ACTIVITY OF RAT DORSAL STRIATAL MEDIUM SPINY NEURONS DURING ODOUR SAMPLING IN A WORKING MEMORY CAPACITY TASK

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ABSTRACT

Working memory is an important cognitive function that allows us to perform everyday tasks including language comprehension and reasoning. It is, therefore, unsurprising that working memory dysfunction has been detected in many neurodegenerative and psychiatric disorders, such as schizophrenia. The striatum is an important brain region for working memory in both humans and rats, but its role remains unclear. While increased striatal activity has been shown during information updating in human working memory tasks, assessment of striatal activity during information maintenance, the retention of information for cognitive processes, have generated mixed results. No previous studies were examined striatal activity in rats completing a working memory task, although medium spiny neurons have been shown to increase activity after a reward was received in short-term memory tasks. Therefore, in the present study, I examined the activity of medium spiny neurons of the dorsomedial striatum when receiving a reward and also, when rats approached familiar and novel odours during the odour span task. Rats were then administered MK801, an *N*-Methyl-D-Aspartate receptor antagonist, and observed for any putative changes in neural activity during the odour span task. I observed significantly increased activity in a population of medium spiny neurons after a reward was received, while no changes of activity were detected in response to rats approaching a familiar odour. The results from the activity of medium spiny neurons in response to novel odours was inconclusive. Neural activity was recorded 30 minutes following MK801 administration. During the last 10 minutes of the recording, medium spiny neurons significantly increased activity compared to a baseline recording pre-injection. By increasing our knowledge regarding the neural activity underlying working memory and how it is affected by psychoactive drugs, our understanding of how to treat working memory dysfunction may improve.

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid
AP	Action potential
A2A	Adenosine type 2A receptor
Capp	Novel approach with the correct choice to lid flip
Cflip	Correct choice to lid flip
CE	Central executive
CPu	Caudate-putamen complex
DA	Dopamine
dlPFC	Dorsolateral prefrontal cortex
dlSTR	Dorsolateral striatum
dmSTR	Dorsomedial striatum
D1	Dopamine type 1 receptor
D2	Dopamine type 2 receptor
D5	Dopamine type 3 receptor
Eflip	Incorrect choice to lid flip
EN	Entopeduncular nucleus
Fapp	Familiar approach with the correct choice not to lid flip
fMRI	Functional magnetic resonance imaging
FSI	Fast spiking neuron
GABA	γ-aminobutyric acid
GP	Globus pallidus
GPe	Globus pallidus internus
GPi	Globus pallidus externus
HS	Head stage pre-amplifier
IP	Intraperitoneal
LFP	Local flied potential
LTM	Long term memory

LTD	Long term depression
LTP	Long term potentiation
LTS	Low threshold spiking
MEA	Multi-electrode array
MK801	Dizocilpine
mPFC	Medial prefrontal cortex
MSN	Medium spiny neuron
M1	Muscarinic type 1 receptor
M2	Muscarinic type 2 receptor
M4	Muscarinic type 4 receptor
nAChR	Nicotinic acetylcholine receptor
NMDA	*N*-Methyl-D-Aspartate
OST	Odour span task
PCA	Principal component analysis
PFC	Prefrontal cortex
PPD	Paired pulse depression
PPF	Paired pulse potentiation
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STM	Short term memory
STR	Striatum
SU	Single unit
TAN	Large tonically active cholinergic neuron
vSTR	Ventral striatum
WM	Working memory
2AFC	Two alternative forced choice task

1.0 GENERAL INTRODUCTION

Working memory (WM) is an important cognitive function that allows us to perform everyday tasks including language comprehension and reasoning (Baddeley, 1992; Cowan, 2009; Aben et al., 2012). Dysfunction of WM has been found in neurodegenerative and psychiatric disorders including Parkinson's disease (Cools and D'Esposito, 2011) and schizophrenia (Barch and Smith, 2008). While models of WM have been purposed in humans (Baddeley, 1992; Barch and Smith, 2008), they are difficult to transfer to rats, as the rat brain is anatomically less complex than a human brain. Nonetheless, WM in rats has attributes similar to those in humans, such as independent WM systems and capacity (Bratch et al., 2016). The striatum (STR) has an important role in WM in both humans (Cools and D'Esposito, 2011) and rats (Floresco et al., 1999; Howland et al., 2014). Since the STR is considered homologous between humans and rats (Devan, 1997; Joel and Weiner, 2000; Voorn et al., 2004), research on the STR's role in WM has a translatable potential between these two species. Using electrophysiological techniques, the neural activity of the STR can be studied in freely moving rats during a WM task. Manipulation of neural activity can also be observed by administration of dizocilpine (MK801), an *N*-Methyl-D-Aspartate (NMDA) receptor antagonist (Foster and Fagg, 1987). MK801 is also used in animal research to induce a state that may resemble schizophrenia and shows similar cortical activations in humans and rats (Jackson et al., 2004; Homayoun and Moghaddam, 2007; MacQueen et al., 2011). To investigate the neural activity related to WM in rats, the odour span task (OST), a non-spatial WM task, was employed to examine changes in medium spiny neuron (MSN) firing rates during the approach to familiar and novel odours in control and MK801-influenced states.

1.1 Working Memory: Humans and Rats

According to current literature regarding humans, there are three main types of memory: long-term memory (LTM), short-term memory (STM) and WM (Cowan, 2009; Aben et al., 2012). LTM is thought to possess unlimited capacity and consists of a vast store of knowledge and records of prior events that can be retained for years (Cowan, 2009; Aben et al., 2012). STM holds the same information as LTM, but it has limited capacity and information will decay over a short period of time (Cowan, 2009; Aben et al., 2012). In contrast, WM is a multi-component system that provides temporary storage and allows the

manipulation of information necessary for a wide range of cognitive tasks including language comprehension, learning, and reasoning (Baddeley, 1992; Cowan, 2009; Aben et al., 2012). WM is also described as a subcategory of STM necessary for cognitive functions and the use of attention to manage STM (Cowan, 2009; Aben et al., 2012). Like STM, WM memory has a limited capacity where only a finite amount of information can be maintained (Cowan, 2009; Aben et al., 2012; Dudchenko et al., 2013). Human performance of higher level cognitive abilities has been shown to correlate to WM capacity, but training to increase WM capacity does not ensure increased fluid intelligence (Harrison et al., 2013).

In 1974, Baddeley and Hitch proposed a model for WM that consists of four main components: the phonological loop, visuospatial sketchpad, episodic buffer and the central executive (CE) (Baddeley, 1992, 1996, 2000, 2003; Baddeley et al., 2011). The phonological loop is responsible for storing acoustic or speech-based information, while imagery and spatial orientation are encoded by the visuospatial sketchpad (Baddeley, 1992; Baddeley et al., 2011). Representations of the integrated information from the phonological loop and visuospatial sketchpad are then held in the episodic buffer, which serves as the interface between temporary and LTM storage (Baddeley, 1992, 2000; Baddeley et al., 2011). The fourth component, the CE, controls the other three components by coordinating the flow of information (Baddeley, 1992, 1996, 2000). Overall, several areas of the brain are involved in WM including the prefrontal cortex (PFC) (Baeg et al., 2003; Baier et al., 2010; de Saint Blanquat et al., 2010; Davies et al., 2013a, 2013b) and the STR (Baddeley, 1992; Cools et al., 2008; Baier et al., 2010). Impaired WM has been implicated in many neurodegenerative and psychiatric disorders including attention deficit hyperactivity disorder (Levy and Farrow, 2001), Parkinson's disease (Cools and D'Esposito, 2011), schizophrenia (Barch and Smith, 2008), and Alzheimer's disease (Baddeley, 1992).

In order to better understand the neurobiology underlying cognitive processes, many researchers opt to use rats, as these models allow for more extensive experimentation compared to the limited procedures possible in human models. Although the Baddeley and Hitch model is applicable to humans, it is difficult to transfer across species to rats, as rats do not have the same anatomical or cognitive complexity, and the components controlled by the CE are not obvious in rodent cognition (Dudchenko, 2004). In rodents, WM is considered a type of STM for stimuli or spatial locations that are used within a testing session, but not between sessions (Dudchenko, 2004; Bratch et al., 2016). More specifically, the duration of

WM in rodents is believed to depend on the time required for the animal to use the information (Dudchenko, 2004). Dudchenko suggests that WM is a STM that should be forgotten or ignored after use, whereas other types of STM may not require the information to be forgotten (Dudchenko, 2004). WM is distinguished from reference memory, which is information stored over several days of repeated training, such as when forming a memory for the rules of a task (Dudchenko, 2004). Because reference is stored for long periods it is categorised as a LTM. WM is typically a delay-dependent representation of stimuli used to guide an animal's behaviour within a task (Dudchenko, 2004).

A fundamental attribute of human WM is the ability to work with information in one domain while simultaneously maintaining information in another without between-domain interference causing performance issues in dual-task paradigms (Bratch et al., 2016). Bratch and colleagues (2016) were able to show rats also possessed this ability by using two established STM approaches – the two alternative forced choice task (2AFC) and the radial arm maze. The 2AFC is a non-spatial task that requires olfactory memory, while the radial arm maze requires spatial memory to complete the task. The 2AFC required a rat to correctly pick the novel odour from a choice of two scents (one novel and one familiar) to receive a reward. The familiar odour was pseudo-randomly picked from a list of odours that the rat had been exposed to earlier that day (Bratch et al., 2016). To show that spatial WM did not interfere with olfactory WM, the rats performed 10 trials of the 2AFC followed by a trial in the radial arm maze (Bratch et al., 2016). The radial arm maze consisted of two phases: the study phase and the test phase. In the study phase, 4 of the eight arms were baited and was completed once the rat had consumed of four rewards, after a delay rewards were only placed in previously non-baited arms (Bratch et al., 2016). After the radial arm maze, the rats then performed 10 more 2AFC trials in which the odours from the first 10 trials were used as the familiar scents (Bratch et al., 2016). As a control, the rats had a delay period equivalent to the time needed to complete the radial arm maze (Bratch et al., 2016). To evaluate if olfactory WM interfered with spatial WM, the rats performed a study phase of the radial arm maze task to learn which arms were baited (Bratch et al., 2016). Once the study phase was completed, they performed 10 2AFC trials followed by the trial phase of the radial arm maze in order to test their memory of the baited arms from the study phase (Bratch et al., 2016). As a control, the rats had a delay period between the study and test phases of the radial arm maze (Bratch et al., 2016). Through comparison of the control trials with the dual-task

experiments, Bratch et al found no significant difference in any of the experimental outcomes (Bratch et al., 2016). The lack of interference between domains provides evidence for the existence of independent WM subsystems in rodents (Bratch et al., 2016).

Another attribute of WM in humans is capacity. Studies of WM capacity in humans have revealed that WM capacity relates to the performance of higher-level cognition (Harrison et al., 2013), age (Light and Anderson, 1985), and cortical activations (Callicott et al., 1999). Higher capacities in WM allows for more available information while cognitive tasks are being performed. Although WM capacity has been studied in rats, further investigations are required to better understand its significance given the minimal findings currently published to this topic. Despite some structural differences between rats and humans, there remains translatability between these species in terms of results obtained from WM experiments. Considering the greater feasibility of WM studies in rats compared to humans, such investigations have been instrumental in gaining a better understanding of the STR in humans, (Cools et al., 2008; Baier et al., 2010), non-human primates (Levy et al., 1997), and other animals – including rats (Floresco et al., 1999).

1.2 The Striatum and Working Memory

The STR is the main input centre of the basal ganglia and receives inputs from all over the cortex (Charara et al., 2003; Yin and Knowlton, 2006; Kreitzer and Malenka, 2008; Purves et al., 2011). In humans, the STR is generally divided into two main functional areas, the putamen and the caudate nucleus, each of which receives projections from different areas (Purves et al., 2011). The putamen predominately receives inputs from the primary and secondary somatosensory regions of the parietal cortex, and from the motor and premotor regions of the frontal cortex (Purves et al., 2011). On the other hand, the caudate nucleus receives inputs from association areas throughout the cortex (Purves et al., 2011).

In rats, the STR is divided into three functional areas: associational, motor, and limbic (Joel and Weiner, 2000). The dorsomedial STR (dmSTR) and the dorsolateral STR (dlSTR) are the associational and motor areas, respectively, and together make up the caudate-putamen complex (CPu) (Devan, 1997; Joel and Weiner, 2000; Voorn et al., 2004). The CPu is homologous to the caudate nucleus and putamen in other mammals including cats, dogs, non-human primates and humans (Devan, 1997; Joel and Weiner, 2000). The third area, the

limbic functional area, is made up of the nucleus accumbens and the olfactory tubercle, which together form the ventral STR (vSTR) (Voorn et al., 2004).

In terms of function, the associational area (dmSTR) is the region of the STR involved in WM. It receives glutamatergic innervation from the mPFC (Voorn et al., 2004; Woolley et al., 2013; Antzoulatos and Miller, 2014), an area analogous to the dorsolateral prefrontal cortex (dlPFC) in humans (Uylings et al., 2003). The role of the motor-control system of the STR is well known; it acts as a gating system that controls when movements are initiated or ceased (Charara et al., 2003; Purves et al., 2011; Jin et al., 2014). As a result, in the event of damage to the STR or over the course of some neurodegenerative diseases, such as in Parkinson's disease, patients have trouble initiating movements (Charara et al., 2003; Purves et al., 2011; Jin et al., 2014). In contrast, the role of the STR in WM is not as well known; it is proposed to also act as a gating mechanism that, in this case, selects when information is to be maintained or updated (Frank et al., 2001; Lewis et al., 2004; Lustig et al., 2005). The gating mechanism is achieved by switching feedback loops on and off in the cortical-striatal-thalamic pathway (Frank et al., 2001; Lewis et al., 2004; Lustig et al., 2005). When striatal MSNs are activated by information sent from the mPFC, they inhibit neurons in the globus pallidus internus (GPi) (Frank et al., 2001; Lustig et al., 2005; Kreitzer and Malenka, 2008) (*Figure 1-1*). The GPi is normally tonically active and inhibits activity in the thalamus, while the STR acts to disinhibit the thalamic neurons (Frank et al., 2001; Lustig et al., 2005) (*Figure 1-1*). Thalamic projections are then received by the PFC whose activity enables the updating of information, whereas its inactivity promotes the maintenance of information (Frank et al., 2001; Lustig et al., 2005) (*Figure 1-1*). Information maintenance refers to intentionally retaining of information in STM that would otherwise decay and be forgotten. In humans, functional magnetic resonance imaging (fMRI) studies have reported increased neural activity in the caudate nucleus when manipulating information during WM tasks compared to information retrieval (D'Esposito et al., 1999; Lewis et al., 2004). In comparison, studies investigating differences between neural activity during maintenance and manipulation have found mixed results as to whether the differences of neural activation are significant or not (D'Esposito et al., 1999; Lewis et al., 2004)

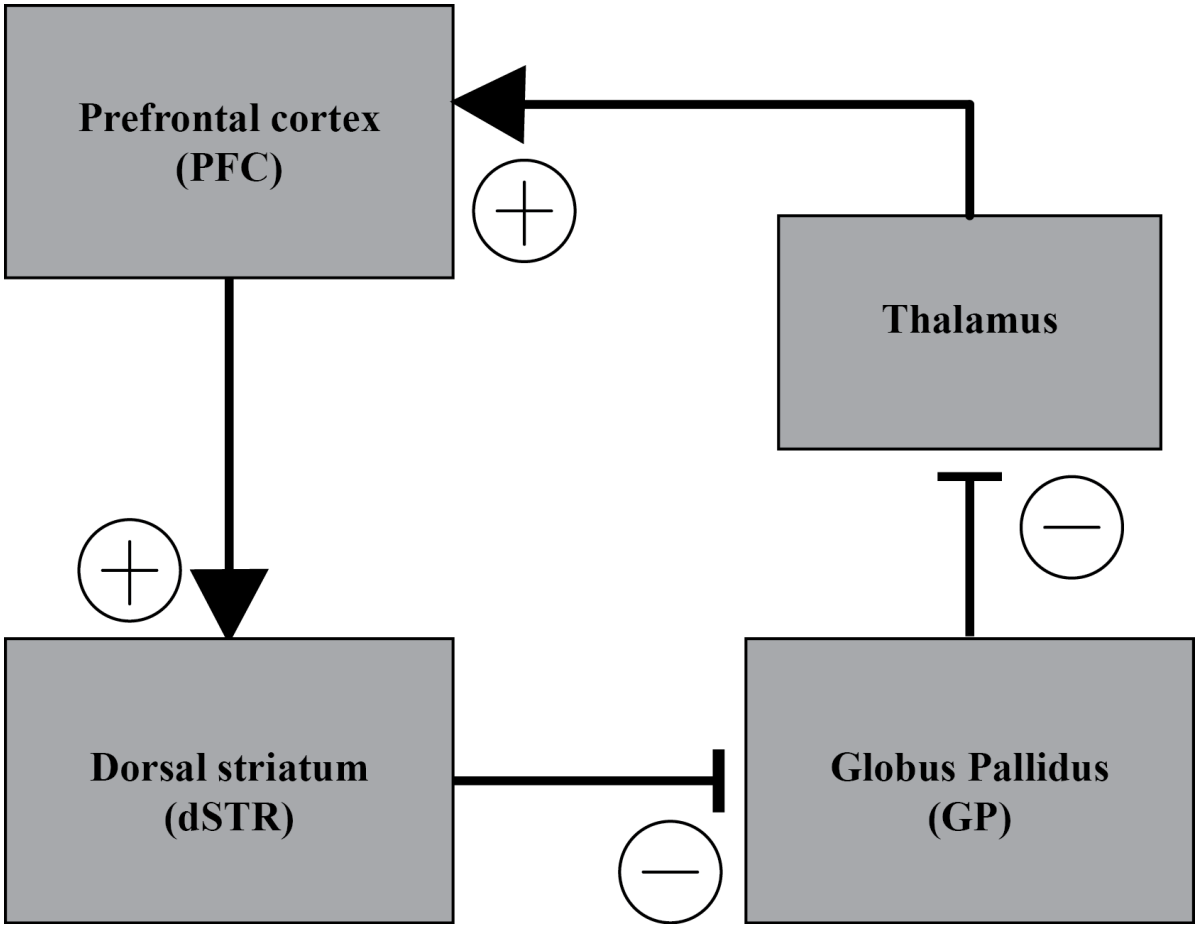

Figure 1-1. Illustration of the cortical-striatal-thalamic feedback loop. Circles with positive and negative symbols represent excitation and inhibition respectively.

1.3 Striatal Cytoarchitecture

MSNs comprise 90-95% of the STR neurons, while several classes of interneurons form the remainder (Frank et al., 2001; Voorn et al., 2004; Lustig et al., 2005; Kreitzer and Malenka, 2008; Kreitzer, 2009). Morphologically, MSNs have a small soma (10-18μm) and an extensive dendritic tree that has a high density of spines (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009). These neurons receive glutamatergic projections and express NMDA and alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid (AMPA) receptors post-synaptically. MSNs typically fire at lower frequencies (roughly 5 Hz) and when activated they release the neurotransmitter γ-aminobutyric acid (GABA) (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009).

As the sole output of the STR, MSNs are divided into two classes based on both the main neuromodulator dopamine (DA) and the DA receptor they contain, either DAergic type 1 (D1) or DAergic type 2 (D2) (Kreitzer and Malenka, 2008; Kreitzer, 2009). D1 MSNs project directly to the output nuclei of the basal ganglia, the GPi – entopeduncular nucleus (EN) in rats – and the substantia nigra pars reticulata (SNr) (Kreitzer and Malenka, 2008; Kreitzer, 2009). Although both classes of neurons contain acetylcholine (Ach) muscarinic type 1 receptors (M1), only D1 MSNs contain ACh muscarinic type 4 receptors (M4) (Kreitzer and Malenka, 2008; Kreitzer, 2009). On the other hand, D2 MSNs contain adenosine type 2A (A2A) receptors and indirectly send signals to the basal ganglia output nuclei via projections to the globus pallidus externus (GPe) – globus pallidus (GP) in rats (Kreitzer and Malenka, 2008; Kreitzer, 2009). These two MSN classes form parallel pathways; the direct pathway (D1 MSNs) promotes thalamic activity and the indirect pathway (D2 MSNs) promotes the inhibition of unwanted thalamic activity that may otherwise interfere with the intended outcome (Kreitzer and Malenka, 2008; Kreitzer, 2009). The activity of MSNs is regulated by a number of projections: glutamatergic projections from the PFC, DA projections from the substantia nigra pars compacta (SNc) (Bolam et al., 2000; Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009), and three main classes of interneurons – fast-spiking (FSI), low-threshold spiking (LTS), and large cholinergic tonically active neurons (TANs) (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009).

FSI are GABAergic neurons that contain the calcium-binding protein parvalbumin (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009). FSI dendritic trees do not contain spines and are less extensive than those of MSNs (Kreitzer and Malenka, 2008; Kreitzer, 2009). Nonetheless, FSIs resemble MSNs in that they express NMDA and AMPA receptors, as they receive numerous glutamatergic excitatory projections from different cortical areas and the thalamus (Kreitzer and Malenka, 2008; Kreitzer, 2009). FSI also receive some input from the GP (Kreitzer and Malenka, 2008; Kreitzer, 2009). FSI are unique to other striatal neurons, as they interconnect with each other via dendritic gap junctions and exhibit electronic coupling, which can lead to synchronised firing in local populations (Kreitzer, 2009). Gap junctions are protein channels that form an electrical synapse connecting two neurons together allowing electric current to pass between the neurons, which produces faster signalling. FSI produce short-duration action potentials (AP)

at higher frequencies than MSNs (roughly 2-60 Hz), with the normal frequency in awake animals lying between 10-30 Hz (Gage et al., 2010; Berke, 2011; Russo et al., 2013). The main function of FSI is the inhibition of MSNs by means of axodendritic and axosomatic synapses connecting the two neuron types at multiple sites (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009). Similar to MSNs, FSIs are modulated by DA and ACh, as they contain DA type 5 (D5) receptors and nicotinic ACh receptors (nAChR), both of which increase FSI excitability (Kreitzer and Malenka, 2008; Kreitzer, 2009).

LTS neurons are GABAergic interneurons that express somatostatin, neuropeptide Y, and nitric oxide synthase (Calabresi et al., 2000; Kreitzer, 2009). These interneurons exhibit a more depolarised cell membrane potential at -60mV compared to -80mV in MSNs and FSIs. LTS neurons also possess a lower threshold for inducing neuronal firing (Kreitzer and Malenka, 2008). Like FSI neurons, they receive glutamatergic innervation from cortical and thalamic projections, and synapse to MSNs to induce inhibition (Calabresi et al., 2000; Kreitzer, 2009). However, LTS dendritic trees are less extensive than FSI and yet they are more arborized (Kreitzer, 2009). A second class of LTS expresses calretinin, but these neurons seem to lack thalamic innervation (Calabresi et al., 2000; Kreitzer, 2009). A large proportion of innervation of these interneurons stems from DA neurons, via DAergic D5 receptors (Kreitzer, 2009). LTS neurons also receive cholinergic innervation via M1 and muscarinic type 2 (M2) receptors (Kreitzer and Malenka, 2008; Kreitzer, 2009; Do et al., 2012).

TANs differ from other STR cells in two ways; they are larger in size and they are excitatory rather than inhibitory (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009; Do et al., 2012). As the largest of the STR neurons, the soma of TANs ranges from 20-50μm, while the other neural types have somas of roughly the same size as those of MSNs (i.e. 10-18μm) (Calabresi et al., 2000; Kreitzer, 2009; Do et al., 2012). Tonically active (>10Hz) TANs receive sparse glutamatergic innervations from the thalamus and some input from the cortex (Kreitzer, 2009). They also receive inhibitory input from MSNs (Calabresi et al., 2000; Kreitzer and Malenka, 2008; Kreitzer, 2009; Do et al., 2012). With a widespread axonal field, TANs synapse mainly onto MSNs and some FSIs (Calabresi et al., 2000; Kreitzer, 2009; Do et al., 2012). TANs express DA receptors (D2 and D5), and ACh muscarinic receptors (M2 and M4) (Calabresi et al., 2000; Kreitzer and Malenka, 2008;

Kreitzer, 2009; Do et al., 2012). TANs are also the main source of ACh in the STR (Do et al., 2012)

Altogether, MSN, FSI, LTS, and TAN neurons make up most of the neural population of the STR and dictate the output of information received from the cortex. Many electrophysiological techniques have been used in order to understand the different properties of the neurons and how they respond to neural input as individuals and as populations.

1.4 Electrophysiological Techniques

Electrophysiology is the study of electrical activity caused by the exchange of ions between the intracellular and extracellular space of a cell or cells. Different electrophysiological techniques have made it possible to study numerous facets of neural activity. Examples include determining the influence of an ion channel on the firing pattern of a neuron, and the correlation between the neural activity of a population of neurons and a particular outcome (e.g., movement of a finger). Two main categories of electrophysiological techniques have been developed – intracellular and extracellular recording (Wickenden, 2014). The technique used in a particular study depends on the research question to be investigated.

Intracellular recordings involve examining currents and voltages across the cell membrane of a single neuron both *in vitro* and *in vivo* (Wickenden, 2014). This technique allows researchers to study detailed properties of a cell, such as current/voltage relationships, membrane resistance, and membrane potentials (Wickenden, 2014). Detailed properties of ion channels and how they affect the properties of a cell can also be studied using this technique (Wickenden, 2014). The main disadvantage to intracellular recordings is their limitation to studying a single neuron (Wickenden, 2014).

Extracellular techniques can be used to observe net ionic fluxes of a single neuron or a population of neurons both *in vitro* and *in vivo* (Buzsáki et al., 2012; Wickenden, 2014). Neural activity is observed by measuring the electrical potential in the extracellular fluid (Buzsáki et al., 2012; Wickenden, 2014). The size of the recording electrode and its placement vary by experiment, as each factor affects the recording obtained (Wickenden, 2014). The size of the electrode affects the recording resolution, which dictates the neural activity that can be detected (i.e. the activity of an individual neuron, or the net activity of a

population) (Wickenden, 2014). With a sufficiently small electrode, it is still possible to record the activity of 2-3 neurons (Wickenden, 2014). However, it is more suitable for distinguishing the activity of each individual neuron (Wickenden, 2014). The distance between the electrode and the neuron(s) is also influential, as it changes the magnitude of the signal; the closer the probe is to the site of activity, the stronger the signal will be (Buzsáki et al., 2012; Wickenden, 2014).

When recording populations of neurons in a specific brain area, the neural activity recorded is referred to as a local field potential (LFP), which reflect the sum of all of the neural activity that the electrode is capable of sensing (Buzsáki et al., 2012; Wickenden, 2014). LFPs can be recorded *in vitro* in tissue samples or *in vivo* in either an anesthetised or conscious animal. This may involve using a stimulating electrode in one area while recording neural activity in another (Buzsáki et al., 2012; Wickenden, 2014). LFP recordings are commonly used to look at the synaptic plasticity of neurons in different brain regions. This technique has been widely used to understand the mechanisms underlying long-term potentiation (LTP) and long-term depression (LTD), which are processes that cause persistent strengthening or reduction in efficacy of neuronal synapses based on recent activity (Buzsáki et al., 2012; Wickenden, 2014).

Single unit (SU) recording captures neural activity in the form of APs and, like LFP recordings, this technique can be used both *in vitro* and *in vivo* (Cousens and Muir, 2006; Buzsáki et al., 2012; Wickenden, 2014). SU recordings are performed with either a single electrode or a multi-electrode array (MEA) (Cousens and Muir, 2006; Buzsáki et al., 2012; Wickenden, 2014). MEAs are used to record SU activity in multiple neurons from a single brain region, a technique that presents a number of advantages (Cousens and Muir, 2006; Buzsáki et al., 2012; Wickenden, 2014). For example, fewer test animals are required to gain the same amount of data and more extensive analyses are possible. A single neural recording of an event in an animal allows researchers to observe both its response to a stimulus and the correlation between its activity and a behavioural outcome. In contrast, the recording of multiple neurons allows for the elucidation of the neural response(s) to an input, any relationship(s) to an output, and overall information processing of neural populations (Buzsáki, 2004; Cousens and Muir, 2006; Buzsáki et al., 2012). *In vivo* recordings of neural activity can be obtained using multiple techniques: restrained/tethered animals that are subjected to stimuli, or freely moving animals that can be monitored to observe either their

reaction to stimuli or their neural activity during the completion of a task. SU recordings have helped develop a better understanding of sensory, motor, and cognitive processes, and sensorimotor integration. Examples of such experiments include: using visual cues to understand collision avoidance in locusts (Gabbiani et al., 1999; McMillan and Gray, 2012); using video capture of wing movements and measuring the torque of the turn on the horizontal axis (yaw) for determining the relation between motor signals and flight controls in moths, (Sponberg et al., 2015); using insect-machine hybrid systems to understand behaviour outcomes of moths based on pheromone cues (López, 2005; Kanzaki et al., 2013); and using rats in a radial arm maze to observe their neural activity during decision-making, reward, and spatial processing (Berke et al., 2009).

In previous electrophysiology experiments utilizing freely moving test animals, three types of STR neurons have been identified – MSN, FSI, and TAN (Berke et al., 2009; Berke, 2011; Yarom and Cohen, 2011; Yael et al., 2013; Benhamou et al., 2014; Jin et al., 2014). In a radial arm maze task (Win-Stay), MSNs showed increased activity when the rat was in the centre of the maze, as it had to decide which arm to explore (Berke et al., 2009). A sharp increase in MSN activity was also seen when it received a reward from a food port (Berke et al., 2009). MSN activity did not seem to correspond to specific spatial cues defined by extra-maze cues, or the speed or trajectory of the rat during the experiment (Berke et al., 2009). In another experiment, rat TANs in the dlSTR and vSTR showed increased firing at the initiation of a trial and when they received an unexpected reward, while firing was briefly paused with an expected reward and following a Go tone cue (Benhamou et al., 2014). Although this trial detected MSN and FSI neurons, no analysis was performed with them. A third study found that FSIs increased activity in the dlSTR immediately prior to decision-making, where the test animal had to choose a nose poke port in order to receive a reward (Gage et al., 2010). This was correlated with inhibition of the GP (Gage et al., 2010). Although STR activity has been investigated in STM and spatial tasks, a review of the current literature did not yield any studies on SU activity in the STR during WM tasks. The combination of a WM memory task with freely moving electrophysiology would be useful for investigating the role of the STR in WM. A comparison of the neural activity of healthy rats to rats induced with symptoms to resemble neurological disorders (i.e. schizophrenia) would also help improve our understanding of WM in both healthy and diseased states.

1.5 Schizophrenia and Working Memory

Schizophrenia is a psychiatric disorder affecting 1% of the human population. Its symptoms are characterised into three groups: positive, negative, and cognitive (Bozikas et al., 2004; Carbon and Correll, 2014). Positive symptoms are defined as behaviours not normally present in a healthy individual that result in the inability of the patient to distinguish what is reality. Such symptoms include hallucinations and delusions (Bozikas et al., 2004). On the other hand, negative symptoms are behaviours that are absent in patients, but that are normally found in healthy people. Negative symptoms included reduced speech and lack of the following: facial expression, vocal tones, pleasure in everyday life, and motivation (Buzsáki et al., 2012; Carbon and Correll, 2014). Negative symptoms can be harder to recognise as signs of schizophrenia and can easily be mistaken for clinical depression (Carbon and Correll, 2014). Finally, cognitive symptoms are reductions in cognitive functions in several areas of the brain, such as decreased attention/vigilance, reasoning, problem-solving, and WM (Young et al., 2009; Carbon and Correll, 2014). Key aspects of WM thought to be affected in schizophrenia include maintenance of information over time, the ability to update information, and capacity (Barch and Smith, 2008; Gold et al., 2010).

In animal research, MK801 is a non-competitive NMDA receptor antagonist that is used to induce a state that may resemble schizophrenia (Jackson et al., 2004; Homayoun and Moghaddam, 2007; MacQueen et al., 2011). MK801 binds to the PCP site of NMDA receptors, which results in the receptor's inability to open and a subsequent reduction in neuron excitability (Foster and Fagg, 1987). Acute infusions of MK801 produce schizophrenia-like behavioural changes, which manifest as social withdrawal, hyper-locomotion, and decreased prepulse inhibition (Rung et al., 2005). NMDA receptor antagonism by MK801 has also shown similar cortical activation in rats to healthy human volunteers injected the NMDA antagonist ketamine (Breier et al., 1997; Krystal et al., 2003; Homayoun and Moghaddam, 2007). The increase of neural activity is caused by disinhibition of glutamatergic pyramidal neurons by FSIs (Breier et al., 1997; Krystal et al., 2003; Jackson et al., 2004; Homayoun and Moghaddam, 2007). In a search of the literature, no publications were found to show the effects of MK801 on MSN activity in freely moving rats during a WM task. In rodents, MK801 has been shown to disrupt WM during the OST, a non-spatial WM task that involves WM capacity (MacQueen et al., 2011). More specifically, MK801 injections produced a decrease in WM capacity and yet did not interfere any other

aspect of the task (MacQueen et al., 2011). Since NMDA receptors are found on the main information inputs to the basal ganglia (i.e. on MSN, FSI, and TAN striatal neurons), MK801 would be a good candidate for investigating alterations in the neural firing of the STR during the performance of a WM task that may affect maintenance, updating, or WM capacity.

1.6 The Odour Span Task

In 2000, Dudchenko published a paper on his investigation of the effect of hippocampal damage on WM capacity. In order to conduct his study, Dudchenko developed a novel non-spatial task – the OST (Dudchenko et al., 2000). Although it was found that the hippocampus was not involved in the non-spatial WM task (Dudchenko et al., 2000) the OST became a useful tool for investigating WM. The OST requires that rats remember previous odours (maintenance) and learn new ones (updating) while increasing the number of items to be stored during the task (capacity). The OST requires rats to dig in a bowl of scented sand to retrieve a food reward. Rats are first trained on a delayed non-matching-to-sample test, where they must dig in a bowl baited with a novel scent to receive the reward. WM span is then assessed by presenting rats with serial delayed non-matching-to-sample trials in a single test session. All odours/spatial locations from a given day's trials are possible choices along with one novel stimulus. Rats typically demonstrate a WM span of approximately 6-8 odours. Lesions of the dorsal STR, mPFC, or the blockade of two subtypes of glutamate receptors impair OST performance (Young et al., 2009; MacQueen et al., 2011; Davies et al., 2013a, 2013b; Howland et al., 2014). In addition, work has been done in the Howland lab to investigate how neural activity correlates with events during the OST in the mPFC (An et al., 2015).

1.7 Hypotheses

The primary goal of my research was to examine the patterns of neural activity in the dmSTR that may mediate OST performance. These experiments were conducted using freely moving rats whose neural activity from the STR was being recorded while they performed the task. No studies to date have assessed patterns of brain activity in rats from the STR during the OST; thus these results are novel. Modifications were made to the current OST protocol used in Howland's laboratory when a number of issues were encountered during the analysis of previous video recordings of the OST. It was found that the black floor of the

OST table made it more difficult to follow the movement of the rat's head. The rats used were Hooded Long Evans, which have black shoulders, a black head, and a black stripe running down the centre of a white body. The lack of contrast between the rat's head and the table made it difficult to obtain timestamps of events for comparison of neural activity, as the video recording was synchronised to the neural activity. The main events tracked were the rat's approach to a bowl, the rat's making of a choice (i.e. when it started to dig), the retrieval of the reward, and the start and finish of consuming the reward.

The orientation of the table layout also made it difficult to retrieve timestamps, as the camera was shooting from behind the housing, which was made of clear plastic. Due to refraction of the plastic, it was occasionally difficult to know when or if the rat approached the bowl, particularly if the bowl was placed in a position where the camera observed it through a corner of the housing/door. Another disadvantage to the camera angle was that for the majority of the time, the camera captured the rat from behind. This made it difficult to see when the rat started to dig or when it retrieved the reward. In 2011, McQueen and colleagues published a paper where the OST was modified so that the rat had to flip a scented lid on a bowl to receive a reward rather than dig in the sand (MacQueen et al., 2011). With modifications to the table and its orientation to the camera, I also adapted the current lab protocol to have the rats flip scented lids based on the McQueen procedure to make it easier to discriminate when the rats made a choice when performing the following experiments.

Experiment 1. The purpose was to investigate the neural activity of MSNs during the following events: a rat's approaching of a novel odour, making a correct choice to flip, and receiving a reward(Capp) compared to when approaching a familiar odour and making a correct choice not to flip (Fapp). My hypothesis was that MSN firing rates would increase when a rat made a Capp compared to a Fapp. It has been proposed that the striatum acts like a gating mechanism in WM switching from maintenance of information to manipulation (Frank et al., 2001; Lewis et al., 2004; Lustig et al., 2005). If the STR does, in fact, act as a gating mechanism and the closing of the basal ganglia feedback loop allows for information updating, a novel scent should cause an increase in firing rate when approaching a novel odour causing disinhibition of the thalamus. The novel odour provides new information to the rat that it will need to remembered to perform the task. On the other hand, a familiar scent should encourage information maintenance with an open feedback loop, which would be reflected by no change in firing rate of MSN. Since the GP will not be inhibited by the

increased activity from the MSNs in the dmSTR the thalamus will be inhibited, ceasing firing to the prefrontal cortex and opening the feedback loop. MSNs in the dmSTR have been shown to increase in activity when receiving a reward in rats during STM tasks (Berke et al., 2009). Using a radial arm maze, Berke and colleagues showed that MSNs increased in firing rate to receiving a reward and decision making (Berke et al., 2009). Since WM and STM are considered to be related in rats (Dudchenko, 2004; Bratch et al., 2016), the increased firing rate by MSNs in response to reward should also be seen during a WM task.

Experiment 2. The purpose was to investigate the effects of MK801 on MSN activity during Capp and Fapp events while performing the OST. My hypothesis was that rats injected with MK801 would exhibit a reduced increase in firing during Capp compared to control rats, as MK801 is a NMDA antagonist. Since NMDA receptors are part of the post-synaptic response, MSNs would be less responsive to inputs. Additionally, no change in neural activity would be seen during Fapp in MK801-injected rats compared to controls, since no change in firing was expected when approaching a familiar odour. Another prediction was that basal MSN firing rates would increase during baseline recordings before the rat performed the OST. The reduced inhibition from interneurons would decrease regulation of MSN firing, which would be reflected by an increase in basal firing from MSNs.

2.0 MANUSCRIPT

2.1 Title Page

Activity of rat dorsal striatal medium spiny neurons during the approach of familiar and novel odours in the odour the span task

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***** The following sections (2.1 - 2.7) are the text of a manuscript to be submitted to the Journal of Neuroscience or a similar venue. The manuscript contains all of the experiments described and completed for this Master of Science thesis. *

2.2 Abstract

Working memory is an important cognitive function that allows us to perform everyday tasks including language comprehension and reasoning. Dysfunction of working memory has been identified in many neurodegenerative and psychiatric disorders, such as schizophrenia. The striatum is considered an important brain region involved in working memory in both humans and rats. Although its role in working memory is not well understood, increases in striatal activity have been shown during information updating in humans. In rat models, studies of striatal neural activity have revealed increased medium spiny neuron firing when receiving a reward during a short-term memory task. However, no previous studies seem to have examined this neural activity in rats during a working memory task. The current study involved the implantation of multiple electrode arrays into 6 rats. This allowed for the recording of medium spiny neuron activity from the dorsomedial striatum when the test animals received a reward or approached familiar and novel odours during the odour span task, a working memory task. After 12 days of odour span task trials, rats were administered MK801, an *N*-Methyl-D-Aspartate receptor antagonist, to observe changes in neural activity. Medium spiny neurons showed a significant increase of activity after receiving a reward, while no response was shown in medium spiny neurons when rats approached a familiar odour. Activity in response to novel odours was inconclusive. Finally, MK801 resulted in a 25% increase in medium spiny neuron firing when neural activity pre- and post-administration was compared.

2.3 Introduction

In humans, working memory (WM) is a multi-component system that provides temporary storage and allows the manipulation of information necessary for a wide range of cognitive tasks including language comprehension, learning, and reasoning (Baddeley, 1992; Cowan, 2009; Aben et al., 2012). Impaired WM has been implicated in many neurodegenerative and psychiatric disorders including Parkinson's disease (Cools and D'Esposito, 2011), schizophrenia (Barch and Smith, 2008), and Alzheimer's disease (Baddeley, 1992). On the other hand, WM in rats is considered a delay-dependent representation of stimuli used to guide an animal's behaviour within a task (Dudchenko, 2004). Although a rat's brain is not as anatomically complex as a human brain, there is evidence of independent WM subsystems in rats, which are fundamental attributes of human cognition and WM (Bratch et al., 2016).

The striatum (STR) is a brain region present in both humans and rats that is implicated in WM (Floresco et al., 1999; Cools and D'Esposito, 2011; Howland et al., 2014). Although the STR likely acts as a gating mechanism that controls when movements are initiated or ceased for the motor-control system (Charara et al., 2003; Purves et al., 2011; Jin et al., 2014), its role in WM is not clear. It is proposed, however, that the STR acts as a gating mechanism like in the motor-control system, but rather than initiating and ceasing movement it switches from the maintenance of information to the manipulation of information (Frank et al., 2001; Lewis et al., 2004; Lustig et al., 2005). When striatal medium spiny neurons (MSNs) in the direct pathway are activated, thalamic activity increases (Frank et al., 2001; Lustig et al., 2005; Kreitzer and Malenka, 2008). Thalamic projections are then received by the prefrontal cortex (PFC) whose activity enables the updating of information, whereas its inhibition of the thalamus promotes the maintenance of information (Frank et al., 2001; Lustig et al., 2005). In humans, fMRI studies have reported increased neural activity in the caudate nucleus during manipulation of information within WM tasks compared to information retrieval (D'Esposito et al., 1999; Lewis et al., 2004). However, mixed results have been found between studies examining if neural activation is significantly different during information maintenance and manipulation (D'Esposito et al., 1999; Lewis et al., 2004). A review of the current literature did not yield any studies on neural activity in the STR in rats during WM tasks. Moreover, neural activity in STM and spatial tasks has been

investigated, which revealed that MSNs show an increase in firing rates during decision-making and reward events (Berke et al., 2009).

The odour span task (OST) is a non-spatial WM capacity task, with capacity referring to the finite amount of information that can be maintained. The OST requires rats to remember previous odours (i.e. maintenance) and learn new ones (i.e. updating) while increasing the number of items to be stored during the task (i.e. capacity). The Howland lab has published a series of paper that examined the neural substrates of a common rodent task in order to assess WM capacity using the OST (Davies et al., 2013a, 2013b; Howland et al., 2014). The OST procedure was published originally by Dudchenko et al, who trained rats to dig in scented bowls to receive a reward (Dudchenko et al., 2000). MacQueen et al have since published a modified procedure of the OST where rats are now required to flip scented plastic lids on bowls to receive a reward (MacQueen et al., 2011). In addition to the lid flip modification, the present study performed modifications to the OST apparatus in order to improve viewing of video playback when recording event times.

MSN activity was observed in the dmSTR while rats were freely moving during the OST. It was hypothesised that MSN activity should increase during the approach to a novel odour with the correct choice not to dig (Capp), as the novel odour is new information that would need to be updated. Contrarily, there should not be a change in MSN firing during the approach to a familiar odour with the correct choice not to dig (Fapp), as familiar odours are considered information already known that only requires maintenance.

I also wanted to examine MSN activity when WM was dysfunctional. In this particular study, MK801 was administered to investigate the changes in neural activity of MSNs in rats performing the OST compared to neural activity in a control state. MK801 is used in animal research to induce a state that may resemble schizophrenia (MacQueen et al., 2011), and WM has been shown to be dysfunctional in schizophrenia (Young et al., 2009; Carbon and Correll, 2014). It was hypothesised that changes in neural firing during Capp would decrease since NMDA receptor inhibition would reduce MSN responsiveness to neural input, while neural activity during Fapp should not change. A second predicted observation was an increase in basal MSN firing rates after administration of MK801 compared to baseline activity. The reduced inhibition from interneurons (which also contain NMDA

receptors) would decrease regulation of MSN firing, which would be reflected by an increase in basal firing from MSNs.

2.4 Materials and Methods

2.4.1 Modifications

The OST apparatus had a cream corrugated plastic sheet fitted over the original black covering. The housing was moved to the opposite corner of the table so that the door opened towards the camera. The position of the table was also moved in order to maximise the area of the table viewed by the camera. Red masking tape was put on the table to mark the camera's visual boundaries in order to prevent the accidental placement of bowls out of view of the camera. Although the placing of bowls outside camera coverage was not a common occurrence before the modifications were made, it had occasionally posed problems if the span was over 10. The contrast between the cream flooring and the rat's black head made it much easier to see where the rat was and where it was looking. There was also enough contrast between the white cups and the flooring to be able to discriminate between them without difficulty (*Figure 2-2*). The improvement in contrast between objects reduced the time spent replaying videos to ensure accurate time-stamping. In addition, the use of the lid flip task as opposed to the digging task simplified and quickened marking the event time Capp; when the rat was digging, its body often made it difficult to see if it had started to dig while with lid flipping, the researcher time-marking the video simply had to watch for the lid to move if the rat's body obscured vision. The only disadvantage to the lid flip modification was some difficulty recognising when the rat got the reward and consumed it. During the digging OST, rats would receive a Froot Loop as their reward, which is considerably larger than a sucrose pellet. This resulted in distinctive head movements when a rat grabbed the Froot Loop out of the sand with its mouth. Given the size of the Froot Loop, rats would take it back to the housing before eating it. In contrast, sucrose pellets are small enough that a rat could pick them up and eat them right away without any noticeable change in movement

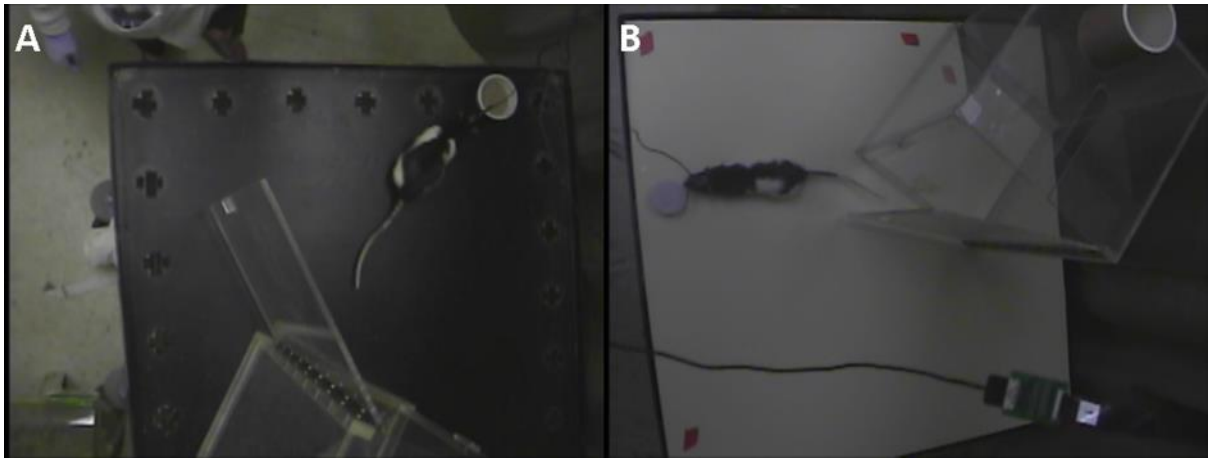

Figure 2-2. Photographs comparing before and after modifications to the OST.

2.4.2 Subjects

Six adult male hooded Long Evans rats (Charles River Laboratories, Quebec, Canada) were individually housed in clear polycarbonate cages with ad libitum access to food and water. Rats were housed in a temperature and humidity controlled vivarium under a 12:12 hour light/dark cycle. All experiments were conducted during the light phase. After arrival to the facility, the rats were given at least 5 days to acclimatise before experimentation was conducted. Following acclimatisation, food was restricted to maintain 85% of the rat's free feeding weight while conducting behavioural experiments. All experiments were conducted in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.4.3 Apparatus

Training and testing occurred on a 91.5 cm^2 platform covered with cream corrugated plastic and a 2.5 cm tall border around the outer edge. The platform was secured to a metal frame with casters and stood 95 cm above the floor. The platform was surrounded by a beige curtain to block visual cues in the room. A clear plastic housing (32 cm W x 50 cm H x 35 cm D) with a hinged door on the front was used to house the rat between trials. A slot was cut out from the top to allow the tether to connect to the rat.

2.4.4 Odours

Plastic Dixie cup lids were stored in airtight plastic containers (n=24) with household spices and flavouring, with each container holding one of the following: allspice, anise seed, basil, caraway, celery seed, cinnamon, cloves, cocoa, coffee, cumin, dill, fennel seed, garlic, ginger, lemon and herb, marjoram, mustard powder, nutmeg, onion powder, orange, oregano, paprika, sage, and thyme. All spices and flavouring were purchased from a local grocery store a couple of days before the beginning of training. The lids were left for at least 24 h in order for the plastic to absorb the odour of the spice. Plastic Dixie cups (59 mL) were half filled with unscented sand and the lids were placed on top. The sequence of the odours used each day was selected randomly and rats were regularly exposed to all odours. The sand-filled cups with scented lids were placed on the platform as needed for each trial.

2.4.5 Training on the Odour Span Task

Lid flip training. First, rats were trained to flip an unscented lid for a food reward (sucrose pellets) in the cup half-filled with unscented sand. Rats were placed opposite to a cup on the platform for four separate phases. In the first phase, the food reward was placed on top of the sand with no lid. In the second phase, the lid covered half the cup. In the third phase, the cup was mostly covered with a small gap, and in the fourth phase, the cup was completely covered with the lid sitting on top. Rats were trained until they would consistently flip the lid for the food reward regardless of the cup's position on the platform.

Odour non-matching-to-sample. Once the rats reliably flipped the lids on unscented bowls, they moved onto the non-matching-to-sample task. In the sample phase of a trial, the rat was presented with a cup and scented lid randomly located on the platform. After the rat flipped the lid and consumed the food reward, the rat was placed in the housing on the platform and lids were changed behind a curtain. The researcher then placed the cup at a random position on the platform and added a second cup with a different odour on the platform beside it. In the choice phase of the trial, the door to the housing was unlocked and the rat would open the door, allowing access to both cups. A food reward was only placed in the cup with the novel odour lid. A correct choice was scored if a rat attempted to move the novel lid, and an error was scored if a rat attempted to move a lid of a previously rewarded scent. The rats completed six non-matching-to-sample trials each day with a 45 second delay

between trials until they chose the novel odour on at least five of the six trials for three out of four days to meet criterion.

Odour span task. After attaining criterion on the non-matching-to-sample task, the rats were introduced to the OST. A cup was placed at a random position on the table with novel scented lid and a reward underneath placed on the sand, span 0 (*Figure 2-3*). The housing door was then unlocked to allow the rat to open the door itself, the rat was then required to sniff and flip the lid on the cup to receive a reward. Once the rat obtained the reward it was then placed back into the housing and the door locked. A second cup was then placed on the table with a new novel odour, which contained a reward. For span 1, the housing door was then unlocked and the rat had to then open the door and choose between the odour from span 0 (familiar) and the new odour (novel) (*Figure 2-3*). An additional cup with a new odour was placed on the table each time the rat chose the novel odour correctly (*Figure 2-3*), while the rat was in the housing. Once the rat incorrectly flipped a lid the trial was over. The measure of the span was considered the amount of correct choices during the trial minus 1 to account for span 0. During a trail lids from the previous span of the trial were removed and replaced with lids of the same odour and randomly placed on cups. This was done to ensure that the rats were not marking the lids to know where they had already been. Also between each span all cups on the table, including the new novel odour, were 'shuffled' and then place at a random location. Thus, rats could not use marking, spatial, and/or odour cues to guide their response. Each rat performed 3 spans per day with a 45 s break between trials until the rat could get a span of 6 or higher, 2 days in a row to meet criterion.

2.4.6 Probe Sessions

To determine if the rats were using the odour to solve the task, one type of probe testing was conducted. The probe session tested if a scent from the sucrose pellets guided behaviour. In this session, the rats were presented with cups as described in the OST with the exception that the reward was absent from all cups. When the rat made a correct choice, the researcher dropped a sucrose pellet on top of the sand in the correct cup.

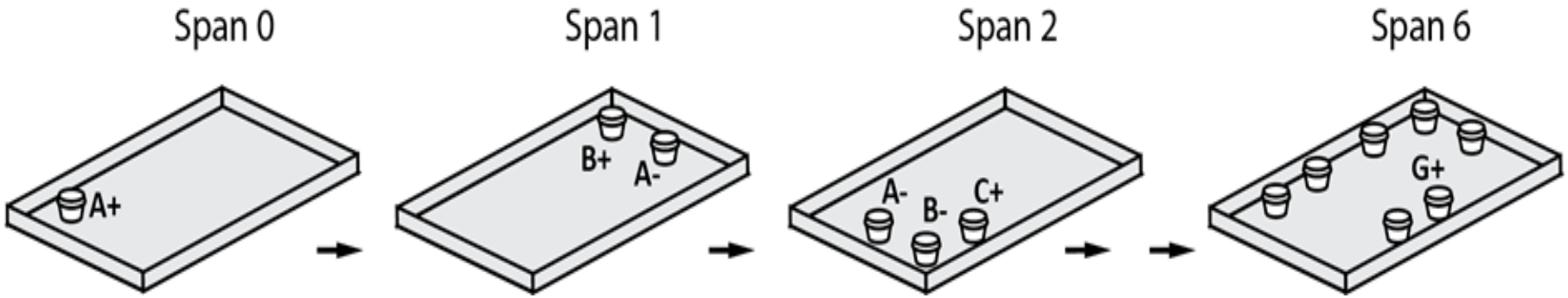

Figure 2-3. A diagram of the OST. In span 0, only the bowl with a novel scent is placed on the table with a food reward (+) for the rat to choose. Span refers to when a new bowl with a novel odour is introduced with a food reward, while the familiar odour from span 0 is placed back on the table with no reward (-). Every time the rat chooses the correct bowl with a reward, another bowl that is novel with a reward will be added until the rat chooses a familiar bowl.

2.4.7 Recording Electrodes

Polyimide tubing (0.203 mm inner diameter) was cut to 10 mm lengths (*Figure.2-4.A*). The polyimide tubing was then adhered together to make 1x8 arrays, which were then cut to 8 mm lengths (*Figure 2-4.B*). Four 1x8 arrays were adhered together to form a 4x8 array (*Figure 2-4.C*). The 4x8 tubing array was attached to a small piece of rubber cut into a rectangle (*Figure 2-4.D*). Each tube was threaded individually with an insulated tungsten wire (0.001 mm) (*Figure 2-4.E*). Tubing was then mounted to a 32-channel electrical interface board (Omnetics from Neuralynx, Bozeman, MT) in a position that allowed for easy mounting to the rat's head. The ends of the electrode wires at the EIB side were individually pulled into circuit holes (*Figure 2-4.F*). All circuit holes were then pinned and the wires trimmed back to the board, while the free end of wires coming out of the open side of the tubing were trimmed to extend 3.0mm from the ends of the polyimide tubes (*Figure 2-4.G*). Electrodes had 2 ground screws attached by separate stainless steel wires to the grounding terminals, and all exposed wiring on the EIB was then covered with epoxy glue to protect the wiring. Testing of electrodes was performed with a test current of 1 nA at 1 kHz with a NanoZ impedance tester (Whiter Matter LLC from Neuralynx, Bozeman, MT). The output values were in ohms (Ω) and the values were between 200 kΩ to 5000 kΩ.

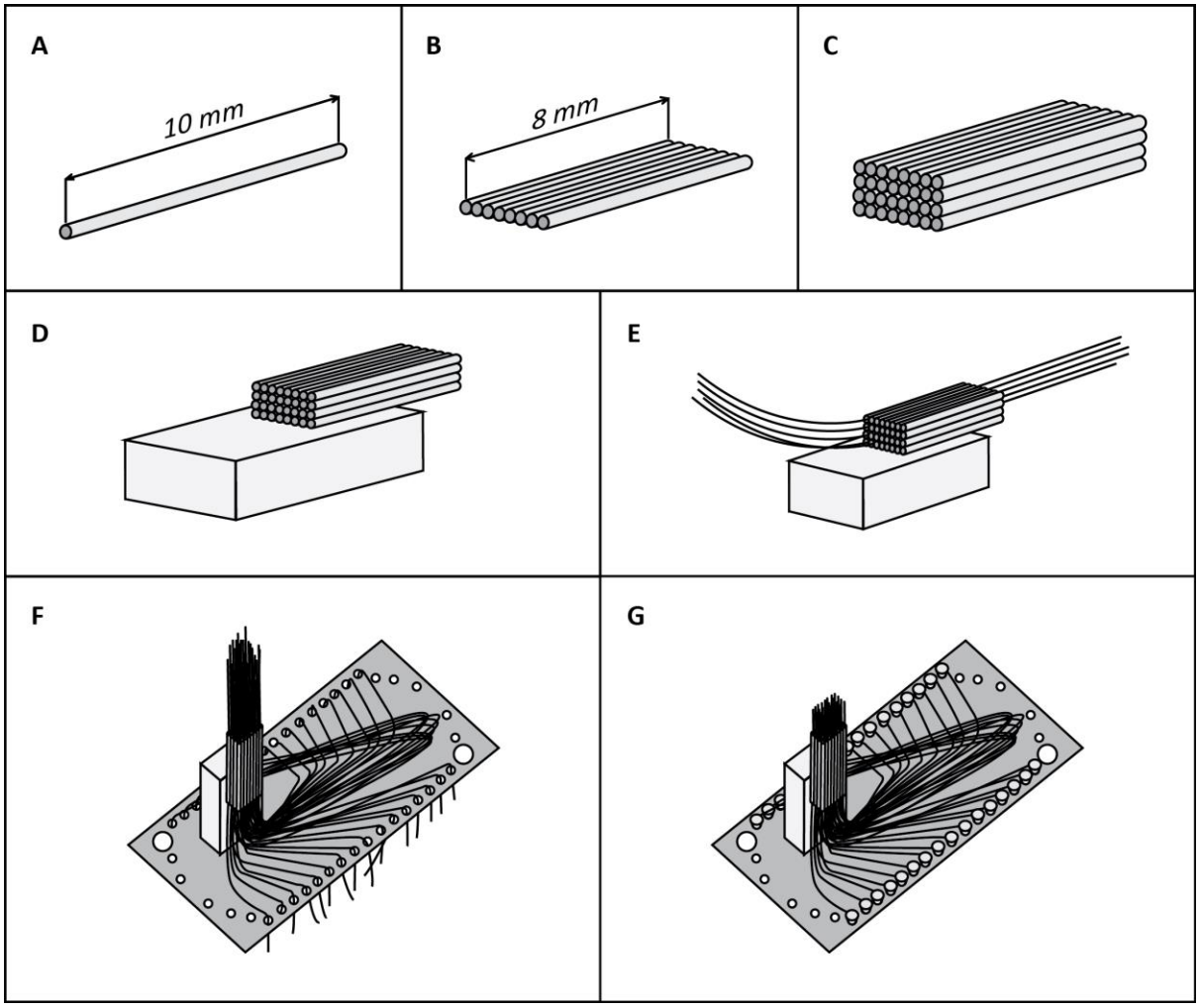

Figure 2-4. An instructional diagram on how to make an 8x4 electrode array. See text for details.

2.4.8 Surgeries

Each rat was anaesthetised with isoflurane (5% induction, 2% maintenance), and monitored periodically for response during the surgery. The rat was then mounted in a stereotaxic frame (David Kopf, CA). A grounded homeothermic temperature control unit (Harvard Instruments, MA) was used to maintain the rat's temperature, monitored via a rectal thermometer, at 37 °C $\pm$ 1 °C for the duration of the procedure. The skull was then exposed and flat skull was found. Six screw holes were marked and drilled - four for screws to help secure the head cap, and two for the grounds from the electrode probe. The four screws for securing the head cap were seated before lowering the electrodes. Next, the electrodes were lowered into the dmSTR through a bored hole (the electrodes closest to the midline were ML = 2.0 mm, the electrode array fell between AP = 0.0 mm and AP = +1.8 mm, and the final

depth was DV = -4.4 mm from bregma). Grounds screws were then seated into the skull. A head cap was made using dental acrylic to secure and protect the probe. An antifungal was used to clean the exposed bone and tissues before suturing the skin closed around the head cap. A 5 mg/kg dose of Anafen was administered to the rat subcutaneously for pain relief when the rat first awoke. The rats were then monitored for 7 days with ad libitum access to food and water before being food restricted to maintain 85% of their free feeding weight while conducting behavioural experiments.

2.4.9 In Vivo Electrophysiological Recordings

Following a 7-day recovery period from surgery, rats were handled for 5 days in order to habituate them to the study set-up, where their head cap electrode array was attached to the head stage pre-amplifier (HS) (Omnetics from Neuralynx, Bozeman, MT). In addition, these 5 days allowed the rats to practise the OST. Once the rats were accustomed to the HS attachment process, 12 days of OST trials were performed during which neural activity was recorded. On each day prior to beginning the trials, the HS was tested to check the channel outputs by attaching a signal mouse (Nueralynx), a 50Hz was then produced. The grounding switches on the signal mouse were then flipped on and off to check if each channel was working. Rats would then be connected to a multichannel acquisition machine (Digital Lynx SX system, Neuralynx, Bozeman, MT) via a tether connected to the HS, which allowed the rats to move freely on the platform while their neural activity was simultaneously being recorded. Before a rat performed an OST trial, it was fed a sucrose pellet while the data acquisition software (Cheetah, Neuralynx, Bozeman, MT) was turned on to observe if the consumption of a reward produced background noise in the recording channels (i.e. if the noise produced in the ground channel was altered). Once the grounding was appropriate, the recording of neural activity began and the rat performed the OST. All behavioural data was recorded on a video camera synchronised with timestamps generated by the Digital Lynx System.

2.4.10 MK801 Treatment and Testing

Once all rats had completed 12 days of OST with electrophysiological recordings, MK801 treatments commenced. Aliquots were made from one MK801 solution batch one day prior to the beginning the MK801 experiments, and stored in a -20°C freezer. MK801

was thawed as needed prior to starting trials. The vehicle used in all experiments was saline. The method of administered was by an IP injection with a 0.1 mg/kg dose of MK801. Before drug administration rats were firstly placed in the housing on the platform and their neural activity was recorded for ten minutes. After the ten minutes, rats received an IP injection of the drug or vehicle. Their neural activity was recorded for an additional 30 minutes before starting span 0 of the OST. Each rat only performed 1 span trial per day.

2.4.11 Histology

Once the rats had completed all recording experiments, they were anaesthetised with urethane via an IP injection (1.5-2.0 mg/kg). A direct current (2 mA, 20 s) was then administered through 8 of the 32 electrodes to create electrolytic lesions. Next, rats were transcardially perfused with 100 mL of physiological saline, followed by the perfusion of 80 mL of 10% formalin. The brains were removed and stored in formalin-10% in a fridge for 2-3 days before being stored in a 10% sucrose solution. Electrode placements were verified by sectioning the brains using a sliding microtome, mounting the slices on slides for viewing with a compound microscope (Fisher Scientific), and using a rat brain atlas to identify the affected area (Paxinos and Waston, 1997). The slides were subsequently stained with cresyl violet and photographed.

2.4.12 Data Analysis

Statistical tests were conducted using SPSS Version 21 (IBM, Armonk, NY) for Windows and Graphpad Prism 6.0 (GraphPad Software, CA). All descriptive values are reported as means ± standard error of the mean (SEM).

For behavioural data, days to criterion was collected for each training phase and the average span calculated for each day of OST once neural recording began. Comparisons between the digging and lid flip OST used independent t-tests. A one-way ANOVA was used to analyse differences between days during the 12 day OST, as well as comparisons between control, saline and MK801 spans. Post hoc analysis used Fisher's LSD for individual comparisons. After the experiments were completed the video recordings were examined to mark event times: start of each span (Start), Capp, Fapp, correct lid flip (Cflip), incorrect lid flip (Eflip), reward, and end of each span (End). Latency times were calculated for the time between approaches (including Capp events), time took from approaching a

novel odour to lid flip (Capp to Cflip), and time taken to make a correct choice (Start to Cflip). Differences in latencies when rats were performing the OST with and with MK801 used independent t-tests.

Raw neural activity data was bandpass filtered at 300-6000 Hz and continuously sampled at a frequency of 32 kHz using the digital lynx SX system. Offline sorting was performed (SpikeSort 3D) where neurons were sorted using the KlustaKwik algorithm program based on peak, valley, and energy (Kadir et al., 2014). After the KlustaKiwk algorithm, clusters where manually checked and if multiple clusters were found on a channel isolation distances and L-ratios were checked. Parameters for isolation distance and L-ratio were taken from Friend at el, isolation distance had to be larger than 40 and L-ratio smaller than 10^{-2} to be considered separate neurons (Friend et al., 2015). Interspike interval (ISI) and cross-correlation analysis were performed to remove any false clusters using Neuroexplorer (Nex Technologies, AL). Clusters were then identified based on the waveform peak-valley duration and firing rates. MSN peak-valley durations could not be shorter than 0.35 μs, and FSIs maximum was 0.30 μs. TANs were considered by longer peak-valley duration where the waveform showed no positive valley. Firing rates for the neurons were set at MSN 0 – 10 spikes/s, FSI 5 – 30 spikes/s, and TAN 5 – 15 spike/s. Video timestamps of event times were collected for each trial session – Start, Fapp, Capp, Cflip, reward, Eflip, and End. MATLAB (Mathworks) was used to calculate the average firing rate (Hz) of different bin sizes and time durations around Capp and Fapp event times for each MSN neuron. To analyse a cell's response to an event, MATLAB was used to perform a one-way ANOVA comparing 0.1s bins over a 2s period (-1 to +1) around the event within each cell (statistics not shown). The results of the analysis gave the cell, P value, and time of the significant P value. Any values that showed significance between -1.0 s to +1.0 s were manually verified, as cells were excluded if any increase in activity was only seen at these times. Two-way ANOVAs with repeated measures (GraphPad 6.0) were used to analysis differences between event times and baseline firing. Post hoc analysis used Tukey's multiple comparisons test to determine specific times and events of significance. Comparison of MSN activity during baseline and after MK801 administration was analysed using a paired t-test.

2.5 Results

2.5.1 Behavioural Training and Task Comparisons

All six rats completed lid flip training in 3 days, and ranged from 7 - 10 days to complete the odour non-matching-to-sample training. The criterion for the OST task before surgeries was achieved within 10 days for all rats, except one that required 13 days. After recovering from the surgeries, one rat was euthanized after the head cap and recording probe were detached. In order to determine if the modified task reduced the duration of the training period, lid flip OST rats were compared to a group of rats (n = 6) previously trained on the digging OST. Rats performing the digging task took 4 - 6 days with an average of 5.16 ± 0.40 days to learn to dig for a reward rather than the maximum of 3 days (with an average of 2.50 ± 0.22 days) for lid flipping, significance was revealed with an independent t-test ($t(10) = 5.80$, $p < 0.01$). The lid flip training may have been completed in a shorter time period, but the odour non-matching-to-sample and OST criterion took slightly longer. For odour non-matching-to-sample and OST criterion, lid flipping rats took 7.50 ± 0.56 and 8.50 ± 1.18 days, respectively, while digging OST rats averaged 6.67 ± 0.42 and 6.83 ± 1.01 days, respectively. Independent t-tests showed no significance between lid flipping and digging for either odour non-matching-to-sample ($t(10) = 1.19$, $p = 0.26$) or OST criterion ($t(10) = 1.07$, $p = 0.31$). The digging odour non-matching to sampling training ranged from 6 to 8 days, while criterion for the digging OST was achieved by all rats within 10 days. However, the rats that performed the digging OST only completed the 12 days of trials and were not trained to meet a criterion like the lid flipping OST rats. These rats' OST spans over the 12 days were retrospectively examined and the time to criterion was marked using the same standards as with the rats that performed the lid flipping task. Overall, no change was observed in the training period with the lid flipping OST, as the average training time for lid flipping and digging were similar (18.50 ± 1.31 and 18.67 ± 1.15 days, respectively).

2.5.2 OST Behaviour

The average span on day 1 was 0.60 ± 0.24 (n = 5), which jumped to 3.60 ± 0.81 on day 2. The average span steadily increased to 5.80 ± 1.36 by day 12 (*Figure 2-5.A*). A one-way ANOVA revealed there was a significant difference of spans between days, $F(11, 48) = 2.07$, $p < 0.05$. However, Tukey's multiple comparisons test showed no statistical difference

between the average spans from days 2 to 12 ($p > 0.05$). On day 1 the rats performed significantly lower than the rest of the trials ($p < 0.05$) and obtained lower spans compared to the training sessions after surgery. Over the 12 days, a total of 60 trials were performed by the rats. Out of the 60 trials the rats obtained spans of ≤ 5 for 45 trails, while 15 trials had spans of ≥ 6 (*Figure 2-5.C*). Average latencies were calculated for three event periods: time taken between approaching bowls (0.76 ± 0.34 s), the average time between Capp to lid flip (0.99 ± 0.12 s), and from the start of the trial to lid flip (9.74 ± 2.26 s). Times between approaches were not included if a rat had stopped performing the task and was wandering around or observing its surroundings. Probe sessions were performed on the one day prior to the start of the 12 days of trials and on day 8. The rats' performance was not affected by the absence of the sucrose pellet in the Dixie cup.

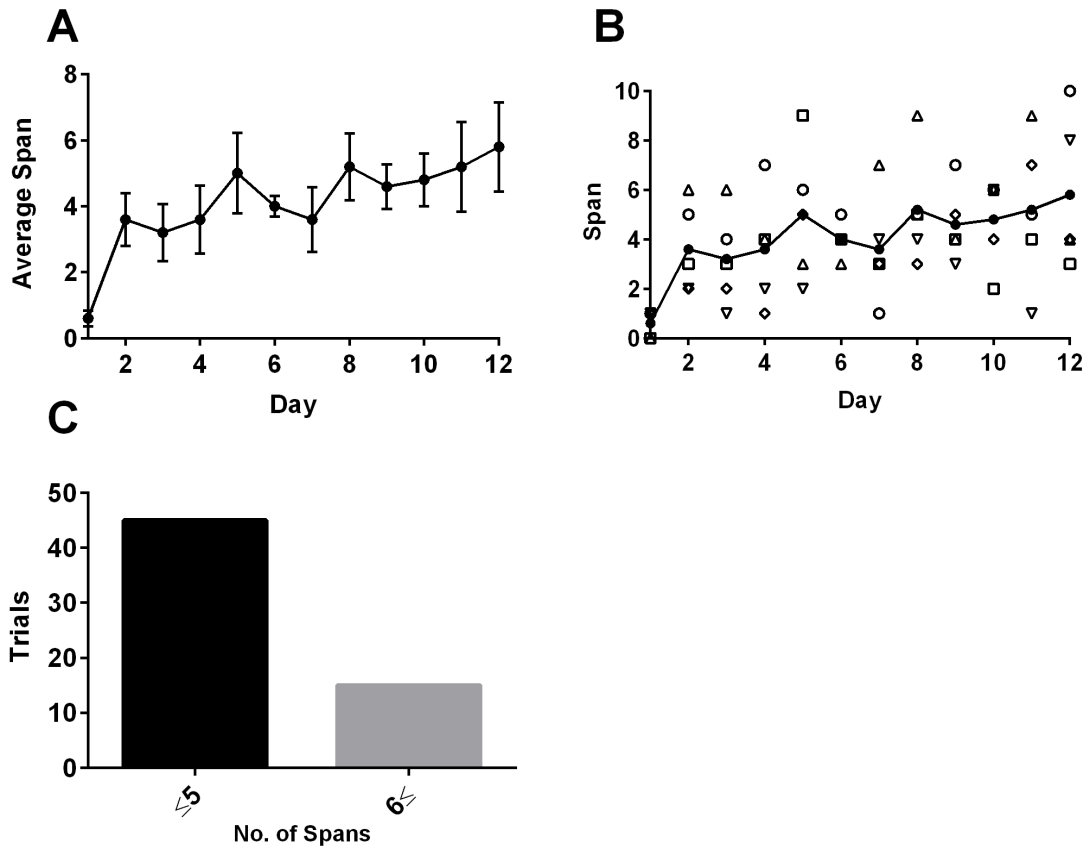

Figure 2-5. Behavioural data obtained from the OST. A) An XY scatter-graph representing the average span per day (n = 5). No significant difference was found between days 2-12. B) An XY scatter-graph illustrating the span achieved per day for each rat represented by symbols, with the average span per day overlayed. C) A column graph representing the numbers of trials with spans ≤5 and ≥6. Error bars represent SEM.

2.4.3 Histology

Four of the five brains were sectioned, mounted, and stained. One was lost due to fracturing during the sectioning process. Electrode placements were generally found in the dmSTR (*Figure 2-6*). In one rat, the electrical lesions did not seem to work compared to the others (*Figure 2-6.D*), as only a small area was found to be lesioned. One rat was found to have an anomaly (*Figure2-6.C*), as a part of its corpus callosum had an extra bundle extending vertically medial to the position of the electrode probe. This rat was not excluded as it still performed well during the OST trials.

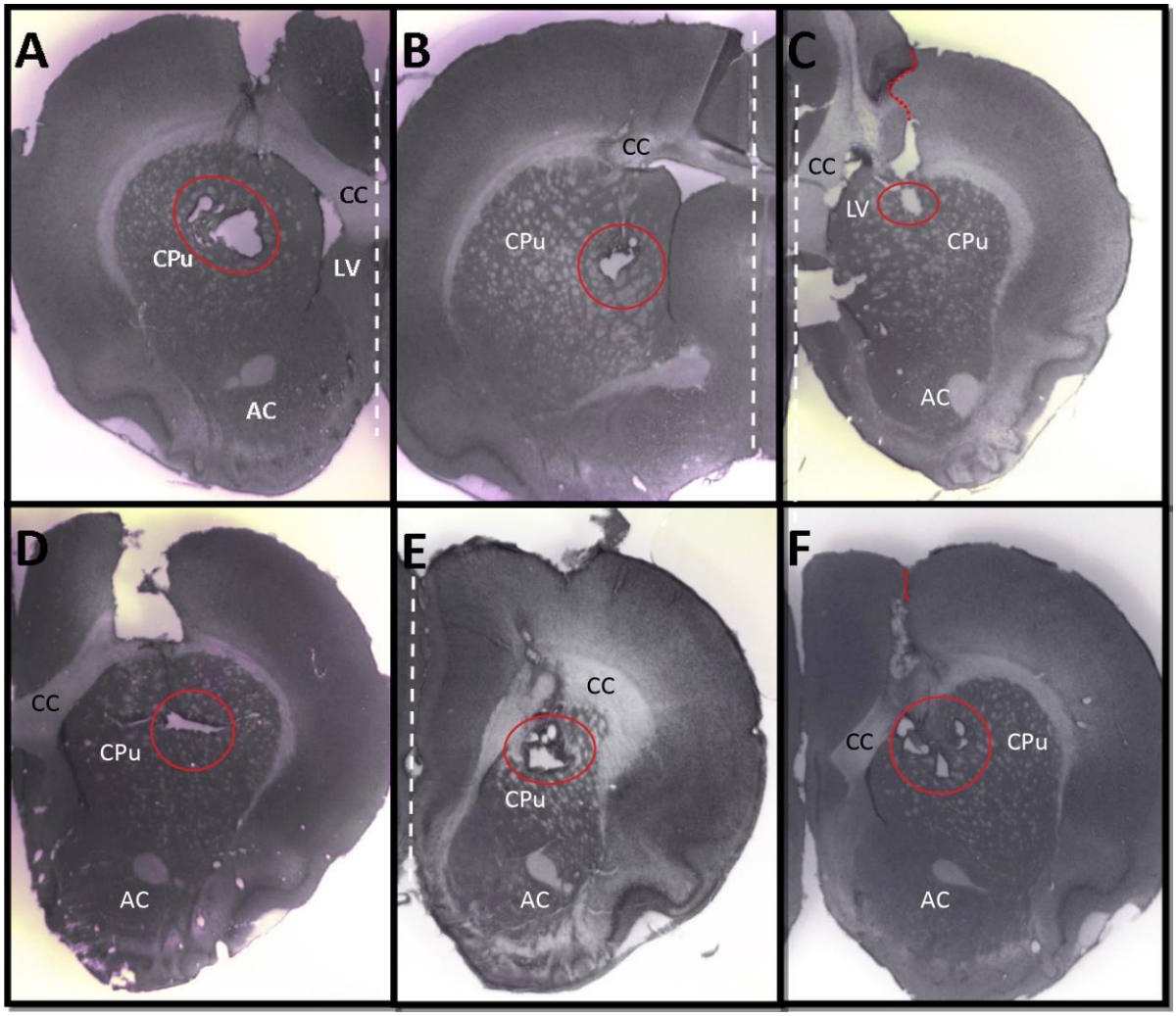

Figure 2-6. Brain section pictures showing the location of electrodes. The pictures represent four of the rats (rat 2 = A and B, rat 3 = C, rat 4 = D, and rat 5 = E and F). AC = anterior commissure, CC = corpus callosum, CPu = caudate-putamen complex, and LV = lateral ventricles. Long white dashes signify the division of the two hemispheres of the brain. Short white dashes reflect where brain slices were separated by the electrode probe, but had fit back together when mounted on the slide. Lesions are circled by the red lines.

2.5.5 OST Neural Activity and Classification

*Spikes were extracted from the neural activity recorded with a minimal cut-off potential of 60 μV (3 times background) before being clustered (*Figure 2-7*). A total of 612 putative cells were found after spike extraction, which was then trimmed to a total of 438 neurons following ISI, cross-correlation, and neural classification (explained in methods). When these neurons were classified into their respective neural classes, the following distribution was found: 233 MSN (53%), 108 FSI (25%) and 53 TCN (12%) (Table 2-1*).

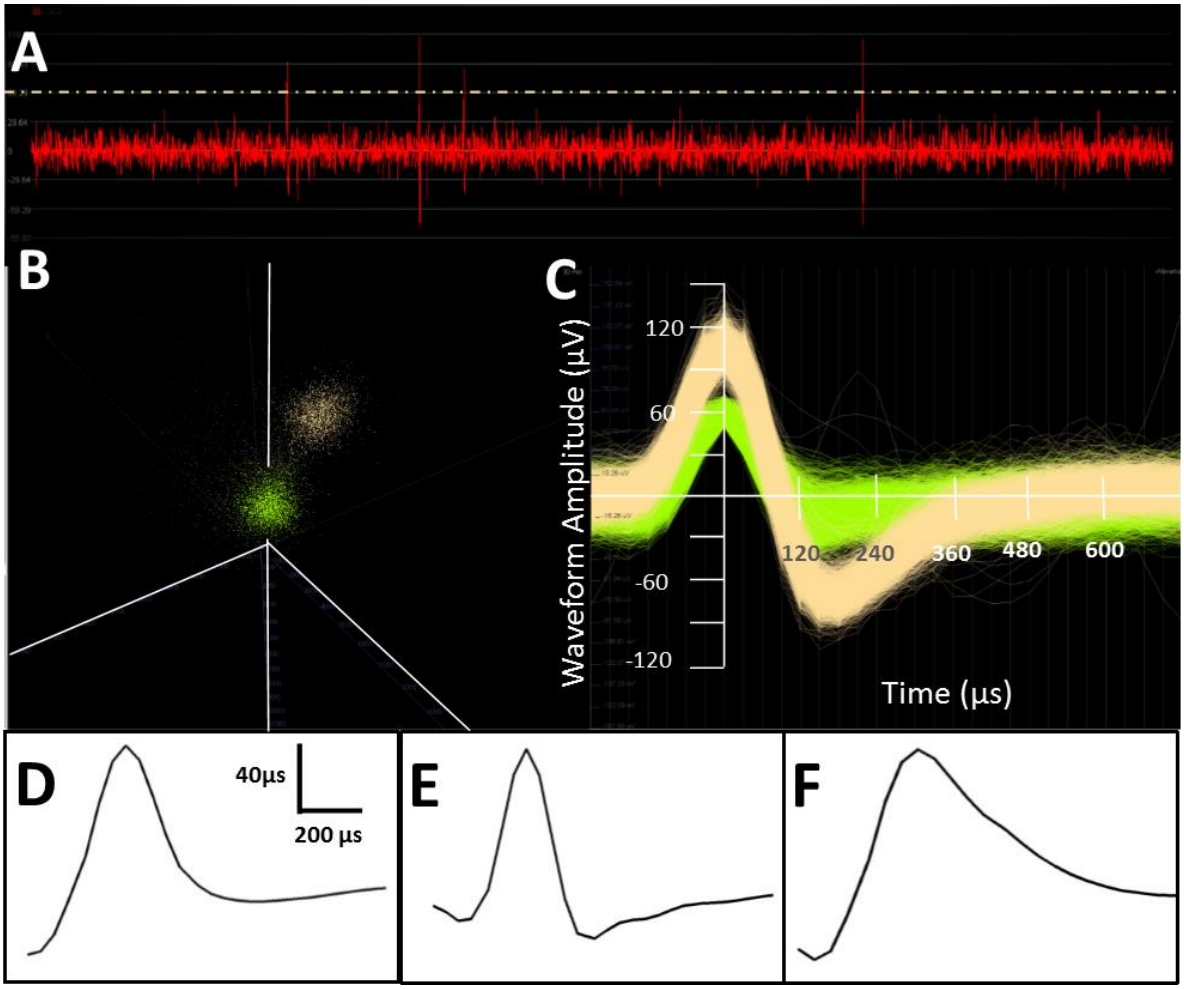

Figure 2-7. Neural clustering and spike sorting. A) A screenshot from Neuraview of a neural recording from the STR. The dashed line represents the minimal value (60 μV) of a spike to be extracted from the recording. B) A screenshot from SpikeSort 3D of two spike clusters after using KlustaKwik. C) Two waveforms representing the two spike clusters from B with respective colours. MSN (D), FSI (E), and TCN (F) neuron representations after neural classification. Axis scale in panel D in the same for E and F panels.

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	MSN	FSI	TCN	Total
OST	233	108	53	438

Table 2-1. The total number of MSN, FSI, and TCN neurons recorded over the 12 days of OST.

The remaining 44 neurons were either considered multiple units unable to be separated into single neurons (24, 5%) or unclassified (20, 5%). Since rats on day 1 had performed significantly worse compared to all other days, only days 2-12 were used when examining event times. There were also three sessions for which no data was usable – the video data was unplayable for rat 3 day 5, there were no MSNs recorded for rat 3 day 10, and the neural recording data was corrupted for rat 4 day 9 (*Table 2-2*). Overall, 210 MSNs were analysed.

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
2	4	3	10	9	4
3	6	4	5	10	5
4	5	4	1	3	6
5	2	4	N/A	8	6
6	2	2	3	5	3
7	2	2	7	8	3
8	3	2	11	7	2
9	3	4	3	N/A	3
10	1	4	0	9	3
11	2	3	2	3	4
12	5	2	2	2	2
Total	35	34	37	64	41

Table 2-2. The numbers of MSNs recorded each day of the OST per rat. A total of 210 MSNs were recorded from all rats from day 2-12.

The baseline recording was considered the average firing rate of each MSN measured during the middle 30 s of the first delay between span 0 and 1. The first delay period was chosen to represent baseline, as the rat was not performing any tasks during this period. The average neural activity at baseline was then compared to during span 0 and during the entire

recording session for each MSN using a one-way ANOVA. There was no significant difference in the neural activity of MSNs between baseline, span 0, or the entire recording session $F(2, 626) = 0.09$, $p = 0.94$ (4.03 ± 0.22 spikes/s, 3.99 ± 0.20 spikes/s, and 4.10 ± 0.21 spikes/s, respectively).

In order to ensure that some neurons did, in fact, respond to events, neural activity was examined for a response to reward. Since it was not possible to accurately mark the exact time rewards were received, neural activity was instead examined after correct lid flips (Cflip) and incorrect lid flips (Eflip). Cflip would proceed a rat receiving a reward and Eflip would not, so increased MSN firing should only be seen after Cflip if changes in MSN activity is related to reward. The neural activity of MSNs (n = 210) was aligned with Cflip events times, and the average firing rates were calculated using MATLAB -1 s to +3 s before and after the events using four different bin widths: 0.01 s, 0.05 s, 0.1 s, 0.2 s, and 0.3 s *(Figure 2-8*). The varying bin widths were used to determine how to best represent the data, from which it was decided to use bin widths of 0.1 s.

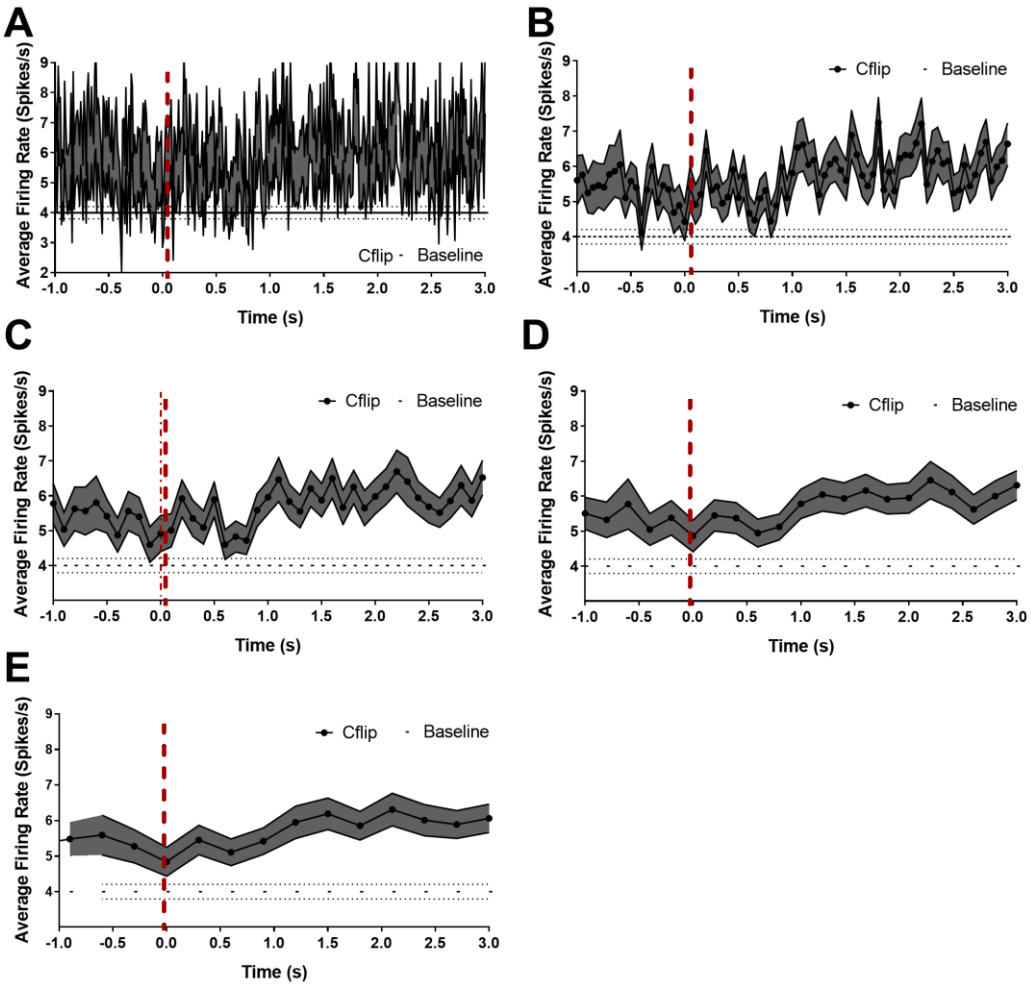

Figure 2-8. Average firing rates of MSNs (n = 210) aligned with Cflip from -1 s to +3 s around events times. Each XY scatter-graph represents the average firing rates for Cflip and baseline using varying bin widths to calculate the average firing rate for each time point: (A) 0.01 s, (B) 0.05 s, (C) 0.1 s, (D) 0.2 s, (E) 0.3 s. Grey shaded areas represents values within ± SEM. Red dashes represent time of event.

Activity of all MSNs (n =210) aligned to Cflip and Eflip was observed between -1 s and +3 s around event times and compared to baseline activity (*Figure 2-9*). A two-way ANOVA with repeated measures found a main effect for firing rates between Cflip and Eflip ($F(2, 418) = 50.78$, $p < 0.01$), but no main effect for time points was seen ($F(40, 8360) = 9.58$, $p = 0.55$). No interaction effect was revealed between firing rates and time points, $F(80, 16720) = 1.98$, $p = 0.11$.

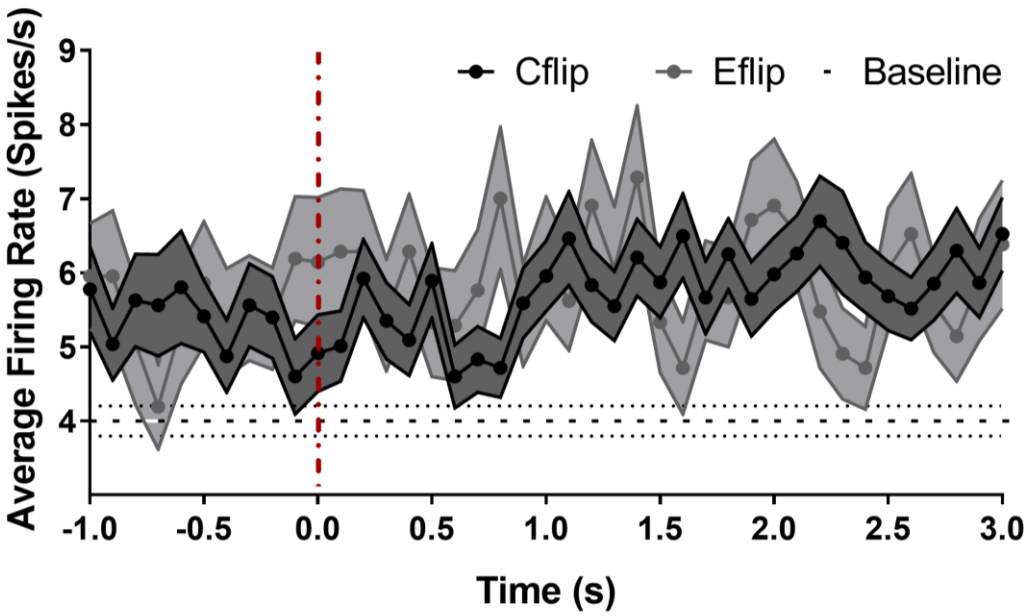

Figure 2-9. Average firing rates of MSNs (n = 210) from -1 s to +3 s from an event (0.1 s bins). A) An XY scatter-graph representing the firing rates of MSNs aligned with Cflip, Eflip, and the average firing rate during baseline. Grey shaded areas represents values within ± SEM. Red dashes represent time of event.

The analyses using MATLAB for responding neurons found 84 MSNs whose activity had changed in response to the Cflip event. Baseline activity was then calculated and compared to neural activity aligned to Cflip and Eflip with a two-way ANOVA with repeated measures, using only the 84 Cflip-responding MSNs (*Figure 2-10*). Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.48$). Analysis found a main effect for firing rates ($F(2, 166) = 14.00$, $p < 0.01$) and an interaction effect between firing rates and time points ($F(38.67, 4814.75) = 1.53$, $p = 0.02$), but no main effect for time points ($F(19.22, 2407.38) = 1.53$, $p = 0.06$),. Bonferroni's multiple comparisons test reveal a significant increase in activity was seen 1.2 s after the lid flip compared to baseline with Cflip alignment (*Figure 2-10.A:* $p < 0.05$), and significance at 7 time points were found when comparing Eflip to baseline (*Figure 2-10.B:* $p < 0.05$). When comparing firing rates of MSNs aligned with Cflip and Eflip several time points appeared to be significant from 1.0 s after the lid flip (*Figure 2-10.C:* $p < 0.05$).

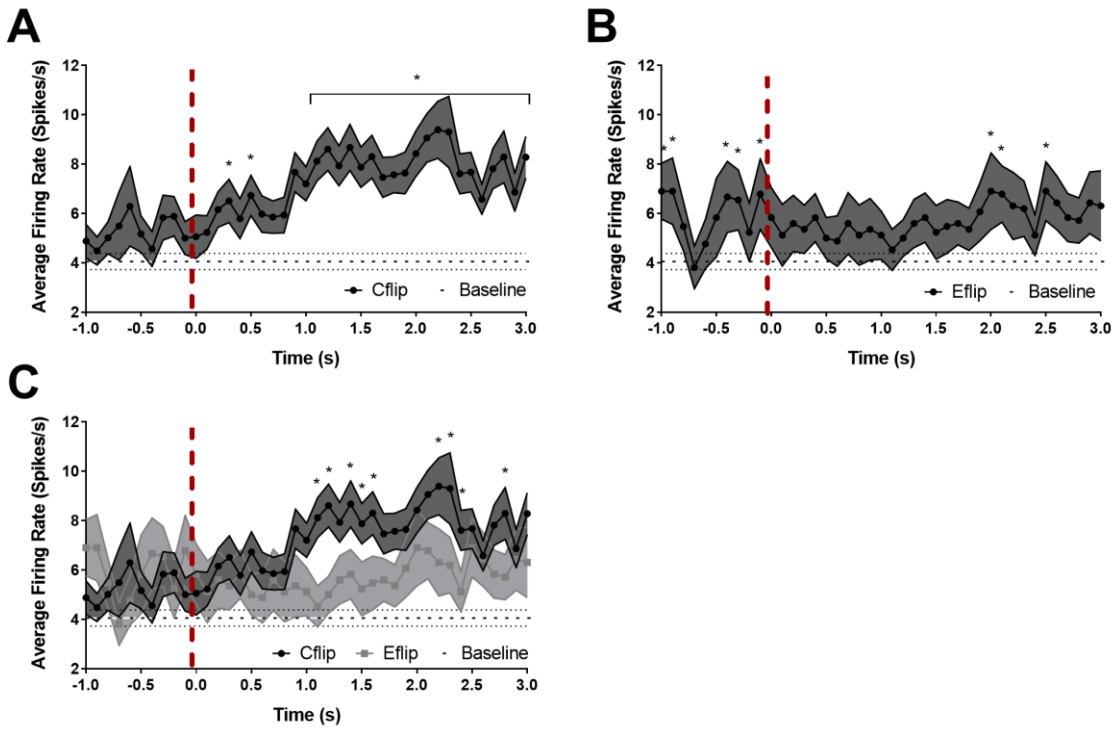

Figure 2-10. MSN activity (n = 84) analysed for responsiveness to Cflip and Eflip events. A) An XY scatter-graph representing the average MSN firing activity aligned to the Cflip events compared to baseline. B) An XY scatter-graph illustrating the average MSN firing rates comparing Eflip to baseline activity. C) An XY scatter-graph comparing Cflip, Eflip, and baseline MSN activity, values of significance are only comparing Cflip to Eflip. Grey shaded areas represents values within ± SEM. Red dashes represent time of event. (= $p < 0.05$).*

To examine overall MSN activity during novel and familiar approaches neuron firing (n = 210) was aligned with Capp and Fapp, -1 s to +1 s around the events were observed (*Figure 2-11*). A two-way ANOVA with repeated measures revealed a main effect for firing rates ($F(2, 418) = 11.11$, $p < 0.01$), time points ($F(20, 4180) = 1.59$, $p = 0.04$), and an interaction effect between firing rates and time points ($F(40, 8360) = 1.54$, $p = 0.02$). Tukey's multiple comparisons test showed no significant difference was found between Capp and Fapp activity ($p > 0.05$), but activity between Capp-baseline, and Fapp-baseline was found to be significant (*Figure 2-11*). The firing rates at all time points during Fapp were found to be significantly higher than at baseline (*Figure 2-11.B:* $p < 0.05$), while only Capp time points -1, -0.9, -6 to 0.1, 0.6, and 0.7s were found to be significantly different from baseline (*Figure 2-11.A:* $p < 0.05$).

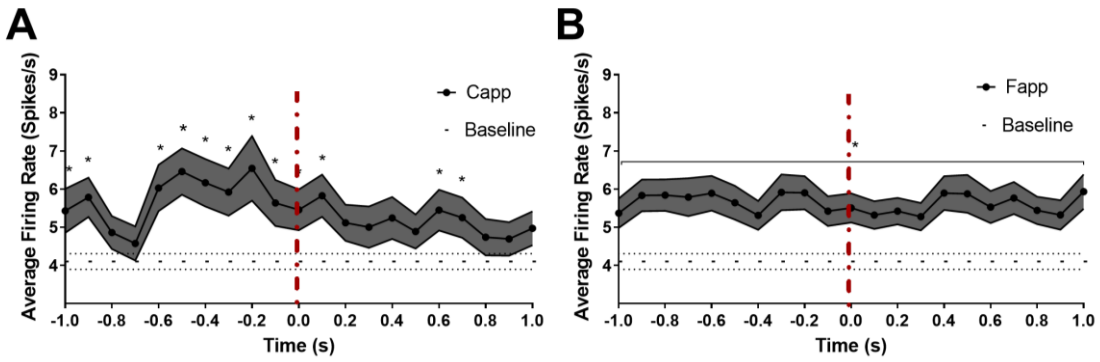

Figure 2-11. Average firing rates of MSNs (n = 210) from -1 s to +1 s from an event (0.1 s bins). A) An XY scatter-graph representing the firing rates of MSNs aligned with Capp and the average firing rate during baseline. B) An XY scatter-graph comparing the firing rates of MSNs aligned with Fapp and the average firing rate during baseline. Grey shaded areas represents values within ± SEM. Red dashes represent time of event. (= $p < 0.05$).*

The neural activity over the 2 s around the Fapp event time did not vary, with the average firing rate being 5.62 ± 0.14 spikes/s. While MSN activity aligned with Capp increased at -0.6 s (6.02 ± 0.61 spikes/s) and peaked at -0.2 s (6.54 ± 0.85 spikes/s), MSN firing decreased to 5.46 ± 0.54 spikes/s at the event time (0 s) and slightly rose to 5.81 ± 0.56 spikes/s at 0.1 s. After 0.1 s, the firing rate decreased with a small increase at roughly 0.6 s to 5.44 ± 0.54 spikes/s. The time period before and after the events (Capp and Fapp) was also extended to observe if there was any other corresponding activity (*Figure 2-12*). The time period before Capp was only extended to -2 s as every trial had a varying amount of events prior to Capp that could cause interference between trials. On the other hand, the period after the Capp event was extended to 10 s, as this was the time where the rat would flip the lid, receive the reward, and eat, after which the span was declared over. An increase in neural activity was observed between 1.6 s - 5.0 s before it decreased and started to fluctuate (*Figure 2-12.A*). The increased activity is presumed to signify when the rats received the reward, since the lid flip occurred on average at 0.99 s after Capp and no other event occurred until the rat was picked up to be put back into the housing (the shortest time recorded that the rat was handled after receiving a reward was 5 seconds). When the time period before and after Fapp was extended to -12 s and 12 s, the activity gradually increased after -7.2 s where it generally flattened out after -2 s and stayed relatively static for the rest of the period (*Figure 2-12.B*).

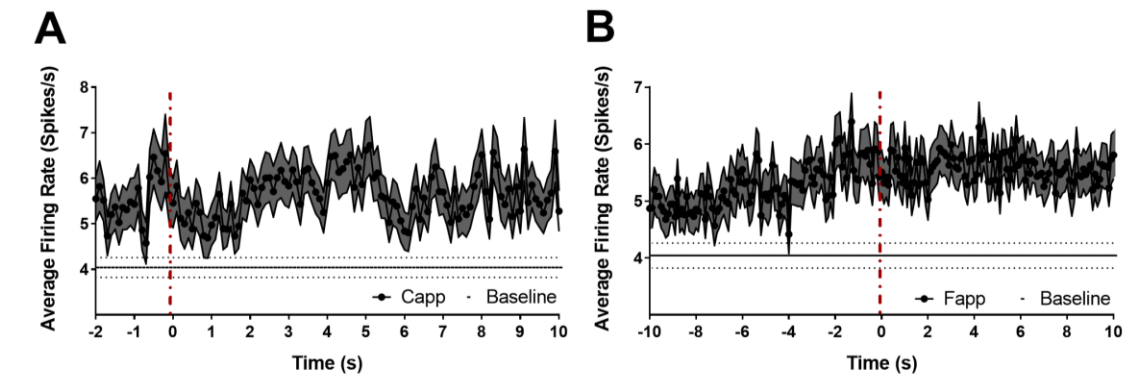

Figure 2-12. MSN firing (n = 210) aligned with Capp (A) and Fapp (B) events over different time periods around the event compared to baseline. Grey shaded areas represents values within ± SEM. Red dashes represent time of event.

Next, each of the 210 neurons were analysed with the use of MATLAB to distinguish if individual neurons responded to the two events. The results found that 19 MSNs responded to the Capp event while 21 MSNs responded to the Fapp event. The MSN firing rate aligned with the Capp event was then compared to the average firing rate of the same neurons at baseline (3.94 ± 0.66 spikes/sec) (*Figure 2-13.A*). A two-way ANOVA with repeated measures was preformed and Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.26$). After adjustment a main effect for time points ($F(5.10, 183.49) = 2.42$, $p = 0.04$) and an interaction effect between firing rates and time points ($F(5.10, 183.49) = 2.42$, $p = 0.04$), with no main effect seen for firing rates ($F(1, 18) = 0.68$, $p = 0.52$). Bonferroni's multiple comparisons test found firing at -0.2 s, 0.1 s, 0.2 s to be significant (*Figure 2-13.A:* $p < 0.05$). When MSN firing aligned with Fapp was compared to baseline *(Figure 2-13.B*) with a two-way ANOVA with repeated measures Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.28$). Adjustments only showed a main effect for firing rates ($F(1, 19) = 6.57$, $p < 0.05$). No significant effects were found for time periods ($F(5.76, 218.86) = 2.01$, $p = 0.07$) and interaction between firing rates and time points ($F(5.76, 218.86) = 2.01$, $p < 0.07$).

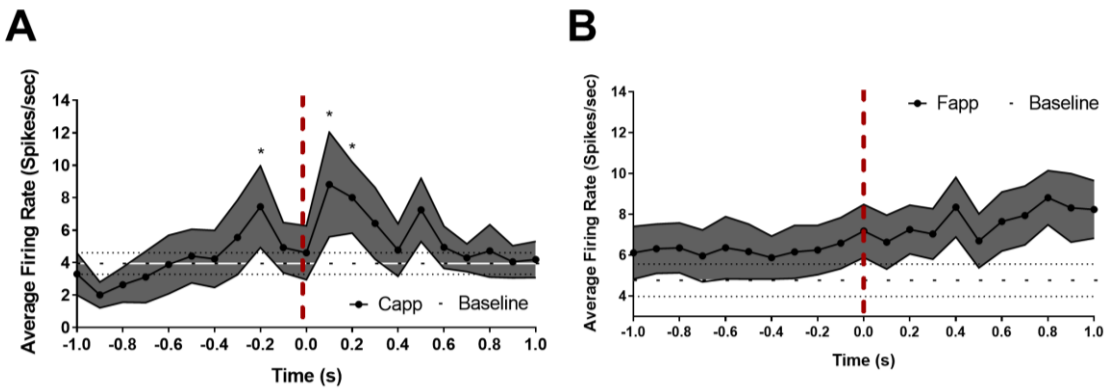

Figure 2-13. MSN activity analysed for responsiveness to Capp and Fapp events. A) An XY scatter-graph representing the firing rate of MSNs (n=19) to the approach of a novel odour compared to baseline (= P<0.05). B) An XY scatter-graph that illustrates MSN firing rates (n = 21) during an approach to a familiar odour. Grey shaded areas represents values within ± SEM. Red dashes represent time of event. (* = p < 0.05).*

2.4.5 OST+MK801 Behaviour

Overall, three days of OST were performed with MK801 injections and one day with saline injections in order to evaluate the effects of MK801 on MSN activity. This testing period was short, as the recording electrodes had been in the rats for a month by this stage and fewer neurons were being recorded with an increase of artefacts being produced. The last day of the 12 OST trials was used as a control (5.80 ± 1.36). The saline trials (n = 5) averaged a span of 7.00 ± 1.02 while average span for the combined three days of MK801 injections (n =15) was 3.73 ± 0.86, one-way ANOVA showed no significance, $F(2, 22) = 2.359$, $p = 0.12$ comparing saline, MK801, and control. However, a post hoc analysis with Fisher's LSD found saline to MK801 was not significance ($t(18) = 2.01$, $p = 0.06$), while control to saline was $t(8) = 0.67$, $p = 0.53$. When comparing control and saline to individual days with a one-way ANOVA, no significance was shown $F(4, 20) = 1.524$, $p = 0.23$). This time however, a post hoc analysis using Fisher's LSD comparing saline to MK801 day 1 significance was seen ($t(8) = 3.13$, $p = 0.03$). Three latency periods were measured to see if MK801 triggered any behavioural effects in the rats: time between approaches, time taken from Capp to dig, and time from start of trial to dig. The latency periods during MK801 trials were compared against latencies the rats obtained during the 12 days of OST trial, controls. The average time between approaching bowls post MK801 was 0.72 ± 0.06 s, which is not significantly different from the 0.76 ± 0.34 s observed during the control trials with an independent t-test (*Figure 2-14.B: $t(8) = 0.46$, $p = 0.65$*). The time from Capp to dig slightly

increased in MK801 trials at 1.08 ± 0.13 s from 0.99 ± 0.12 s in control rats, however, this is not significant in an independent t-test (*Figure 2-14.C: t(8) = 0.53, p* = 0.88). Latency from start to dig increased to 12.0 ± 3.81 s in the MK801 trials but failed to gain significance in an independent t-test from control data of 9.74 ± 2.27 s (*Figure 2-14.D: t(8) = 0.51, p* = 0.62). Observed changes in rat behaviour post-MK801 including stumbling in three rats on MK801 day 1 and two on MK801 day 3 were, as they would trip on their back legs when getting out of the cage and would also get their head caught on the door frame with the head cap. The two rats on day 3 were two of the rats that stumbled on day 1. In addition, on span 0 of MK801 day, 1 rats generally took longer to initially exit the cage (from 22 s to 177 s), which resembles their behaviour when they were getting acclimated to the task. Nonetheless, after the initial span on day 1, the rats did not show this again.

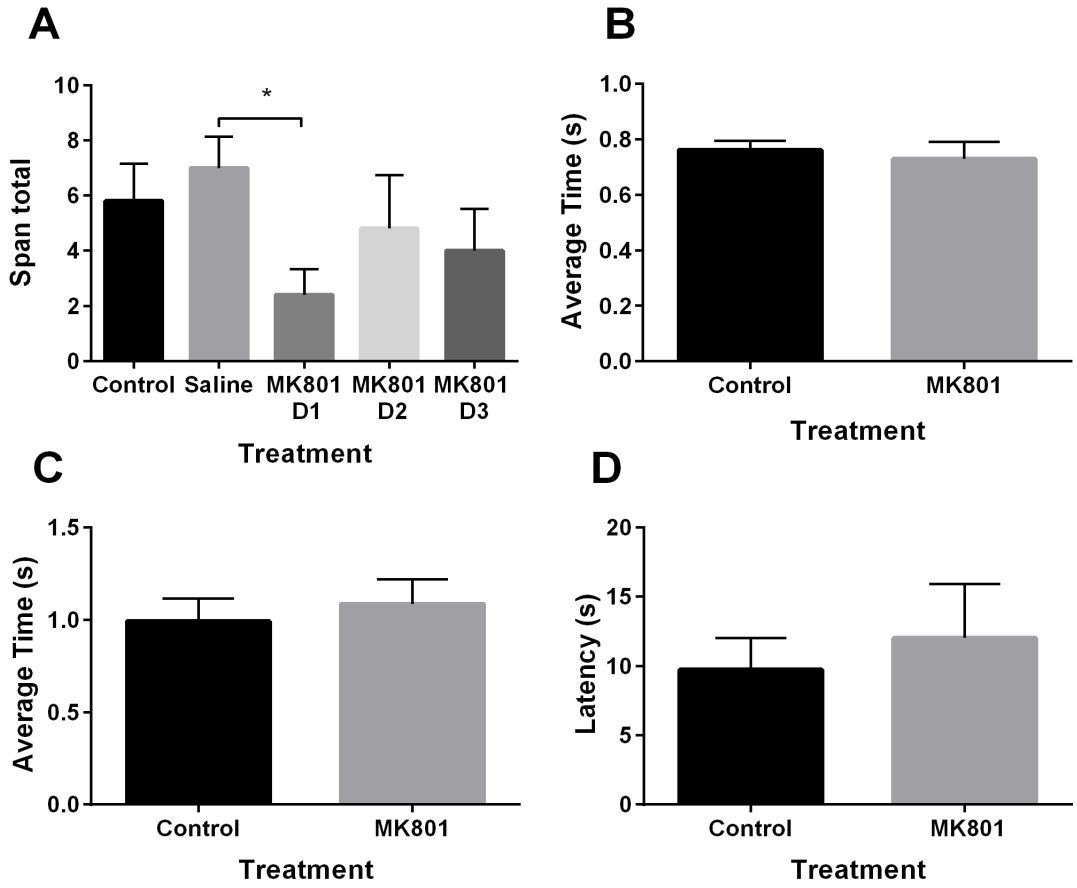

Figure 2-14. Behaviour data obtained during the OST during control, saline and MK801 trials with error bars showing SEM. A) A column graph comparing average spans between control, saline and MK801 days 1 - 3 (= p < 0.05). B) A column graph comparing average times between approaching bowls between control and MK801 trials. C) A column graph showing average times between Capp and Cflip comparing control to MK801 trials. D) A column graph representing latency from the start of a span to Cflip comparing control to MK801.*

2.4.6 OST+MK801 Neural Activity and Classification

The same procedure was used for identifying neurons for saline and MK801 trials as the previous 12-day trials. A total of 105 neurons were found and classified 37 MSNs (34%), 37 FSIs (34%), 20 TANs (19%), and 13 unidentified neurons (12%) (*Table 2-3*).

Unfortunately, the video file for rat 5 MK801 day 1 was corrupted and was not usable. Furthermore, there were three trials where rats had a span of 0 and there were no events to analyse (rat 2 MK801 day 3, rat 3 day 1, and rat 4 MK801 day 2). This reduced the neurons available for analysis to 27 MSNs. All 37 MSNs were used to evaluate changes in baseline recordings when MK801 was administered.

	MSN	FSI	TCN	Total
OST+M801	37	37	18	105
OST+Saline	6	14	5	33

Table 2-3. The total number of MSN, FSI, and TCN neurons recorded over OST trials with the administration of MK801 and saline.

The average MSN firing rate was calculated from the 10 minutes of baseline measurements prior to the MK801 injection, and the last 10 minutes of the 30-minute period post-MK801 before the rat performed the OST. The average MSN firing rate increased from 5.55 ± 0.53 spikes/s to 6.94 ± 0.83 spikes/s post administration (*Figure 2-15.A*). The change in MSN baseline firing was then checked in the 27 neurons to be used for event analysis and the resulting firing rates were very similar; in these select neurons, the average MSN firing rate before MK801 administration was 5.55 ± 0.57 spikes/s, which increased to 6.83 ± 0.92 spikes/s after injection. A paired t-test was used to compare the mean increase in MSN firing post-MK801 compared to baseline in both the 27-neuron sample and the 37-neuron sample, both were found to be significant ($t(26) = 3.41$, $p < 0.01$ and $t(36) = 2.98$, $p < 0.01$, respectively). However, during the MK801 OST, firing rates were compared to MK801 baseline between Capp, Fapp and MK801 baseline using a two-way ANOVA with repeated measures (*Figure 2-15.B*). No main effects were seen for firing rates ($F(2, 52) = 0.68$, $p =$

0.51), time points ($F(20, 520) = 0.75$, $p = 0.77$) or interactions between firing rates and time points ($F(40, 1040) = 0.95$, $p = 0.57$).

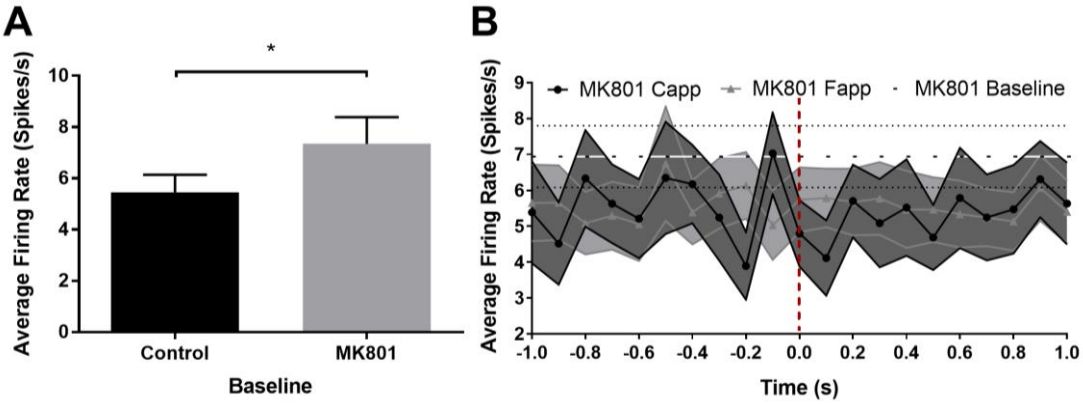

Figure 2-15. MK801 effects on MSN activity (n = 27) during baseline and OST events. A) A column graph illustrating average firing rates of MSN before and after administration of MK801. B) An XY scatter-graph comparing MSN firing rates between events (MK801 Capp, MK801 Fapp) and baseline. Grey shaded areas represents values within ± SEM. Red dashes represent time of event. (= $p < 0.05$).*

2.6 Discussion

Modifications to the task revealed that rats learnt to lid flip more quickly than to dig for a reward, while the overall training period for the OST remained the same. The neural activity of MSNs during the OST was examined during the approach to familiar and novel odours in order to observe if MSNs responded to these events. Subsequently, MK801 (0.1 mg/kg) was administered by IP injection prior to the OST to test for changes in MSN firing before and after the MK801 injection as well as during the rats' approach of familiar and novel odours. A significant increase of MSNs activity was observed after receiving a reward. Although no MSNs responded when rats approached a familiar odour, there may be evidence of a response by a small number of MSNs during the approach to a novel odour in the OST. MK801 was found to influence MSN activity through an increase in the basal firing of MSNs before the OST, however, no other conclusions could be made.

2.6.1 MSN Response to Familiar and Novel Odours During the OST Were Not Seen

The STR is thought to enable a gating mechanism in WM where its activation correlates with information updating, while its inactivity corresponds to information maintenance. Although WM and STM are related in rats (Dudchenko, 2004; Bratch et al., 2016), MSNs have only been shown to be responsive during decision making and rewards in rats performing a STM task (Berke et al., 2009), no studies have been published examining MSN activity during a WM task. In humans, fMRI studies have shown increased activity in the STR corresponding to information manipulation during WM tasks (D'Esposito et al., 1999; Lewis et al., 2004). However, contrasting results have been found regarding whether neural activity differs between information maintenance and manipulation (D'Esposito et al., 1999; Lewis et al., 2004). MSNs were first analysed to observe response of activity to reward, which was expected to show increased firing (Berke et al., 2009). The neural activity revealed a response to reward, with 84 neurons showing a significant difference in firing rates 0.9 s after flip when comparing correct with incorrect choices and correct choices with baseline firing. My results reported here show a significant increase in neural activity when MSN firing was aligned to Fapp events with the inclusion of all MSNs recorded over the 2 s period, but no change in activity was seen during to the approach to the familiar odour itself. An analysis to examine if any of the 210 MSNs responded to Fapp found that 21 MSNs, but no significant change in firing rats were seen on further analysis. The increased activity of all

neurons could be related to other simultaneous events occurring since the rats averaged 0.76 s ± 0.34 s between bowls. Confidence of MSNs not to respond to a familiar odour is increased as MSNs firing was shown to increase in response to a reward. When this same analysis was repeated for MSN activity aligned to Capp events, a significant increase in neural activity was observed 0.6 s prior the approach to a novel odour, which lasted to 0.1 s after the event. When cellular responses to Capp were analysed, 19 MSNs corresponded to the novel odour. These corresponding neurons showed short but significant increases in activity at 0.2 s prior to approach and another from 0.2 s to 0.3 s after Capp. Due to the small sample of neurons (9%) compared to the overall MSN population further analysis is required before making any conclusions to confirm that the observed increase in activity in these 19 neurons was not coincidental.

2.6.3 MK801 Increases MSN Basal Firing

MK801 injections produced a significant increase in the firing of 27 neurons from 5.55 Hz to 6.83 Hz. Increased neural activity induced by MK801 has already been shown in pyramidal cells of the prefrontal cortex in rats, which is due to the reduced inhibition of pyramidal cells by FSIs (Homayoun and Moghaddam, 2007). Although MSNs would be expected to have reduced sensitivity from cortical inputs due to NMDA antagonism, the reduced inhibition from FSIs would also be expected, as NMDA receptors are involved in receiving glutamatergic input from cortical areas. However, since the MK801 was administered systemically the increase MSN firing could be due to dysfunction in other brain areas rather than dysregulation by FSIs. The increase in MSN activity was seen when the rats were not performing the task, whereas, during the OST, MSN activity during Capp and Fapp events was not reduced significantly. However, when comparing neural activity during Capp and Fapp events in control-state versus MK801-state rats, the overall neural activity was similar. This could result from sufficient regulation of MSNs by cognitive input to striatal FSIs when a rat is focusing on a task. Unfortunately, due to a lack of neurons and events, no further conclusions can be drawn at this point. To do so would require more animals to be trained to perform the OST and to be injected with MK801.

2.7 Conclusions

The results showed an increase of MSN activity in response to a reward during the OST as seen in STM tasks, but suggests that MSNs are non-responsive to familiar odours during the OST. While MK801 causes an increased MSN basal firing in freely moving rats. The results are novel findings for MSNs in rats during WM tasks. More experiments need to be conducted before any other conclusions can be made with MSN activity in response to approaching a novel odour or MK801 effects on MSN firing during the OST.

3.0 GENERAL DISCUSSION

In order to investigate the role of MSNs during a WM task, the present study sought to characterise neural activity corresponding to familiar and novel approaches in the OST. Increased MSN response to a reward was also replicated to show increased firing as seen in STM tasks. Since WM dysfunction is found in schizophrenia, MSN activity during dysfunction of WM was examined after administration of MK801, which used to antagonise NMDA receptors while inducing schizophrenic-like symptoms. This allowed me to test for putative changes in MSN activity or response to events during the WM task. Over the 12 days of trials, 84 MSNs were found to increase in activity in response to a reward, while all 210 MSNs were found to be non-responsive to a familiar odour during the OST. However, a small number of MSNs may have shown a response to a novel odour. The administration of MK801 resulted in an increase of firing in 27 MSNs before performance of the OST, but unfortunately, no other conclusions could be drawn due to a low sample size.

3.1 MSN Response to Events during OST

Compared to its role in the motor-control system, the function of the STR in WM is not well understood. It is thought to be a gating mechanism where increased MSN firing activates a thalamic feedback loop that allows for information updating, while inhibition of the thalamic feedback loop results in information maintenance (Frank et al., 2001; Lewis et al., 2004). In fMRI studies, striatal activity was increased when information was updated, but findings on the differences between information updating and maintenance have varied (D'Esposito et al., 1999; Lewis et al., 2004). In the OST, a novel odour represents new information, that requires updating any current information used to perform the task, while a familiar odour represents current information only requiring maintenance.

Given that MSNs have previously been shown to respond to reward in the dmSTR (Berke et al., 2009), activity in the recorded MSNs were first examined to see if they showed a similar reaction. Due to accurate timing of the rats receiving a reward was not possible, two other events were compared to gain an appreciation for any changes in neural activity occurring around rewarding: neural activity of a rat making a correct choice and receiving a reward (Cflip) compared to a rat making an incorrect choice and not receiving a reward (Eflip). For increased activity from a reward to be confirmed, the increase of activity seen in

a reward trial would need to be significantly reduced in an incorrect choice. 84 MSNs were considered to be responsive to a reward and activity around the event was compared to an error flip and the baseline activity. All activity from 0.9 s after correct flip was found to be significant between Cflip and baseline average, while time points 1.1, 1.2, 1.4 - 1.6 and 2.0 – 2.4 s were found to be significant between Cflip and Eflip. Although significance was found between Eflip and baseline average, the majority of time points found with significant were before the event time (-1.0, -0.9, -0.4, -0.3, and -.01) with three after the event (2.0, 2.1, and 2.5). Overall there was a large increase in MSN activity 1.0 s after Cflip that was significantly higher compared to Eflip and baseline. By replicating a known response to reward from a population of the MSNs recorded, it was confirmed that event related changes in MSN activity were observed.

On approach of familiar odours, no changes in neural activity were seen, although activity was shown to be significantly higher when all MSN activity was accounted for. However, the separation distance between the cups could have resulted in overlapping activity when viewing the data. The average time between bowls was 0.76 s with a standard error of 0.34 s, reducing the window to view activity to 0.4 s on either side of the event before the possibility of interference from neural activity of other events. A larger separation between bowls would be required to widen the gap for more accurate analysis. This would also allow for the determination of whether overall activity during the OST is increased or if the activity increase observed during alignment of the Fapp events was manufactured by closely related activities. However, the results revealing MSNs respond to reward increases the confidence in the current data that MSNs do not respond to familiar odours during the OST.

In response to approach of a novel odour, there initially seemed to be a significant increase of activity 0.6 s before the rats were considered to approach the novel scented lid, which eventually decreased by 0.2 s after the event. After a cell response analysis, this increase was drastically changed when only 19 neurons were found to be significantly responsive to approaching the novel odour. Increases in activity were seen at 0.2 s before the event and from 0.1 s - 0.2 s after the event. Although a response to the event was shown, the limited number of cells (19 out of 210) responding to a novel odour was disconcerting as the neurons found to respond could be a type 1 error. A one-way ANOVA had been performed on each individual neuron to sort them into those that were responsive to an event and those

that were not. By comparing each 0.1 s bin between -1 s and +1 s of the event time, and all neurons that did not show significant variation in firing during this period were excluded. However, there are drawbacks to using this method for analysing neuron responses. False positives in the time period being analysed are possible, as a neuron's firing could increase within the 2-second window and yet may not be related to the event. The analysis was manually checked to ensure significant activity was not isolated only to the start or finish of the period. Overall, the 19 neurons found from the final analysis did show an increase in activity during the event period, but more work would need to be done to verify if they actually responded to the novel odour itself.

The analysis used to search for responding neurons in relation to an event can produce type 1 errors and, given the large number of neurons being sorted (n = 210), the family-wise error rate could also be high. This could explain why the 21 neurons were analysed to be responsive to the Fapp event by showed no significant increases in firing rates. A potential method for reducing error while more accurately sorting responding from non-responding neurons could be the use of principle component analysis (PCA). PCA is a non-parametric method used to extract components that explain variability in a data set. In order to apply PCA for identifying responding neurons, each time point during a chosen time period surrounding an event would be considered a variable. The analysis would generate components that explain the variability; the first component explains the most variability, the second component explains the second largest, and so forth. The first two to four components are usually the only ones necessary to analyse, as the remaining components generally explain little of the variability. Overall, if the variables representing the time points in question show little contribution to the variability in a component, it is likely that no response was seen from the neurons investigated. On the other hand, if a large contribution is seen, neurons are more likely to have responded to the event. This analysis can then be used to sort neurons based on the weighted scores produced. When graphed, the neurons that showed an increase in response to the event should produce a distinct grouping compared to non-responsive neurons, neurons whose firing decreased, and neurons whose firing was constantly changing. Altogether, PCA would more accurately represent the data, as it would reduce the family-wise error rate while revealing more about the neuronal activity.

A potential experiment for verifying the results would be to perform an odour non-matching-to-sample task with rats implanted with electrodes, a procedure that is already used

when training the rats for the OST. The two phases of the task would be ideal; the first phase presents a novel odour and the second presents a new novel odour with the now familiar odour from the first phase. With adequate distance between the two odours, it would be possible to distinguish any response to the novel odour with less potential of overlapping activity. The same task would work to verify non-response to a familiar odour. The non-matching-to-delay task also allows for rats to perform multiple trials per day, as one rat could perform the task six times in roughly 20 minutes, instead of completing to six OST trials over six days. The 2AFC task would also be a good candidate to test MSN response to familiar and novel odours, as it differs from the non-matching-to-sample task in that the first phase occurs only once. After the first phase of the 2AFC task, rats are always presented with two options including a new novel odour and a familiar odour randomly selected from odours previously presented during that session. The 2AFC also requires WM capacity, as the trial continues until the rat makes a mistake, while the non-matching-to-sample task only requires the rat to remember the novel and familiar odour for that specific trial. These two tasks have the advantage of being more directed, compared to the OST where the rat can pick and choose which odours to sniff and in what order. Although you cannot force a rat to always approach both bowls on the table, the choices that the rat can make are more limited.

3.2 MK801 Increases Basal Activity of MSNs

NMDA receptor antagonism by MK801 has shown disinhibition of glutamatergic neurons in the mPFC of rats, while ketamine has been used to show the same effect in humans (Breier et al., 1997; Krystal et al., 2003; Homayoun and Moghaddam, 2007). Although the drug reduces the responsiveness of the pyramidal cells, the reduced inhibition of FSI resulted in the increased basal firing of the pyramidal cells (Homayoun and Moghaddam, 2007). Since MSNs are regulated by FSIs in the STR, it was hypothesised that an increase in basal activity should also be seen in the STR. After administration of MK801, a 25% increase in MSN activity was seen before the rats started the OST. Increased MSN activity would inherently up-regulate thalamic activity, which could potentially cause problems if the STR is acting as a gating mechanism in WM as the thalamic activity would enable information manipulation, thereby decreasing information maintenance and potentially also decrease storage. The up-regulation of MSN activity from MK801 administration in freely moving rats is a novel finding. However, since MK801 was administered systemically the increase of firing could be due to dysregulation of a number of

brain areas. Further studies using intracranial infusions for site specific administration will be required to confirm while the MSN increased in firing.

Unfortunately, due to the fact that electrodes had already been implanted in the rats for a month, this did not allow for the performance of many trials with MK801. As a result, only a small amount of MSNs were found and few events were available to analyse for changes in neural responses. To fill this gap in knowledge, more rats could be trained to perform the OST followed by repeated MK801 and saline administration to increase the number of neurons and events.

A logical first step would be to confirm response of MSNs to events via the non-matching-to-sample or 2AFC tasks before proceeding with MK801 trials. Subsequently, the effects of MK801 on STR activity should also be observed through these same tasks, as these procedures have fewer confounding factors than does the OST. To reduce the time electrodes are implanted before observing MK801 effects, rats could be trained to higher span before surgery. As rats have been seen to reach a span of over 16, with higher spans translating to fewer days required to observe a rat naturally performing the task compared to its performance under the influence of MK801. Once the rats have recovered from surgery, a couple of days should be spent reacquainting them to the task, after which control and MK801 trials could begin. In addition, higher spans would allow for investigation of STR activity concerning WM capacity.

3.3 General Conclusion

MSNs showed an increase of firing to a reward during the OST, but no response to approaching familiar odours during the OST. However, more data will need to be collected to confirm the response to novel odours seen by 19 neurons. The administration of MK80 was shown to cause an elevation in MSN firing rates 20 mins after injection.

3.4 Future Directions

The current data set for this experiment was not fully analysed, with data of MSN response to lid flipping and to starting a trial still needing to be examined. Furthermore, no data pertaining to FSIs and TANs have been investigated. A FSI response to MK801 administration could be observed through comparison of the pre-MK801 injection data, as

FSIs should show a decrease in firing activity, as previously seen within the mPFC (Homayoun and Moghaddam, 2007). The decrease in FSI activity will validate why the MSNs increased in basal activity.

Further experiments should be conducted to confirm whether MSNs truly respond to novel odours and do not respond to familiar odours. To do so, future experiment should be performed with the 2AFC and odour non-matching-to-sample tasks, as well as larger distances between bowls to ensure little if any overlap in activity between events. The 2AFC task could also be used to follow any changes in neural activity in rats as the spans increase.

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