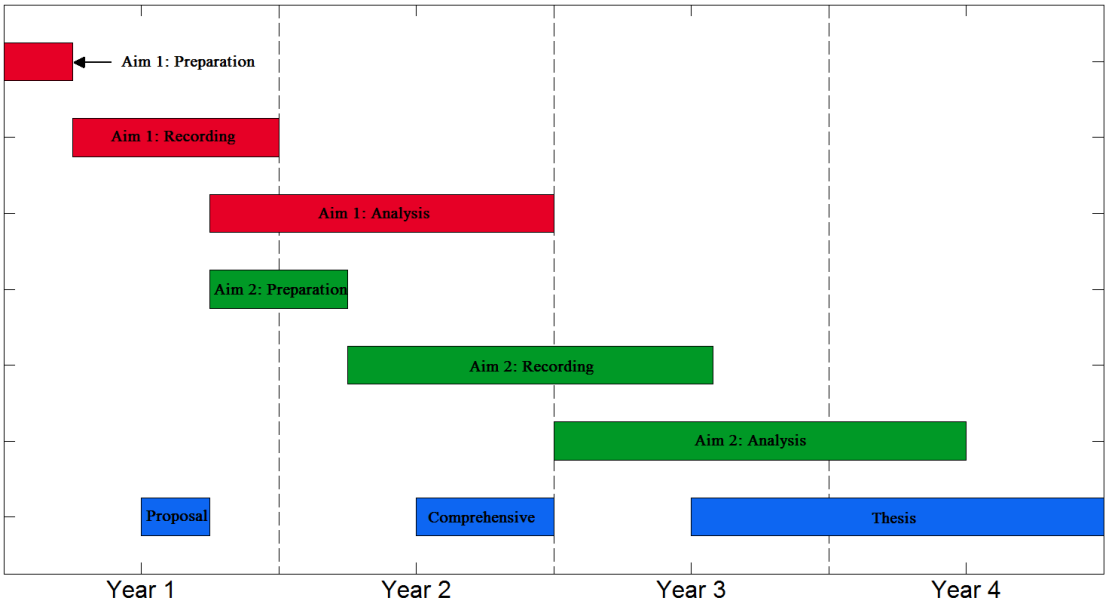

Figure 15: Proposed timeline for the experiments in aims 1(red) and 2 (green), as well as the PhD milestones (blue). All the preparations for the first experiment have already been carried out and recording of the third subject (out of 5) is already underway. The preparation phase for the second inactivation experiments will vary depending on the method of inactivation, with electrode or pharmacological blockage taking less time than the optogenetic approach due to acquisition of the virus and laser/cables. Note this timeline is quite generous with respect to the second experiment and the thesis writing phases.

(which seems unlikely) or within the LFP the NAc, which though not has not been present in unit recordings could still exist at the population level, most likely in the gamma band. Third, the value of experience on behaviourally relevant LFP changes will also be tested. Though experience has been shown to alter the LFP signals in the beta and gamma bands, thus far experiments have not been able to determine the degree of change as a subject acquires the task from an agnostic state and how a shift in the reward expectations would alter the power of these signals. By facilitating both naive learning and an abrupt shift in the reward patterns (and possible cue versus place paradigms) this question of experience based LFP changes can be addressed. Finally the influence of the HC inputs on theta modulated cells in the vStr as well as any interactions with striatal delta, beta or gamma oscillations can be tested. Inactivation and dual recordings will allow for the persistence of theta modulation on reward ramping cells in the vStr and any greater influences on the vStr LFP can be investigated. Together these objectives address both internal changes in the vStr as well as one

its main afferents during both goal directed behaviours and changes in learned action-reward contingencies.

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