The Role of the Striatum in Working Memory

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Abstract

Several lines of evidence suggest that the striatum has an important role in spatial working memory. The neural dynamics in the striatum have been described in tasks with short delay periods (1-4s), but remain largely uncharacterized for tasks with longer delay periods. We collected and analyzed single unit recordings from the dorsomedial striatum of rats performing a spatial working memory task with delays up to 10s. We found that neurons were activated sequentially, with the sequences spanning the entire delay period. Surprisingly, this sequential activity was dissociated from stimulus encoding activity, which was present in the same neurons, but preferentially appeared towards the onset of the delay period. These observations contrast with descriptions of sequential dynamics during similar tasks in other brains areas. Ongoing experiments using temporally precise perturbations at different phases of working memory are aimed to clarify the contribution of the striatum in spatial working memory.
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2 Organization of the thesis

This thesis presents the results of experimental work investigating how the striatum contributes to working memory in the brain. In Chapter 2, I will review some background on what we know about working memory and its implementation in the brain, and motivate the work done in this thesis. In Chapter 3, I introduce the behavioral assay that I use for studying working memory in rat in all the experiments that follow. In Chapter 4, we establish that performance in our spatial working memory task, requires neuronal activity in the dorsomedial striatum, the brain region of interest. Once, a causal relationship is demonstrated, we then investigate the neuronal correlates of the task in Chapter 5. Chapter 5 includes results from extracellular recordings of neuronal activity in the dorsomedial striatum. We employ various analytical methods to characterize spiking patterns of striatal neurons and show that it contrasts with other brain regions implicated in working memory. Importantly, we demonstrate that these results hold even when controlling for motor/positional confounds. In Chapter 6, I will describe ongoing experiments that are aimed at clarifying the role of the striatum using temporally precise perturbations while rats perform the spatial memory task. Finally, in Chapter 7 we discuss our interpretation of the results presented in the thesis and the future directions for this line of research.
3 Introduction

3.1 Working Memory and Its Implementation in the Brain

Working Memory (WM) is one of the most fundamental components of cognition. It refers to the ability to mentally store and manipulate information over short periods of time, typically over periods less than a minute, and use that information to guide ongoing behavior (Baddeley, 1992, 2003; Cowan, 2008). Working Memory is typically conceptualized in different phases. First the memory is loaded, either from external stimuli or from information generated by mental processes. Then the “delay period” follows, during which the memory must to be mentally stored without the assistance of external stimuli. And finally, the memory is retrieved. (See Figure 1).

Figure 1 - The phases of working memory. The horizontal axis represents time. Loading and retrieval are separated across a delay period during which the memory must be mentally stored.
What is intriguing about working memory is that it operates at time scales too short for it to be mediated through long-term synaptic plasticity, which requires protein synthesis (Kandel, 2001; Sutton and Schuman, 2006). Without a mechanism for implementing working memory, all memories would need to be consolidated before they can influence behavior and experiences cannot be used to immediately guide behavior over short time scales. This would make a simple task, like remembering a phone number only to dial it seconds later, impossible to implement. Given its fundamental nature, much effort has been invested in studying the underlying neural mechanisms of working memory.

One of the most important findings in this regard is that neurons encode memories over short time scales by modulating their spiking activity, with different memories corresponding to different spiking patterns. This was first demonstrated in the 1970s from single unit recordings in the prefrontal cortex of monkeys performing a delayed response task (Funahashi, 2015; Niki, 1974) and has since been observed in many brain regions across many organisms (Major and Tank, 2004). Neural correlates for memory have typically been characterized as persistent changes in firing rates. However, it is important to note that firing rates of individual neurons do not necessarily have to be persistently modulated throughout the entire delay period, to carry information forward in time. For example, neurons in the posterior parietal cortex of mice performing a T-maze memory task have been shown to be sequentially activated
during the delay period, with different sequences corresponding to different memories (Harvey et al., 2012).

### 3.2 The Role of the Striatum in Working Memory

Most studies of working memory have focused on prefrontal cortical regions of the brain, partially due to the relative abundance of neurons that are persistently activated during the delay period. (Arnsten, 2011; Baeg et al., 2003; Clark et al., 2012; Erlich et al., 2015; Funahashi et al., 1989; Fuster and Alexander, 1971; Guo et al., 2014; Hanks et al., 2015; Harvey et al., 2012; Horst and Laubach, 2012; Jung et al., 1998; Kojima and Goldman-Rakic, 1982a; Lak et al., 2014; Powell and Redish, 2014; Romo et al., 1999; Schoenbaum and Eichenbaum, 1995; Shadlen and Newsome, 2001; Spellman et al., 2015; Wang et al., 2013b; Yoon et al., 2008). However, working memory is not implemented merely in cortex, but instead emerges from the interaction between cortical and subcortical areas (Floresco et al., 1997; Kopec et al., 2015; Parnaudeau et al., 2013), with the striatum as a key subcortical region. For example, human imaging studies have noted increased activation of the striatum during working memory tasks (Chang et al., 2007; Lewis et al., 2004; Olesen et al., 2004; Postle and D’Esposito, 1999). In addition, in the primate caudate, metabolic activity and single cell recordings point to elevated activity during spatial working memory (i.e. tasks that involve a memory for location) (Kermadi and Joseph, 1995a; Levy et al., 1997a). Finally, electric stimulation or
lesions of the primate caudate, as well as pharmacological silencing of the analogous region in rats, the dorsomedial striatum (DMS), leads to disruptions of spatial working memory (Balleine and O’Doherty, 2010; Cohen, 1972; Mordvinov, 1981; Rosvold and Delgado, 1956a; Spencer et al., 2012; Stamm, 1969).

Different theories have emerged as to how the striatum contributes to implementing working memory. These theories rely on the known anatomical connections from cortex to striatum to thalamus and back to cortex, referred to as the “cortico-striato-thalamic loop” (See Error! Reference source not found.). These loops are organized in the form of distributed parallel pathways maintaining their separation all throughout the loop (Alexander et al., 1986, 1990; Houk and Wise, 1995a; Middleton and Strick, 2000a, 2002a). One theory is that the striatum is involved in the maintenance of memories throughout the delay period. Distributed cortico-striato-thalamic loops may provide a neural substrate for memory maintenance by generating positive feedback that sustains the delay-period activity widely observed in cortical areas.
(Alexander et al., 1986, 1990; Houk and Wise, 1995b; Middleton and Strick, 2002b; Wang, 2001). An alternative theory suggests that the striatum is involved in initiating the storage of new memories in cortical networks, an idea often referred to as “gating” (Baier et al., 2010; Frank et al., 2001; Gruber et al., 2006; O’Reilly and Frank, 2006; T. S. Braver, 2000). According to this idea, the striatum plays a role in selecting cortical memory buffers that are to be updated and determines the time memory updating occurs, similar to the role the striatum is thought to play in selecting and initiating motor actions (Bailey and Mair, 2006; Bhutani et al., 2013; Grillner et al., 2005; Kropotov and Etlinger, 1999; Mink, 1996). More specifically, by activating in the loading phase of memory, the striatum disinhibits thalamus through the direct pathway and transiently provides positive feedback to cortex, taking the network to a state of stable elevated activity that can be maintain during the delay period independent of the striatum, i.e. through local reverberations or through cortico-thalamic loop feedback.

3.3 Motivation

In this thesis we attempt to elucidate the role of the striatum in working memory. To this end, we chose to study working memory in the rat striatum, a model system capable of acquiring complex behaviors that also has a wide range of available tools and methods for circuit interrogation. We train rats on a spatial working memory task (see Chapter 4) that requires remembering a lever side (one of two possibilities) for a
variable delay length and making a binary choice at the end of the delay period. We use the rat choice accuracy on the task as a readout of how well the rat is able to mentally store a memory. Different theories about the striatum’s role in working memory posit differences in activity patterns and differences in the temporal causal role of the striatum in a working memory task. Using in vivo extracellular recordings, pharmacology, and transient optogenetic perturbations, we hope to clarify how the striatum contributes to working memory, and confirm, refute, or reform the existing theories.

We focus on the dorsomedial striatum (DMS) a region implicated in spatial working memory and other related aspects of cognition (Corbit and Janak, 2007a; Jin et al., 2014; Kimchi and Laubach, 2009; Ragozzino et al., 2002; Stalnaker et al., 2010; Wang et al., 2013a; Yin et al., 2005). The DMS receives the most projections from the prefrontal cortex (Mailly et al., 2013) and it is a homologue of the primate caudate (Balleine and O'Doherty, 2010) that has been previously implicated in working memory (Cohen, 1972; Kermadi and Joseph, 1995b; Levy et al., 1997b; Mordvinov, 1981; Rosvold and Delgado, 1956a; Stamm, 1969). This decision was reinforced by the studies that compare the rat dorsomedial striatum (DMS) with the dorsolateral striatum (DLS) and find that the DMS is involved in higher order cognitive functions, e.g. planning, and model based learning, versus the DLS which is involved in lower level functions, e.g.
motor planning and habitual learning (Balleine and O’Doherty, 2010; Burton et al., 2015; Ito and Doya, 2015; Kim et al., 2013; Stalnaker et al., 2010; Yin and Knowlton, 2006).

In addition, striatal activity during working memory needs to be thoroughly characterized. Neural correlates of working memory in the striatum have been characterized to some extent in the case of relatively short delay period (1-4s) (Antzoulatos and Miller, 2011; Chiba et al., 2015; Hikosaka et al., 1989; Histed et al., 2009; Kawagoe et al., 1998; Kermadi and Joseph, 1995a; Pasupathy and Miller, 2005) but less is known about striatal dynamics in the case of longer delay periods. This is a significant knowledge gap, given that animals (and humans) can remember stimuli over many seconds in real world situations. We sought to answer several questions by examining firing patterns of striatal neurons while rats perform this task. First, is sustained delay-period activity in the striatum a feature of activity in the case of long delay periods (>4s), as has been observed in primates for short delay periods (1-4s)? (Hikosaka et al., 1989; Kawagoe et al., 1998; Schultz and Romo, 1988; Schultz et al., 1994) If so, does that activity encode the memory of the stimulus throughout the delay period?

Another possibility is that there is sequential transient activation of neurons in the striatum during the delay period. This is a reasonable hypothesis, given that 1) memory-encoding sequences have been observed in cortical and hippocampal areas in working
memory tasks (Fujisawa et al., 2008; Harvey et al., 2012; Horst and Laubach, 2012; McDonald et al., 2013; Pastalkova et al., 2008), and 2) the striatum is known to exhibit sequential activity in tasks that do not directly involve working memory (Lustig et al., 2005; Matell and Meck, 2000, 2004; Mello et al., 2015). If we do observe sequences during a working memory task, do those sequences encode the memory of the stimulus throughout the delay period, as has been observed in other brain regions?

To address these questions and characterize striatal dynamics during working memory, we trained rats to perform a spatial working memory task that involved long delay periods (up to 10s). We recorded single unit activity during this task from the DMS, a region involved in spatial working memory and other related aspects of cognition (Corbit and Janak, 2007b; Jin et al., 2014; Kimchi and Laubach, 2009; Ragozzino et al., 2002; Stalnaker et al., 2010; Wang et al., 2013a; Yin et al., 2005). We used information theoretic analyses and population decoding to characterize the neural dynamics in the recorded population.
4 The Delayed Non-Match to Position Task (DNMP)

4.1 Introduction

In this chapter we introduce the Delayed Non-Match to Position task (DNMP) (Dunnett et al., 1988), which is the central paradigm used in this thesis to study working memory, and we will characterize the behavior of rats trained on this task. The behavioral characterizations will be focused solely on the recording sessions from the rats implanted with multielectrode arrays (see Chapter 0).

Rats are placed in an operant chamber with two retractable levers (see schematic in Figure 2). Each operant session consists of hundreds of trials. At the beginning of each trial, a sample lever appears in one of two possible positions, i.e. either the left side or right side of the front wall of the chamber. The rat was required to press the sample lever, at which point the lever retracts into the wall and the delay period begins. The rat must remember the sample lever side for the duration of the delay period (1s, 5s, or 10s - randomly interleaved), because at the end of the delay period both levers are extended and the rat must press the opposite lever to be rewarded, i.e. the lever with the “non-matching” position to the sample lever. To prevent rats from using motor bridging strategies (more on this in section 4.3) we required the rat to move to the
of the chamber during the delay period. Specifically, the end of the delay period is signaled by the illumination of the nose-port on the back wall of the chamber (left side of schematic, see Figure 2). The rat must then poke his nose into the illuminated nose port for both levers to extend from the front wall of the chamber, and then choose one of the two levers. Following a correct lever press, i.e. a choice lever press that does not match the initial sample lever, the rat is rewarded with 40μl of milk in the reward. Rats were kept on a limited food diet to motivate behavior. The rats were given 30s to respond to the sample lever, 5s to respond to the activated at the end of the delay period, and 5s to respond to the choice lever. An incorrect lever press or a failure to respond within the time limit, resulted in a 5s time-out penalty, during which the house-light is turned off. Trials were followed by 10s long inter-trial interval (ITI).

It is difficult to train rats to maintain their nose in a nose-port for the entirety of the delay period. Thus, we only required them to make a nose poke at the end of the delay period. However, the task was designed to encourage the rats to spend the delay period at the nose port. Rats did not know the length of the delay period in advance, so they almost immediately move to the nose port after a sample lever press and spent the majority of the delay period in the nose-port (Figure 2 bottom panel and Figure Y).
Figure 2 - Delayed non-match to position (DNMP) task. A. Schematic illustration of the task structure. A trial starts with the presentation of the sample lever at one of two locations ("sample press"). By pressing the sample lever, the rat initiates the delay period (1s, 5s, or 10s duration). At the end of the delay period, the nose-port on the back wall of the chamber is activated, and by entering the port (i.e. nose-poking), both levers are presented. By pressing the lever that does not match the sample lever at the beginning of the trial (i.e. choice press), the rat receives a reward; pressing the other lever results in a timeout (T/O). B. Top: Schematic illustration of the chamber and the rat’s position at the time of the sample lever press (left), the delay period (middle), and the choice lever press (right). Bottom: Occupancy-map of the rats’ head position for right-sample trials at the time of the sample lever press (time window from 250ms before to 250ms after lever press), delay period (center, time window is the entire delay period for 10s delay period trials only), and choice lever press (right, time window from 250ms before to 250ms after lever press). Occupancy-maps were generated by averaging the occupancy-maps of the 9 rats used in the electrophysiology experiments. Individual occupancy-maps were calculated by binning the head positions over the respective time windows into 0.5" × 0.5" tiles (covering the 9.5" × 12" chamber).
4.2 Behavioral Performance

Of note, the rats’ accuracy in this task declined with the length of the delay period (p < 10^{-4} repeated measures ANOVA, Figure 3A). This delay-dependence provides validation that the short-term memory component of the task played a role in the rats’ performance, as expected in a working memory task.

Figure 3 – **A.** Accuracy is delay dependent, decreasing as the duration of the delay period increases (p < 10^{-4}). Solid black line represents mean accuracy, and dotted grey lines represent the accuracy for each individual rat. Accuracy was calculated from the final recording session. **B.** Rate of incomplete trials (defined as percentage of trials in which the rat failed to respond to either the sample lever, nose port, or choice lever, within the allowed time-limit) are less than 10% across all delay periods. Dashed lines are for individual rats and solid line is the average across all rats.
In addition, omission rates and response latencies during the task revealed that the rats were highly engaged during each trial. Omission rates were low regardless of the length of the delay period (less than 10%, Figure 3B). In addition, median response latencies were short for all subjects for all 3 types of response (median sample press latency < 3.5s, median nose-poke latency < 1s, median choice press latency < 3.5s; Figure 4).

Figure 4 - Response latency for each individual rat for sample press (time from sample presentation to sample press), nose-poke (time from nose-port activation to nose-poke), and choice press (time from choice presentation to choice press). Center of each boxplot represents the median, edges correspond to 25th and 75th percentile, and whiskers correspond to 5th and 95th percentile.
4.3 Controlling for Motor Mediating Strategies

Working memory refers to the ability to the *mental* storage of values. Tasks that require working memory can be solved using strategies that exploits external stimuli, physical position, posture, or motor actions for carrying information forward in time. (Think of using your fingers to count). The DNMP task is susceptible to motor mediating strategies because the rat is permitted to freely move during the delay period. We introduced a nose port at the other end of the chamber to prevent rats from staying in front of a lever during the delay period. Our task was designed such that a nose poke was required only at the end of the delay period, but since rats could not predict the

![Graph](image)

Figure 5 - Average percentage of time spent waiting at nose port from the delay period onset, averaged across the 9 rats of the electrophysiology experiment. The shading is ±1 SEM.
length of the delay (either 1s, 5s, or 10s determined randomly) they typically moved to the nose port immediately after pressing the sample lever (see Figure 5). This modification in the task was intended to reduce that chance that rats adopt a motor mediating strategy to bridge the delay period. The delay dependency in their performance (see Figure 3) also indicate a mental component to the memory.

In conclusion, is important to control for motor mediating strategies to the extent possible. Using video tracking of LED lights installed on the head-stage of rats performing the task, we track the head position of rats during the entire session. We show that the conclusion that we draw from the striatal recordings hold even when controlling for head position (See Chapter 6.2.3).
5 Striatal Activity has a Causal Role in Spatial Working Memory

5.1 Introduction

In primates, a number studies have causally linked the caudate with performance in working memory tasks. These studies include electric stimulations and lesions of the caudate (Cohen, 1972; Mordvinov, 1981; Rosvold and Delgado, 1956b; Stamm, 1969). To the author’s knowledge, there has only been one study in rats that show a causal dependency to dorsomedial striatal activity in a working memory task (Spencer et al., 2012). Thus, it becomes crucial to establish that there is a causal relationship between the rat dorsomedial striatum (DMS) and the spatial memory task at hand.

To this end, we show that pharmacological inactivation of the DMS impairs performance in the delayed non-match to position (DNMP) task, leading to more incorrect choice lever presses. We conclude that DMS activity has a causal role in spatial working memory.

5.2 Methods

All procedures were performed in accordance with the university-approved IACUC protocol.
5.2.1 Behavioral Assay

7 Male Long Evans rats were trained on a delayed non-match to position (DNMP) spatial working memory task in operant chambers. (See Chapter 4). Sessions were one to two hours long. The rats were kept on a limited food diet and fed once a day after the training session to motivate behavior.

5.2.2 Surgical Procedure

For the cannula implantations, 10 adult Long Evans rats (> 300g) that were previously trained in the DNMP task were deeply anesthetized and a double guide cannula was implanted bilaterally above the dorsomedial striatum (DMS) (A/P: 1.2mm, M/L: ±1.9mm, D/V: -5.25mm, taking into account the 1.25mm or 2.75mm projections of internal cannulas used during injections).

5.2.3 Muscimol and Saline Infusions

After a week of recovery time post-surgery, the rats were retrained on the DNMP task until behavior re-stabilized. Before each testing session, rats were anesthetized in an induction chamber and were then moved to a nose cone to maintain anesthesia (with 2% isoflurane) for a total duration of 20-25 minutes. During this period the cap and dummy cannulae were removed and internal cannulae with appropriate projection
length were inserted into the guide cannula. 600nl of saline or muscimol solution was infused at a rate of 200nl/min. The internal cannula was removed and dummy cannulae were inserted 4 minutes after each infusion, to allow the solution to diffuse. Rats were given 20-25 minutes of recovery time after the infusion and anesthesia, before being placed in the operant chamber to begin behavioral testing. To identify the muscimol concentration that was used for testing each rat, a range of muscimol infusion concentrations (37.5ng-75ng) was tested on different days, and the largest concentration for which each rat performed a sufficient number of trials during a session was identified (at least 50 trials per session). Once the concentration was identified, the rats underwent two days of additional infusion sessions. On the first day they received an infusion of saline, and on the second day they received an infusion of the muscimol concentration selected as described above. Concentration and volume were similar to values used in previous studies (Spencer et al., 2012; Yin et al., 2005). The protocol for saline infusions was identical except saline was used instead of muscimol.

Of the 10 implanted rats, 3 rats were not included in the final data set. One rat became sick over the course of the study. One rat did not perform the task under the range of muscimol concentrations that we infused. And one rat had a brain infection that was evident in the post-mortem histology analysis.
5.2.4 Histological Analysis

After the infusion experiments, rats underwent a transcardial perfusion of PBS followed immediately by perfusion of 4% PFA in PBS. Brains were placed in 4% PFA in PBS solution for 24 hrs. Then they were transferred to a solution of 30% sucrose in PBS. 40um thick sections were generated with a microtome. The relevant DMS slices were stained with DAPI. The depth of the guide cannula implants were visualized with a stereomicroscope (Leica) and the infusion sites were determined by adding the length of the internal cannula projections. All 7 rats included in this report had accurate targeting.

Figure 6 - Red circles with blue fillings represent location of injection cannula tips as revealed by post-mortem histological analysis. Anterior/Posterior coordinate indicated along each coronal slice.
in the DMS (See Figure 6).

5.3 Results

To determine if neural activity in the DMS has a causal role in the DNMTP task, we inactivated the DMS using the GABA_A agonist muscimol, while rats performed the task. We found a significant decline in choice accuracy with the infusion of muscimol in comparison to when the same rats received an infusion of saline (See Figure 7A; p < 0.001 effect for infusion day, repeated measures ANOVA, n=7). The accuracy impairment was also significant when taking the subset of trials with 10s or 5s delay periods (See Figure 7B) Importantly, DMS inactivation had no significant effect on the response latencies for rats, suggesting a lack of motor impairment (See Figure 7C). Other behavioral measures were also not significantly affected with the infusion of muscimol (See Figure 8).

Given that activity in the DMS is needed for performing the task, we believe that characterizing the neural dynamics during the task is inherently interesting. The next chapter pertains to characterizing the neural dynamics in the DMS.
Figure 7 - A. Muscimol infusion in DMS impairs accuracy when compared to infusion of saline (p < 0.001; effect of muscimol repeated measures ANOVA, no significant interaction effect between delay length and infusion day). Error bars are ±1 SEM across 7 rats. B. Effect of DMS inactivation on accuracy of each individual rat for each delay length. Accuracy was significantly impaired for the 5s and 10s delay trials (p < 0.05; Wilcoxon signed rank test) but not for 1s delay trials (p = 0.38; Wilcoxon signed rank test). C. Effect of DMS inactivation on median response latency of each individual rat for sample press (top), nose-poke (middle), and choice press (bottom). No significant effect was found for any of the three response latencies (p=0.58, p=0.28, and p = 0.92, respectively; Wilcoxon signed rank test).
Various behavioral measures were not significantly affected across rats with the infusion of muscimol. **number of trials**: total number of trials completed (correct trials and error trials) within the session (n = 7, Wilcoxon signed rank test, p = 0.22). **sample omission rate**: percentage of all trials where the rat omitted the sample lever press (n = 7, Wilcoxon signed rank test, p = 0.38). **trial abort rate**: percentage of trials where the rat omitted the nose-poke or the choice lever press (n = 7, Wilcoxon signed rank test, p = 0.11). **sample bias**: defined as the absolute value of the difference between left sample omission rate and right sample omission rate (n = 7, Wilcoxon signed rank test, p = 1.00). **choice bias**: defined as the absolute value of the difference between accuracy on left sample trials and accuracy on right sample trials (n = 7, Wilcoxon signed rank test, p = 0.16).
6 Neuronal Correlates of Spatial Working Memory in the Dorsomedial Striatum

6.1 Introduction

Given that the neuronal activity of the dorsomedial striatum (DMS) has a causal role in the delayed non-match to position (DNMP) spatial working memory task, we would like to know what firing patterns in the DMS look like. Do we observe sustained elevated activity during the delay period? Do the firing patterns encode the memory during the delay period? If so, is this done through sequences or sustained changes in firing rates? These are all questions that would help us further characterize the role of the striatum in working memory.

Primate in vivo physiology has led to some degree of characterization of striatal firing patterns during working memory tasks (Antzoulatos and Miller, 2011; Chiba et al., 2015; Hikosaka et al., 1989; Histed et al., 2009; Kawagoe et al., 1998; Kermadi and Joseph, 1995a; Pasupathy and Miller, 2005). But those studies typically used shorter delay periods (at most 4 seconds long). The findings we present in this chapter would have been hard to identify had we used short delay periods, and thereby provide new insights to striatal dynamics in working memory tasks.
6.2 Results

Spiking activity for 105 neurons was isolated from the DMS of 9 rats (summary of electrode localizations in Figure 9A). Between 5 and 25 units were recorded in each subject (Figure 9B, inset). The average firing rates of these units tended to be low (population mean < 6Hz) (Figure 9B), consistent with previous reports of medium spiny neurons in the dorsal striatum (Berke, 2008; Berke et al., 2004; Mallet et al., 2005).
6.2.1 Sequential Activation of Neurons

To determine if and how the neurons’ firing rates evolved during the course of a trial, we examined the firing rate trajectory of each neuron relative to the onset of the delay period. The recorded units displayed a diversity of firing rate trajectories; for

Figure 10 - Sequential transient spiking activity spans the delay period. A. Raster plot (top) and smoothed peristimulus time histogram (bottom) aligned to the onset of the delay period for an example neuron. Shaded area represents ±1 SEM. B. Heat-map representing the z-score of firing rates for all units for the 10s, 5s, and 1s delays, aligned to the onset of the delay period. Each row is a single unit. Rows in all three plots were sorted by ascending order of peak time in the 10s delay trials (left-most plot).
instance, the example unit depicted in Figure 10A showed a transient peak in its firing rate a few seconds into the delay period. In order to visualize the activity across the neural population, we ordered the units by the time of the peak firing rate for the 10s delay trials, and then generated firing rate heat-maps for 1s, 5s, and 10s delay-duration trials (Figure 10B). Neurons displayed sequential peaks of activity that spanned the entirety of the 10s long delay period (Figure 10B, left panel). The firing rates for the shorter delay trials displayed similar sequential activity throughout the delay period (Figure 10B, middle and right panel), when ordered based on the peak times for the 10s delay-duration trials \((p<10^{-7}, \text{Spearman correlation test between peak times of 5s-delay trials and 10s-delay trials})\). Note, by design, only neurons that were engaged during the first 1s or 5s of the 10s-long delay period could be part by the sequence for the shorter delay trials, which is why the sequences involved fewer neurons for the shorter delay periods.

To further validate and characterize the finding of sequential firing rate peaks, we repeated the same analysis after randomly shifting the time of the recorded spikes and the behavioral timestamps for each neuron (Figure 11). The randomly shifted data (Figure 11) differed dramatically from the real data (Figure 10B), confirming that the activity sequences that we observed were not an artifact of ordering the activity based on the peak response. In the shifted data, sequences were non-existent in the 5s and 1s duration trials and there was no correlation between the order of the peak firing rate
for the 10s delay-duration trials and for the 5s delay-duration trials ($p=.29$, Spearman correlation test). As another method to quantify the presence of sequential firing rate activity, we calculated the ridge-to-background ratio (Harvey et al., 2012). Ridge to-background levels were significantly greater than chance level for all delay period lengths (Figure 12, $p < 0.001$; one tailed test using the ratios from the randomly shifted data as the null distribution). In addition, in comparison to the shifted data, the

![Graph showing random shifted activity sorted by peak time in 10s delay](image)

**Figure 11 – Sequential activity expected by chance.** A. Same as Figure 10B, but spike times were randomly shifted within the session. Rows were sorted according to peak time of randomly shifted activity in the 10s delay trials (left-most plot). The narrow peaks in the left-most plot and the absence of sequences in the middle and right-most plots contrast with Figure 10B. B. Histogram of peak times for 10s-delay trials for non-shifted data (Figure 10B) and randomly shifted data (panel A). Comparison of histograms reveals the peaks were biased towards the beginning of the delay period ($p < 0.02$; Wilcoxon rank sum test).
distribution of peak firing rate times in the real data revealed a bias towards the onset of the delay period (See Figure 11B). In other words, although the sequences spanned the delay period, there were more peaks towards the beginning of the delay period relative to what would be expected by chance ($p<.02$, Wilcoxon rank sum test).

6.2.2 Transient Encoding of the Sample Stimulus

To determine how firing rate modulation during the delay period relates to the sample lever selectivity of the neurons, we compared neural activity for trials when the sample lever was in the right versus the left location, excluding error trials and omission

![Figure 12 - Ridge-to-background ratios of delay period activity during 1s delay trials (left), 5s delay trials (middle), and 10s delay trials (right). Green bars represent ridge-to-background ratios from the real data and correspond to the three heat-maps in Figure 10. Blue bars represent the mean ridge-to-background ratio obtained by circularly shifting the spike times by random values 1,000 times. For all three delay periods, ridge-to-background ratio was significantly larger than that expected by chance ($^* p < 0.001$; one tailed test using the ratios from the randomly shifted data as the null distribution).](image)
Individual neurons exhibited different patterns of sample selectivity (Figure 13). One example neuron displayed no choice-selective modulation (left panels, Figure 13), another neuron was only activated during right sample trials (middle panels, Figure 13), and finally a neuron displayed different patterns of activation for left and right sample lever trials (right panels, Figure 13).

Figure 13 – Example neurons vary in how they encode the sample stimulus. A. Each column represents data from an example neuron, with neural activity aligned to the onset of the delay period, and activity color-coded in red for right sample trials and left for blue sample trials. Top: raster plot, with each dot representing a spike. Middle: firing rate trajectories for left and right sample trials. Shaded area represents ±1 SEM. Bottom: Information about sample as a function of time. Shaded area represents the 99th percentile of the maximum information expected by chance across the entire time interval, calculated by shuffling the sample labels for the trials.
Given the diversity of responses in individual neurons, we turned to mutual information as a method to quantify the amount of information about the sample stimulus encoded in the firing rate as a function of time (Borst and Theunissen, 1999; Dayan and Abbott, 2001; Rieke et al., 1999). We calculated the time-varying mutual information between the sample stimulus and the spike trains of individual neurons (within a 1s sliding window), by assuming that spiking of each neuron is a Poisson point process with a time varying rate determined by the sample stimulus and time from the delay onset. We found many neurons showed transient information peaks (e.g. example neurons 2 & 3 in Figure 13). To determine if these peaks were statistically significant, we calculated the distribution of the maximum information across the entire delay period that would be expected by chance for each neuron by repeatedly shuffling the labels specifying whether a trial had a left or a right sample and recalculating the mutual information. A neuron was considered to significantly encode the sample stimulus during the delay period if its peak sample information was larger than the 99th percentile of that distribution (shaded area in the bottom panels of Figure 13). By this criteria, 53 of the 105 neurons showed significant encoding of the sample stimulus at some point during the delay period (p < 0.01) with peak information values ranging between 0.07 to 0.69 bits (average peak information was 0.34 bits).

To compare firing rate sequences to stimulus encoding across the recorded population, we compared the time-varying firing rate alongside the sample information
for the subset of neurons that encoded the stimulus significantly at some point in the delay period (See Figure 14A). Surprisingly, the sample information peaks did not display a sequential organization to match the peak firing rate sequence. The time of the peak sample information did not correlate significantly with the peak firing rate time (See Figure 14).

**Figure 14 - Sample lever information peaks are clustered towards the beginning of the delay period.** **A.** Left: Heat-map showing information about sample as a function of time for the 53 neurons that had significant information peaks within the 10s delay period (p < 0.01; one-tailed test using shuffled data for null distribution of peak information within the 10s delay period). Rows are sorted based on the peak firing rate times to match right heat-map. Right: Heat-map showing z-score of firing rates of the same neurons depicted in the left plot. Neurons in both the left and right panels are sorted by the peak firing rate in the delay period, such that a neuron on the left plot appears in the same row in the right plot. **B.** Time of peak sample information plotted against the time of peak firing rate for 53 sample encoding neurons (where 0 corresponds to the onset of the delay period). The data reveals sample information peaks occur earlier than firing rate peak times (p < 0.001; Wilcoxon signed rank test), and a lack of correlation between the time of peak firing rate and time of peak sample information (p = 0.49; Pearson correlation test). All panels are calculated using 10s-delay correct trials only.
Figure 14, Pearson correlation test, $p = 0.49$). In fact sample information most often peaked towards the onset of the delay period, with more than 80% of the sample encoding neurons (45/53) displaying peak information within the first 2s of the delay period. On average, peak sample information was significantly earlier than peak activity ($p < 0.001$; Wilcoxon signed rank test. Only neurons with significant information peaks were considered for this analysis).

We found that individual neurons encode the sample stimulus at specific times in the delay period, and that for many neurons sample encoding was strongest towards the beginning of the delay period (See Figure 14). To quantify how sample encoding in the DMS changes throughout the course of the trial we constructed a population decoder that takes spiking activity of all the recorded neurons within a 500ms sliding window and evaluated its performance using leave-one-out cross validation (Brown et al., 2004; Gerwinn et al., 2009; Ma et al., 2006; Pouget et al., 2003; Quiroga and Panzeri, 2009). In order to combine neurons across rats, we create pseudo-trials (see Chapter 6.3.8 for Methods). This provided us with a time varying sample decoding accuracy representing how well DMS neurons encode the sample stimulus at different parts of the delay period (Figure 15). In agreement with our findings from the mutual information analysis (Figure 14), sample decoding was highest at the onset of the delay period (at the time of sample lever press) and declined over the course of the delay period (Figure 15A).
In order to compare the neural code in DMS for correct and error trials, we calculated the output of the decoder on error trials (after the decoder was trained on correct trials). This allowed us to determine when in the trial neural activity encoded the identity of the sample versus the identity of the choice. This distinction is difficult to make without analysis of error trials, because in correct trials the sample and choice levers perfectly predict one another. Given that the decoder for error trials is trained on correct trials only, a significantly lower than chance level decoding accuracy means

![Figure 15 - Performance of population decoder for sample identity. A. Accuracy of decoding sample lever from population spiking data within a 1s-wide sliding window in 10s-delay correct trials. Decoder was trained on 10s-delay correct trials and evaluated using leave-one-out cross validation. B. Same as A but decoding 10s-delay error trials. Decoder was trained on all 10s-delay correct trials. In both panels, the dotted lines marked with “nose-poke” marks the median time of nose-poke relative to the onset of the delay period, and the dotted line marked as “choice press” marks the median time of choice press. Shaded area represents ±1 SEM.](image-url)
higher than chance level encoding the choice lever. The accuracy of decoding error trials was high towards the beginning of the delay period, quickly dropped and stayed at chance level throughout most of the delay period, and fell below chance near the choice lever press time (Figure 15B). This means that neural activity around the time of the sample lever press encodes the sample lever identity, as opposed to the rat’s future choice. In contrast, at the time of the choice lever press, neural activity encoded the identity of the choice lever, as opposed to the sample identity.

6.2.3 Controlling for Motor/Position Confounds

Since the rats were allowed to move freely during the delay period, it is crucial that we examine motor/position confounds in the results presented above. Here, we use head tracking data to show that our conclusions stand even when controlling for movement during the delay period. Specifically, we show the following. 1) The sequential activity presented in Figure 10, cannot be explained in terms of motor/positional correlates, and exist even when the rat is stationary at the nose port. 2) The strong sample selectivity in individual neurons at the onset of the delay period (presented in Figure 14) cannot be explained as merely selectivity for motor actions or position (e.g. selectivity for pressing the left lever versus pressing the right lever), and that the sample information in neuronal spike patterns are dependant on the structure of the task. 3) Striatal activity during the delay period actually encodes the mental
representation of memory rather than encoding the possible differences in their movements and positions between left and right sample trials.

In this thesis, we only control for head position, and not body posture or orientation. This means that our controls are not perfect, but it is important to recognize that controlling for all possible motor/positional confounds is difficult as even muscle tension may mediate the delay period.

Figure 16 – Sequential activity is present when controlling for position, aligned to delay period onset. 
A. Z-score for firing rates when aligned to delay period onset (sample press). Each row corresponds to one neuron. Left-most plot was generated by only using data from times that the rat was at the nose port. White area corresponds to when the rat was not at the nose port for a sufficient amount of time (see Methods). Middle plot was generated using data from all times regardless of the rat position, but time bins were masked to match masking of left plot. Right-most plot was generated using all data without any masking. Rows are ordered based on the time of their peak firing rate in right-most plot. B. Peak activity time when rat was in the nose port (left plot of panel A) was significantly correlated with peak activity time calculated using all data (middle plot of panel A) ($r = 0.74$, $p < 10^{-6}$ Pearson correlation test). Neurons that had their peak activity fall in the masked time bins (white area in middle heat-map of panel A) were excluded ($n = 35$).
Because the rats were not stationary during the entirety of the delay period, the possibility arises that the sequences were in fact a byproduct of neural selectivity for position or movement. To control for this possibility, we re-calculated the sequences while using only the subset of time during which the rats were positioned in front of the nose port (Figure 16). We found that even when controlling for the rats’ position by masking times that they were not at the nose-port, sequences were evident that closely matched the sequences based on the full dataset (0.74 correlation coefficient, p < 10^{-6}).

**Figure 17 - Sequential activity is present when controlling for position, aligned to nose port arrival time.**

A. Z-score for firing rates when aligned to first nose port arrival. Each row corresponds to one neuron. Left-most plot was generated by only using data from times that the rat was at the nose port. White area corresponds to when the rat was not at the nose port for a sufficient amount of time (see Methods). Middle plot was generated using data from all times regardless of the rat position, but time bins were masked to match masking of left plot. Right-most plot was generated using all data without any masking. Rows are ordered based on the time of their peak firing rate in right-most plot. 

B. Peak activity time when rat was in the nose port (left plot of panel A) was significantly correlated with peak activity time calculated using all data (middle plot of panel A) (r = 0.87, p < 10^{-6} Pearson correlation test). Neurons that had their peak activity fall in the masked time bins (white area in middle heat-map of panel A) were excluded (n = 37).
Pearson correlation test). This correlation was even stronger when aligning to the moment they arrived at the nose port after pressing the delay period, rather than aligning to the delay period onset, i.e. the moment they press the sample lever (See Figure 17). In fact, sequences are more pronounced when aligning to the nose port arrival time, as revealed by our ridge-to-background ratio analysis (See Figure 18). We conclude that sequential activations of DMS neurons appear even when the rat is stationary and therefore cannot simply be a result of selectivity for movement or position.

As an additional approach to control for the possibility that sequential firing activity might be due to position and locomotion selectivity, we demonstrate that time in the delay period is a significant predictor of neural activity, even when taking into account position/locomotion variables as alternative predictors. More specifically, we modeled each neuron’s spiking activity using two generalized linear models (GLMs), one with position related predictors only (i.e. head position, head direction, and velocity – all calculated using head tracking) and the other with both the position-related predictors as well as time from the delay period onset as a predictor (see Methods for details). Time from delay onset significantly improves the model in 80% of the neurons (84/105) in comparison to a model with only the position/locomotor variables (p < 0.0001, likelihood ratio test comparing models with and without time from delay period as
predictor). This demonstrates that the time-dependent changes in firing rates during the delay period cannot be accounted for by position or the other variables we tested.

We next considered the possibility that high sample information at the onset of the delay period may be due to the fact that the rat is simply in different locations or performing different actions in the two conditions, and that striatal activity correlates with the motor action and position. To determine if neurons are encoding the identity of the sample lever rather than motor actions or location in the chamber, we took advantage of the task design in that the rats performed the same lever pressing action at the same location but in different contexts of the task, i.e. either in the sample phase or the choice phase. More specifically, if striatal neurons only encode the action or the location, we would not to expect to see differences in the neural coding for sample lever presses and choice lever presses. Note that the rats’ head position is very similar at the time of sample and choice lever presses (Figure 19).

We found that many neurons differentiate between sample and choice presses in their encoding of lever identity. For instance, the example neuron in Figure 19B responds to right sample presses but not right choice presses. Such a dramatic difference cannot be explained merely as a result of encoding of motor actions or position since the rat performs the same action at a similar location but we observe very different neural responses. Much like the example neuron (Figure 19B), across the
population of recorded neurons (Figure 19C), some neurons preferentially encoded lever identity during the sample press, while others during the choice press. In fact, there was significantly more dispersion in the difference between sample and choice information than expected by chance (inset in Figure 19C, $p < 10^{-5}$; two sample F-test for equality of variance for (sample information - choice information) in real vs shuffled data). This analysis supports the conclusion that DMS firing patterns are not merely reflecting position or motor actions.
Figure 19 - Context-Dependent Neural Encoding of the Lever Press. **A.** Left plots show similarity of head position during left lever press for sample lever press (top) and choice lever press (bottom). Right plots are same as left plots except for right lever press. Average head position occupancy-maps from 250ms before to 250ms after the lever press. Occupancy-maps were generated by averaging individual occupancy-maps of all 9 rats (0.5"×0.5" tiles covering the 9.5"×12" chamber). **B.** Firing rates of an example neuron during sample lever press and choice lever press. This neuron displays dramatic difference in its encoding of lever side between the sample and choice presses. **C.** Neurons differentiate between sample and choice presses in the amount of information conveyed about the lever identity. Each neuron’s information about lever side was calculated by taking the mutual information between lever identity and spiking patterns within a 500ms time window centered at the lever press (to match time window used in panel A). Orange circles represent sample lever information plotted against choice lever information. Green circles are obtained by recalculating the information after shuffling sample and choice labels for lever presses. Insets are histograms of choice information minus sample information for non-shuffled data (orange) and shuffled data (green). Neurons showed significantly greater dispersion in the difference between sample and choice information than expected by chance ($p < 10^{-6}$; two sample F-test for equality of variance for (sample information - choice information) in shuffled versus non-shuffled data).
Given that rats are allowed to move freely during the delay period, we next considered the possibility that sample encoding during the delay period (Figure 15) may simply be a result of encoding of different movement trajectories for left and right sample trials. To address this, we examined a time point during the delay period during which the movement trajectories converge for left and right sample trials. Following the sample press, rats typically move to the opposite wall and wait in front of the nose port for the delay period to end (Figure 5). Instead of aligning trials to the beginning of the delay period (i.e. the sample lever press), we aligned trials to the moment the rats first arrived at the nose port, and excluded the small subset of trials in which they left the nose port within 1.5 seconds of the nose port arrival. We show that head position is very similar across left and right sample trials following nose port arrival (Figure 20A), yet our population decoder was able to decode the sample stimulus with high accuracy, even during the first 1.5s when the rat strictly maintains its head position in front of the nose port (Figure 20B). This is important as it provides evidence of memory encoding within striatal neurons during the delay period while controlling for the rats’ position on left vs right sample trials.

In an alternative approach to determine if the sample memory is present in DMS activity in a form beyond encoding of position/locomotion, we sought to test how well the sample stimulus can improve predictions of firing patterns when including other variables as predictors. Towards this end, we modeled each neuron’s spiking activity
using two generalized linear models (GLMs), one with position/locomotion related predictors only and the other with both task-related and position-related predictors. The position/locomotion predictors we used were head position, head direction, and head velocity and the task-related variables were time from delay period onset, sample stimulus of current trial, and the interaction between time and sample stimulus. We performed a nested model likelihood ratio test for
each neuron to compare the two models, to determine if the addition of the sample memory related variables improved the model (see Methods for details). For more than 90% of neurons (95/105), the model significantly improved when including the sample memory related variables (p < 0.0001; Pearson’s chi-squared test). These neurons included 51 of the 53 sample encoding neurons identified through the mutual information analysis (Figure 14).

6.3 Methods

All procedures were performed in accordance with the university-approved IACUC protocol.

6.3.1 Behavioral Assay

Male Long Evans rats were trained on a delayed non-match to position (DNMP) spatial working memory task in operant chambers. (See Chapter 4). Sessions were one to two hours long. The rats were kept on a limited food diet and fed once a day after the training session to motivate behavior.

6.3.2 Surgical Procedure

9 adult Long-Evans rats (> 300g) that had previously undergone training in the DNMP task were deeply anesthetized and electrode arrays were implanted either unilaterally or bilaterally over the DMS. The implants were omnetics based TDT micro-
wire arrays, composed of 1-2 rows of 8 polyimide insulated tungsten wires (50μm diameter, with 175μm spacing within a row and 500μm spacing between rows). The DMS was targeted stereotactically (A/P: 1.2mm, M/L: ±2.0mm, D/V: -4.0 to -5.0mm) and electrodes were oriented so that the length of the rows went along the anterior/posterior axis. A ground screw was implanted in a posterior location on the skull and connected to the ground wire from the array.

6.3.3 In-vivo electrophysiological recordings

After a week of recovery time, the rats were retrained on the DNMP task while simultaneously being habituated to having wires connecting the implant arrays to the TDT 32 channel recording system through a motorized commutator. After 2 weeks of retraining and habituation, each rat underwent a single recording session of 1.5-3.5 hrs in, in which 200-520 trials were recorded. Video tracking of the head position and orientation was performed and digitized simultaneously (RV2, TDT) using two head-mounted LEDs.

6.3.4 Head Tracking

All head tracking data was obtained by using the TDT RV2 system, linked to a camera directly above the chamber. The RV2 system was trained to detect two LEDs mounted on top of the rat head stage (one green and one red). Linear interpolation was used to fill in the gaps for the periods when the signal was lost for either of the LEDs. To
determine when a rat was “at the nose port” (Figure 17 and Figure 20) we first found the median head position coordinates for when the rat made nose pokes. Whenever a rat was within 2.5” of that position, that rat was considered to be “at the nose port”. The nose port arrival time was calculated accordingly.

6.3.5 Histological Analysis

After the recording experiments, under deep anesthesia, electrolytic lesions were generated at each electrode tip to enable the locations of the electrode tips to be visualized. Immediately after the lesions were generated, the rats received a transcardial perfusion of PBS followed immediately by perfusion of 4% PFA in PBS. Brains were place in 4% PFA in PBS solution for 24 hrs. Then they were transferred to a solution of 30% sucrose in PBS. 40um thick sections were generated with a microtome. The relevant DMS slices were stained with DAPI and the lesions were visualized with a stereomicroscope (Leica).

6.3.6 Data Analysis

Spikes were detected online with amplitude thresholding. Units were isolated on each channel using Plexon Offline Sorter. Waveforms were analyzed using the software’s built-in feature extraction and principal component analysis. Clustering was performed manually. Any cluster that was recognizably distinct from the noise cluster
with reasonable spike amplitude/waveform and inter-spike interval histograms, was identified as a single unit.

The firing rate z-score heat-maps (Figure 10) were generated by first calculating the peri-stimulus time histograms (normalized to equally weigh left and right sample trials) triggered on the onset of the delay period using 0.1s time bins, and then using the mean and standard deviation of spike counts per bin from 50s before the onset of the delay to 50s after, to calculate z-scores. The heat-maps in Figure 11 were generated with the same process but with shifted time-stamps for the delay-onset, where all time-stamps were circularly shifted by the same randomly determined amount. In Figure 16A and Figure 17A, to calculate firing rates for the left-most plots, we only used data from times that the rat was at the nose port. For some time bins, not enough data was available as a result of excluding data from when the rat was not at the nose port. Time bins that had less than 25 left sample trials or 25 right sample trials were masked by white. Firing activity in all three plots are normalized to z-score values, with mean and standard deviation for firing rate determined by taking spike counts per 0.1s bin from $t=-50s$ to $t=50s$, using all data (regardless of rat head position).

To quantify and statistically test the presence of sequences in firing activity, we use an approach previously used by (Harvey et al., 2012). For each neuron, we first calculate the peri-stimulus time histogram using a 0.1s time bin (again normalizing for
unequal number of left and right trials). We define the ridge-to-background ratio to be the mean value of the ridge activity divided by the background activity averaged across all neurons. Ridge activity is the mean firing rate of the 11 bins (a 1.1s-wide window) centered at the peak activity time. Background activity is defined to be the mean firing rate of all the other time bins. We used a large window for calculating background activity, i.e. from 50s before to 50s after the aligning point. In Figure 18, those time bins that were masked were not included in the background activity calculation or ridge activity calculation. We tested the statistical significance of the sequential activity by a one-tailed test comparing the ridge-to-background ratio against the null distribution of ratios expected by chance. The null distribution was calculated by circularly shifting spike times by random values, 1000 times to test for p < 0.001.

In Figure 10B and Figure 11, firing activity peaks were chosen from 1s before the onset of the delay period to 1s after the end of the delay period. Figure 24 firing activity peaks were chosen from 5s before to 15s after the desired event. Likewise, the ridge-to-background analysis in Figure 25 was carried out on a time window spanning 5s before to 15s after the desired event. For all other heat-maps and scatter plots (Figure 14, Figure 16 and Figure 17, and ridge-to-background ratio analyses (Figure 18) firing activity peaks were chosen from the onset to the end of the delay period.
To estimate time dependent firing rates (Figure 10A and Figure 13) we calculate the peri-stimulus time histogram using 50ms time bins and then smoothed the estimates using a 1s wide Gaussian kernel spanning ±3 standard deviations (σ = ⅖ s). This method was used when plotting firing rates of example neurons in.

6.3.7 Mutual Information

Information about the sample stimulus identity in neural data was quantified by calculating the mutual information between spike trains and the sample side (Borst and Theunissen, 1999; Dayan and Abbott, 2001; Quian Quiroga and Panzeri, 2009; Rieke et al., 1999). We first made the two following simplifying assumptions: 1) the spiking of each neuron is a Poisson process (i.e. spiking at different time bins across neurons are independent of one-another) and, 2) the only factors involved in determining the spike rate is the sample stimulus of the current trial and time relative to the delay onset. Our calculations make use of the firing rates for left and right sample trials only, which are estimated using the method described above. The firing rate trajectories are then used to generate 500 random spike trains and the average pointwise mutual information with the sample stimulus for each randomly generated spike train is calculated to obtain the mutual information. Spike trains are generated by assuming a sample stimulus x (randomly chosen to be left or right) and determining the number of spikes in every 50ms time bin of the desired time segment from the firing rate trajectory of when
<sample> = x. A 1s wide sequence \( s = s_1, s_2, \ldots, s_k \) of spike counts from the spike train is then analyzed. The pointwise mutual information between that sequence and the sample stimulus is derived as follows:

\[
\log \left( \frac{Pr(<\text{sample}>=x \& <\text{spike train}>=s_1s_2\ldots s_k)}{Pr(<\text{sample}>=x) Pr(<\text{spike train}>=s_1s_2\ldots s_k)} \right) \\
= \log \left( \frac{Pr(<\text{spike train}>=s_1s_2\ldots s_k | <\text{sample}>=x)}{Pr(<\text{spike train}>=s_1s_2\ldots s_k)} \right) \\
= \log \left( \frac{Pr(<\text{spike train}>=s_1s_2\ldots s_k | <\text{sample}>=x)}{\sum_{\ell \in \{\text{left}; \text{right}\}} Pr(<\text{spike train}>=s_1s_2\ldots s_k | <\text{sample}>=L) \} \right)
\]

From independence between bins, we have:

\[
Pr(<\text{spike train}>=s_1s_2\ldots s_k | <\text{sample}>=x) = \prod_{i=1}^{k} Pr(<\text{spikes in bin } i >= s_i | <\text{sample}>=x)
\]

And the term on the right can be calculated from the estimated firing rates in the case <sample> = x.

To determine if mutual information peaks are statistically significant, we compared mutual information peaks from the data to that obtained with shuffled data (Panzeri et al., 2007). Towards this end, we randomly shuffled the left sample and right sample trials and recalculated time-varying sample information using the approach explained above. For each neuron, this process was repeated 200 times and the
maximum value of sample information within the delay period was taken each time, to obtain a distribution of the sample information peak value expected by chance. The peak information of the non-shuffled data was compared to that distribution to test for significance ($p < 0.01$, one-tailed test). All neurons that had significantly large peaks were considered to be significantly encoding the sample stimulus (Figure 14A). The green shadings on the bottom panels of Figure 13 are the thresholds for significance of the peak value across the entire delay period interval, obtained by taking the 99th percentile of distribution for sample information peak expected by chance.

Information about lever identity for choice and sample presses was calculated in a similar fashion, with the modification that we did not use a sliding window, but rather calculated mutual information for the entire 500ms window centered at a lever press. This gives us a single measure of information about lever side for each neuron, for each of the sample and choice phases.

6.3.8 Population Decoder

We used a maximum likelihood estimator to decode the sample stimulus from population data (Brown et al., 2004; Gerwinn et al., 2009; Ma et al., 2006; Pouget et al., 2003). Our decoder was constructed based on the assumption that spiking of each neuron is a Poisson process with a time-varying spike rate function. To obtain a time-dependent evaluation of the decoder, we use a 500ms wide sliding window. For each
neuron the desired 500ms segment of the spike train is binned into 50ms bins. The decoder is given an N×k matrix $S$, where $S_{i,j}$ is the spike count of neuron $i$ for bin $j$ of that segment ($N$ denotes the number of neurons, and $k$ is the number of bins). The decoder then finds $x$ to maximize $\Pr(<\text{sample}>=x | <\text{spike data}>=S)$. Assuming equal probability for left vs. right lever we have:

Assuming a uniform prior over the possible values for $x$, we have:

$$\Pr(<\text{sample}>=x | <\text{spike data}>=S) \propto \Pr(<\text{spike train}>=S | <\text{sample}>=x)$$

with the right term being the likelihood function. From independence between bins across neurons we know the likelihood function can be calculated as follows:

$$\Pr(<\text{spike train}>=S | <\text{sample}>=x) = \prod_{i=1}^{N} \prod_{j=1}^{k} \Pr(<\text{spikes in bin } i \text{ of neuron } j>=s_{i,j} | <\text{sample}>=x)$$

The term on the right can be calculated using the Poisson probability mass function and the spike rate of the corresponding bin under condition $<\text{sample}>=x$. We train the sample decoder by calculating firing rate trajectories for left sample and right sample trials separately, from the training data set. The sample decoder knows what time segment of the trial the spike train belongs to and uses firing rate estimates
from that segment. Decoder is trained using correct trials only (i.e. trials in which the rat responded by choosing the correct lever).

Since the neurons were not all recorded simultaneously from the same rat, we randomly combined different trials from each neuron with one another into pseudo-trials. Trials were drawn with replacement, within a determined condition of the four trial conditions (i.e. \([\text{left sample, right sample}] \times [\text{correct, incorrect}]\)). 500 pseudo-trials were generated from the training set. We used leave-one-out cross validation in to assess the accuracy. Specifically, when testing for a pseudotrial we did not use any of the trials that it was composed of in calculating the mean firing rates for left sample trials and right sample trials. The error bars in Figure 15 are the SEM determined by the number of pseudo-trials if trials were drawn without replacement (i.e. the minimum number of trials for a specific condition across neurons), which is an overestimate for the actual SEM.

### 6.3.9 Generalized linear models of spiking activity

Generalized linear models (GLMs) were constructed to predict the number of spikes of each neuron in each 10ms time bin using various predictors related to the task and the animal’s behavior. The GLMs modeled number of spikes with a Poisson distribution and a log link function). Positional predictors included: 9 binary predictors for head position, 8 binary predictors for head direction, and 3 numerical predictors for
velocity (x component, y component, and magnitude of velocity vector). For the head position predictors, the chamber was divided into nine tiles (3 equal segments along each axis) and each binary predictor represented whether the head position was located in the corresponding tile. For the head direction predictors, the range of possible head directions (0-360 degrees) was divided into 8 equal segments and binary predictors for head direction represented which segment the head direction was in. For the task related predictors, the 10 binary predictors denoted time within the delay period and one binary predictor denoted the identity of the sample stimulus. For the time related predictors, time from delay onset was divided into 1s long time bins and each of the 10 binary predictors signified whether the current time was in the corresponding 1s time bin. Nested Model Comparison of GLMs were performed by first fitting the GLM to the data using R function glm() and then performing a likelihood ratio test using the R function anova(model1, model2, test="Chisq").
7 Towards Optogenetic Perturbations to Clarify the Striatum’s Role

7.1 Introduction

In this chapter I motivate and describe an ongoing project that I believe will ultimately clarify the nature of the striatum’s contribution in implementing working memory. This chapter includes data from anesthetized recordings showing that optogenetics is an efficient method of silencing striatal neurons.

7.2 Motivation and Background

The theories that have been proposed about the role of the striatum in working memory, are distinguishable based on the time epoch they claim the striatum is important. One theory claims that the striatum provides positive feedback to cortical areas through the cortico-striato-thalamic loop, emphasizing continuous engagement throughout the delay period. The other claims that the striatum gates working memory, emphasizing the onset of a delay period when the memory is being loaded. The pharmacological inactivations using muscimol (Chapter 5) strongly support the hypothesis that DMS activity has a role in spatial working memory, but those perturbations lacked the temporal precision required to tease out these two theories
apart. Optogenetics, however, provides us with the fine temporal resolution needed to reversibly inactivate the DMS during sub-epochs within a trial (Zhang et al., 2007).

The approach put forth here is to perturb activity during different epochs of a randomly selected subset of trials and compare choice accuracy in those trials with accuracy in non-perturbed trials. This can be done by using viral vectors to infect the DMS of rats and by delivering light via fiber optic implants. This approach has been used in several behavioral cohorts by the author, although results are preliminary and are not reported in this thesis.

Studies done in primates show that electric microstimulation of the primate caudate during working memory leads to working memory impairments. This makes excitatory perturbations seem more promising. However, our ongoing experiments are designed for inactivations as opposed to activations for the following reasons. 1) Long-term inactivations of striatum have been shown to impair performance on this task. (See Chapter 5). 2) Preliminary results using ChrismsonR - an excitatory opsin (Klapoetke et al., 2014) - show behavioral results which are difficult to interpret. More specifically, optogenetic activations of the rat striatum during the DNMP task leads to trial abort and is shown to be rewarding (preliminary results using intra-cranial self-stimulation) and these interfere with our ability to measure working memory deficits in the DNMP task.
The behavioral assay for this project is the same delayed non-match to position (DNMP) spatial working memory task described in Chapter 4, but with a small modification. In addition to having to make a nose poke at the end of the delay period, the rat must make a nose poke after pressing the sample lever to initiate the delay period (See Figure 21). This will allow more precise control of the lasers for event triggered perturbations.

Figure 21 – Schematic diagram showing the restructured delayed non-match to sample task. The top panel shows the different phases of the task and is identical to that drawn in Figure 2A, except a new nose poke is introduced after the sample lever press. The three lines below represent the different epochs for light delivery on perturbed trials: during memory loading, during memory maintenance, and during memory retrieval. We hypothesize that specific epochs will lead to memory impairments, informing us on which of the theories about the striatum’s role in implementing working memory is correct.

7.3 Experimental Design and Projected Results

The behavioral assay for this project is the same delayed non-match to position (DNMP) spatial working memory task described in Chapter 4, but with a small modification. In addition to having to make a nose poke at the end of the delay period, the rat must make a nose poke after pressing the sample lever to initiate the delay period (See Figure 21). This will allow more precise control of the lasers for event triggered perturbations.
Once a rat is fully trained on the modified DNMP task and habituated to behaving while plugged in to an optical fiber head stage, we put the rat in a test session. During a test session, a subset of trials (20% - determined randomly) will be perturbed trials, in which the striatum is inactivated with precisely timed delivery of light through the optic fiber implants. Three epochs are of interest: 1) The **memory loading phase**, defined as from the time the rat presses the sample lever to the time the rat makes the first nose poke at the other end of the chamber. This time durations depends on the rat response time, and typically takes 1-3 seconds. 2) The **maintenance phase**, which we only define for 10s-delay trials, as the interval starting at 4 into the delay period and lasting for 2s long. 3) The **retrieval phase**, define as from 1s before the delay period ends and lasting for 1s after the rat makes the final nose poke. The duration of this period depends on the response time of the rats, but will typically take 1-3 seconds.

A perturbed trial will have light delivered during one of the three epochs of interest defined above. (See Figure 21). The specific epoch will be determined randomly, but will be such that the rat could not possibly predict the length of the delay period from the light stimulation it receives at any point of the trial (making the loading epoch three times more likely than the maintenance epoch). This design will aid us in interpreting the behavioral results, as the stimulation protocol carries no information about the trial type to the rat (or an ideal observer).
Additionally we will control for the memory component of the task by introducing guided trials, where the correct choice is signaled by a light above the incorrect lever. Rats have already been trained on guided and non-guided trials, and perform at near 100% during guided trials regardless of the delay period. This provides us with trials that have no memory component and can be used to control for non-memory related deficits that can impair accuracy.

In these experiments we will target different regions of the striatum, including the DMS, DLS, and anterior striatum. Optic fiber implants and viral injections will be bilateral, one region targeted per rat. We expect to find differential effects of stimulation since each region lies on a different parallel cortico-striato-thalamic pathway (Alexander et al., 1986, 1990; Mailly et al., 2013). Differences in how the perturbations impair performance may be illuminating in understanding which areas within the striatum are involved in different aspects of implementing working memory. For example, inactivation of the anterior dorsal regions of the striatum may lead to memory impairments, since they receive projections from prefrontal areas and are thought to be involved in higher order cognitive functions (Balleine and O’Doherty, 2010; Burton et al., 2015; Ito and Doya, 2015; Kim et al., 2013; Stalnaker et al., 2010; Yin and Knowlton, 2006), whereas inactivation of the dorsolateral areas may only lead to motor deficits.
According to the gating hypothesis, inactivating during the memory loading phase, but not the maintenance phase, will lead to an accuracy impairment in this task. According to the memory maintenance hypothesis, inactivating during any of the three epochs are equally disruptive to working memory. Results from these experiments will be instrumental at arriving at a complete and cohesive theory for how working memory is implemented in the brain.

### 7.4 Characterization of Jaws in the Striatum

In the ongoing optogenetic experiments we use the red-shifted crux-halorhodopsin, Jaws, to silence neurons in the striatum (Klapoetke et al., 2014). We express Jaws in the striatum with viral injections of AAV5-hSyn-Jaws-GFP-WRPE-hGh. Human synapsin is a universal neuronal promoter (Kügler et al., 2003). The expression of the green fluorescent protein (GFP) will allow us to assess the accuracy of our targeting in post-mortem histological imaging. In this section we will characterize Jaws in the striatum using anesthetized recordings.

#### 7.4.1 Efficacy of Optogenetic Perturbations

In order to evaluate the efficacy of silencing striatal neurons using this method, we injected rats with AAV5-hSyn-Jaws-GFP-WRPE-hGh in the striatum. After waiting 5 weeks for expression of virus, we anesthetized them and recorded from the striatum.
using an optrode, an optical fiber glued to an electrode. This setup allowed us to record from neurons and observe how their activity is modulated with light. Of the 11 neurons recorded across 2 rats, all but two were silenced using light (See Figure 23 & Figure). This data demonstrates a strong inactivation effect that works across long distances. Many of the neurons had near perfect silencing.

Figure 22 – Normalized firing rates of all 9 neurons that were tested with a 5s long 10mW red light. Blue shades indicate units with 800nm distance and red shades indicate units with 1100nm distance.
Figure 23 — Summary of raster plots and peri-stimulus time histograms for all 11 recorded units that were tested with 10mW red light. Light durations were 5s or 10s (red bar). Light was emitted at a distance of 800nm or 1100nm from electrode tip (annotated). Greater number of trials were used to compensate low firing rates.
7.4.2 Inactivation Length

Different lengths of inactivation were tested, ranging from 2 – 60 seconds. We found that some neurons are able to maintain their silence for as long as 60 seconds. Crucially, long or repeated inactivations did not kill cells in any of the cells that we tested.

7.4.3 Sensitivity of Jaws to Different Light Wavelengths

One of the advantages of using Jaws relative to eNpHR3.0 is that one can use red light to inactivate neurons (Klapoetke et al., 2014). Red light is preferable in our experiment for two reasons: 1) light in the red wavelength spreads further in brain tissue because it is less absorbed by hemoglobin, and 2) rats cannot see red light, making it easier to control for unwanted reflections out of the head implant (Szél and Röhlich, 1992). However, we compared red and green light at different light intensities for an example cell and found that, contrary to what is expected from Jaws, green was more effective than red at the same light intensities for a distance of 1.1mm.

7.4.4 Ramping Light

One of the concerns with using chloride pumps like Jaws for inactivations is that the perturbation may affect neuronal spiking after the perturbation. Specifically, neurons tend to fire more intensely after an inactivation, as seen in our data (see Figure
This may be due to cell-intrinsic mechanisms such as the intracellular accumulation of chloride (Raimondo et al., 2012) or may be a result of network dynamics such as the removal of inhibitory drive of the network.

One way to address this issue is to use a ramping light, that gradually turns on or off, instead of a square light pulse. To investigate this question we tested ramping vs square light on one cell that had a strong post-inhibitory rebound. We found that ramping light had the effect of decreasing the maximum firing rate by spreading the post-inhibitory spikes across a longer time duration. This data is preliminary and more cells need to be tested in order to make a conclusive statement about the effectiveness of ramping.

7.5 Methods

All procedures were performed in accordance with the university-approved IACUC protocol.

7.5.1 Surgical Procedure

3 adult Long-Evans rats (> 300g) were deeply anesthetized with 2% isoflurane and bilaterally injected with 1000nl of AAV5-hSyn-Jaws-GFP-WRPE-hGh (3.69e13 parts/ml) in four striatal coordinates (A/P: 0.5, M/L: ±3.0, D/V: -5.0 and A/P: 1.2, M/L: ±2.0, D/V: -5.0). Injection needle was lowered to D/V: -5.4 and raised back to D/V: -5.0 before injection,
to provide a pocket for the viral fluid. Injections were done at a rate of 100nl/min and were followed by 10 minutes of waiting time for the virus to diffuse, before retracting the injection needle. Rats were sutured and placed back in their home cage after surgery.

7.5.2 Anesthetized recordings

After 5 week of viral expression time, the rats were anesthetized using an injection of urethane and head-fixed in a stereotax. A 300μm fiber optic (similar to the ones used in the implants for our behavioral cohort) was glued to a tungsten electrode to construct an “optrode”. Craniotomies and durotomies were performed over the injection sites and optrodes were lowered in the brain. All cells were recorded within 1.25mm of the injection site. For light stimulations, a green and red lasers (with wavelengths of 532nm and 638.7nm was used).
8 Overview

8.1 Summary of Results

We investigated the role of the striatum in the rat model system using a spatial working memory task. The study reported in this thesis leads us to four main conclusions:

1) **Striatal activity in causally involved in spatial working memory.** This was established by showing that pharmacological inactivations of the dorsomedial striatum to an impairment in choice accuracy.

2) **Striatal neurons are sequentially activated throughout the delay period.** This was established using in vivo extracellular recordings while rats performed the delayed non-match to sample task.

3) **Individual striatal neurons transiently encode the sample stimulus during the delay period, with the sample information tending to be strongest earlier in the delay period.** This was shown using information theoretic analysis and a Bayesian maximum likelihood estimator to decode population activity.

4) **Sample information peaks are not correlated with firing rate peaks.** (See Figure 14B). In other words, sequential activity and sample encoding activity appear in the same neurons. But the time that a neuron most strongly
encodes the sample stimulus during the delay period is not predictive of the
time that it is most active.

8.2 Discussion

In this thesis we first established that DMS activity is needed to perform our spatial working memory task. Then, we characterized neural dynamics in the DMS and found that neurons were sequentially activated throughout the course of the delay period. We employed information theoretic analysis and population decoding to reveal that sequential activity and stimulus encoding are dissociated in the neural dynamics of the striatum during spatial working memory. Specifically, neurons transiently encoded the stimulus during the onset of the delay period, whereas the same neurons encoded time with sequential activity throughout the delay period. These dynamics are consistent with a role for the striatum in initiating the encoding of new memories into short-term memory buffers elsewhere in the brain.

8.2.1 Insights into the striatal dynamics underlying working memory

We employed two different methods to quantify the encoding of the sample stimulus during the delay period. The first method was to quantify the mutual information between the sample stimulus and the firing patterns for each individual neuron (See Figure 14B), which revealed strong stimulus encoding at the onset of the
delay period and less encoding throughout the delay period. The second method (See Figure 15) was to measure our ability to decode the sample stimulus from the entire neural population as a function of time during the delay period. The second method revealed perfect decoding accuracy at the onset of the delay period and greater than chance level accuracy during most of the delay period. Both approaches demonstrated that the sample stimulus is encoded strongly at the onset of the delay period and that stimulus encoding declines noticeably with either measure by 2s into the delay period. Stronger stimulus encoding toward the onset of the delay period has been reported in studies of primate cortical regions, although those studies did not compare stimulus encoding to sequential firing activity (Brody et al., 2003; Bruce et al., 1985; Clark et al., 2012; Colby et al., 1996; Gregoriou et al., 2012; Romo et al., 1999).

Our experiments differed from most previous work in that we employed relatively long delay periods (up to 10s). Considering that sample information in the DMS decreased substantially within several seconds (Figure 14B and Figure 15), the transient nature of stimulus encoding would have been less obvious if our task had only employed substantially shorter delay periods. Instead, the activity would have been better described as sustained memory-encoding activity. Previous recordings in primates have reported sustained delay period activity that encodes short-term memory in a small fraction of caudate neurons with delay periods of 2-4s (Hikosaka et al., 1989; Kawagoe et al., 1998; Schultz and Romo, 1988; Schultz et al., 1994). In our study, we observed
some neurons with stimulus encoding during the delay period that persisted for several seconds, in agreement with prior work. However, no single neuron was found to encode the sample stimulus for the entire duration of the delay period (See Figure 14B).

8.2.2 Relevance to Theoretical Models of Striatal Contribution to Working Memory

Different theories have been proposed to explain how the striatum contributes to working memory. For example, it has been suggested that the striatum may be involved in initiating the storage of new memories in cortical networks, an idea often referred to as “gating” (Baier et al., 2010; Frank et al., 2001; Gruber et al., 2006; O’Reilly and Frank, 2006; T. S. Braver, 2000). According to this idea, the striatum plays a role in selecting cortical memory buffers that are to be updated and determines the time memory updating occurs, similar to the role the striatum is thought to play in initiating and selecting motor actions (Bailey and Mair, 2006; Bhutani et al., 2013; Grillner et al., 2005; Kropotov and Etlinger, 1999; Mink, 1996).

The short-term memory gating model ascribes a crucial role to the striatum at the onset of the delay period, when new information must be stored in short-term memory buffers (Baier et al., 2010; Frank et al., 2001; Gruber et al., 2006; O’Reilly and Frank, 2006; T. S. Braver, 2000). Interestingly, our data emphasizes the importance of the striatum at the beginning of the delay period, as the stimulus is preferentially encoded during that period.
However, another possibility is that DMS is contributing to maintaining the short-term memory. Although the accuracy of our population decoder decreases substantially during the course of the delay period, the decoding accuracy near the end of the delay period was roughly comparable to behavioral performance even at the 10s delays (Figure 3 and Figure 15A), indicating that activity in the DMS might be sufficient to support the animal’s behavior. As another possibility, the DMS could assist cortical areas in maintaining the memory throughout the delay period, irrespective of holding the content of the memory. Memory encoding activity in cortex often appears as persistent elevated activity or sequential activations of neurons (Baeg et al., 2003; Cromer et al., 2011; Fujisawa et al., 2008; Fuster and Alexander, 1971; Hanks et al., 2015; Harvey et al., 2012; Horst and Laubach, 2012; Kojima and Goldman-Rakic, 1982b; MacDonald et al., 2013; Pastalkova et al., 2008; Powell and Redish, 2014; Schoenbaum and Eichenbaum, 1995), which may be implemented using positive feedback through distributed cortico-striato-thalamic loops (Alexander et al., 1986; Houk and Wise, 1995b; Middleton and Strick, 2000b, 2002b; Wang, 2001). In this view, the sequential activity that spans the delay period in DMS could play a role in exciting cortical memory buffers and enabling memory encoding activity there. As yet another possibility, the stimulus memory may be stored in the striatum in a form that would not be fully detectable in the spiking activity, perhaps in short-term synaptic plasticity, as suggested by recent theoretical models (Lundqvist et al., 2010; Mongillo et al., 2008; Stokes, 2015). Future experiments
employing transient perturbations of the striatum with sub-trial temporal precision will help address the question of whether the DMS is more important for updating versus maintaining short-term memories.

### 8.2.3 Advantages and Limitations of the Delayed Non-Match to Sample Task

A strength of our task design is that it allowed us to compare the same motor action (i.e. lever pressing) in different contexts within the trial (Figure 19). This revealed that striatal neurons differentially encode the sample press and the choice press, and that encoding of the same action is dependent on the context of the action within the task. This supports the idea that the striatum is involved in encoding memories of task-related variables, and not merely encoding the rats’ position or movement during the task.

Unlike most prior work performed in head-fixed primates, our rats were freely moving. Thus, we performed several analyses to control for the possibility that our conclusions of sequential firing rate activity or transient stimulus encoding was a result of motor or position confounds. This included analyzing data from times that the rat was in the nose port (Figure 16, Figure 17, & Figure 20), as well as showing that task-relevant variables remained a significant predictor in the vast majority of neurons, even when accounting for movement or position with a generalized linear model (GLM). However, motor and position confounds may have been even more strongly excluded had we
instead used a task design that restricts movement of the rat during the delay period. Additionally, in our task design the correct choice of a trial can be determined from the onset of the delay period, making it difficult to distinguish encoding of the memory of the sample stimulus from encoding of future motor actions (“retrospective” versus “prospective” memory). A more sophisticated task design that enabled dissociation of stimulus encoding and pre-motor activity could have allowed us to make even stronger conclusions about memory encoding.

8.2.4 Relationship to Neural Dynamics Reported in Other Tasks

Sequential neural activity dynamics have been reported in the striatum in tasks that do not involve spatial working memory (Lustig et al., 2005; Matell and Meck, 2000, 2004; Mello et al., 2015). Even in our task, we observed similar sequential activation of

![Figure 24](image)

Figure 24 - Normalized firing rates of all neurons triggered to different events of the task. Rows in each plot are sorted by time of peak firing rate. Notice that aligning to sample press time is the same as aligning to delay period onset for all delay period lengths.
neurons when time-locking to other task events, although those sequences did not span out as far as the sequences following the delay period onset (See Figure 24 and Figure 25). Altogether, this suggest that the delay-period spanning sequences in DMS might support a general role for the striatum in time keeping, rather than a specific role in working memory.

Figure 25 - Ridge-to-background ratios of activity aligned to sample lever press, nose-poke, choice lever press, and reward port entry. Green bars represent the ridge-to-background ratios and correspond to the four heat-maps in Figure 24. Blue bars represent the mean ridge-to-background ratio obtained by circularly shifting the spike times by random values 1,000 times. For all four cases ridge-to-background ratio was significantly larger than that expected by chance (* p < 0.001; one tailed test using the ratios from the randomly shifted data as the null distribution). Ridge-to-background analysis was calculated on a time window spanning 5s before event to 15s after event.
The neural dynamics we observed in the DMS underlying spatial working memory provide an interesting contrast with previous observations of sequential activity dynamics in other regions during spatial working memory. In particular, cortical (Astrand et al., 2015; Cromer et al., 2011; Crowe et al., 2010; Fujisawa et al., 2008; Harvey et al., 2012; Horst and Laubach, 2012) and hippocampal (MacDonald et al., 2013; Pastalkova et al., 2008) areas display stimulus encoding sequences that span the delay period. This suggests a different role for the striatum in working memory relative to these other regions. Specifically, cortex and hippocampus may be involved in maintaining the memory throughout the delay period, whereas the striatum may be involved in initiating memory storage.

8.3 Future Directions

One remaining question that needs to be addressed is whether the memory encoding in striatal activity pertains to prospective memory (e.g. motor planning) or retrospective memory (e.g. memory of the sample press). A first approach to address this question would be to use spiking data in the delay period to decode the sample press and see how well it does in comparison to a decoder that decodes the choice press. We were limited in this approach due to the low number of error trials in our data that led to large error bars for the decoder performance on erroneous trials (see Figure 15B). However, even if this analysis was done with more data it cannot provide us with a
definitive answer regarding prospective versus retrospective memory. In other words, even if striatal activity during the delay period completely correlate with, say, the choice lever and not the sample lever, one may be compelled to conclude that those neurons are encoding motor actions rather than remembered stimuli from the past. However, we cannot rule out the possibility that they are encoding a misremembered sample stimulus from the past and have not yet planned an action for the future. A more definitive approach at answering this question would be to use a task which the rat cannot determine the correct choice press until the end of the delay period. For example, in a task where two stimuli need to be compared to determine the correct choice with the two stimuli being separated across a delay period, rats cannot know what the correct action will be before observing the second stimulus. In this situation, delay period activity that correlate with sample or choice are more likely to be encoding stimuli from the past (retrospective memory) rather than motor actions. Additionally, even with the delayed non-match to sample task used in this project, many rats show stereotypical behavior that can be used to dissociate motor planning memory from other forms of memory. For example, a rat may always go to the left lever after making a nose-poke, and then deliberate and decide to press that lever or to switch to the right lever. For that rat, delay period activity that correlates with the trial type is very unlikely to be related to motor planning, because the motor action following the delay period is always to immediately go to the left lever.
Another interesting expansion of this project would be to differentiate between striatal neurons in the direct and indirect pathways that are generally thought to have opposing roles due to the opposing excitation/inhibition of the thalamus that they cause (see Figure 26) (Lanciego et al., 2012; Parent and Hazrati, 1995; Yager et al., 2015). In motor control, it is thought that the direct pathway is responsible for initiating selected motor actions and the indirect pathway is responsible for withholding motor actions (Bailey and Mair, 2006; Bhutani et al., 2013; Grillner et al., 2005; Kropotov and Etlinger, 1999; Mink, 1996). Similarly, it is proposed that in cognitive control, the same pathways

![Figure 26 - Schematic of cortico-striato-thalamic loop distinguishing the direct and indirect pathways that have opposite signed effects. Abbreviations are as follows. MSN: medium spiny neurons, GPe: globus pallidus external, GPi: globus pallidus internal, SNR: substantia nigra, STN: subthalamic nucleus](image-url)

Figure 26 - Schematic of cortico-striato-thalamic loop distinguishing the direct and indirect pathways that have opposite signed effects. Abbreviations are as follows. **MSN**: medium spiny neurons, **GPe**: globus pallidus external, **GPi**: globus pallidus internal, **SNR**: substantia nigra, **STN**: subthalamic nucleus
may be used to initiate the action of updating working memory with selected stimuli and suppressing the influence of distractive stimuli on working memory (O’Reilly and Frank, 2006). In our ongoing experiments explained in Chapter 7, we target all striatal neurons using the human synapsin promoter, and assume that the direct pathway will be the dominant pathway. However, the gating theory posits an active role of suppressing distracting stimuli during the delay period through the indirect pathway. It may be the case that suppression of the indirect pathway during the delay period leads to memory impairment, thus confounding are interpretation of the projected results (see Chapter 7).

The type of dopamine receptor expressed in medium spiny neurons are highly predictive of whether they contribute to the direct or indirect pathway (Yager et al., 2015). Thus, the indirect and direct pathways can be targeted optogenetically by using Cre lines that are specific for D1 and D2 receptor neurons and injecting them with a Cre dependent virus to express the intended opsin. This will allow a handle to experiment with the effect of distractors in a working memory task. According to the gating theory, optogenetic activation of the direct pathway simultaneous with a distracting stimulus should increase the distracting effect and lead to an accuracy impairment. But activating the indirect pathway during the delay period should reduce the effect of distracting stimuli in a memory task.
8.4 Conclusion

We employed information theoretic analysis and population decoding to reveal that sequential activity and stimulus encoding are dissociated in the neural dynamics of the striatum during spatial working memory. Specifically, neurons transiently encoded the stimulus during the onset of the delay period, whereas the same neurons encoded time with sequential activity throughout the delay period. Temporally precise perturbations will further elucidate the role of the striatum in working memory.
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