The medial prefrontal cortex and the dorsomedial striatum are necessary for working memory in rats: role of NMDA receptors

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Abstract

Working memory is a form of short-term memory involved in the storage (maintenance) of information over time and reorganization (manipulation) of a memory set necessary for complex cognition. The human frontal cortex and striatum are involved with working memory; however, the mechanisms through which these structures contribute to working memory are incompletely understood. Given the similarities between cortical and striatal areas in the human and rodent brain, I used rats to elucidate the contrbutions of N-methyl-D-aspartate (NMDA) receptors in medial prefrontal cortex (mPFC) and dorsomedial striatum (dmSTR) using two working memory tasks. The trial unique non-match to location (TUNL) task is a delayed-non-match-to-sample visual working memory task performed in touchscreen equipped operant conditioning chambers. TUNL enables the concurrent assessment of delay-dependent and "pattern separation" effects that were not possible with previous delayed-non-match-to-sample-tasks. The odour span task (OST) measures working memory capacity using an incremental delayed-non-match-to-sample paradigm that involves the addition of stimuli (scented bowls) after each correct response. Results obtained following systemic treatment of rats with a broad spectrum NMDA receptor antagonist showed that NMDA receptors contribute to performance of both tasks. Given the contribution of cortical GluN2B-containing NMDA receptors to working memory in primates, we tested the role of these receptors in the TUNL task and OST. Systemic injections of the GluN2B-containing NMDA receptor antagonist Ro 25-6981 impaired OST but not TUNL accuracy. Additional experiments with intracranial infusions showed NMDA receptors in mPFC or dmSTR contribute to TUNL task accuracy. Ro 25-6981 infusions into dmSTR, but not mPFC impaired OST. These experiments contribute to our understanding of the role NMDA receptors perform in mPFC and dmSTR in working memory.

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List of Abbreviations

AC	anterior cingulate
AI	agranular insular cortex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
AP5	aminophosphonovaleric acid
CA3	Cornu Ammonis region 3
cAMP	cyclic AMP
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNTRICS	cognitive neuroscience treatment research to improve cognition in schizophrenia
CPP	3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid
DLPFC	dorsolateral prefrontal cortex
dlSTR	dorsolateral striatum
dmSTR	dorsomedial striatum
EEG	electroencephalography
ERP	Event-related potential
fMRI	functional magnetic resonance imaging
GABA	gamma-Aminobutyric acid
IL	infralimbic cortex
LO	lateral orbital cortex
LTD	long term depression
LTP	long term potentiation
MDMA	3,4-Methylenedioxymethamphetamine

mGlu	metabotropic glutamate
MO	medial orbital cortex
mPFC	medial prefrontal cortex
MSN	medium-size spiny neurons
Mus/Bac	muscimol and baclofen
NMDA	N-methyl-D-aspartate
OST	odour span task
PCP	phencyclidine
PFC	prefrontal cortex
PL	prelimbic cortex
PrCm	precentral cortex
PSD	postsynaptic density
Ro	Ro 25-6981
STR	striatum
TUNL	trial unique non match to location
VLO	ventrolateral orbital cortex
VO	ventral orbital cortex
YM90K	6-(1H-imidazol-1-yl)-7-nitro-2,3(1H,4H)-quinoxalinedione hydrochloride

Chapter 1: Introduction

1.1 Working memory

For over 50 years, scientists have accumulated a wealth of knowledge about working memory at a psychological and neurological level. Working memory is a form of short-term memory involving maintenance (storage of information across time) and manipulation (reorganization or updating of a memory set). Maintenance and manipulation allow processing of multiple items of information and guide behaviour with information that is no longer in the environment (Barch and Smith, 2008; D'Esposito and Postle, 2015). Many activities involve working memory such as remembering a phone number, performing mental math, and planning actions. Most researchers agree that functional networks formed by multiple brain regions are activated during working memory tasks (Eriksson et al., 2015; D'Esposito and Postle, 2015; Nyberg and Eriksson, 2015; Kyllonen, 2002).

1.1.1 History of working memory

The term working memory was first coined in a 1960 text (Miller et al., 1960); however, working memory experiments date back to the 1880's when Ebbinghaus used nonsense syllables as neutral stimuli to study learning and forgetting (Ebbinghaus, 1885). James introduced the term "primary memory" to define the construct of the temporary maintenance of information in memory (James, 1890). Miller pioneered the term "immediate memory" and showed that working memory capacity is limited to 7 ± 2 items of information, which is consistent with Ebbinghaus's research on working memory capacity (Miller, 1956).

1.1.2 Early models of working memory

Influenced by cognitive psychology, Atkinson and Shiffrin introduced a memory model, which contained three components, which include a sensory store, a short term store and a long term store. Within this memory model, incoming information is first held in a sensory store. Only a limited amount of information can be attended to and then transferred into the capacity limited working memory. Information is maintained for further processing, and when rehearsed is transferred to long term memory (Atkinson and Shiffrin, 1968). While components of Atkinson and Shiffrin's model related to working memory were overly simple, Baddeley and Hitch proposed a model of working memory containing a phonological loop, visuospatial sketch pad and central executive. The Baddeley model has stimulated working memory research over the past 4 decades (Baddeley and Hitch, 1974; Baddeley, 1992; Baddeley, 2012). More specifically, the components of the Baddeley model function as: 1) the phonological loop consists of auditory memory and rehearsal that maintains the information in working memory; 2) the visuospatial sketch pad manipulates visual and spatial information in working memory; and 3) information from the phonological loop and visuospatial sketch pad are coordinated via the central executive, an attention controlling system. The phonological loop, visuospatial sketch pad, and central executive form the larger concept of working memory.

1.1.3 Current models of working memory capacity

Working memory capacity is severely limited (Cowan, 2010), and capacity limits correlate to measures of intelligence and academic achievement (Alloway and Alloway, 2010; Fukuda et al., 2010). Current research is focused on the factors that contribute to working memory capacity limitations (D'Esposito and Postle, 2015). The two dominant models of

working memory capacity assume that items are either stored in slots or within a shared pool of resources. One foundational experimental study (Luck and Vogel, 1997), in particular, has led to many subsequent studies on capacity limitations (Brady and Alvarez, 2015; Li et al., 2013; van den Berg, and Ma, 2014) because it fostered the slot model. The slot model assumes capacity is restricted by a limited number of discrete slots, with each slot able to store all the features of one item. Luck and Vogel (1997) used a change detection task to develop the slot model of working memory. Participants are presented with a target array of squares (varying from 1-12) for a few hundred milliseconds, followed by a 1 s delay, then presented with a probe array containing the same number of items with 1 item of a different colour or orientation for half of the trials (Luck and Vogel, 1997). Using a yes/no recognition procedure participants indicate if there was a change in 1 item. In one condition, participants were instructed to look for a colour change and only colour would vary between the sample presentation and the test phase. In another condition, participants were instructed to look for an orientation change and only orientation would vary. In the final condition, participants were instructed to look for colour and orientation changes and either colour or orientation would vary. Critically, the participants remembered double the information in the final condition and showed the same capacity in all conditions. Since capacity was the same regardless of whether participants remembered 1 or 2 features of the items, all the features of an item are stored within working memory.

In contrast, the resource pool model proposes that a single pool of resources are divided among all items stored in working memory. Since a single pool is divided among all items, increased items within memory results in decreased precision of memory. Support for the resource pool model comes from working memory capacity experiments that measured the precision of a memory by using continuous variables such as colour, orientation and spatial

frequency (Bays et al., 2009; Huang and Sekuler, 2010; Prinzmetal et al., 1998; Wilken and Ma, 2004). For example, participants were asked to remember the orientation of multiple items followed by a delay phase and then asked to indicate the direction of rotation for a test item. The participants specified on a spectrum the item's specific feature. The precision of the retrieved memory is assessed using the variance in responses (Swan and Wyble, 2014). These experiments indicated that precision declined as the set size increased (D'Esposito and Postle, 2015). In other words, the number of items in a resource pool is negativity related to the memory precision (i.e. more precise memory with deeper encoding and fewer items). These results contested the slot model and led to the resource pool model, predicting that working memory involves a single fixed pool of resources that are divided across the items stored in working memory. While the slot model predicts when slots are full the brain is unable to encode new information, the resource pool model suggests some information about the item can be encoded but would be imprecise or unlikely for conscious perception.

Both the slot model and the resource pool model of working memory are supported in the literature, and aspects from each may be correct for different reasons as shown in the Buschman et al. (2011) study in monkeys. Their findings supported the slot model by showing that a monkey's overall working memory capacity was composed of two independent slots, one in the right and left visual fields. However, by adding one object on the same side of the visual field, performance declined, demonstrating the left and right hemispheres operate independently in a visual working memory capacity task. Since information was shared and spread among items within each visual field, this supported the resource pool model (Miller and Buschman, 2015). These findings led Miller and Buschman (2015) to conclude that the two hemispheres act as independent slots and within each hemisphere neural information is divided among the items like

a resource pool. While limited information exists regarding the neural mechanisms underlying the different models of working memory, neural activity in monkey frontal and parietal cortex supports the slot model and resource pool model (Buschman et al., 2011).

1.2 The frontal cortex and striatum function in working memory

The brain regions involved in working memory include the parietal lobe, hippocampus, sensory cortex, PFC, and striatum (STR). The PFC and STR, and the interaction between the two substrates support working memory. The PFC controls voluntary allocation of attention to certain items for working memory. The STR allows relevant information into working memory and inhibits irrelevant information from entering working memory. The mechanisms of the interaction between the PFC and STR to support working memory are unknown. The PFC and STR function related to working memory is described below.

1.2.1 The prefrontal cortex's function in working memory

The function of the PFC is one of the most intensely researched areas in neuroscience and psychology (Roberts et al., 1998; Thierry et al., 2011). The PFC mediates diverse cognitive functions including working memory, information processing, behavioural organization, attention, and judgement. The role of PFC in working memory was first proposed by Pribham et al. (1964) since they found PFC lesions produced deficits on various tasks that imposed a delay between the target stimulus and the subsequent target response. Further research in monkeys confirmed and extended these initial findings with extracellular electrodes in the PFC that recorded persistent neural activity during a delay, which requires maintenance of information for the future (Fuster and Alexander, 1971; Kubota and Niki, 1971). The delay phase occurs

between the sample presentation and the test phase of a working memory task. The persistent firing during the delay phase of a working memory task that occurs in the absence of external stimuli suggests a neural basis for working memory (Funahashi et al. 1989; Fuster and Alexander, 1971; Kubota and Niki, 1971; Miller et al., 1996).

With the use of functional magnetic resonance imaging (fMRI), the relationship between working memory and the PFC has been examined further in humans. Studies confirmed that persistent neural activation within the PFC occurred for relevant information during working memory tasks (Courtney et al., 1997; Zarahn et al., 1997). This neural activation seemed consistent with the hypothesis that persistent activity is involved with the maintenance of representations since the magnitude of an fMRI signal reflects the accuracy of behavioural responses (Curtis et al., 2004; D'Esposito and Postle, 2015).

While persistent neural activation is one correlate of working memory, a recent experiment showed activity-silent neural states that correlate with working memory (Wolff et al., 2015). The activity-silent neural state is measured with electroencephalography (EEG) in humans. A novel EEG method, analogous to echo location where an impulse is used to reveal a hidden structure, can be used to probe the hidden state of a neural network (Wolff et al., 2015). Participants were instructed to remember the orientation of sine-waves in a working memory task. Wolff et al. (2015) presented and measured a high-energy visual impulse stimulus during the delay phase designed to activate the visual system. Multivariate analysis showed that the impulse response had information about the orientation of an item in working memory. Both the persistent neural activity and an activity-silent neural state may be essential for working memory.

For decades, the dominant view in the working memory literature has been that the PFC maintains items via persistent neural activity. However, Riggall and Postle (2012) obtained

evidence that the PFC controls top-down attention rather than maintaining items. In their experiment, participants viewed a sample display of dot motion, then during the delay phase they were cued to whether they would be asked for the speed or direction of the sample dot motion. The PFC represented an abstract level of performance during the delay phase (Riggall and Postle, 2012). This study and others suggest that the PFC represents complex information such as rule learning, goals and representation of categories, whereas sensory cortex may represent specific items (Lee et al., 2013; Meyers et al., 2008, Freedman et al., 2001, Freedman et al., 2003; Chen et al., 2012; Sreenivasan et al., 2014). These abstract representations in the PFC are essential for the mediation of events divided in time but are linked to one another (Fuster, 1990; Miller and Cohen, 2001). The PFC is involved with top-down control over neural substrates where items are stored, which implies that the PFC does not store items within working memory (Smith and Jonides, 1999; D'Esposito et al. 2000, Petrides 2000). Therefore, the PFC is a hub within a large network of neural substrates that guides behaviour during working memory.

Top-down signalling can take many forms. For example, the PFC provides feedback to cortical areas that process sensory inputs. This enhances the search for a target stimulus while supressing irrelevant information, and then involves motor outputs for achievement of a task (Miller and Cohen, 2001). Event-related potentials (ERPs), which are electrophysiological measures of a brain's response to a stimulus, and fMRI showed that there are two types of top-down signals in humans, one that enhances target information and another that supresses target irrelevant information (Gazzaley et al., 2005; D'Esposito and Postle, 2015).

It is important to simultaneously record neural activation in both the PFC and sensory cortex to understand the nature of their interactions to produce top-down attention for a specific stimulus. Liebe et al. (2012) examined this interaction and showed that when monkeys

performed a working memory task with recording electrodes in lateral PFC and V4 there was enhanced theta phase locking during the delay phase (a measure of synchrony between PFC and V4). Spike times were locked to the phase of delta-band oscillations in the more distal area such that PFC spikes were phase locked to V4 delta-band, and vice versa. Furthermore, spikes phase locked to the delta-band in the more distal area was stronger in trials where the monkey correctly maintained information, and weaker in incorrect trials. Overall, this demonstrates that the PFC and sensory cortex interact for task relevant behaviour in working memory tasks, and further support that the PFC may be uninvolved in the information storage in working memory. Instead, the PFC uses top-down attention signalling to focus on the relevant sensory representation and inhibit the irrelevant sensory representations to select information that is necessary for achievement of a goal (Postle, 2006).

The top-down attentional role of the PFC also contributes to the temporal integration of working memory, which is lateral PFC dependent in monkeys and humans (Fuster, 2001). There is considerable support for the contribution of the PFC in temporal integration for both a retrospective role and a prospective role in memory (Kutas and Donchin, 1980; Brunia et al., 1985; Singh and Knight, 1990; Niki and Watanabe, 1979; Fuster et al., 1982; Boch and Goldberg, 1989). Some PFC neurons are active for retrospective tasks, whereas others are active for prospective tasks, suggesting different neural circuits may be involved for each task in the PFC (Quintana and Fuster, 1999). The PFC is part of a larger network for temporal integration, which includes the thalamus and basal ganglia (Fuster, 2001).

1.2.2 Delay cells in the prefrontal cortex of rodents

The majority of experiments examining increased neural activity during a delay phase in a working memory task come from monkeys or humans. In rat medial PFC (mPFC), neurons that have increased activity during a working memory delay phase in the absence of external task stimuli are termed delay neurons. In a delayed alternation y-maze task, three different types of delay neurons were observed in the mPFC (Yang et al., 2014). Delay neurons showed a characteristic transient firing either early, middle, or late in the delay phase, but it is unclear if there are different functions among the different transiently firing neurons. One hypothesis suggests the early delay neurons may relay information to the middle delay neurons, which relays it to the late delay neurons (Yang et al., 2014). There was strong synchronization of neural firing during the delay phase, which suggests that some mPFC neurons are organized into functional assemblies (Yang et al., 2014). Rats that performed an eight-arm radial maze task had increased mPFC firing during a delay phase, but few cells discharged differentially, suggesting delay neurons were not involved during the task (Jung et al., 1998; Jung et al., 2000).

Other experiments using a delayed response or delayed-matching-to-sample task revealed that 30–50% of neurons in mPFC increase firing during the delay phase (Batuev et al., 1990; Chang et al., 2002). In a figure-8 maze, only 10% of rat PFC neurons displayed continuous activity throughout the delay phase, while 21% showed increases during specific periods of the delay phase with neurons conveying information for future goal locations (Baeg et al., 2003). This is similar to the interpretations of neural activation in primate PFC that have a gradual increase in neural activity while a few neurons display continuous delay activity (Fuster, 1973; Rainer and Miller, 2002; Durstewitz and Seamans, 2006; Shafi et al., 2007). The gradual increase of neural activation may reflect an anticipation of future experiences, or responses

(Quintana and Fuster, 1999; Rainer et al., 1999; Durstewitz, 2004). Activity of mPFC neurons were similar for correct and incorrect trials early in the delay, whereas firing rates increased with approach to the goal, suggesting an anticipation of reward (Jung et al., 1998; Cowen and McNaughton, 2007). Active neurons during a delay phase in rat mPFC represent actions and rewards, suggesting the mPFC is essential for top-down attention selection.

1.2.3 The striatum's function in working memory

The STR is connected to many different brain regions including, but not limited to, the PFC, hippocampus, amygdala, visual cortex, and sensory-motor cortices (Fuccillo, 2016). The STR supports many behaviours including addiction, decision making, motivation, attention, working memory, and motor planning (Emond et al., 2009; Eriksson et al., 2015; Yager et al., 2015; Yin, 2016). The STR receives input from the frontal cortex, which is transformed into outputs that regulate working memory, learning, and decision making. Experiments in computational models, humans, monkeys, and rats show that the basal ganglia and, more specifically, the STR is involved in working memory. According to a computational model, the basal ganglia interacts with the PFC by allowing relevant and inhibiting irrelevant information from entering working memory (O'Reilly, 2006). Specific neurons within the STR are responsible for allowing relevant information into working memory and activating the PFC, which results in rapid updating of representations (O'Reilly, 2006). The open gate effect that updates working memory occurs when neurons in the STR inhibits the substantia nigra pars reticulata and disinhibits the excitatory loops in the thalamus and PFC. The close gate effect for an irrelevant item prevented from entering working memory results in STR neurons firing, which excites the substantia nigra pars reticulata and inhibits excitatory loops to the basal ganglia and

PFC through the thalamus. Theses circuits show that the STR acts as the gatekeeper into working memory.

Neuroimaging studies in humans using positron emission tomography (PET) and fMRI showed that the STR was active during working memory (Wager and Smith, 2003). Patients with unilateral basal ganglia lesions showed behavioural and electrophysiological visual working memory deficits regardless of the hemifield that the stimulus was presented, indicating a global deficit (Voytek and Knight, 2010). When Parkinson's disease patients were impaired on a verbal two-back working memory task (asked to recall a stimulus presented two items ago), patients had reduced activation in bilateral STR and frontal regions relative to controls (Ekman et al., 2012). In patients with Parkinson's disease and mild cognitive impairment, working memory was impaired and presynaptic dopamine uptake in the right caudate was lower relative to patients with Parkinson's disease only (Ekman et al., 2012). Therefore, dopamine within the STR contributes to working memory.

A computational model developed by O'Reilly (2006) with the STR updating working memory is empirically supported in human Parkinson's disease patients off dopamine medication, which results in reduced updating of working memory but improvements with distractor stimuli (Cools et al., 2010). In Parkinson's disease patients, the gating mechanisms from the STR are in a close state more relative to controls, and allows less relevant or irrelevant information into working memory. McNab and Klingberg (2008) showed that PFC and basal ganglia activity was positivity correlated with increased visual span capacity. McNab and Klingberg (2008) also showed that the basal ganglia was responsible for the relevant information that entered into working memory since basal ganglia activity increased in preparation for selection of information that would be stored in working memory.

Studies in humans are mostly limited to using non-invasive neuroimaging techniques. These techniques suffer from a lack of direct access to the neural substrates, making research in humans limited. Direct access to neural substrates is possible in rodents, making them a great model system because various tools may be used to examine behavioural properties, such as lesions, pharmacology and electrophysiology. The following section will examine working memory in rodents.

1.3 Lesions, function, and anatomy of the prefrontal cortex and dorsal striatum in rodents

Lesions of specific neural substrates are used to examine different behaviours in rodents including working memory, attention, and decision making (Kesner and Churchwell, 2011; Robbins, 2002; Walton et al., 2002). In the following section, I review the literature regarding whether damage to the PFC or dorsal STR in rodents alters working memory. This section will also compare the DLPFC of humans and monkeys to the mPFC in rats and argue that the two areas have many similarities. Therefore, information gained from experiments conducted with rats involving mPFC should serve as a model for research in monkey and human DLPFC. The neuroanatomical structure of the STR is mostly conserved across humans, monkeys, and rodents with similar inputs and outputs of the STR and similar behavioural properties related to STR sub-regions. The neuroanatomical section on the STR argues that the dorsal-ventral divide of the STR should be refined to a dorsolateral-ventralmedial divide based on evidence of inputs and outputs to the STR.

1.3.1 Effects of lesions within the prefrontal cortex on working memory in rodents

The rat PFC can be divided into sub-regions including the anterior cingulate cortex, precentral cortex, prelimbic cortex, infralimbic cortex, orbital cortex, medial PFC, ventral medial PFC, lateral PFC, and ventral PFC (Figure 1.1). The rodent anterior cingulate cortex and precentral cortex is involved in working memory for motor response information. Lesions of the anterior cingulate cortex and precentral cortex that spared the surrounding cortex including prelimbic and infralimbic cortex reduced working memory motor response of turning either left or right (Kesner et al., 1996; Ragozzio and Kesner, 2001; Kesner and Churchwell, 2011). However, lesions of the anterior cingulate cortex and precentral cortex did not affect visual working memory (Ennaceur et al., 1997; Kesner et al., 1996; Shaw and Aggleton 1993) or appetitive information for a food reward (DeCoteau et al., 1997; Ragozzino and Kesner, 1999). The majority of studies that reported lesions of the anterior cingulate cortex and precentral cortex show no effect in spatial working memory using delayed non-matching to position, delayed spatial alternation, or non-matching to sample in a t-maze, 8 arm maze, and continuous spatial recognition memory tasks (Ennaceur et al., 1997; Harrison and Mair, 1996; Joel et al., 1997; Kesner et al., 1996; Kolb et al., 1983; Neave et al., 1994; Passingham et al., 1988; Ragozzino et al., 1998; Sanchez-Santed et al., 1997; Shaw and Aggleton, 1993; Silva et al., 1986; van Haaren et al., 1985; Winocur, 1991; Wolf et al., 1987). The anterior cingulate cortex and precentral cortex processes motor response information for working memory tasks but not visual, spatial or appetitive information.

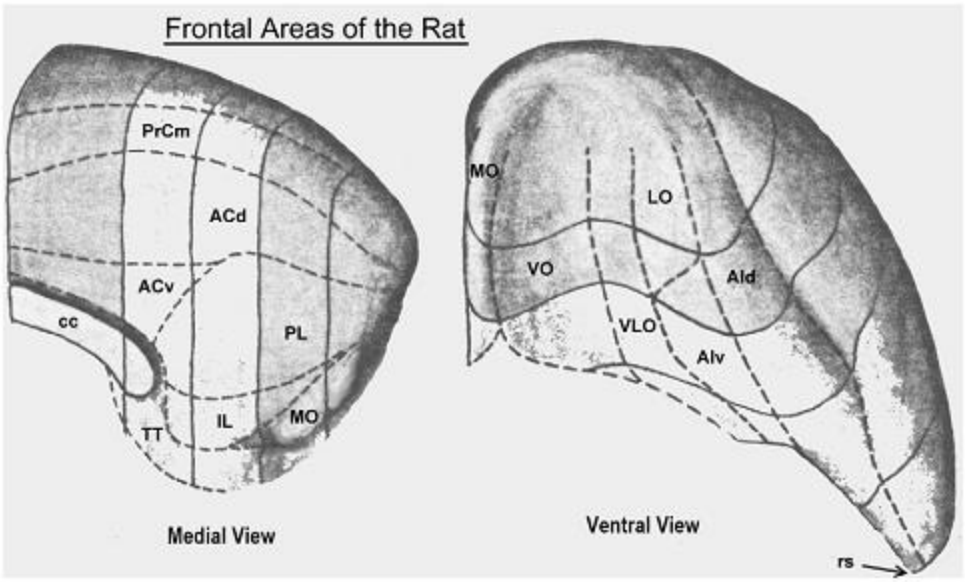

Figure 1.1: Frontal regions of the rat. (Left) medial view; (Right) ventral view. Abbreviations: PrCm – Precentral cortex; AC – Dorsal and ventral anterior cingulate; PL-IL – Prelimbic and infralimbic cortex; MO – Medial orbital cortex; AI – Dorsal and ventral agranular insular cortex; LO – Lateral orbital cortex; VO – Ventral orbital cortex; VLO – Ventrolateral orbital cortex. Reprinted with permission from "An analysis of rat prefrontal cortex in mediating executive function" Kesner and Churchwell. 2011. Neurobiol Learn Mem. 96: 417-431.

The prelimbic, infralimbic and medial orbital cortex contribute to visual and spatial working memory (Brito and Brito, 1990; Delatour and Gisquet-Verrier, 1996; Fritts et al., 1998; Granon et al., 1994; Horst and Laubach, 2009; Ragozzino et al., 1998; Seamans et al., 1995; Van Haaren et al., 1988; Di Pietro et al., 2004; Kesner et al., 1996; Ragozzino et al., 2001). However, some studies show that prelimbic, infralimbic and medial orbital cortex lesions do not produce a deficit in working memory for appetitive information such as a food reward (DeCoteau et al., 1997; Ragozzino and Kesner, 1999). Odour and taste working memory information is regulated by the agranular insular and lateral orbital cortex (Kesner and Churchwell, 2011). Lesions to these regions produced impairments on working memory tasks with odour and taste (DeCoteau et al., 1997; Di Pietro et al., 2004; Otto and Eichenbaum, 1992; Ragozzino and Kesner, 1999) but did not affect visual or spatial working memory (DeCoteau et al., 1997; Di Pietro et al., 2004; Horst and Laubach, 2009; Ragozzino and Kesner, 1999). The role of ventral orbital and ventrolateral orbital cortex in working memory tasks is unclear. Ventral orbital and ventrolateral orbital cortex lesions did not affect spatial location information on a working memory task (Janis and Kesner, 1995). There are connections from the posterior parietal, medial extrastriate and lateral extrastriate cortices to the ventral orbital and ventrolateral orbital cortices (Reep et al., 1996). Visual and spatial information may be retrieved from the ventral orbital and ventrolateral orbital cortex, and these regions may contribute to other types of learning and memory rather than working memory.

1.3.2 Neuroanatomy and function of the prefrontal cortex

The majority of information on working memory models stems from studies on humans and monkeys; therefore, it is necessary to detail homologies between the primate DLPFC and the

rat mPFC. The following criteria is taken into account when assessing homologies in neural substrates across species: the pattern of specific connectivity and the density of the connectivity; the electrophysiology and behavioural properties of specific neural substrates; the presence of neurotransmitter receptors; the developmental characteristics; and for closely related species, cytoarchitectonic features (Uylings et al., 2003). The more similarities shared between neural substrates, the greater the likelihood neural substrates are homologous.

The PFC may have evolved from the older archicortical and paleocortical (Pandya and Yeterian, 1990). The archicortical gave rise to the proisocortical, which is a type of neocortex. The proisocortical areas are composed of the anterior cingulate cortex, infralimbic and prelimbic, which gave rise to the DLPFC in the primate. The prelimbic area in rats may be considered a primitive version of the DLPFC in primates (Pandya and Yeterian, 1990; Seamans et al., 2008). Although, the monkey and human DLPFC is more complex than the rat mPFC, they share anatomical and behavioural features.

The lack of an operational definition of the PFC led to different views on the existence of a PFC in rats (Uylings et al., 2003). In the past, some have questioned whether rats have a PFC that is comparable with the DLPFC in humans and monkeys. The anterior frontal lobe, termed the PFC, is composed of medial, lateral, and orbital components. The DLPFC in humans and monkeys is connected with the posterior parietal cortex, head of the caudate nucleus, and the dorsomedial thalamic nucleus (Siddiqui et al., 2008). The structural, behavioural, and electrophysiological evidence, outlined below, strongly suggests that the rat mPFC has features analogous to the primate DLPFC.

The frontal cortex receives its main input from the basal ganglia through a relay in the thalamus (Middleton and Strick, 2000). The frontal cortex, parietal, occipital and temporal cortex

projects to the STR (Middleton and Strick, 1996). Primates and rats have five similar basal ganglia-thalamocortical circuits: a motor, an oculomotor, an anterior cingulate/medial orbitofrontal, a lateral orbitofrontal, and DLPFC circuits (Alexander et al., 1990; Alexander et al., 1986; Uylings et al., 2003). The connections in frontal cortex to basal ganglia to thalamocortical areas display similarities between rats and primates, specifically the similarities between the rat dorsal anterior cingulate and Fr2 and the primate DLPFC. Reciprocal cortical to cortical connections show three subtypes in rat mPFC, which are the dorsal shoulder region (dorsal anterior cingulate and Fr2 areas), an anterior part of mPFC, and the prelimbic and infralimbic cortices.

Specific neural substrates should have similar behavioural correlates across species to demonstrate homology. Lesions of the DLPFC in monkeys results in reduced working memory and executive functioning such as planning behaviours (Fuster, 1997; Sawaguchi and Goldman-Rakic, 1994), and damage to DLPFC in humans produces similar impairments (Uylings et al., 2003). Rats with mPFC lesions have impaired working memory on delayed response (Kolb et al., 1974), delayed alternation (Divac, 1971; Kolb et al., 1974; Wikmark et al., 1973), delayed-nonmatching-to-sample (Broersen et al., 1995; Dunnett, 1990; Otto and Eichenbaum, 1992), and related tasks (Kesner and Holbrook, 1987; Ragozzino, 2000). The mPFC in rats and the DLPFC in monkeys and humans share common functions for working memory. Cytoarchitectonic characteristics are similar in homologous neural substrates among akin species. Cytoarchitectonic features show that rat mPFC is agranular cortex that lacks a layer IV and is similar to the anterior cingulate cortex in primates (Uylings et al., 2003).

1.3.3 Effects of lesions within the dorsal striatum in working memory in rodents

The dorsal STR is composed of the dorsolateral striatum (dlSTR), which receives projections from the sensory-motor region of the PFC, and the dorsomedial striatum (dmSTR), which receives projections from the prelimbic and infralimbic cortex. Infusions of amphetamine, a dopamine agonist, into dlSTR improved win-stay performance (a task that consists of repeated reinforcement of responding to a light stimulus; Packard and White, 1991). However, it did not alter performance on a win-shift task that depends on working memory (rats learn to enter the baited arms once; Packard and White, 1991). Packard and White's (1991) data shows that stimulus-response learning may be consolidated in the dlSTR, which is consistent with the inputs of a corticostriatal circuit and the output of a striatonigral circuit (described in section 1.4.2). Stimulus-response learning is associated with sensory-motor information in the dlSTR (White, 2009).

Using a 12 arm radial maze, containing 7 arms that always contained a food pellet (not replaced after it was consumed), Colombo et al. (1989) examined the effects of dorsal STR lesions in this working memory and reference memory task. Rats learned to enter the arms that contained food once. This demonstrates intact working memory since it requires maintenance of the specific arms that have been entered. The avoidance of empty arms demonstrates reference memory. Dorsal STR lesions impaired reference memory while sparing working memory (Colombo et al. 1989). However, lesions targeting the dorsal STR may have spared some of the dmSTR since the researchers were not examining the distinction between dmSTR and dlSTR.

The presence or absence of food reward affects working memory in rats with dorsal STR lesions. Dorsal STR lesions impaired working memory when rats had access to food in the maze arms during the initial adaption to the maze, whereas no deficit occurred when adaption is

performed without food (Packard et al., 1992). White (2009) proposed that eating near the ends of arms during adaptation results in a dlSTR modulated stimulus-response learning to enter all arms. Therefore, the early adaption to enter arms indiscriminately may interfere with the win-shift working memory task. The win-shift task measures working memory in the radial arm maze and half of the arms are open in the sample phase. All arms are open during the test phase and rats that enter only the arms that were blocked in the sample phase show correct performance. Re-entering the same arm in the test phase are within phase errors and entering an arm on the test phase that was open during the sample phase are across phase errors.

While the previously mentioned studies examined dorsal STR or dlSTR, Winocur (1980) lesioned the dmSTR and the anterior dorsal STR prior to a working memory task. Rats with dmSTR and anterior dorsal STR lesions were impaired on a win-shift working memory task (Winocur, 1980). Lesions of dmSTR impaired working memory while lesions of dlSTR did not affect reference memory. DeCoteau et al. (2004) measured working memory in a 3 arm maze that contained food in one arm, and after a 30 second delay, a correct choice involved entering the same arm as the sample trial. Kesner and Gilbert (2006) found similar results by using a motor working memory task that involved rats displacing objects that glow with phosphorescent paint to receive food reward in complete darkness. The sample trial consisted of a phosphorescent object randomly positioned to cover a baited food port. The rat would displace the object to receive food and then return to the start box. The box was rotated to face another direction with an identical baited phosphorescent object in the same position relative to the start box and a second identical object was positioned to cover a different unbaited port. To display correct behaviour on the choice phase, the rat reproduces the motor response made on the sample phase. Rats with dmSTR and partial dlSTR lesions were impaired on the task (Kesner and

Gilbert, 2006). These studies show that the dmSTR contributes to spatial working memory in rats.

1.3.4 Neuroanatomy and function of the striatum

Approximately 40 years ago the concept of a dorsal and ventral subdivision within the STR was proposed. However, the dorsal and ventral subdivision may not be an accurate representation of the structure (Voorn et al., 2004). The terms dorsal and ventral STR are widely used, but a well-accepted boundary between these structures is not defined. Some researchers demarcate the dorsal-ventral border between the nucleus accumbens and the caudate-putamen but this border does not differ in histological or immunohistochemical properties (Voorn et al., 2004). Other researchers used connectivity of hippocampal, amygdaloid and PFC to demarcate the border (Voorn et al., 2004). However, other studies suggest that the dorsolateral-ventromedial divide of the STR is correct given behavioural and neural connectivity studies (Voorn et al., 2004).

The STR sub-regions are involved with different behaviours. For example, the dlSTR is involved with procedural or stimulus-response learning, and the dmSTR and ventral STR with working memory. However, the dmSTR and ventral STR may have distinct roles in working memory. The dmSTR and ventral STR are responsible for inhibitory control of behaviour. In rats with dmSTR lesions performing attention tasks, there were increased omissions on a go task, whereas rats with ventral STR lesions had increased preservative responding on the 5-choice serial reaction time task (Eagle and Robbins, 2003; Christakou et al., 2004). Different facets of cognitive flexibility in monkeys and humans are mediated by dorsal and ventral STR with set-shifting being mediated by dorsal STR and reversal learning mediated by ventral STR (Crofts et

al., 2001; Cools et al., 2002; Cools et al., 2001). The distinction between dlSTR on one hand and dmSTR and ventral STR on the other hand may be valid when examining cognitive function.

Cytology shows the similarity between the dorsal and ventral STR, characterized by same neural cell types distributed throughout the STR. The medium-size spiny neurons (MSNs) compose 95% of the neural cell population, and several classes of interneurons compose a minority of the cell population (Gerfen, 2004). The proportions of different cell types vary little between STR areas; however, the interaction between pallidal neurons and MSN increase in the ventral STR (Heimer, 2000). The CART peptides are a putative neurotransmitter that are increased in the ventral STR relative to the dorsal STR (Kuhar and Dall Vecchia, 1999). However, the increased complexity in ventral STR does not allow for a clear demarcation of dorsal-ventral STR.

MSNs show similar membrane properties throughout the STR, such as relatively hyperpolarized resting potentials more negative than -65mV and strong inward rectification of electrical currents (Dorris et al., 2015; Voorn et al., 2004). The STR has fast excitatory inputs to MSNs that are mediated by glutamate receptors, with AMPA and kainate receptors mostly working at potentials near membrane resting potential, while NMDA receptors active at more depolarized membrane potential. Synaptic transmission onto MSNs in the STR usually leads to glutamate-mediated subthreshold depolarized potentials (Wilson and Kawaguchi, 1996), or $GABA_A$-mediated hyperpolarized potentials (Tunstall, et al., 2002; Taverna et al., 2004). Fast spiking GABAergic interneurons contribute to GABA-mediated inhibition of MSNs in the STR (Koos and Tepper, 1999). Generally, the features of the STR are similar throughout, although dorsal-ventral differences do exist. These differences are subtle, for instance dopamine attenuates

glutamatergic inputs to ventral but not dorsal STR, and in dorsal STR dopamine receptors attenuate AMPA receptor function but increase NMDA receptor function (Cepeda et al., 1998).

A dorsolateral-ventromedial divide is seen in humans based on the different calbindin concentrations, and the pattern of primate corticostriatal inputs (Haber, 2003; Karachi et al., 2002; Namba, 1957). The pattern of PFC inputs in the rat suggests that the dorsolateral-ventralmedial STR divide is a general pattern across humans, non-human primates and rats (Willuhn et al., 2003). The dlSTR receives substantial sensorimotor information from the PFC, whereas the ventromedial STR processes visceral afferents, and STR regions between the dlSTR and ventromedial STR receive higher order information (Figure 1.2; Voorn et al., 2004). The dmSTR processes higher order information and receives inputs from the prelimbic area within the PFC of rats. The dorsolateral-ventromedial gradient receives further support from results of single unit recordings in awake animals, in which sensorimotor manipulation correlated with activity in dlSTR and reward delivery was correlated with ventromedial STR (Hollerman et al., 1998; Carelli and West, 1991). The STR outputs are mostly organized on the dorsolateral-ventromedial gradient, projecting to pallidal and nigral substrates (Gerfen, 2004; Zahm, 2000). The inputs and outputs of the STR and the specific behaviours related to different areas of the STR support a dorsolateral-ventromedial gradient rather than a dorsal-ventral STR divide.

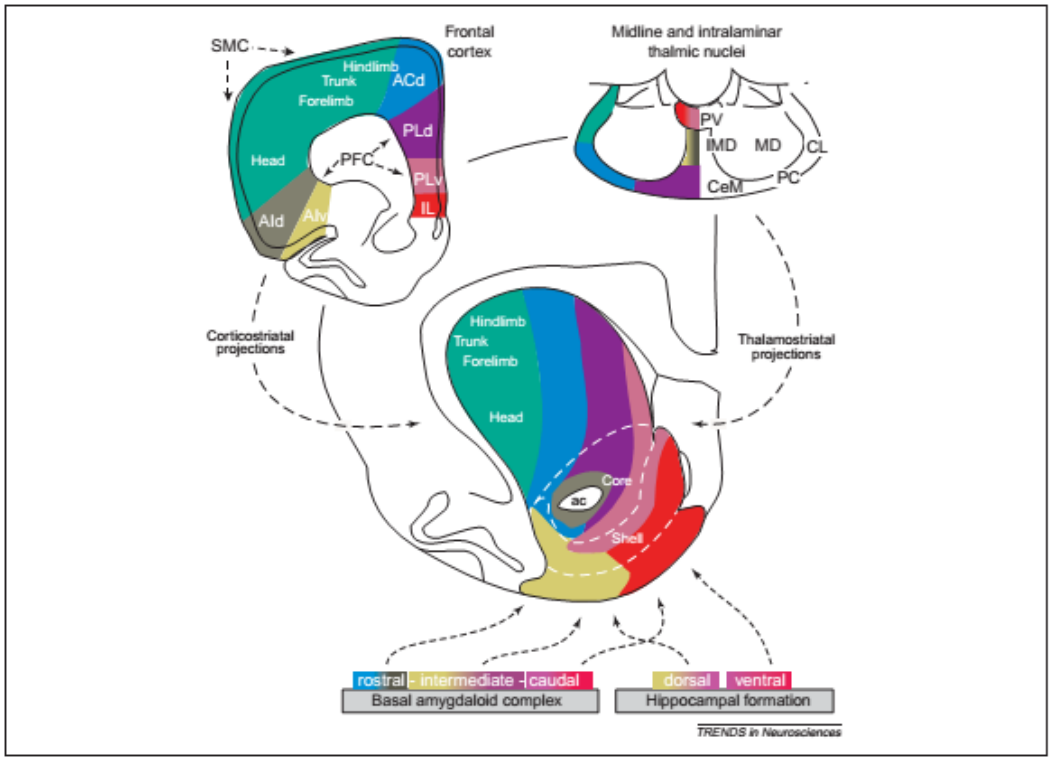

Figure 1.2: Cortical and thalamic projections into the STR distribute into dorsomedial-ventrolateral zones. The arrangement of STR afferents from the frontal cortex (upper left), midline and intralaminar thalamic nuclei (upper right), basal amygdaloid complex (lower left) and hippocampus (lower right) are displayed. Frontal cortex regions and its corresponding STR projection zones are displayed in the same colours. The dlSTR receives somatotopically organized sensorimotor information (green), and the ventromedial region of the STR collects viscerolimbic cortical afferents (red and pink), and the STR regions between these zones receive information from higher order cortical areas (blue and purple). Reprinted with permission from Voorn et al. 2004. "Putting a spin on the dorsal-ventral divide of the striatum." Trends Neurosci. 27: 468-474.

1.4 The role of glutamate receptors in working memory

1.4.1 Glutamate receptors

Glutamate receptors are classified in either the metabotropic or ionotropic family (Ozawa et al., 1998). Metabotropic glutamate receptors are G-protein coupled receptors that utilize secondary messenger cascades and modulate glutamate release (Schoepp and Conn, 1993). Ionotropic glutamate receptors include NMDA, AMPA, and kainate receptor subtypes. NMDA receptors consist of four domains: a ligand-binding domain that binds agonists such as L-glutamate, transmembrane domain that forms the ion channel, carboxyl terminal domain that involves synaptic targeting, and an N-terminal extracellular domain (Regan et al., 2015). In all glutamate receptors, the ligand-binding domain connects with three short linkers to the transmembrane domain (Traynelis et al., 2010). The transmembrane helices from each of the four subunits influence the formation of the ion channel (Wo and Oswald, 1995). The carboxyl terminal domain varies in sequence and length among glutamate receptor subunits (Traynelis et al., 2010). The extracellular domain accounts for approximately 80% of an ionotropic glutamate receptor with distinct organization since the N-terminal domain forms two pairs of stable dimers that associate into tetramers (Krieger et al., 2015). NMDA receptors have been shown to play a central role in working memory, particularly in tasks such as the radial arm maze (Li et al., 1997), delayed-match-to position task (Doyle et al., 1998; Smith et al., 2011), delayed-non-match-to position task (Aura and Riekkinen, Jr. 1999), and t-maze (Moghaddam and Adams, 1998).

NMDA receptors have a central role at glutamatergic excitatory synapses in the central nervous system. Ionotropic glutamate receptors co-localize mainly at the postsynapses and regulate excitatory postsynaptic potentials (Tovar and Westbrook, 2016). In addition to

ionotropic glutamate receptors, adenosine and the endocannabinoid system influence synaptic transmission. Adenosine controls the action of modulators for synaptic function and operates as a metamodulator of synaptic transmission (Sebastiao and Ribeiro, 2015). The endocannabinoid system controls synaptic transmission by limiting glutamate (Rossi et al., 2015).

1.4.2 Structure and function of NMDA receptors

Seven different NMDA receptor subunits have been identified: the GluN1 subunit, the GluN2 subunits (GluN2A, GluN2B, GluN2C, and GluN2D), and GluN3 subunits (GluN3A and GluN3B; Paoletti et al., 2013). NMDA receptors are heterotetrameric (protein containing four non-covalently bound subunits) compositions that typically have GluN1 subunits with GluN2 subunits or a combination of GluN2 and GluN3 subunits (Traynelis et al., 2010; Cull-Candy and Leszkiewicz, 2004; Paoletti, 2011). The GluN1 subunit is encoded by one gene and has eight distinct isoforms, which are different versions of a receptor subunit. The GluN2 or GluN3 subunits can be identical or different, termed di-heteromeric and tri-heteromeric receptors, respectively (Sheng et al., 1994). Di-heteromeric receptors contain two distinct subunits in the receptor complex such as GluN1/GluN2B, whereas tri-heteromeric receptors contain three distinct subunits in the receptor such as GluN1/GluN2A/GluN2B. In the GluN1/GluN2 receptor complex, the two GluN1/GluN2 dimers have alternating subunits around the pore GluN1/GluN2/GluN1/GluN2 (Riou et al., 2012; Salussolia et al., 2011). The GluN1 and GluN3 subunits have glycine binding sites, and the GluN2 subunits have glutamate binding sites (Furukawa et al., 2005).

The co-binding of glutamate and glycine induce a conformational change in the NMDA receptor that gates the channel and allows the flux of ions though the channel. In addition to

glycine as an agonist at the GluN1 subunits, the D- and L-isomers of serine and alanine function as agonists (Pullan et al., 1987; McBain et al., 1989). While D-cycloserine is a partial agonist of GluN2A, GluN2B, and GluN2D subunits, the responses of GluN2C subunits are greater in D-cycloserine than those induced by glycine (Sheinin et al., 2001; Dravid et al., 2010). Endogenous GluN2 subunit agonists include glutamate, D- and L-aspartate, homocysteate and cysteinesulfinate (Benveniste, 1989; Do et al., 1986; Traynelis et al., 2010). Glycine and D-serine bind in the GluN3 subunit cleft (Yao et al., 2008). Competitive GluN1 antagonist (competes with available agonists) include 7-chlorokynurenic acid and its analog 5,7-dichlorokynurenic acid (Birch et al., 1988; Kemp et al., 1988; McNamara et al., 1990). The GluN2 competitive antagonist (R)-2-amino-5-phosphonopentanoate are used to selectively block NMDA receptor activity distinct from AMPA and kainate receptor activity (Davies et al., 1986; Lester et al., 1990). 3-((*R*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid shows an approximately 50-fold preference for GluN2A over GluN2D and intermediate affinity for GluN2B and GluN2C (Ikeda et al., 1992; Kutsuwada et al., 1992; Feng et al., 2005). The competitive antagonist 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) binds to the glutamate site of the NMDA receptor (Monaghan and Jane, 2009). Noncompetitive antagonists bind to an allosteric site on the receptor to prevent activation. The noncompetitive antagonist ifenprodil binds to GluN2B-containing NMDA receptors with high affinity (200-400-fold more potent) for GluN1/GluN2 receptors than for GluN1/GluN2A, GluN1/GluN2C, or GluN1/GluN2D receptors (Williams, 1993; Hess et al., 1998). Ifenprodil and its more potent derivative Ro 25-6981 interact with the GluN1/GluN2B N-terminal domain (Karakas et al., 2011).

The localization of specific NMDA receptors depends on various factors. GluN2B-containing NMDA receptors can move in and out of synapses more frequently relative to GluN2A-containing NMDA receptors (Groc et al., 2006). The localization of NMDA receptors depends on a postsynaptic density (PSD), which is a protein-dense attachment to the postsynaptic membrane of excitatory synapses. A PSD contains many proteins including glutamate receptors, scaffold proteins, actin cytoskeleton components, and signalling molecules. The PSD-95 interacts with the GluN2A subunit and results in differential postsynaptic locations (Paoletti et al., 2013). Among the four PSD-95-like membrane associated guanylate kinases (PSD-95, PSD-93, SAP102, and SAP97), PSD-95 is the most extensively examined in the context of synaptic plasticity (Xu, 2011). PSD-95 is highly enriched in the PSD and regulates synaptic AMPA receptor function (Elias and Nicoll, 2007). PSD-95 mutant mice have learning and memory deficits on the Morris water maze (Migaud et al., 1998).

There are two hypotheses for how PSD-95 regulates NMDA receptor dependent synaptic plasticity. The slot protein for anchoring AMPA receptor hypothesis proposes that PSD-95 acts as the target of signaling during plasticity since changes in the levels of PSD-95 influence the levels of synaptic AMPA receptors. A signaling cascade from long term depression (LTD) induction is linked to the removal of synaptic PSD-95 and AMPA receptors (Schnell et al., 2002; Colledge et al., 2003; Xu, 2011). The signaling scaffold hypothesis involves PSD-95 bringing intracellular signaling complexes close to NMDA receptor channels. LTD is disrupted from the removal of PSD-95, mutants destabilizing PSD-95 in PSD, and mutants disrupting intercellular interaction (Xu et al., 2008; Xu, 2011). The two hypotheses form a larger hypothesis about the function of PSD-95 (Xu, 2011). PSD-95 contributes to LTD in two stages, first as a signaling scaffold that mediates the dynamic interaction in signaling events during the induction of LTD,

and second as an AMPA receptor anchoring protein that is downregulated to maintain the decrease of synaptic AMPA receptor content during LTD expression.

Phosphorylation is a fundamental mechanism that regulates the function of proteins and lipids (Pawson and Scott, 2005; Blume-Jensen and Hunter, 2001; Salter et al., 2009). Phosphorylation and dephosphorylation are reversible processes that active and deactivate enzymes. Phosphorylation by serine/threonine kinases and protein tyrosine kinases is a mechanism for regulating NMDA receptors (Ali and Salter, 2001; Chen and Huang, 1991). The serine/threonine kinase Cdk5 is critical for regulating synaptic plasticity through phosphorylation (Lai and Ip, 2015). Protein kinase A intracellularly administered has been shown to increase NMDA receptor current (Cerne et al., 1993). Electrophysiological studies show that protein kinase C regulates NMDA receptor current (Salter et al., 2009). NMDA receptor currents are determined by a balance between tyrosine phosphorylation and dephosphorylation (Salter et al., 2009). The Src family of protein tyrosine kinases are critical for the enhancement of NMDA receptor function (Wang and Salter, 1994). The five Src family members, Src, Fyn, Lck, Lyn, and Yes, are responsible for upregulation of NMDA receptor function and are found in the PSD. Src, Fyn, Lyn, and Yes are components of the NMDA receptor complex and all are at appropriate locations to potentially regulate NMDA receptor function (Salter et al., 2009). The basal activity of Src is normally in a low state but can be enhanced by upstream signaling cascades. Src family kinases act as a molecular hub through which many signaling cascades converge to control NMDA receptors (Salter and Kalia, 2004). Src is regulated by three PSD proteins within the NMDA receptor: RACK1, H-Ras, and PSD-95 (Yaka et al., 2002; Thornton et al., 2003; Kalia et al., 2006).

1.4.3 NMDA receptors in learning and memory

NMDA receptors are critical for synaptic plasticity, which contribute to learning and memory (Salter et al., 2009). Long-term potentiation (LTP) is a lasting form of synaptic plasticity and is a major cellular model of learning and memory (Malenka and Nicoll, 1999). The induction of LTP requires a substantial increase of calcium through NMDA receptors. The Src family kinases are important for the induction of LTP in CA1 (Grant et al., 1992; Lu et al., 1998). It is hypothesized that kinase induced increase of NMDA receptor function results in production of LTP at low stimulation frequencies. Dephosphorylation and striatal-enriched tyrosine phosphatases suppress NMDA receptors, and higher stimulation frequencies may be necessary for induction of LTP (Salter et al., 2009). LTP is induced by AMPA receptors from glutamate release in a presynaptic terminal, which depolarizes the AMPA receptor (Blundon and Zakharenko, 2008). The magnesium block from the NMDA receptor is removed and sodium, potassium, and calcium enter the channels. Calcium, a second messenger, phosphorylates AMPA receptors, which facilitates AMPA receptor conductance. This strong synapse results in a greater number of AMPA receptor channels opening, which allows NMDA receptor channels to open.

Many studies show that NMDA receptor function contributes to working memory (Monaco et al., 2015; Wang et al., 2013). The NMDA receptor antagonist ketamine administered in humans reduced spatial working memory and the connectivity of the prefrontal cortex (PFC) network (Driesen et al., 2013). This reduced connectivity suggests that the PFC had impaired ability to select the target representation stored within working memory (Driesen et al., 2013). Working memory in monkeys was also impaired following chronic NMDA receptor blockade with MK-801 (Tsukada et al., 2005).

Wang et al. (2013) showed that NMDA receptor function is essential for persistent firing in monkey's dorsolateral PFC (DLPFC) neurons during a delay phase of an oculomotor delayed response task. The oculomotor delayed response task is a spatial working memory task where a cue is present in 1 of 8 locations, and then the cue is removed (delay phase). A correct response is when a monkey makes a saccade to the location of the cue. The broad spectrum blockade of NMDA receptors or the selective blockade of GluN2B-containing NMDA receptors in DLPFC reduced delay cell firing. The AMPA receptor mediates fast excitatory synaptic transmission (Ozawa et al., 1998). Blockade of AMPA receptors showed a lag effect in that activity was reduced later during the delay phase. AMPA receptors may have a supporting role for persistent firing, whereas NMDA receptors are essential for the persistent firing during the delay phase (Wang et al., 2013; Monaco et al., 2015). Therefore, NMDA and AMPA receptors are important for persistent firing in the delay phase of a working memory task.

GluN2B-containing NMDA receptors within the frontal cortex contribute to working memory in monkeys and rodents (Cui et al., 2011; Wang et al., 2013). GluN2B-containing NMDA receptors overexpressed in the frontal cortex and striatum in mice resulted in enhanced span capacity on the odour span task (OST), which measures working memory capacity in rodents using an incremental delayed-non-match-to-sample paradigm (Cui et al., 2011; Tang et al., 1999). This enhancement of span capacity may be due to the extended NMDA receptor channel opening and enhanced NMDA receptor activation, suggesting a strong connection between NMDA receptor activation and working memory.

Relative to the GluN2A subunit, the GluN2B subunit conducts a large quantity of calcium and sodium ions while in its open channel state, which prolongs depolarization and synaptic summation (Wang, 1999; Wang, 2001; Cull-Candy et al., 2001; Wang et al., 2008).

Temporal summation brings neurons closer to the threshold of spike firing, which may enhance working memory (Kumar and Huguenard, 2003). The delay phase during a working memory task employs active engagement of neurons, and the GluN2B subunit affects NMDA receptor channel kinetics and contributes to persistent firing within the PFC (Goldman-Rakic, 1995; Wang et al., 2013; Wang, 1999).

An influx of calcium into post synaptic neurons contributes to persistent firing within the PFC (Monaco et al., 2015). Calcium accumulation in the dendritic spine regulates through signalling molecules such as protein kinase A, cyclic AMP (cAMP), IP3-dependent intracellular stores, metabotropic glutamate receptors, and monoamines (Arnsten and Jin, 2014; Arnsten et al., 2012; Snyder and Gao, 2013). Increases of cAMP activity results in reduction of neural firing during a delay phase in a working memory task (Wang et al., 2011; Wang et al., 2007). The increase duration of channel opening results in a greater influx of calcium into post synaptic neurons and contributes to persistent firing, but incurs a risk for excitotoxicity (Lett et al., 2014; Wang et al., 2013). This excitotoxicity from the GluN2B-containing NMDA receptor may be involved with neurodevelopmental disorders such as schizophrenia (Monaco et al., 2015). The role of the GluN2A subunit is less well known than the GluN2B subunit. The GluN2A subunit may protect against the excitotoxic effects of large amounts of calcium; however, this has not been examined experimentally (Monaco et al., 2015).

1.5 Pattern separation

Pattern separation is “the process of reducing interference among similar inputs by using non-overlapping representations” (Bussey, 2013). The hippocampus performs pattern separation at the time of encoding and storage to make similar information distinct (Hunsaker and Kesner,

2013). There is debate of whether the term pattern separation is appropriate in behavioural science, and whether the term discrimination is more appropriate (Santoro, 2013). Pattern separation involves discrimination, and behavioural studies show that different neural substrates are engaged when discriminating similar compared to dissimilar stimuli, which suggests that pattern separation is a unique process. Recently, many studies have examined the neural substrates involved with pattern separation (Kent et al., 2016). Marr developed a computational model of the hippocampus memory system that retained detail and precision during encoding and introduced the concept of pattern separation (Marr, 1971; Marr, 1969). Computational models show that the process of pattern separation amplifies the discrepancies between similar patterns (Marr, 1969; Torioka, 1979; Gibson et al., 1991). These computational models led to experimental data in support of pattern separation from electrophysiology, immediate early-gene, behaviour, and imaging studies.

Electrophysiological experiments provide strong evidence for pattern separation and are consistent with predictions of computational models (Kent, 2015). An *in vivo* patch-clamp experiment showed that single mossy fibres connecting a dentate gyrus neuron and CA3 pyramidal neuron have fast and repetitive firing of the presynaptic granule neuron that can fire a downstream CA3 neuron (Henze et al., 2002). This downstream firing of a CA3 neuron may be referred to as a detonator potential of the mossy fibres, which is a powerful synapse that repeatedly causes depolarization and firing of a post synaptic neuron (McNaughton and Morris, 1987). Electrophysiological experiments measuring the ability of neurons to keep small differences distinct in cortical input patterns provide additional evidence for pattern separation. Dentate gyrus cells significantly alter neural activity from gradually morphing enclosures with a circle to a square or vice versa (Wills et al., 2005; Leutgeb et al., 2007). Wills et al., (2005)

suggest that these results provide evidence for pattern separation since highly similar stimuli created very different representations. Neunuebel and Knierim (2014) provided more evidence to support pattern separation occurring in the dentate gyrus. Measuring input and output representations allowed for the test of whether outputs are more similar or less similar to inputs, which is a measure of pattern completion or pattern separation, respectively. Pattern completion is the ability to retrieve a memory from partial or degraded input cues. To assess pattern completion and pattern separation, rats were trained to navigate a track with local and global cues near the testing area. Rotation of cues resulted in gradual changes in sensory input while simultaneously recording single unit activity from the dentate gyrus and CA3. This shows the dentate gyrus sends degraded input to CA3, and that CA3 produces an output that reflects the originally stored representations. The dentate gyrus region contributes to pattern separation whereas the CA3 region contributes to pattern completion.

Behavioural experiments provide additional evidence of the dentate gyrus supporting pattern separation. Behavioural experiments employ tasks where the similarity of stimuli is varied parametrically to assess pattern separation. Behavioural experiments provide a broader picture for the construct of pattern separation by integrating literature from computational models and electrophysiology. Pattern separation is measured in many different tasks such as contextual fear conditioning (McHugh et al., 2007; Sahay et al., 2011; Nakashiba et al., 2012; Tronel et al., 2012), novel context exploration (Hunsaker et al., 2008), radial arm maze (Clelland et al., 2009), object place association (Lee and Solivan, 2010), touchscreen location discrimination (McTighe et al., 2009; Clelland et al., 2009; Creer et al., 2010), spontaneous location recognition (Hunsaker et al., 2008; Bekinschtein et al., 2013; Bekinschtein et al., 2014; Kent et al., 2015), delayed match to sample spatial mazes (Gilbert et al., 1998; Gilbert et al.,

2001), and the TUNL task (Oomen et al., 2013). Subjects in these tasks resolve overlapping inputs that require discrimination of stimuli (Oomen et al., 2014).

There is strong evidence that pattern separation occurs in the hippocampus during behavioural tasks (McDonald and White, 1995; Frankland et al., 1998; Gilbert et al., 1998; McTighe et al., 2009), and within the dentate gyrus (Gilbert et al., 2001; Hunsaker et al., 2008; McHugh et al., 2007; Bakker et al., 2008; Lee and Solivan, 2010). Dentate gyrus plasticity (McHugh et al., 2007; Bekinschtein et al., 2013), and neurogenesis seems important for pattern separation (Clelland et al., 2009; Creer et al., 2010; Sahay et al., 2011; Nakashiba et al., 2012; Tronel et al., 2012; Kheirbek et al., 2012; Bekinschtein et al., 2014; Kent et al., 2015).

To specifically assess pattern separation in rats a spontaneous location recognition task was developed, which uses a rat's innate preference for novelty (Hunsaker et al., 2008; Kent, 2015). In comparison to rats with CA3 lesions, rats with dentate gyrus lesions spent less time exploring objects moved to a novel location (closer together than during the sample phase) and a novel environment (change from circular to a square area). Lower levels of novel object or location exploration time in rats with dentate gyrus lesions suggest that spatial pattern separation is processed in the dentate gyrus. The location discrimination task is an automated task in touchscreen equipped operant conditioning chambers, which assesses spatial pattern separation in rodents that varies locations of stimuli systematically (McTighe et al., 2009). Rats with dorsal hippocampal lesions had reduced performance when discriminating between similar locations on the touchscreen. Rats with sham-lesions were unaffected by spatial separation. Pattern separation is hippocampal dependent as assessed by the spontaneous location recognition task that measures the rat's innate preference for novelty and the location discrimination task that involves weeks of training to shape the desired behaviours.

1.7 General hypothesis

The general hypothesis of this thesis is that NMDA receptors within the mPFC and dmSTR of rats contribute to working memory as assessed by the TUNL task and OST. The TUNL task is a visual working memory and pattern separation task that uses a delayed-non-match-to-sample paradigm with visual stimuli presented in 1 of 14 different locations. The OST is an incremental delayed-non-match-to-sample paradigm that measures working memory capacity and requires olfaction to guide correct performance.

1.8 Thesis objectives

1. Demonstrate that NMDA receptors contribute to working memory (Chapters 2, 4). This was achieved by systemic administration of an NMDA receptor antagonist, or a GluN2B-containing NMDA receptor antagonist.

2. Demonstrate that the mPFC contributes to working memory (Chapter 3). This was achieved by intracranial infusions with $GABA_A$ and $GABA_B$ agonists to induce inactivation of the mPFC.

3. Demonstrate that NMDA receptors within mPFC and dmSTR contribute to working memory (Chapters 2, 5). This was achieved by intracranial infusions of an NMDA receptor antagonist or a GluN2B-containing NMDA receptor antagonist.

Chapter 2: Medial prefrontal cortex and dorsomedial striatum are necessary for the trial-unique, delayed nonmatching-to-location (TUNL) task in rats: role of NMDA receptors

The content of this chapter is in preparation for submission to *Learning & Memory*. I gratefully recognize the contributions of Jessica L. Hurtubise, Quentin Greba, John G. Howland to this work. Any redundant information provided elsewhere in the dissertation has been removed.

2.1 Abstract

The prefrontal cortex and striatum interact to support attention and working memory. The trial unique non-match to location (TUNL) task is a novel paradigm that measures spatial working memory and spatial pattern separation. Working memory is involved in the storage, maintenance and manipulation of information essential for complex cognition. Pattern separation is the ability to keep similar memories distinct. Limited information exists regarding the neurotransmitters and neural substrates involved in the TUNL task. N-methyl-D-aspartate (NMDA) receptors within the prefrontal cortex and striatum have a critical role in working memory, and NMDA receptors are important for spatial pattern separation. The present experiments tested TUNL task performance following systemic injections of NMDA receptor antagonist CPP, or GluN2B-containing NMDA receptor antagonist Ro 25-6981, and intracranial injections of the NMDA receptor antagonist AP5 into medial prefrontal cortex (mPFC) or dorsomedial striatum (dmSTR). Long Evans rats were trained to nose poke a sample stimulus illuminated in 1 of 14 locations. After a 2 or 6 s delay, the rat was presented with the sample stimulus and an illuminated stimulus in a new location. Nose poking the new stimulus was scored as a correct choice. Rats systemically injected with CPP had impaired accuracy overall and on small separations, suggesting a working memory and pattern separation impairment, while Ro 25-6981 did not affect accuracy. AP5 infused into mPFC or dmSTR reduced overall accuracy but not accuracy on small separations, suggesting a working memory impairment but spared pattern separation. These results suggest that TUNL task performance depends on NMDA receptors within the mPFC and dmSTR.

2.2 Introduction

The function of the prefrontal cortex is one of the most intensely researched areas in neuroscience because of its role in attention and working memory (Roberts et al., 1998; Thierry et al., 2011). The prefrontal cortex projects to the striatum, which is involved with behavioural flexibility, attention, and working memory (Fuccillo, 2016; O'Reilly, 2006). Interactions between the prefrontal cortex and striatum support attention and working memory (O'Reilly, 2006). During working memory, the prefrontal cortex regulates top-down attention to select the correct item within working memory (D'Esposito and Postle 2015), and the striatum allows relevant information into working memory and inhibits irrelevant information from entering working memory (O'Reilly, 2006).

The trial unique non-match to location (TUNL) task in touchscreen-equipped operant conditioning chambers allows for assessment of spatial working memory and spatial pattern separation in rodents. Rodents performing the TUNL task show minimal mediating responses to bridge the delay phase (Talpos et al. 2010). The TUNL task is adapted from touchscreen based tests for humans such as the Cambridge Neuropsychological Test Automated Battery (CANTAB) spatial working memory task (Bussey et al. 2012). Working memory is a form of short-term memory necessary for the storage, manipulation, and maintenance of information for complex cognition (Baddeley 2003; D'Esposito and Postle 2015). Pattern separation is a process of reducing overlap between similar input stimuli to minimize interference between representations (Kent et al. 2016). Working memory and pattern separation is mediated by N-methyl-D-aspartate (NMDA) receptors (Kannangara et al. 2015; Kheirbek et al. 2012; Kumar et al. 2015; Monaco et al. 2015).

Working memory impairments following NMDA receptor blockade are observed in the odour span task (Davies et al. 2013a; Galizio et al. 2013; MacQueen et al. 2016; Rushforth et al. 2011), TUNL task (Kumar et al. 2015), radial arm maze (Li et al. 1997), delay-match-to position task (Doyle et al. 1998; Smith et al. 2011), delayed-non-match-to position task (Aura and Riekkinen, Jr. 1999), and t-maze (Moghaddam and Adams 1998). The role of GluN2B-containing NMDA receptors in working memory is unclear. Systemic administration of a GluN2B-containing NMDA receptor antagonist impaired working memory capacity on the odour span task in rats (Davies et al. 2013a) but did not affect working memory on the TUNL task (Kumar et al. 2015) and an operant delayed-match-to-position task (Doyle et al. 1998; Smith et al. 2011). GluN2B-containing NMDA receptors within monkey prefrontal cortex contribute to the persistent firing observed during the delay phase of a working memory task (Wang et al. 2013).

Limited information exists regarding the effects of glutamatergic manipulation on the TUNL task. In the TUNL task, rats with mPFC lesions had reduced working memory while pattern separation was unchanged (McAllister et al. 2013) and in rats with hippocampal lesions both working memory and pattern separation were impaired (Talpos et al. 2010). NMDA receptors within the prefrontal cortex and striatum contribute to working memory (Monaco et al. 2015; Smith-Roe et al. 1999). The role of NMDA receptors within the prefrontal cortex and striatum has yet to be assessed by the TUNL task. While there is an abundance of information that supports the ventral striatum's involvement in working memory (Ferretti et al., 2007; Takahashi et al., 2011), much less is known about the dorsomedial striatum (dmSTR) in working memory, which receives projections from the prelimbic area of the prefrontal cortex. Spatial working memory is reduced with NMDA receptor blockade in medial prefrontal cortex (mPFC)

during a delayed-non-match-to-position task (Aura and Riekkinen, Jr. 1999) and dmSTR during a radial arm maze paradigm (Smith-Roe et al. 1999). This suggests that NMDA receptors in the mPFC or dmSTR may be critical for the working memory component of the TUNL task. NMDA receptors within the dentate gyrus are involved with pattern separation (Kannangara et al. 2015; Kheirbek et al. 2012; McHugh et al. 2007). Systemic blockade of NMDA receptors would affect the dentate gyrus and may impair pattern separation. In the present study we used systemic injections of NMDA receptor antagonist CPP or GluN2B-containing NMDA receptor antagonist Ro 25-6981 to examine spatial working memory and spatial pattern separation on the TUNL task. We also used direct brain infusions of the NMDA receptor antagonist AP5 into the mPFC or the dmSTR to examine the role of NMDA receptors within these neural substrates during TUNL task performance.

2.3 Methods

2.3.1 Subjects

Two groups of adult male Long Evans rats (Charles River, Quebec, Canada) were tested in the experiments using a within subjects design (total n=32). The rats were individually housed in clear plastic cages in a colony room on a 12 h light/dark cycle (lights on at 07:00) with *ad libitum* access to water. Except for several days after arrival and surgery, rats were restricted to maintain 85% of their free feeding weight. Experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.3.2 Apparatus

Training and testing was conducted in eight touchscreen-equipped operant conditioning chambers (Lafayette Instruments, Lafayette, Indiana, USA). Each operant conditioning chamber is located on a sliding shelf at the base of a sound-attenuating chamber containing a fan to circulate air and create background noise. Another sliding shelf above the operant conditioning chamber holds a pellet dispenser and video camera, which provides live video of the rat's behaviour within the chamber on an external monitor. The operant conditioning chambers are trapezoidal in shape with the wide end consisting of a touchscreen covered with a black polycarbonate mask. The TUNL mask has 14 square windows, which allowed the rats to contact the touchscreen only in areas where the stimuli can be presented. Below the 14 windows is a spring-loaded "response shelf" that the rat presses down to contact the touchscreen thereby making a selection.

2.3.3 Touchscreen habituation and pretraining

Rats were left undisturbed for seven days following arrival to the animal holding facility and then handled for three days before habituation and training. The training of rats to acquire the TUNL task followed steps described previously (Oomen et al. 2013) with some modifications. On day one of habituation, rats were brought into the testing room. Five reward pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, Frenchtown, New Jersey, USA) were placed in their cages and left undisturbed for 1 h with all testing equipment powered on (eight chambers and two computers). On subsequent days of training, rats were left undisturbed for 15 – 20 min following transport to the testing room before being placed into the chambers. On day two and three of habituation, 10 reward pellets were placed into the reward

port and the rats were given 30 min to consume the reward pellets (all rats consumed reward pellets). Following the habituation phase, rats were incrementally trained to touch a white square stimulus presented pseudo-randomly in one of the windows to receive a food reward, and then initiate the next trial by breaking the infrared (IR) beam near the reward port. Rats were trained until they collected 60 reward pellets in 60 min. In the last step of pretraining, a touch made to a blank window was followed by a 5 s time-out signalled by illumination of the house light. The same trial(s) were repeated (correction trials) after an inter-trial interval (ITI) of 5 s until the rat made a correct response. The correction trials were excluded when calculating performance. Reward collection initiated a 20 s ITI for the next trial. After completing 60 trials with 80% or higher correct, the rats moved onto the TUNL task.

2.3.4 TUNL task

A trial of the TUNL task is comprised of two phases (Figure 2.1). First, the sample phase is initiated when the rat breaks the IR beam in the reward port. The sample stimulus (a white square) is then presented in one of the 14 possible locations on the screen. Following a touch to the sample square, the stimulus disappears for a given delay (2 s during training). After the delay, the rat must break an IR beam at the reward port for presentation of the test phase. Breaking an IR beam after the delay phase reduces mediating strategies. In the test phase, two stimuli were presented; one in the sample location (incorrect) and the other in the new (correct) location. A touch to the correct location resulted in delivery of a food reward and a 20 s ITI for the next trial, but a touch to the incorrect location resulted in a 5 s time-out and then correction trial(s). Correction trials followed the same process as normal trials, except that the same sample

and test locations from the previous incorrect trial were presented until the correct choice was selected.

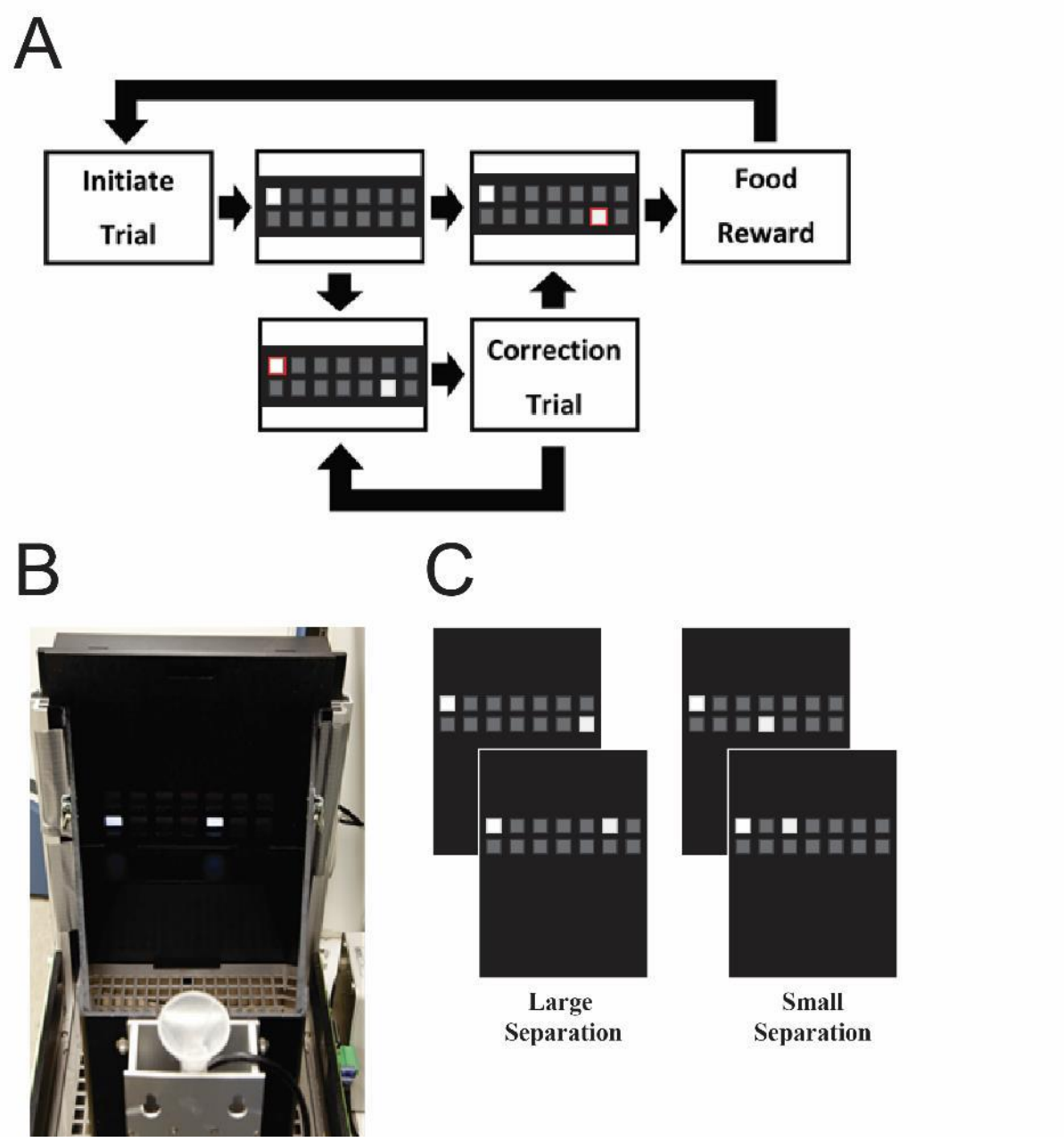

Figure 2.1: (A) Flow chart of the TUNL task. See the methods section for procedural details. Note that red squares denote the correct and incorrect choices possible after initial presentation of the stimuli in this figure. (B) The interior of the chamber as it is set up during the TUNL task. Note the mask with 14 windows open to the touchscreen and spring-loaded response shelf below the windows. The funnel-shaped opening opposite the touchscreen guides the reward pellet to the port when the chamber is closed. (C) Examples of the illuminated squares that could be presented to a rat during the choice phase of the TUNL task for a large separation and a small separation.

The TUNL task acquisition is composed of two phases. Initial TUNL acquisition consisted of rats that received up to 42 trials in 60 min. Once the rats performed 42 trials in fewer than 30 min, they proceeded to the final TUNL acquisition phase in which rats received up to 84 trials and were trained until they attained 80% correct on trials with large separations (4 – 5 blank squares apart between sample and test stimuli). After TUNL acquisition was completed, treatments were administered. Several measures were taken on treatment days. Percent correct on novel trials measured the accuracy on the trials without correction trials. Percent correct for large separations measured accuracy (without correction trials) on trials with separations of 4 – 5 blank squares between sample and test stimuli. Percent correct for small separations measured accuracy (without correction trials) on trials with separations of 1 – 2 blank squares apart. Novel trials measured correct and incorrect trials but not correction trials. Total trials measured the number of novel trials and correction trials. Correct response latency measured the time from when the rat broke the IR beam in the second initiation phase (after the delay phase) to touching the correct stimulus on the touchscreen and incorrect response latency measured the time from when the rat broke the IR beam in the second initiation phase to touching the incorrect stimulus on the touchscreen. Reward collection latency measured the time from when the rat touched the correct stimulus to breaking the IR beam in the reward port. In the CPP and Ro 25-6981 experiments, two 30 min blocks of a 2 s and 6 s delay was used in a counterbalanced order.

2.3.5 Systemic NMDA Receptor Antagonist Administration

Systemic NMDA receptor antagonist experiments occurred in a separate group of rats. Rats were injected 30 min prior to starting the TUNL task. In the CPP experiment, rats (n=8) were injected (i.p.) with either vehicle (Veh;saline) or CPP (10mg/kg) (Whitlock et al. 2006). In

the Ro 25-6981 experiment, rats (n=16) were injected (i.p) with either Veh (20% DMSO; 80% H_2O) or Ro 25-6981 (6 mg/kg or 10 mg/kg) (Davies et al. 2013a; Howland and Cazakoff 2010; Li et al. 2010; Wong et al. 2007).

2.3.6 Surgery and Infusions

Rats were anesthetized with isoflurane and prepared for surgery using previously reported procedures (Davies et al. 2013a; Davies et al. 2013b). Rat were implanted with guide cannula (23 Ga) bilaterally to target the mPFC (AP + 3.00 mm; ML ± 0.70 mm; DV -3.20 mm from bregma) and dmSTR (AP + 0.80 mm; ML ± 2.20; DV -3.40 mm). Obdurators (0.033 cm diameter stainless steel wire) were placed into cannula to prevent obstruction. One rat died during surgery and another rat did not learn the task adequately (did not achieve at least 80% correct on large separations for two consecutive days) and was excluded from testing. Therefore, 14 rats received brain infusions and were tested on the TUNL task. Following surgery, rats were allowed to recover for at least one week before training resumed. Rats were habituated to the infusion procedure on three separate days during the week before infusions were administered (Cazakoff and Howland 2011; Davies et al. 2013a; Davies et al. 2013b). Custom made needles (30 Ga stainless steel tubing) linked via PE-50 tubing to an infusion pump (PHD 2000, Harvard Apparatus, Holliston, Massachusetts, USA) were inserted 1 mm past the end of the cannula. Drugs were infused over 1 min and the infusion needles remained in place for 1 min after the infusion to allow diffusion of the drug. Rats were tested on the TUNL task 5 min following brain infusions. The NMDA receptor antagonist AP5 (10 uM/0.3ul or 30 mM/1.0ul) was infused bilaterally into mPFC or dmSTR (Baker and Ragozzino 2014; Winters et al. 2010).

2.3.7 Histology

After testing on the TUNL task was complete, rats were sacrificed with isoflurane and perfused with saline. Brains were removed and post-fixed in a 10% formalin-10% sucrose solution. Brains were sectioned on a sliding microtome and infusion sites were determined using standard protocols with reference to a rat brain atlas (Paxinos and Watson, 1997).

2.3.8 Data Analysis

The variables measured during the TUNL task were recorded using ABET II Touch (Lafayette). All statistical analysis was done using the Statistical Package for the Social Sciences (SPSS version 19). All descriptive values in graphs are reported as means ± standard error of the mean. Comparisons were performed using paired *t*-tests or repeated measures ANOVAs. Violation of sphericity assessed by Mauchly's test was corrected by the Greenhouse-Geisser epsilon adjustment. The Tukey honest significant test was used if the repeated measures ANOVA was significant. Statistical tests were considered significant if p values were < 0.05.

2.4 Results

2.4.1 NMDA receptor antagonist impairs TUNL performance

We tested 8 rats following systemic administration of CPP, which impaired overall accuracy ($t(7) = 3.52$, $p = 0.01$; Figure 2.2). CPP impaired performance on large separations ($t(7) = 2.65$, $p = 0.03$), but not small separations ($t(7) = 1.23$, $p = 0.26$). CPP impaired overall accuracy on the TUNL task with a 2 s delay ($t(7) = 4.20$, $p = 0.01$). For trials with a 6 s delay there was no significant difference in overall accuracy ($t(7) = 1.56$, $p = 0.16$). CPP impaired percent correct on a 2 s delay for large separations ($t(7) = 5.71$, $p = 0.001$) and small separations

($t(7) = 2.73$, $p = 0.03$). There was no significant difference with the 6 s delay for accuracy on large separations ($t(7) = 1.36$, $p = 0.22$) or accuracy on small separations ($t(7) = 0.17$, $p = 0.87$). CPP did not affect the number of novel trials ($t(7) = 1.35$, $p = 0.22$) or total trials ($t(7) = -1.99$, $p = 0.09$) but increased correction trials ($t(7) = -3.18$, $p = 0.02$). CPP did not affect latency of rats to collect a reward ($t(7) = 0.85$, $p = 0.42$), or correct responses ($t(7) = 0.95$, $p = 0.37$), or incorrect responses ($t(7) = 1.43$, $p = 0.20$).

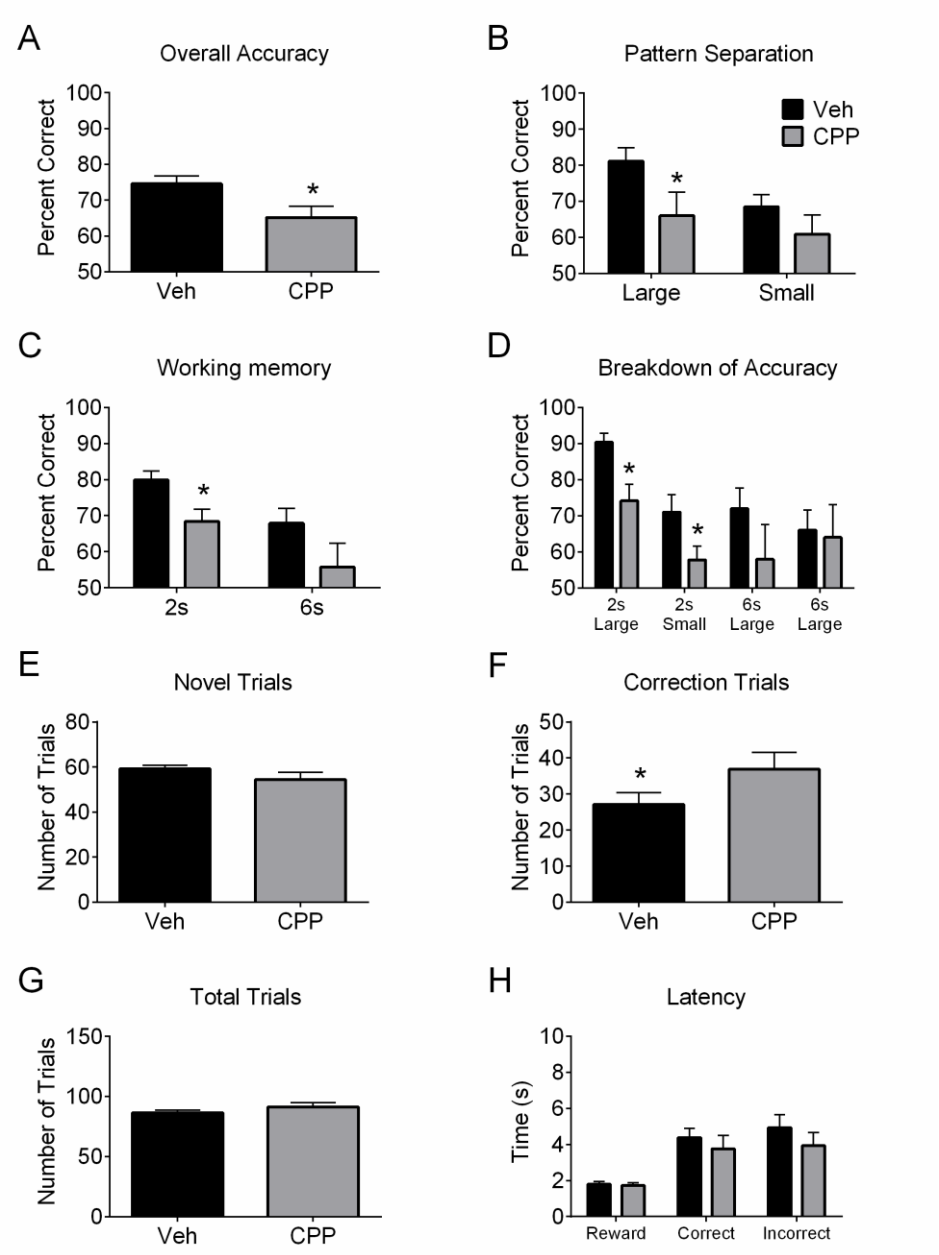

Figure 2.2: Performance of rats in the TUNL task with CPP or Veh treatment. (A) Overall accuracy (percent correct) on novel trials was reduced with CPP treatment. (B) Accuracy on large separations was reduced with CPP treatment but not on small separations. (C) CPP reduced accuracy on a 2 s delay but not a 6 s delay. (D) Accuracy was reduced on large and small separations at a 2 s delay but not on large or small separations at a 6 s delay. (E) CPP treatment did not affect the number of novel trials. (F) CPP treatment increased the number of correction trials. (G) CPP treatment did not affect the number of total trials (novel trials + correction trials). (H) CPP treatment did not affect the reward latency, correct response latency, or incorrect response latency. Asterisk indicates a significant effect.

2.4.2 GluN2B-containing NMDA receptors are not involved in TUNL performance

Blockade of GluN2B-containing NMDA receptors with Ro 25-6981 (in 16 rats) did not affect accuracy on the TUNL task ($F(2,30) = 0.81$, $p = 0.42$; Figure 2.3). Ro 25-6981 did not alter accuracy on large separations ($F(2,30) = 0.12$, $p = 0.89$) or small separations ($F(2,30) = 1.23$, $p = 0.31$). Accuracy on a 2 s delay in large separations was unaltered ($F(2, 30) = 2.30$, $p = 0.12$), and accuracy in small separations was unaffected ($F(2, 30) = 0.88$, $p = 0.43$). Accuracy on a 6 s delay in large separations was unaltered ($F(2, 28) = 0.56$, $p = 0.58$; one rat did not receive any trials on the large separations, therefore the sample size for this measure is 15), and the 6 s delay in small separations was unaltered ($F(2, 30) = 1.87$, $p = 0.17$). Ro 25-6981 did not affect novel trials ($F(2,30) = 1.55$, $p = 0.23$). Ro high (10mg/kg) treatment increased correction trials relative to Veh ($F(2,30) = 3.66$, $p = 0.04$; post hoc $p < 0.05$). Ro low (6mg/kg) and Ro high treatments increased total trials relative to Veh ($F(2,30) = 6.28$, $p = 0.01$; post hoc $p < 0.05$). Ro 25-6981 did not affect reward latency ($F(2,30) = 3.22$, $p = 0.09$), or correct response latency ($F(2,30) = 2.24$, $p = 0.16$). Ro 25-6981 altered incorrect latency but posthoc testing revealed no differences among treatments ($F(2,30) = 6.26$, $p = 0.02$).

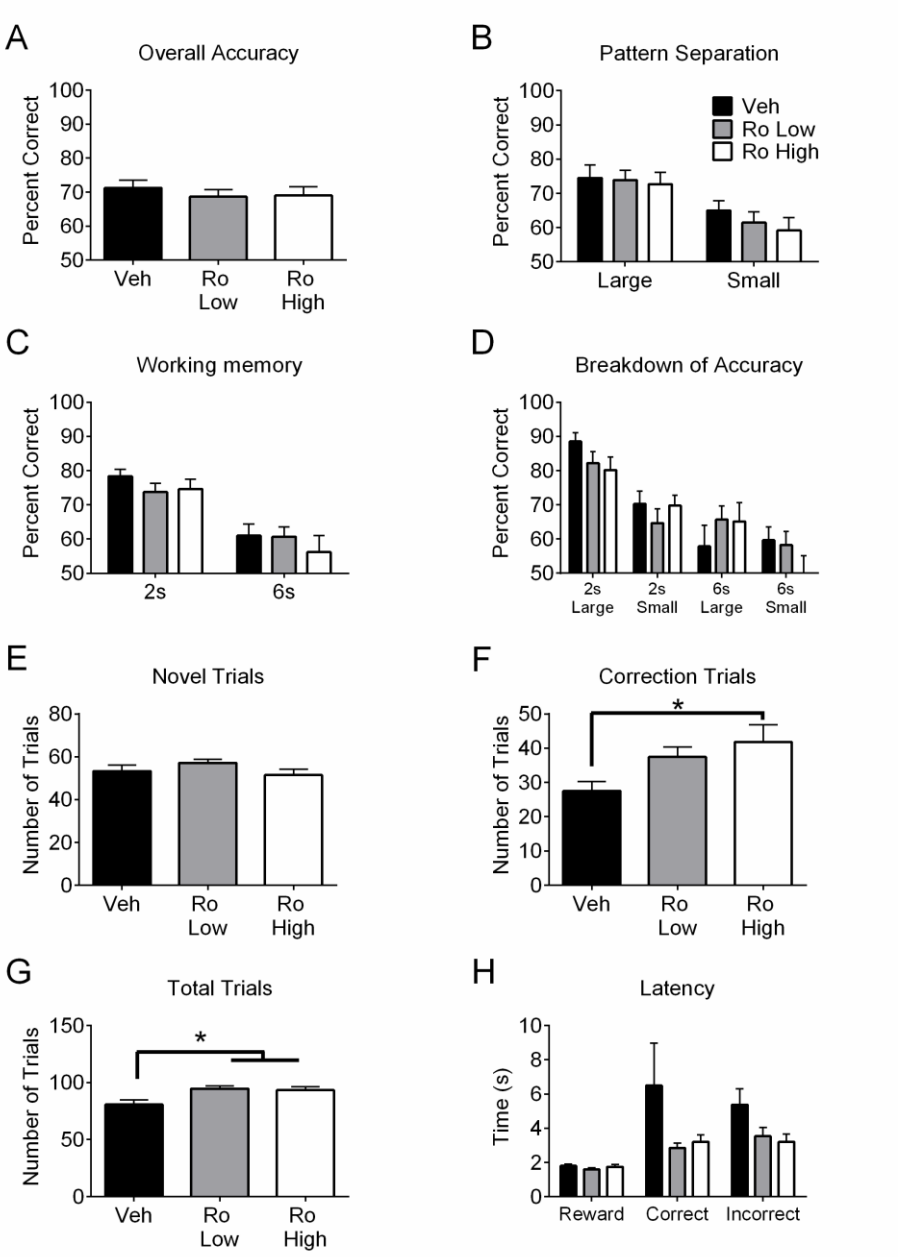

Figure 2.3: Performance of rats in the TUNL task with Ro 25-6981 (Ro Low = 6 mg/kg or Ro high = 10mg/kg) or Veh treatment. (A) Ro 25-6981 did not affect accuracy (percent correct) on novel trials. (B) Ro 25-6981 did not affect accuracy on large or small separations. (C) Ro 25-6981 did not affect accuracy on a 2 s or 6 s delay. (D) Ro 25-6981 did not affect accuracy at large or small separations at a 2 s or 6 s delay. (E) Ro 25-6981 treatment did not affect the number of novel trials. (F) Ro high treatment increased correction trials relative to Veh treatment. (G) Ro 25-6981 (Ro Low and Ro High) increased total trials relative to Veh treatment. (H) Ro 25-6981 did not affect reward response latency, correct response latency, or incorrect response latency. Asterisk indicates a significant effect.

2.4.3 NMDA receptor antagonist infusions into mPFC or dmSTR impair TUNL performance

The 10uM treatment of AP5 in mPFC or dmSTR with a 2 s delay (in 14 rats) did not affect TUNL performance. In the 10uM treatment of AP5 in mPFC overall accuracy was unaffected ($t(13) = 0.55$, $p = 0.59$; Figure 2.4). Accuracy in large separations was unchanged ($t(13) = 0.91$, $p = 0.38$), and accuracy in small separations was unaltered ($t(13) = 0.99$, $p = 0.34$). AP5 10uM did not affect the number of novel trials ($t(13) = 0.44$, $p = 0.67$), correction trials ($t(13) = -0.56$, $p = 0.58$), or total trials ($t(13) = -0.19$, $p = 0.91$). The latency was unaffected for reward collection ($t(13) = -0.88$, $p = 0.39$), correct responses ($t(13) = -0.50$, $p = 0.63$), and incorrect responses ($t(13) = 1.83$, $p = 0.09$). The mPFC infusions occurred in the prelimbic area in 8 rats, and in the infralimbic area in 6 rats.

The 10uM treatment of AP5 in dmSTR did not affect overall accuracy ($t(13) = 0.74$, $p = 0.48$; Figure 2.4). Accuracy in large separations was unchanged ($t(13) = 0.91$, $p = 0.38$) and accuracy in small separations was unaltered ($t(13) = 1.15$, $p = 0.27$). AP5 10uM did not affect the number of novel trials ($t(13) = 1.23$, $p = 0.24$), correction trials ($t(13) = -0.54$, $p = 0.60$), or total trials ($t(13) = 0.73$, $p = 0.48$). Latency was unaffected for reward collection ($t(13) = -0.88$, $p = 0.39$), correct responses ($t(13) = 0.01$, $p = 0.999$), and incorrect responses ($t(13) = 1.31$, $p = 0.21$).

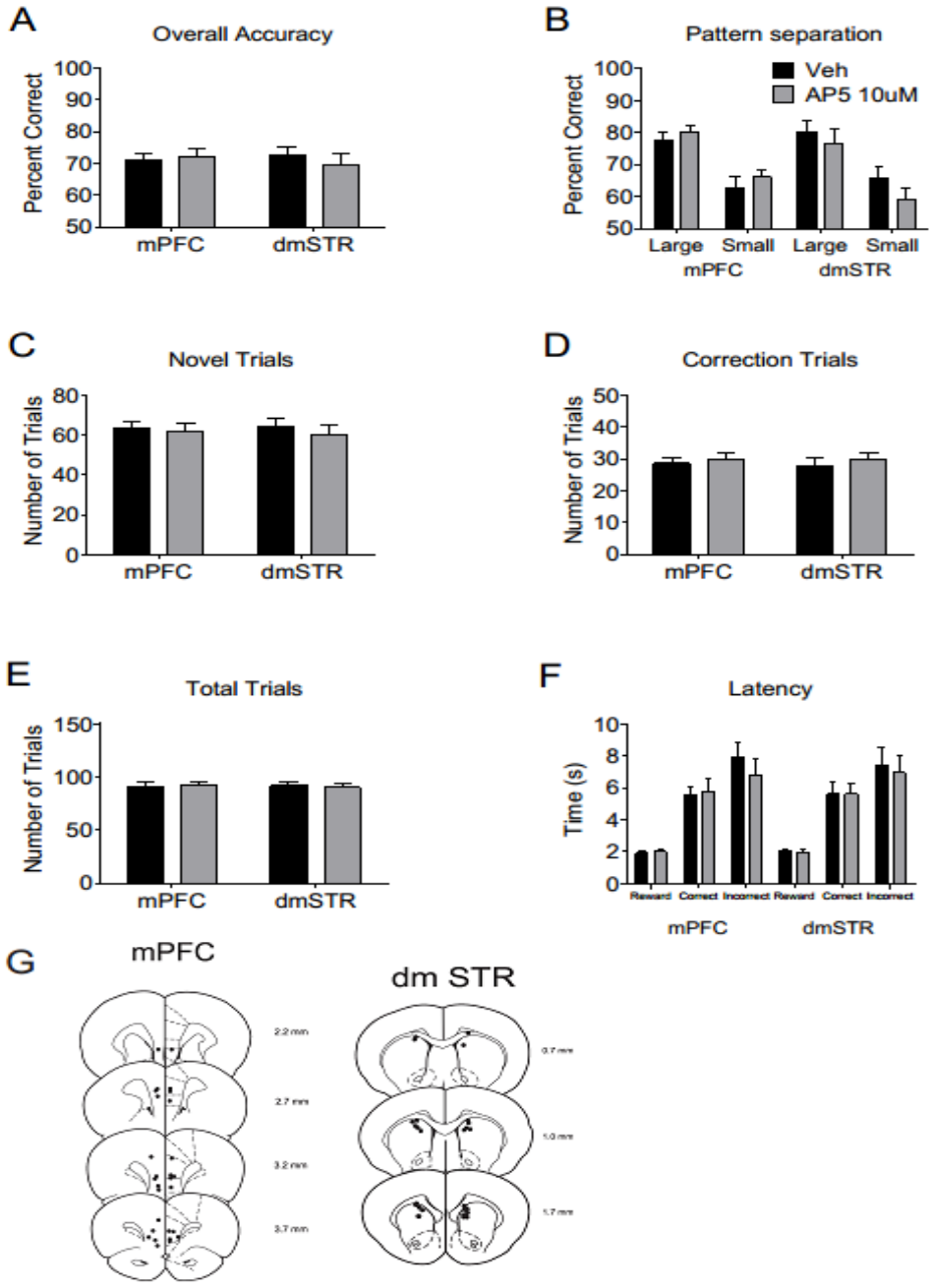

Figure 2.4: Performance of rats in the TUNL task with AP5 10uM or Veh infusions into mPFC and dmSTR. (A) AP5 10uM did not affect accuracy (percent correct) on novel trials. (B) AP5 10uM did not affect accuracy on large or small separations. (C) AP5 10uM did not affect the number of novel trials (D) AP5 10uM treatment did not affect the number of correction trials. (E) AP5 10uM treatment did not affect the number of total trials (novel trials + correction trials). (F) AP5 10uM treatment did not affect reward latency, correct response latency, or incorrect response latency. (G) Representative infusion sites in the mPFC and dmSTR. Numbers refer to the anterior-posterior location of plates relative to bregma.

The 30mM treatment of AP5 in mPFC or dmSTR impaired accuracy on overall trials, and accuracy on large separations but did not affect accuracy on small separations. AP5 30mM treatment in mPFC impaired overall accuracy ($t(13) = 3.56$, $p = 0.01$; Figure 2.5). AP5 30mM treatment impaired accuracy in large separations ($t(13) = 2.31$, $p = 0.04$), but did not affect accuracy in small separations ($t(13) = 1.16$, $p = 0.27$). AP5 30mM reduced novel trials ($t(13) = 4.92$, $p < 0.001$), and total trials ($t(13) = 3.02$, $p = 0.01$). Correction trials were unaffected by AP5 30mM treatment into mPFC ($t(13) = -1.44$, $p = 0.17$). Correct response latency was reduced in the Veh treatment ($t(13) = -2.50$, $p = 0.03$). Latency was unaffected for reward collection ($t(13) = -1.62$, $p = 0.13$), and incorrect responses ($t(13) = -0.38$ $p = 0.71$).

AP5 30mM infused into dmSTR impaired overall accuracy ($t(13) = 2.16$, $p = 0.0498$; Figure 2.5). AP5 30mM impaired accuracy for large separations ($t(13) = 2.33$, $p = 0.04$), but did not affect accuracy in small separations ($t(13) = 0.65$, $p = 0.53$). AP5 30mM treatment in dmSTR decreased novel trials ($t(13) = 2.86$, $p = 0.01$) but increased the number of correction trials ($t(13) = 2.67$, $p = 0.02$). Total trials were unaffected ($t(13) = 0.70$, $p = 0.50$). Latency was unaffected for reward collection ($t(13) = -1.61$, $p = 0.13$), correct responses ($t(13) = -0.84$, $p = 0.42$), and incorrect responses ($t(13) = 1.01$, $p = 0.33$).

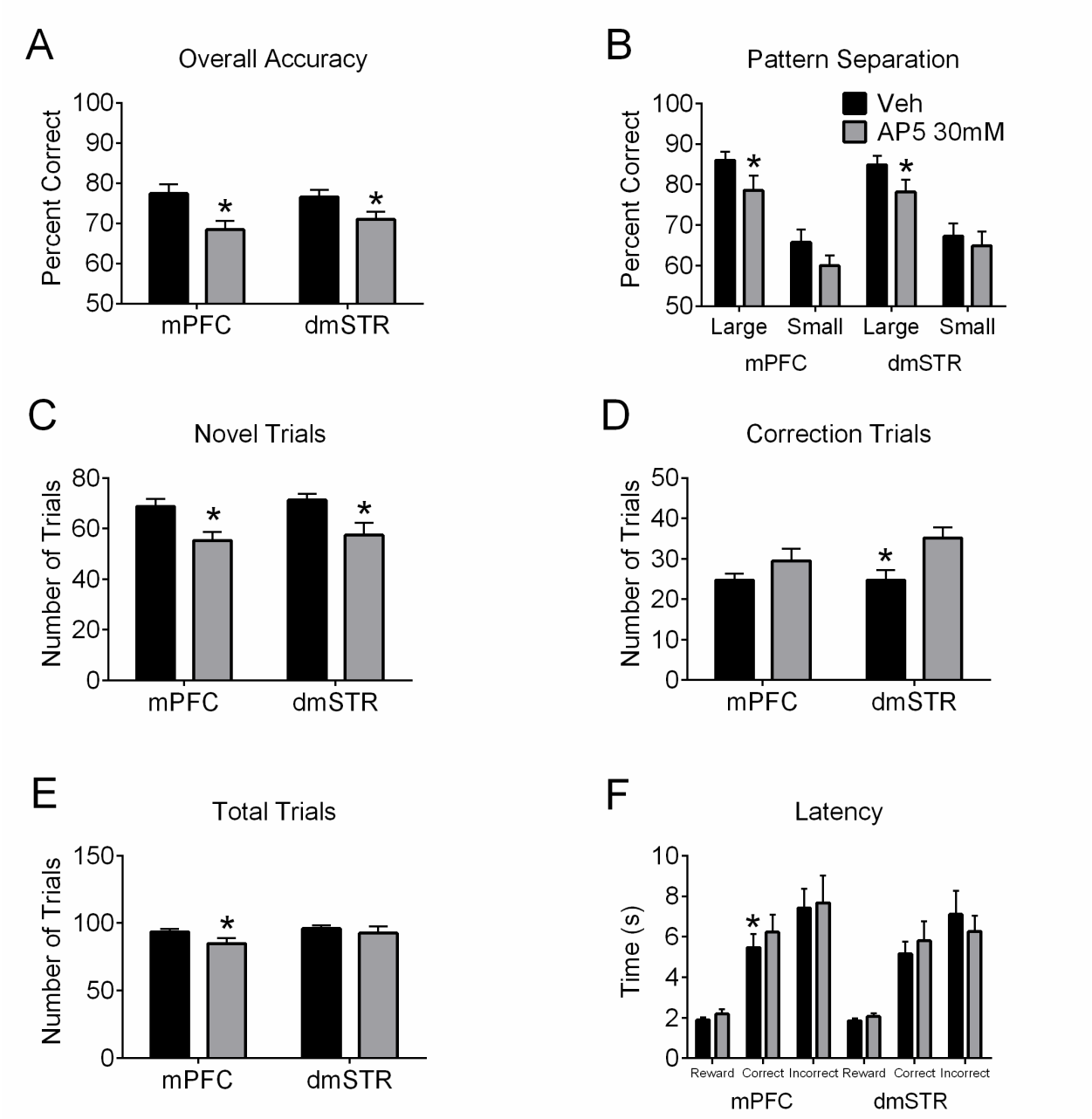

Figure 2.5: Performance of rats in the TUNL task with AP5 30mM or Veh infusions into mPFC or dmSTR. (A) Accuracy (percent correct) on novel trials was reduced with AP5 30mM treatment. (B) Accuracy on large separations was reduced with AP5 30mM treatment. Small separations was not affected with AP5 30mM treatment. (C) AP5 30mM reduced the number of novel trials. (D) AP5 30mM in dmSTR increased correction trials relative to Veh treatment. AP5 30mM in mPFC did not affect correction trials. (E) AP5 30mM treatment in mPFC reduced the number of total trials (selection trials + correction trials). AP5 30mM treatment in dmSTR did not affect the number of total trials. (F) AP5 30mM treatment in mPFC increased correct response latency. AP5 30mM treatment in mPFC did not affect reward latency or incorrect response latency. AP5 30mM treatment in dmSTR did not affect the reward latency, correct response latency, or incorrect response latency. Asterisk indicates a significant effect.

2.5 Discussion

Many studies have shown that the prefrontal cortex and striatum contribute to working memory. NMDA receptors are involved with various forms of memory and pattern separation (Park et al. 2013; McHugh et al. 2007). In the current experiments, we used the TUNL task to assess the effects of systemic NMDA receptor antagonist CPP, and GluN2B-containing NMDA receptor antagonist Ro 25-6981. We also conducted intracranial brain infusions with NMDA receptor antagonist AP5 into mPFC or dmSTR. Working memory and pattern separation can be teased apart in the TUNL task. Working memory can be assessed by comparing accuracy on a 2 s or a 6 s delay, and pattern separation can be assessed by comparing accuracy on large or small separations. Systemic administration of CPP impaired overall accuracy and accuracy on small separations at a 2 s delay, which suggests that working memory and pattern separation were impaired. Systemic administration of Ro 25-6981 did not affect accuracy. AP5 10uM infused into mPFC or dmSTR did not affect accuracy, whereas AP5 30mM infused into mPFC or dmSTR impaired overall accuracy but not on small separations, suggesting a working memory impairment but spared pattern separation.

2.5.1 Systemic NMDA receptor blockade impairs TUNL performance

NMDA receptors are extensively studied since they are implicated in various forms of learning and memory (Park et al. 2013). NMDA receptor blockade using CPP impaired TUNL task performance in the current experiment and is similar to the finding in the TUNL task using MK-801 (Kumar et al. 2015). NMDA receptor blockade impaired working memory odour span with CPP, (Davies et al. 2013a) MK-801, (Galizio et al. 2013; MacQueen et al. 2011) and ketamine (Rushforth et al. 2011). Working memory impairment in other tasks are well

documented following treatment with NMDA receptor antagonists in a delayed-non-match-to-position task, operant delayed-match-to-position task, and delayed alternation task (Aura and Riekkinen, Jr. 1999; Doyle et al. 1998; Li et al. 1997; Moghaddam and Adams 1998; Smith et al. 2011). NMDA receptors within the dentate gyrus are involved with pattern separation (McHugh et al. 2007). Therefore, the impaired accuracy in small separations with systemic CPP and MK-801 (Kumar et al. 2015) administration may have resulted from NMDA receptor blockade within the dentate gyrus.

GluN2B-containing NMDA receptors have received intense investigation since it was discovered that GluN2B receptors mediate LTD (Liu et al. 2004). The role of GluN2B-containing NMDA receptors in working memory is unclear. Blockade of the NMDA receptor GluN2B subtype did not alter working memory in our study and in (Kumar et al. 2015) using the TUNL task, and other working memory studies using operant delayed-match-to-position tasks (Doyle et al. 1998; Smith et al. 2011). However, in a working memory capacity task systemic blockade of GluN2B-containing NMDA receptors impaired capacity on the odour span task (Davies et al. 2013a). Odour information is required to guide accuracy on the odour span task whereas spatial information is required to guide accuracy on the TUNL task and the delayed-match-to-position task. GluN2B-containing NMDA receptors in monkey prefrontal cortex are important for the persistent firing observed during a delay phase in a working memory task (Wang et al. 2013). GluN2B-containing NMDA receptors within the dentate gyrus contribute to pattern separation (Kheirbek et al. 2012). In the current experiment, small separations were not affected with systemic Ro 25-6981 treatment and Kumar et al. (2015) showed similar findings in the TUNL task, suggesting that the GluN2B subunit does not contribute to pattern separation in the TUNL task.

2.5.2 NMDA receptor blockade in mPFC and dmSTR impairs TUNL performance

The prefrontal cortex has received intense investigation because of its involvement with complex cognition such as attention and working memory (Roberts et al., 1998; Thierry et al., 2011). Blockade of NMDA receptors within mPFC impaired overall accuracy on the TUNL task, which is consistent with working memory impairments in a delayed-non-match-to-position task (Aura and Riekkinen, Jr. 1999). The mPFC is involved with other working memory tasks including delayed alternation (Kolb, 1990; Baeg et al. 2003) and the win-shift on a radial arm maze (Seamans et al. 1995; 1998; Aujla and Beninger 2001; Lapish et al. 2008). NMDA receptor blockade with MK-801 in mPFC impaired reversal learning (Watson and Stanton 2009a). NMDA receptor blockade with AP5 in the prelimbic cortex impaired the ability to switch a response choice for an entire trial block in a behavioural flexibly task (Baker and Ragozzino, 2014). In our current experiment, blockade of NMDA receptors in mPFC did not affect accuracy on small separations, suggesting that the mPFC is not involved with pattern separation. NMDA receptor antagonist altered firing properties of mPFC pyramidal cells in rodents by decreasing burst firing (Jackson et al. 2004) while increasing basal firing rate (Homayoun and Moghaddam 2007; Jackson et al. 2004).

The striatum receives substantial projections from the prefrontal cortex, and is involved with allowing relevant information into working memory and inhibiting irrelevant information from entering working memory (O'Reilly, 2006). In the present study, blockade of NMDA receptors within dmSTR reduced overall accuracy on the TUNL task, which is consistent with (Smith-Roe et al. 1999) that found radial arm maze impairments when blockade of NMDA receptors occurred within dmSTR. Lesions of the dmSTR impaired working memory in a

delayed-match-to-sample task (DeCoteau et al. 2004; Kesner and Gilbert, 2006), and a t-maze (Moussa et al. 2011). NMDA receptor blockade in dmSTR impaired reversal learning (Watson and Stanton 2009b) and behavioural switching (Baker and Ragozzino 2014). In the current study, NMDA receptor blockade in dmSTR did not affect accuracy on small separations, suggesting that dmSTR is not involved with pattern separation. Striatal NMDA receptors are enriched in the medium spiny projection neurons and interneurons (Standaert et al. 1994). NMDA receptor blockade in the striatum reduces spontaneous firing of medium spiny neurons in anesthetized rats (Pomata et al. 2008) and awake rats (Sandstrom and Rebec 2003).

2.5.3 Functional implications

Working memory requires allocation of attention to select a relevant item for storage within working memory (Eriksson et al. 2015). NMDA receptor blockade in mPFC of rats performing the five-choice serial reaction time task is associated with reduced accuracy of visual discrimination and enhanced impulsivity (the number of premature responses) and compulsivity (the number of perseverative responses) (Pozzi et al. 2011). Correction trials increased with NMDA receptor blockade in the dmSTR, which may suggest perseverative responses in the TUNL task. However, the increased correction trials may also suggest a memory impairment in the TUNL task. NMDA receptor blockade in dmSTR during the five-choice serial reaction time task is associated with decreased accuracy and increased proportion of omissions with no effect on impulsivity and compulsivity (Agnoli and Carli 2011). These studies raise the possibility that an attentional impairment may have contributed to the reduction in accuracy on the TUNL task in the current experiment.

The TUNL task eliminates orientating towards the to-be-correct location since the rat is unable to predict the to-be-correct location. However, other mediating strategies may be used such as touching the sample location during the delay phase. Talpos et al. (2010) found that rats touched the sample location in the delay phase of the TUNL task, which only accounted for a small improvement in performance. Therefore, the drastic reduction in accuracy induced by NMDA receptor antagonists cannot be accounted for by touching the sample location during a delay phase.

The TUNL task has face validity since the human CANTAB has an automated visual spatial working memory task. In humans, PFC lesions impair performance on the CANTAB spatial working memory task (Chase et al. 2008), and in bipolar patients caudate volumes are enlarged and were correlated with poorer performance (Kozicky et al. 2013). Therefore, the prefrontal cortex and striatum are essential for proper spatial working memory as assessed by the CANTAB working memory task. Our experiments provide back translation since we showed that NMDA receptors within the mPFC and dmSTR is necessary for proper TUNL performance. Therefore, increasing the activation of NMDA receptors in the prefrontal cortex and striatum may result in new therapeutics for disorders such as schizophrenia, which are associated with frontal-striatal dysfunction and working memory impairment (Pantelis et al. 1997).

Chapter 3: Inactivation of medial prefrontal cortex or acute stress impairs odour span in rats

The content of this chapter is published in *Learning & Memory*. I gratefully recognize the contributions of Joel J Molder, Quentin Greba, and John G Howland to this work. Any redundant information provided elsewhere in the dissertation has been removed.

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3.1 Abstract

The capacity of working memory is limited and is altered in brain disorders including schizophrenia. In rodent working memory tasks, capacity is typically not measured (at least not explicitly). One task that does measure working memory capacity is the odour span task (OST) developed by Dudchenko and colleagues. Manipulating the set size in the TUNL task would include a pattern separation component, whereas the OST uses distinct odours, which eliminate pattern separation. In separate experiments, the effects of medial prefrontal cortex (mPFC) inactivation or acute stress on the OST were assessed in rats. Inactivation of the mPFC profoundly impaired odour span without affecting olfactory sensitivity. Acute stress also significantly reduced odour span. These findings support a potential role of the OST in developing novel therapeutics for disorders characterized by impaired working memory capacity.

3.2 Introduction

Working memory, a type of short-term memory for storage and manipulation of information necessary for higher order cognition (Goldman-Rakic 1996; Baddeley 2003; D'Esposito 2007), is impaired in numerous brain-related disorders including schizophrenia (Barch et al. 2009). Thus, preclinical research using the appropriate tasks to measure working memory in rodents may lead to improved therapeutics for these disorders (Dudchenkoet al. 2012). In the case of schizophrenia, working memory capacity is decreased (Chey et al. 2002; Gold et al. 2010) and the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) group has identified capacity as a component of working memory requiring more basic research before being included in the translational battery (Barch and Smith 2008; Dudchenko et al. 2012).

In rodents, span tasks (Dudchenko et al. 2000; Young et al. 2007b; Cui et al. 2011) are argued to offer the best potential for measuring working memory capacity in translational models of schizophrenia (Dudchenko et al. 2012). In rats, odour span tasks (OST) involve a modified serial delayed nonmatch to sample procedure to receive food reward by either digging in small bowls filled with scented sand (Fig. 3.1A; Dudchenko et al. 2000; Rushforth et al. 2010, 2011) or displacing scented lids on bowls (MacQueen et al. 2011; Galizio et al. 2013). Bowls are added one at a time during a "span" and the novel bowl must be chosen for a reward to be received. Systemically administered nicotinic receptor agonists increase odour span (Rushforth et al. 2010) while the NMDA receptor antagonist dizocilpine (MK-801) and γ-aminobutyric acid (GABA) A modulator chlordiazepoxide selectively impair performance of the OST (MacQueen et al. 2011; Galizio et al. 2013). Rats that were administered a subchronic regime of ketamine, to model

schizophrenia symptoms, are also impaired on the OST, an effect prevented by nicotine (Rushforth et al. 2011).

Research regarding the neural substrates involved in the OST is scarce. Previous research indicates that the OST does not depend on the hippocampus, although a variant involving spatial cues does (Dudchenko et al. 2000). As some researchers have speculated that the OST depends on the prefrontal cortex (PFC) (Dudchenko et al. 2012), the first goal of the present study was to assess the role of the PFC in the OST in rats. The medial PFC (mPFC) is involved in working memory in rodents (Kolb 1990; Seamans et al. 1995; Floresco et al. 1997; Aujla and Beninger 2001; Holmes and Wellman 2009), although the tasks used in these experiments do not directly measure working memory capacity (Dudchenko 2004; Dudchenko et al. 2012). In humans, the role of the PFC in span tasks is controversial (D'Esposito and Postle 1999; Bor et al. 2006). Given these findings, we assessed the effect of temporary inactivation of the mPFC (McFarland and Kalivas 2001; St. Onge and Floresco 2010) on the OST in male rats. We also conducted a test of odour sensitivity (Witt et al. 2009; Malkova et al. 2012) following inactivation of the mPFC as odour sensitive neurons are found in the prelimbic and infralimbic subregions (Nikaido and Nakashima 2011). In rodents, acute stress impairs working memory likely via alterations in mPFC function (Diamond et al. 1996; Arnsten 2009; Holmes and Wellman 2009; Butts et al. 2011; Devilbiss et al. 2012). To date, studies have not assessed the effects of acute stress on working memory capacity per se, as measured by the OST. Therefore, the second objective of the present experiments was to test the effects of acute restraint stress (MacDougall and Howland 2012) on the OST.

3.3 Methods

3.3.1 Animals

Three separate groups of adult male Long-Evans rats (220– 380 g; Charles River, Quebec) were used. Rats were pair housed in a colony room with a 12-h light–dark cycle (lights on at 07:00) with ad libitum access to food and water for 1 wk. Rats in the OST experiments were subsequently individually housed and food restricted to maintain 85% of their free feeding weight. The experimenter testing the rats was blind to the treatment status of the rats. Rats in the OST and odour sensitivity experiments had cannulae implanted bilaterally in mPFC (AP +2.60 mm, ML + 0.70 mm, DV 2 3.60 mm, flat skull) using standard procedures (Cazakoff and Howland 2011) and were permitted at least 7 d to recover. Rats were handled for 3 d before testing. Placements of the infusion needles were obtained postmortem using conventional methods (Cazakoff and Howland 2011) with the aid of a rat brain atlas (Paxinos and Watson 1997). All experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

3.3.2 Apparatus

Methods for the OST (Fig. 3.1A) closely followed those described by Dudchenko et al. (2000). A black corrugated plastic platform (91.5 cm2, 2.5-cm border, 95 cm above the floor) surrounded by an off-white curtain was used. Odours (0.5 g of dried spice; allspice, anise seed, basil, caraway, celery seed, cinnamon, cloves [0.1 g], cocoa, coffee, cumin, dill, fennel seed, garlic, ginger, lemon and herb, marjoram, mustard powder, nutmeg, onion powder, orange, oregano, paprika, sage, and thyme) were mixed in Premium Play Sand (100 g; Quikrete Cement

Products) and placed in white porcelain bowls (4.5 cm in height, 9 cm in diameter). The bowls were randomly placed in one of 24 equally spaced locations along the perimeter of the platform marked with Velcro.

3.3.3 Training on the odour span task

Initially, rats were shaped to dig for a cereal reward (Kellogg's Froot Loops) in a bowl filled with 100 g of unscented sand. Rats were trained until they would reliably dig for the reward regardless of bowl placement on the platform (~1 wk). Rats were then trained on the nonmatching to sample task (NMS). In the sample phase of a trial, the subject was presented with a bowl of scented sand randomly located on the platform. After the subject dug and retrieved the reward, it was removed from the platform and placed behind the curtain. The experimenter then moved the bowl to the opposite side of the platform and added a second bowl with a different odour to the platform that contained a reward. In the choice phase, the subject was placed on the platform opposite both bowls and allowed to freely sample them. A choice was scored when the subject dug or placed its paws or nose on the sand. An error was scored if the rats chose the previous odour. The subjects were given six NMS trials daily until the novel odour was selected on five of the six trials for 3 d. Subsequently, rats were trained on the OST. Trials were run as described for the NMS task except that bowls containing novel odours (for that trial) were added until the rat made an error (i.e., dug in any of the bowls except the new one). The previously presented bowls were randomly moved before each novel bowl was added to the platform. The span for a given trial was scored as the number of odour bowls correctly chosen minus one. During training, three or four rats were transported to the testing room together. Each rat performed three spans per day with a break between spans while the other rats

were tested. The average of the three spans is reported. Once performance was stable (7–15 d of training), the effects of mPFC inactivation or acute stress on span were assessed using a within subjects design (see below). On test days, rats were tested on three spans consecutively or for 30 min. To confirm that the subjects were using odour to solve the task, two probe sessions were conducted. The first probe session assessed if the scent of the reward guided choice by omitting the reward from all bowls on a trial. When the subject made a correct choice, a food reward was dropped on top of the sand. The second probe tested if the subjects marked the bowls when they sampled them by replacing all the bowls with new ones containing new, scented sand. Performance of the rats did not deteriorate during either of these probe sessions (data not shown).

3.3.4 Inactivation of mPFC

In the mPFC inactivation experiment, rats were tested on the OST 15 min following three treatments in a counterbalanced order: sham infusions, PBS infusions, and mPFC inactivation using a combination of the GABA receptor agonists muscimol (Sigma-Aldrich Canada) and baclofen (Sigma-Aldrich Canada; Mus/Bac; McFarland and Kalivas 2001; St. Onge and Floresco 2010). The drugs were dissolved separately in PBS (500 ng / mL) and mixed together. Rats were habituated to the infusion process as described previously (Cazakoff and Howland 2011) and trained for at least 2 d between treatments. Infusions (0.5 mL in 1 min via a PHD 2000 infusion pump, Harvard Apparatus) were performed by inserting custom-made needles (30-gauge stainless steel tubing and PE-50 tubing) 1 mm past the end of the cannulae. In the sham condition, shorter needles were used that did not exit the cannulae and no solution was delivered. The infusion needles were left in position for an additional minute after the infusion to allow for diffusion.

3.4 Results

3.4.1 Inactivation of mPFC impairs span

Figure 3.1B shows the infusion sites of the rats in the mPFC lesion (7 infusion sites in the prelimbic; 4 infusion sites in the infralimbic) and OST experiment. Figure 3.1C displays the average span for the 7 d immediately before the first infusions. A relatively stable span of about seven odours was achieved, as reported previously (Dudchenko et al. 2000; Rushforth et al. 2010, 2011). Figure 3.1D shows the effects of sham, PBS, or Mus/Bac infusions on odour span. A dramatic and significant reduction in span was observed in rats following Mus/Bac infusions into the mPFC compared to the other treatments. A repeated measures ANOVA reveals a significant main effect of treatment ($F(2,24) = 24.32$, $p < 0.001$); post-hoc analyses (Newman–Keuls) indicated that the Mus/Bac treatment resulted in significantly lower spans than the other two groups ($p < 0.05$). The day after treatment, spans recovered in all rats (mean span, 8.00 ± 1.4). The latency for subjects to dig in the first bowl was greater for rats following mPFC inactivation (57.31 ± 21.0 sec) than the other two treatments (sham, 2.88 ± 0.6 sec; PBS, 1.92 ± 0.2 sec, $F(2,24) = 8.11$, $p = 0.002$; Newman–Keuls post hoc, $p < 0.05$). Inspection of the latency data following mPFC inactivation (Fig. 3.1E) revealed a bimodal distribution, with some rats ($n = 6$) displaying long latencies (>50 sec) and others ($n = 7$) displaying latencies similar to those following sham and PBS treatments. A correlation performed on latency vs. span length revealed no relationship between the latency for rats to make a choice and span length ($r = 0.11$, $p = 0.73$) (Fig. 3.1E).

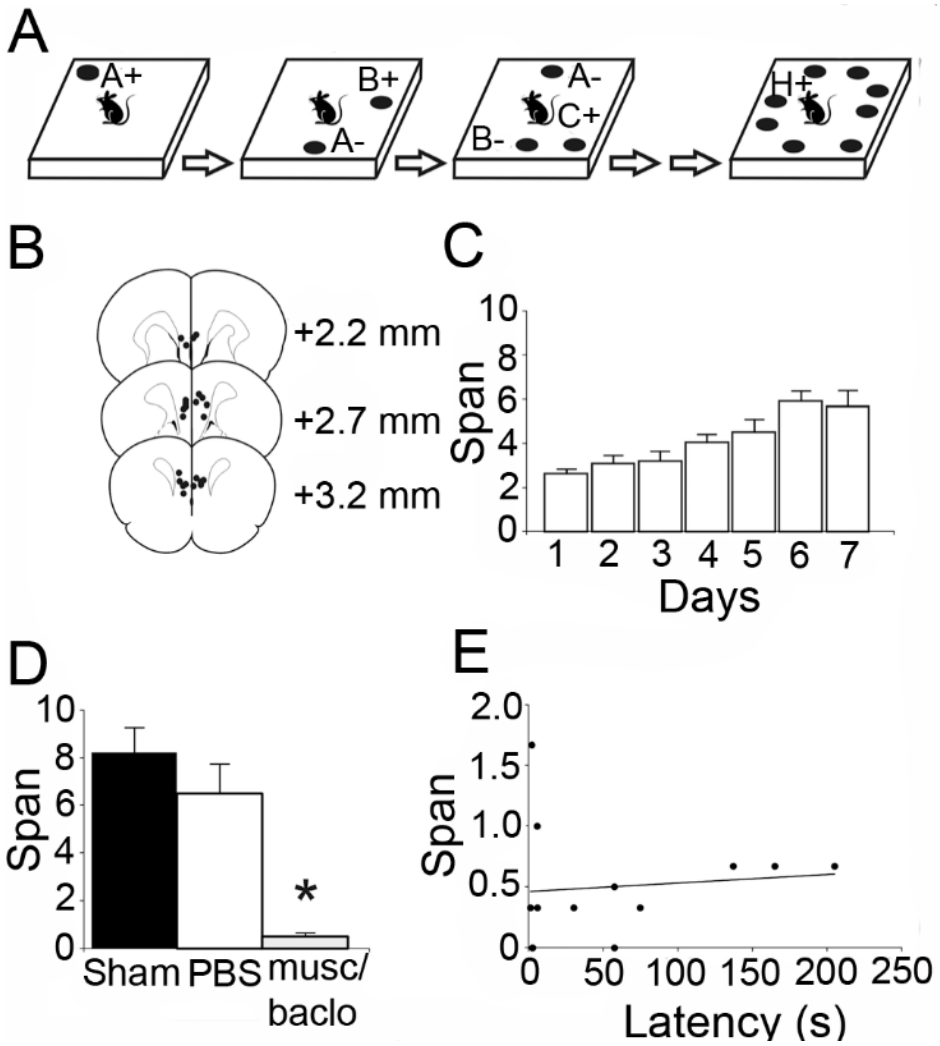

Figure 3.1. Effects of medial prefrontal cortex (mPFC) inactivation on odour span capacity in rats. (A) Schematic of the apparatus and procedure for the odour span task (see text for details; schematic based on Dudchenko et al. 2000, 2012). Odours are denoted with letters. On subsequent trials, the bowl (black circle) containing novel odour is rewarded (+) while the other stimuli are not rewarded (2). Rats are tested on a series of trials until an error is made. The number of bowls on the platform when an error is made minus one is recorded as the span. Note that a given odour is moved to a different position on the platform for each trial so that spatial cues cannot be used to solve the task. (B) Placements (black dots) of the infusion needle tips for rats in the mPFC inactivation and odour span experiment (n = 13). Infusion sites were located in both the prelimbic and infralimbic areas of the mPFC and impinged on the dorsal peduncular cortex in four subjects. The distance each plate is anterior to bregma is indicated in millimeters. (C) Mean (± standard error of the mean) odour spans during the 7 d of training immediately prior to testing for rats in the mPFC inactivation experiment. (D) Mean spans for the rats following each treatment of the mPFC inactivation experiment. Musc /baclo refers to the muscimol /baclofen treatment to temporarily inactivate the mPFC. (E) Correlation between spans and latencies to the first choice in the olfactory span task for rats treated with muscimol /baclofen. (∗) Indicates a significant difference from all other groups ($p < 0.05$).

3.4.2 Olfactory sensitivity test

The olfactory sensitivity test was used to detect deterioration of olfactory function following mPFC inactivation (Witt et al. 2009; Malkova et al. 2012). Testing occurred in a 40-cm × 40-cm × 60-cm (height) white corrugated plastic open field. Two bowls containing 100 g of sand were used for each trial; one was unscented and the other was scented with 0.5 g of a randomly chosen odour. A separate group of rats (n = 6) was infused with either PBS or the GABA agonists and individually placed in the empty open field for 15 min. The bowls were then placed in opposite corners of the box and the behaviour of the rats was recorded for 3 min. All rats were tested once in the PBS condition and once in the GABA agonist condition with 1 wk between the two tests (order was counterbalanced). Latency to approach the first bowl and total exploration time of each bowl was scored in a manner similar to object exploration using stopwatches (Cazakoff and Howland 2011).

Figure 3.2A shows the placements of the infusions for the rats tested in the olfactory sensitivity test (3 prelimbic infusion sites; 3 infralimbic infusion sites). Rats spent significantly more time exploring the scented bowl than the unscented bowl, irrespective of treatment condition (one-sample t-test vs. 0 or equal exploration of the bowls; PBS, $t(5) = 3.64$, $p = 0.015$; Mus/Bac, $t(5) = 3.32$, $p = 0.021$) (Fig. 3.2B). Treatment did not significantly affect the preference of the rats for the scented bowl (paired t-test, $t(5) = -0.20$, $p = 0.85$). Furthermore, there was no significant difference as a result of treatment in the amount of time spent exploring either the scented bowl (PBS = 5.61 ± 1.0 sec, Mus/Bac = 7.47 ± 0.9 sec). In this experiment, mPFC inactivation had no effect on latency in this test of spontaneous olfactory-related behaviour (paired t-test, $t(5) = 0.68$, $p = 0.53$) (Fig. 3.2C).

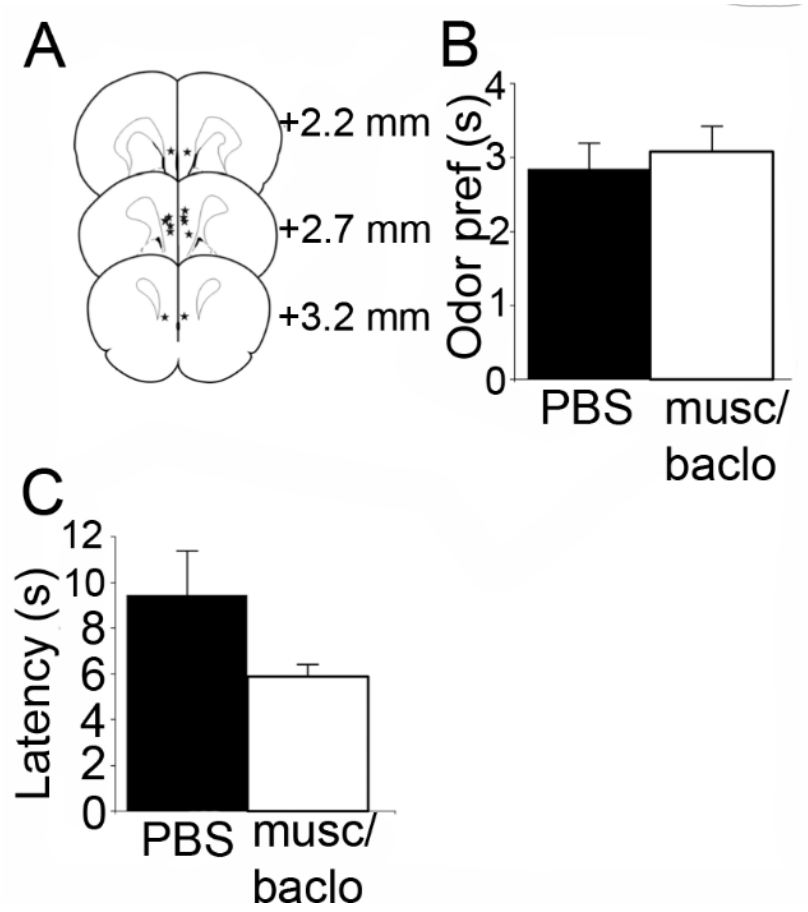

Figure 3.2. Effects of medial prefrontal cortex (mPFC) inactivation on olfactory sensitivity in rats. (A) Placements (black stars) of the infusion needle tips for rats in the medial prefrontal cortex (mPFC) inactivation and olfactory sensitivity experiment (n = 6). The distance each plate is anterior to bregma is indicated in millimeters. (B) Mean (± standard error of the mean) preference for the scented bowl compared to the unscented bowl. Musc /baclo refers to the muscimol /baclofen treatment to temporarily inactivate the mPFC. (C) Mean latency for the rats to explore the first bowl according to treatment. Abbreviation: Pref – Preference.

3.4.3 Acute stress impairs span

The effects of acute stress on the OST were tested in a third experiment. Figure 3.3A shows the mean span of the rats during the 7 d preceding acute stress. Acute stress was achieved by immobilizing rats in a Plexiglas restraint tube (544-RR, Fisher Scientific) in a brightly lit novel room for 30 min (MacDougall and Howland 2012). All rats were transported from the room where stress was administered to the room for the OST and span testing began shortly afterward. Acute stress significantly reduced span when compared to either the day prior or the day after stress (repeated measures ANOVA, $F(2,12) = 4.44$, $p = 0.036$; Newman–Keuls post hoc, $p < 0.05$) (Fig. 3.3B).

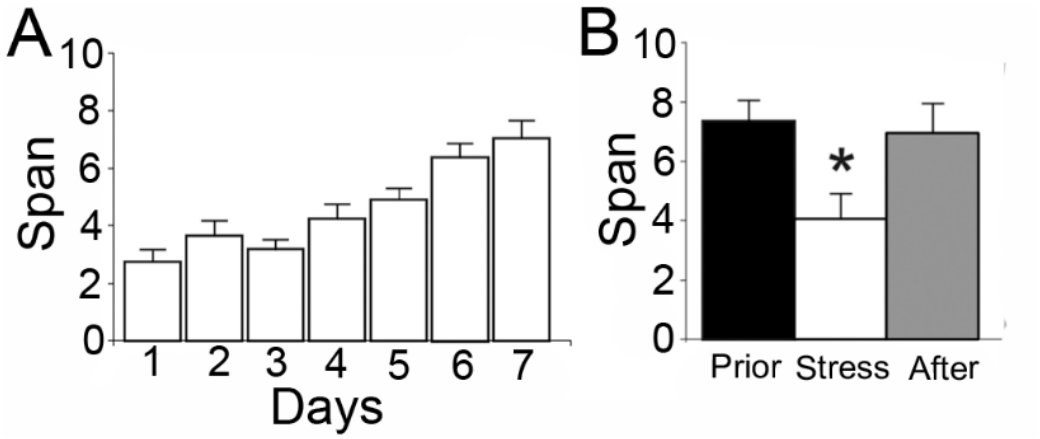

Figure 3.3. Effects of acute stress on odour span capacity in rats. (A) Mean (± standard error of the mean) odour spans during the 7 d of training immediately prior to testing for rats in the acute stress and odour span experiment ($n = 7$). (B) Mean spans for the day before stress (Prior), day of stress (Stress), and day after stress (After). (∗) Indicates a significant difference from all other groups ($p < 0.05$).

3.5 Discussion

The present study yielded a series of novel results. Our first experiment demonstrates that inactivation of the mPFC impairs performance of the OST (Fig. 3.1). Rats tested in our laboratory displayed similar acquisition rates and mean spans to those in previous reports that have used the version of the OST that requires the rats to dig in sand-filled bowls (Dudchenko et al. 2000; Rushforth et al. 2010, 2011). The involvement of the mPFC has been reported for a variety of working memory tasks (Holmes and Wellman 2009) including delayed alternation (Kolb 1990; Baeg et al. 2003) and the delayed win-shift task on the radial arm maze (Seamans et al. 1995, 1998; Floresco et al. 1997; Aujla and Beninger 2001; Lapish et al. 2008), although these tasks do not specifically measure working memory capacity as the OST does. These tasks measure manipulation of a memory set and maintenance of information over time, which are constructs involved with working memory. In addition to manipulation and maintenance, working memory capacity experiments measure the number of stimuli that can be stored in working memory. Working memory capacity is a complex cognitive construct that is incompletely understood (Barch and Smith 2008; Dudchenko et al. 2012) but requires appropriate allocation of attentional resources (Leonard et al. 2013). Rats with mPFC lesions are severely impaired on tasks which measure attention such as the five-choice serial reaction time task (Chudasama and Robbins 2004) raising the possibility that an attentional impairment may underlie the reduction in span capacity observed. However, working memory impairments following mPFC lesions have also been proposed to result from impaired inhibitory response control (Holmes and Wellman 2009).

Inactivation of the mPFC dramatically increased the latency of six out of 13 rats to dig in the first bowl (Fig. 3.1E). While a nonselective effect of the infusion on brain function may have

increased the latency, this is unlikely as latency was unaffected following PBS infusions in the OST or the olfactory sensitivity test following infusions of either PBS or the GABA receptor agonists (Fig. 3.2B). It is worth noting that the rats tested in the OST were subjected to a stereotyped training schedule for days before their infusions while rats in the olfactory sensitivity test were not. In previous reports using other maze tasks that require significant training, mPFC inactivations did not affect response latencies (Floresco et al. 1997), although in operant-based tasks, increases in latencies have been observed (St. Onge and Floresco 2010). Latency was not correlated with performance of the OST (Fig. 3.1E); thus, it is unlikely that this was a critical determinant of the results obtained. Given that odour sensitive neurons are found in the mPFC (Nikaido and Nakashima 2011), another possible explanation for the dramatically reduced span observed is that rats were anosmic following mPFC inactivations. However, rats with mPFC inactivations performed normally on the olfactory sensitivity test (Fig. 3.2B), consistent with results demonstrating that olfactory recognition is intact in mice with lesions of the mPFC (Devito and Eichenbaum 2011).

Immediately following acute restraint stress, performance of the OST was impaired, while 24 h after stress, mean spans returned to levels similar to those before stress (Fig. 3.3B). These findings demonstrate that performance of the OST, like other working memory tasks (Diamond et al. 1996; Arnsten and Goldman-Rakic 1998; Butts et al. 2011; Devilbiss et al. 2012), is sensitive to the short-term effects of acute stress. Acute stress causes rapid changes in the physiology of mammals, including the release of catecholamines and glucocorticoid hormones in a timeframe of minutes (Joels and Baram 2009; Koolhaas et al. 2011) that aligns with the behavioural effects observed on the OST. The actions of both catecholamines (particularly dopamine) and glucocorticoids (corticosterone) in the mPFC have been implicated

in the disruptive effects of acute stress on working memory (Arnsten 2009; Holmes and Wellman 2009; Butts et al. 2011). Thus, given that mPFC inactivation disrupted performance on the OST, the neural substrates mediating the effects of acute stress on the OST may include the mPFC. These data are consistent with the growing literature showing that acute stress impairs a range of executive functions mediated by the mPFC (Holmes and Wellman 2009; Butts et al. 2013). However, it should be noted that some aspects of executive functioning, such as reversal learning, are facilitated by acute stress (Graybeal et al. 2011; Thai et al. 2013).

The CNTRICS group has identified working memory capacity as a construct requiring more basic research before being included in the translational battery for drug development (Barch and Smith 2008; Dudchenko et al. 2012). The results of the present study contribute to this goal by demonstrating that memory capacity, as measured by the OST in rats, is sensitive to inactivation of the mPFC and the short-term effects of acute stress. Future experiments designed to assess the validity of putative cognitive enhancers for the brain disorders such as schizophrenia (Rushforth et al. 2011) may benefit from including the OST in their test battery (Young et al. 2009a; Dudchenko et al. 2012).

Chapter 4: GluN2B-containing NMDA receptors and AMPA receptors in medial prefrontal cortex are necessary for odour span in rats

The content of this chapter is published in *Frontiers in Behavioral Neuroscience*. I gratefully recognize the contributions of Quentin Greba, and John G Howland to this work. Any redundant information provided elsewhere in the dissertation has been removed.

Davies DA, Greba Q, Howland JG. 2013. GluN2B-containing NMDA receptors and AMPA receptors in medial prefrontal cortex are necessary for odor span in rats. *Front. Behav. Neurosci.* **7**:183.

4.1 Abstract

Working memory is a type of short- term memory involved in the maintenance and manipulation of information essential for complex cognition. While memory span capacity has been extensively studied in humans as a measure of working memory, it has received considerably less attention in rodents. Our aim was to examine the role of the N-methyl D-aspartate (NMDA) and α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors in odour span capacity using systemic injections or infusions of receptor antagonists into the mPFC. Long Evans rats were trained on a well-characterized OST. Initially, rats were trained to dig for a food reward in sand followed by training on a non-match to sample discrimination using sand scented with household spices. The rats were then required to perform a serial delayed non-match to sample procedure which was their odour span. Systemic injection of the broad spectrum NMDA receptor antagonist 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) (10 mg/kg) or the GluN2B-selective antagonist Ro 25-6981 (10 mg/kg but not 6 mg/kg) significantly reduced odour span capacity. Infusions of the GluN2B- selective antagonist Ro 25-6981 (2.5 μg/hemisphere) into mPFC reduced span capacity, an effect that was nearly significant (p = 0.069). Infusions of the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (1.25 μg/hemisphere) into mPFC reduced span capacity and latency for the rats to make a choice in the task. These results demonstrate span capacity in rats depends on ionotropic glutamate receptor activation in the mPFC. Further understanding of the circuitry underlying span capacity may aid in the novel therapeutic drug development for persons with working memory impairments as a result of disorders such as schizophrenia and Alzheimer's disease.

4.2 Introduction

Working memory, a type of short term memory, enables the maintenance and manipulation of information needed for complex cognitive functions (Goldman-Rakic, 1996; Baddeley, 2003; D'Esposito, 2007). Working memory is impaired in numerous brain disorders including schizophrenia (Barch et al., 2009) and Alzheimer's disease (Huntley and Howard, 2010); thus, the use of appropriate preclinical working memory tasks in rodents to understand the neural mechanisms underlying working memory provides one approach for the development of novel therapeutics. In animals, working memory has been assessed using a variety of different tasks, many of which relate solely to the short-term storage of information over delays, without assessment of the capacity of working memory (Dudchenko, 2004; Dudchenko et al., 2012). In schizophrenia, working memory capacity is decreased (Chey et al., 2002; Gold et al., 2010) and the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) group has identified capacity as a component of working memory requiring more basic research before being included in the translational battery (Barch and Smith, 2008; Dudchenko et al., 2012).

Working memory capacity has been studied in rodents using span tasks with odours or spatial locations as stimuli. One of these tasks is the OST first developed by Dudchenko et al. (2000) (Figure 3.1A). The OST is an incremental non-match-to-sample task where rats or mice receive a food reward by choosing to dig in a bowl of sand with the novel scent (Dudchenko et al., 2000; Young et al., 2007b; Rushforth et al., 2010, 2011; Davies et al., 2013) or by moving scented lids (MacQueen et al., 2011; April et al., 2013; Galizio et al., 2013). Once the subject chooses the novel bowl, additional bowls are added with the previous bowl(s) rearranged on the platform until the subject chooses a previously rewarded bowl. The number of bowls correctly

discriminated minus 1 is the span of the subject. Mean spans of approximately 7–9 odours have been reported when rats are stopped after their first error (Dudchenko et al., 2000; but see April et al., 2013; Davies et al., 2013b). Span capacity declines following reversible inactivation of the medial prefrontal cortex (mPFC; Davies et al., 2013b), but not permanent lesions of dorsal hippocampus (Dudchenko et al., 2000), in rats. Span capacity is also reduced following exposure to acute stress (Davies et al., 2013b). Further research has demonstrated that odour span capacity is increased by systemically administered nicotinic receptor agonists (Rushforth et al., 2010) while it is transiently reduced following 192 IgG-saporin-induced cholinergic lesions of the basal forebrain (Turchi and Sarter, 2000). Odour span is also impaired by non-competitive NMDA receptor antagonists (MacQueen et al., 2011; Rushforth et al., 2011; Galizio et al., 2013) and the GABA A receptor modulator chlordiazepoxide (Galizio et al., 2013).

Limited information exists regarding the effects of brain site specific modulation of neurotransmitters and their receptors on working memory capacity as assessed by the OST. In one study, working memory capacity was increased in transgenic mice overexpressing the NMDA receptor subunit GluN2B in forebrain areas including the cortex (Cui et al., 2011). A study examining the maintenance, but not capacity, of working memory demonstrates a role of ionotropic glutamate receptors in the dorsolateral prefrontal cortex for working memory in monkeys (Wang et al., 2013). Using the delayed occulomotor response task, Wang et al. (2013) showed that NMDA receptors containing GluN2B subunits in the dorsolateral prefrontal cortex are essential for the maintenance of working memory by regulating the activity of delay period neural activity during the task. Mixed effects were found for the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the same paradigm in monkeys (Wang et al.,

2013) and previous studies examining working memory in rodents (Li et al., 1997; Romanides et al., 1999).

Taken together, these findings suggest that ionotropic glutamate receptors in the mPFC may be critical for span capacity. To test this possibility, we used the OST in rats and first performed systemic injections of the broad NMDA receptor antagonist CPP (Lehmann et al., 1987) and the GluN2B-selective antagonist Ro 25-6981 (Fischer et al., 1997). Subsequently, we used direct intracranial infusions of Ro 25-6918 and the AMPA receptor antagonist CNQX (Honore et al., 1988) targeted to the mPFC to specify a role for receptors in that area in odour span capacity.

4.3 Methods

4.3.1 Animals

Eight adult male Long-Evans rats (270–310 g; Charles Rivers, Quebec, Canada) were tested in the experiments using a within subjects design. For 6 days after arrival to the facility, the rats were paired housed in clear plastic cages in a colony room on a 12 h light/dark cycle (lights on at 07:00) with ad libitum access to food (Purina Rat Chow) and water. Otherwise, the rats were individually caged with ad libitum access to water and were food restricted to maintain 85% of their free feeding weight (except for several days before and after surgery when food was also available ad libitum). All experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board. Training and testing was similar to chapter 2 and other published protocols (Dudchenko et al., 2000; Davies et al., 2013b).

4.3.2 Systemic Drug Administrations

Rats were injected 30 min prior to starting the OST. For the CPP experiment, rats were injected (i.p.) with either vehicle (saline) or CPP (10 mg/kg; i.p.). This dose was chosen on the basis of previous studies (Whitlock et al., 2006). For the Ro 25-6981 experiment, rats were injected (i.p.) with either vehicle (20% DMSO; 80% H2O) or Ro 25-6981 (6 mg/kg or 10 mg/kg; i.p.; Wong et al., 2007; Howland and Cazakoff, 2010; Li et al., 2010).

4.3.3 Surgery and mPFC Infusions

Surgeries for the mPFC and OST experiment were conducted after the systemic Ro 25-6981 (10 mg/kg) injections experiment. Subjects were anesthetized with isoflurane and prepared for surgery using previously reported procedures (Cazakoff and Howland, 2011; Davies et al., 2013). Guide cannulae (23 Ga) were bilaterally inserted above mPFC (AP + 2.60 mm; ML ± 0.70 mm; DV − 3.60 mm; flat skull). Obdurators (0.033 cm diameter stainless steel wire) were placed into the cannulae to avoid obstruction. Following surgery, rats were allowed to recover for 8 days before training resumed. One rat died during surgery. Thus, the *n* for the infusion experiments is seven.

Rats were habituated to the infusion procedure on three different days during the week before infusions were administered (Cazakoff and Howland, 2011; Davies et al., 2013b). Infusions were achieved by inserting custom made needles (30 Ga stainless steel tubing) linked via PE-50 tubing to an infusion pump (PHD 2000, Harvard Apparatus, Holliston, MA) 1 mm past the end of the cannulae. Needles were inserted into both cannula then delivery of Ro 25-6981, CNQX or the vehicle into the mPFC was initiated (0.5 µl in 1 min). The infusion needles were left in place for an additional minute after the infusion to permit diffusion of the drug. Rats

were tested on the OST 15 min following brain infusions. Ro 25-6981 (2.5 μg/0.5 μl; Zhang et al., 2008) and vehicle (12% DMSO; 88% PBS) were given in a counterbalanced order followed by CNQX (1.25 μg/0.5 μl; Ho et al., 2011) and vehicle (PBS) which were also counterbalanced.

Following testing in all conditions, the rats were sacrificed with isoflurane and perfused with saline. Brains were removed and post-fixed in a 10% formalin-10% sucrose solution. Brains were sectioned on a sliding microtome and infusion sites were determined using standard protocols with reference to a rat brain atlas (Paxinos and Watson, 1997).

4.3.4 Data Analysis

Odour spans and latencies to choose the first bowl were manually recorded during testing and entered into Microsoft Excel (2010) and Statistical Package for the Social Sciences (SPSS; version 19.0) for analysis. All descriptive values are reported as means ± standard error of the mean. Comparisons were done using paired t-tests. Analysis of the spans for baseline sessions before the various treatments were compared using a repeated measures ANOVA followed by Neuman Keuls post-hoc tests (Figure 4.1C). Statistical tests were considered significant if p values were < 0.05.

4.4 Results

4.4.1 Training

After dig training, we trained rats in the non-matching-to-sample task until they selected the novel odour in 5/6 trials for three sessions (mean = 7.13 days; range = 6–9 days). Rats were then trained on the OST (Figure 4.1A) for a mean of 12.38 sessions (range, 8–16 days). During the 7 days immediately prior to the first treatment, the rats reached a span of approximately 7

odours (Figure 4.1A), as previously reported when sand-filled bowls are used (Rushforth et al., 2010, 2011; Davies et al., 2013).

Figure 4.1B displays the spans of the rats' baseline sessions. Over the 8 weeks of repeated testing, we noticed a significant increase in span on the baseline sessions ($F(4, 28) = 7.06$, $p < 0.001$). Average baseline spans increased from a mean of 7.00 ± 0.5 the day before CPP treatment to 12.14 ± 1.6 before CNQX treatment. It should be noted that surgery was performed on the rats between the second systemic administration of Ro 25-6918 and first intracranial injection of Ro 25-6981 into the mPFC. Surgery had no measureable effect on the average span observed following the retraining period (two sessions).

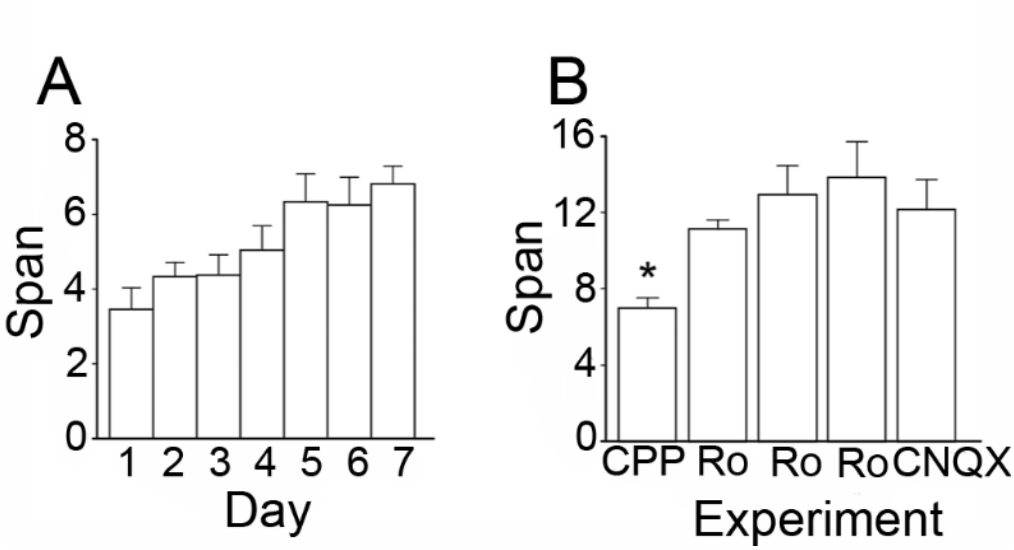

Figure 4.1. (A) Mean odour spans during the 7 days of training immediately prior to the first treatment (3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP); n = 8). (B) Mean odour spans on the baseline sessions before each treatment in the within subjects design. The GluN2B-selective NMDA receptor antagonist Ro 25-6981 was given three times: first, 6 mg/kg (i.p.); second, 10 mg/kg (i.p.); and third, 2.5 µg/hemisphere. * Refers significantly lower span relative to all other conditions ($p < 0.05$). Ro, Ro 25-6981.

4.4.2 Systemic injection of either CPP or Ro 25-6981 impairs odour span

Systemic injections of CPP (10 mg/kg; i.p.) impaired span capacity (Figure 4.2A) without affecting latency to dig in the OST (Figure 4.2B). Following CPP rats had a span of 4.64 ± 0.6 odours which was significantly lower than when the rats were injected with saline (7.65 ± 0.9 odours; $t(7) = 5.19$, $p < 0.001$). Latencies to begin digging in bowls did not differ between treatments (Figure 4.2B; Saline = 5.17 ± 0.9 s; CPP = 4.03 ± 0.4 s; $t(7) = 1.12$, n.s.).

To test the effect of blocking only NMDA receptors containing GluN2B subunits, two doses of Ro 25-6981 were administered systemically (i.p.). A trend of decreased odour span was found following administration of the 6 mg/kg dose (Figure 4.2C; Veh = 12.07 ± 1.8 odours; Ro 25-6981 = 9.14 ± 1.0); however, this difference was not significant ($t(7) = 1.41$, n.s.). A higher dose of Ro 25-6981 (10 mg/kg) significantly reduced span in rats (Figure 4.2E; Veh = 13.19 ± 1.5; Ro 25-6981 = 6.90 ± 2.2; $t(7) = 3.55$, $p = 0.009$). Latency for the rats to begin digging in a bowl did not differ between treatments for either dose (Figure 4.2D $t(7) = 0.32$, n.s.; Figure 4.2F $t(7) = -0.42$, n.s.). Thus, systemic blockade of GluN2B-containing NMDA receptors impaired odour span capacity without affecting latency in the OST.

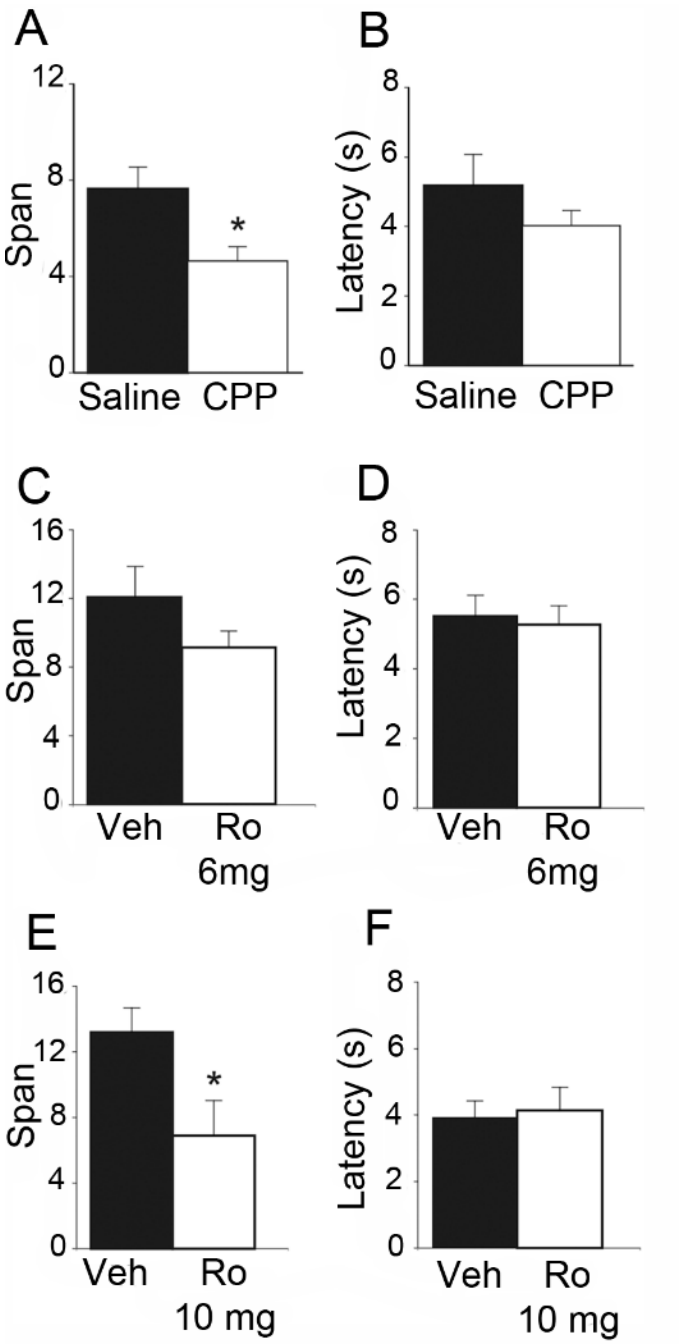

Figure 4.2. Effects of systemic NMDA receptor antagonism on performance of the OST. **(A)** Mean spans of the rats following saline or CPP treatment (10 mg/kg, i.p.). **(B)** The mean latency of the rats to start digging in a bowl (saline or CPP). **(C)** Mean spans for the rats following vehicle (Veh) or Ro25-6981 (Ro) treatment (6 mg/kg, i.p.) **(D)** The mean latency of the rats to start digging in a bowl during the tests conducted in C. **(E)** Mean spans for the rats following each treatment with either Veh or Ro25-6981 (10 mg/kg, i.p.). **(F)** The mean latency of the rats to start digging in a bowl (Veh or Ro, 10 mg/kg). * Refers to a significant difference between treatments ($p < 0.05$). Error bars represent standard error.

4.4.3 Ro 25-6981 or CNQX infusions in mPFC impair performance of the OST

In an effort to delineate the critical brain regions that underlie disruption in odour span observed following systemic administration of NMDA receptor antagonists, mPFC infusions of Ro 25-6981 were performed. Intra-mPFC Ro 25-6981 infusions impaired span capacity but not latency on the OST (Figures 4.3A, B). A robust decrease in span was observed in six out of the seven rats. When all seven rats were considered, spans decreased from 12.43 ± 2.5 for vehicle infusion to 5.95 ± 1.6 for Ro 25-6981 infusion (Figure 4.3A), an approximate 50% decrease in mean span. This difference was close to significant ($t(6) = 2.21$, $p = 0.069$). When the rat that showed the opposite pattern of behaviour was removed, a significant difference was observed (Veh = 14.08 ± 2.3; Ro 25-6981 = 4.95 ± 1.4, $t(5) = 6.32$, $p < 0.001$). The latency for rats to begin digging into bowls did not differ between the two treatments (Figure 4.3B; $t(6) = -0.40$, n.s.).

To test the potential role of mPFC AMPA receptors in span capacity, CNQX was infused into the mPFC (Figures 4.3C, D). A marked reduction in odour span was observed in rats following CNQX infusions (Veh = 9.71 ± 1.5; CNQX = 4.14 ± 1.4; $t(6) = 5.57$, $p < 0.001$). Latency for rats to begin digging into bowls following CNQX infusions into the mPFC was also significantly reduced by CNQX infusions (Veh = 4.29 ± 0.6 s; CNQX = 1.86 ± 0.3 s; $t(6) = 3.23$, $p = 0.018$).

Figure 4.3E shows the infusion sites of the rats in the mPFC OST experiments (4 prelimbic infusion sites; 3 infralimbic infusion sites). Infusion sites were located in the prelimbic, infralimbic, and dorsal peduncular areas of the mPFC.

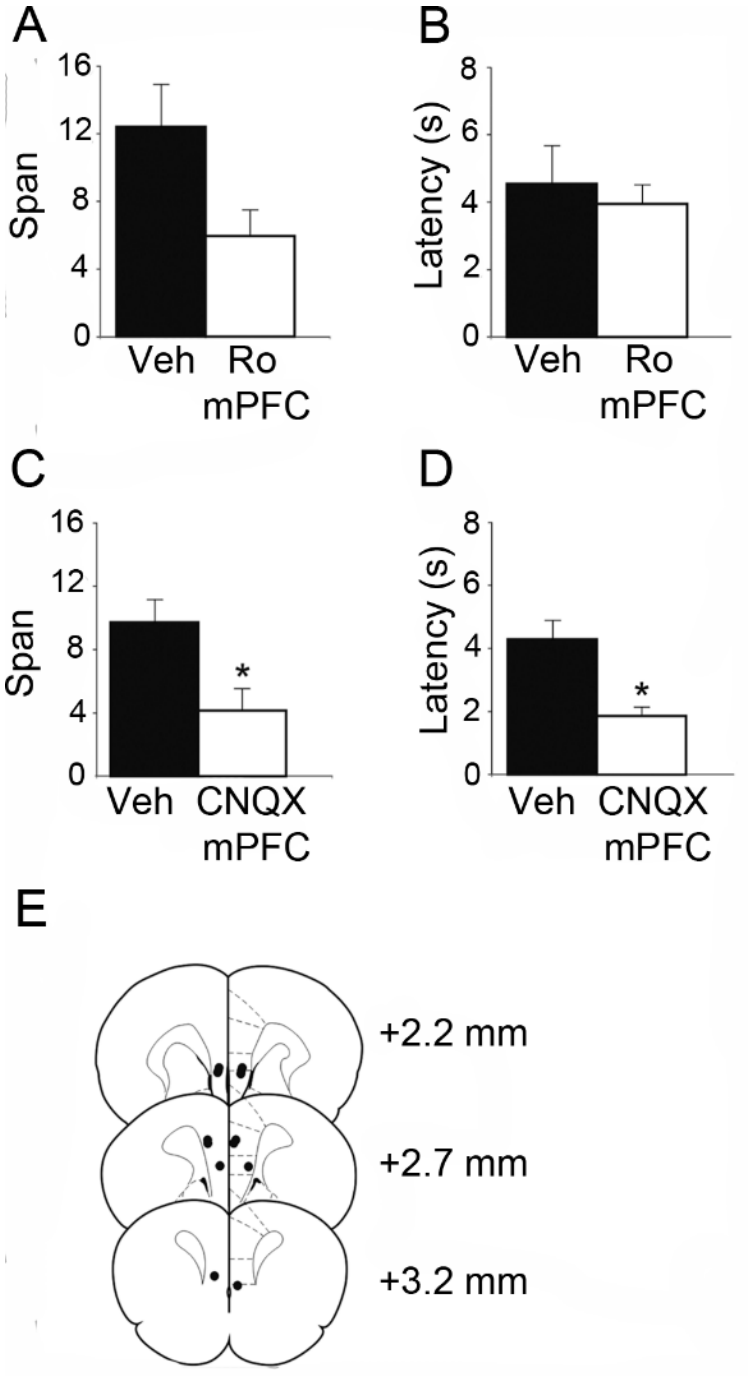

Figure 4.3 OST performance after infusions of either Ro25-6981 or CNQX into mPFC. **(A)** Mean spans of the rats following vehicle (Veh) or Ro25-6981 (Ro) infusions into mPFC. **(B)** The mean latency of the rats to start digging in a bowl for the treatments in A. **(C)** Mean spans for the rats following treatment with either Veh or CNQX into the mPFC ($n = 7$). **(D)** The mean latency for the rats to start digging in a bowl (Veh or CNQX; mPFC infusion). **(E)** Infusion sites in the mPFC. Numbers refer to the anterior-posterior location of the plates relative to bregma. * Refers to a significant difference between treatments ($p < 0.05$). Error bars represent the standard error.

4.5 Discussion

The present study revealed a series of novel findings: (1) span capacity of untreated rats significantly increased from a mean of approximately 7 to a mean of approximately 13 following the first treatment (Figure 4.1B); (2) systemic injections of the broad spectrum NMDA receptor antagonist CPP significantly reduced span capacity (Figure 4.2A); (3) systemically administered Ro 25-6981 dose-dependently impaired odour span (Figures 4.2C, E); (4) GluN2B subunit-containing NMDA receptors in the mPFC may be involved in performance of the OST because Ro 25-6981 infusions into the mPFC marginally impaired span capacity (Figure 4.3A); (5) blocking AMPA receptors in mPFC with CNQX infusions impaired span capacity and reduced latency to dig in the task compared to vehicle infusions (Figures 4.3C, D).

4.5.1 Performance of rats on the odour span task

Rats in the present experiment initially performed similarly to those reported in a previous publication from our group (Davies et al., 2013b) and others using the version of the OST that requires the rats to dig in scented sand (Rushforth et al., 2010, 2011). By testing the rats on 1–3 spans per day for a maximum of 30 min, within - session variability was reduced in our study, a characteristic of the OST that has been discussed previously (Dudchenko et al., 2000). We did not observe a consistent pattern of span length over the spans tested on a given day, although it should be emphasized that rats with high spans would not receive a 2nd or 3rd span on a given day given the time constraint (30 min testing/day) we imposed. The results from the pharmacological experiments include the mean of all spans tested for each animal on a given day. If just the first span is considered for the baseline and treatment days, all results are the same as those reported for the mean daily spans (data not shown).

Unexpectedly, the mean baseline spans of the rats in the present study increased to approximately 12 odours with further training (Figure 4.1B) and as a result performance was not stable before the systemic CPP treatment was given. While it is not clear why a higher span capacity was achieved in the present experiments, probe sessions showed that rats were not using either the odour of the buried food or some unknown feature of the bowls to solve the task. Rats in our previous study (Davies et al., 2013b) underwent the same stereotaxic surgery to implant cannulae in the mPFC; thus, effects related to that procedure are likely not involved. The present experiment was conducted in a new facility (with the identical platform and bowls) as our previous report, although the specific reasons why span capacity would be altered in the new facility are unclear. In any case, performance of the rats in the present study is within the normal range for our method of training the OST. When a testing procedure is used that allows rats to continue sampling odours after an error is committed, spans higher than 15 have been reported (Dudchenko et al., 2000; Turchi and Sarter, 2000). Results using a different version of the OST that limits the number of stimuli available on each trial also suggests that maximum span capacity is higher than the previously reported 7–9 odours (April et al., 2013), a result we would expect with that procedure.

This group of rats may have also become better able to recognize the 24 odours used in the task over the extended training they received. On a given day, the odours used for a given span are randomly selected but the same odours are used repeatedly over the weeks of training. Thus, the experience of the rats with the odours may influence their performance. However, this experiment did not assess if the increase of span capacity was due to familiarly with the odours, continuing rule learning, or strengthening of memory. One future test of this hypothesis would be to introduce rats to a series of novel odours after extended training as we conducted in this

experiment. If the familiarity of the rats with the "well-trained" odours affected their performance, a reduction in span may be observed when the rats were tested with a series of entirely novel odours.

4.5.2 Ionotropic glutamate receptors in mPFC are necessary for odour span

The impairment of odour span following treatment with the NMDA receptor antagonist CPP (Figure 4.2A) is consistent with previously reported effects of the non-competitive NMDA receptor antagonists MK-801 (acute treatment; MacQueen et al., 2011; Galizio et al., 2013) and ketamine (repeated injections) on odour span (Rushforth et al., 2011). Impairments in aspects of working memory other than capacity are well documented following treatment with NMDA receptor antagonists (Li et al., 1997; Doyle et al., 1998; Moghaddam and Adams, 1998; Aura and Riekkinen, 1999; Smith et al., 2011).

The role of NMDA receptor subtypes in cognition has been the subject of intense investigation since it was first shown GluN2B receptors mediate LTD and GluN2A receptors mediate long term potentiation (LTP) in the hippocampus (Liu et al., 2004). For the first time, we demonstrate that blocking NMDA receptors specifically containing GluN2B subunits systemically impairs odour span capacity (Figure 4.2E) while infusions of Ro 25-6981 in mPFC marginally impair odour span capacity (Figure 4.3A). We used the compound Ro 25-6981 which is 5,000 times more selective for GluN2B subunits compared to GluN2A subunits (Fischer et al., 1997). Thus, while we cannot exclude the possibility that some GluN2A-containing NMDA receptors were affected, it is highly likely that the effects we observed on odour span were due to effects of Ro 25-6981 on GluN2B-containing receptors. Previously, we found a systemic dose of 6 mg/kg of Ro 25-6981 was sufficient to prevent the stress-induced disruptions of spatial (Wong

et al., 2007; Howland and Cazakoff, 2010) and object memory retrieval (Howland and Cazakoff, 2010). Differences between the presumed site of drug action (hippocampus vs. mPFC) or the specific cognitive operations examined (spatial and recognition memory vs. working memory) may have contributed to the results observed at the dose of 6 mg/kg. Higher doses, including 10 mg/kg, also cause behavioural changes in the forced swim test (Li et al., 2010). Our results compliment a study from Cui and colleagues showing increased span capacity in mice with overexpression of GluN2B-containing NMDA receptors in the forebrain (Cui et al., 2011). Others have demonstrated a role for mPFC GluN2B receptors in trace fear conditioning, which is similar to working memory as it involves a temporal gap between the conditioned and unconditioned stimuli (Gilmartin and Helmstetter, 2010; Gilmartin et al., 2013). However, at least two reports have failed to observe deficits in working memory as assessed using operant delayed-match-to-position paradigms following systemic administration of GluN2B antagonists (Doyle et al., 1998; Smith et al., 2011).

The role of GluN2B-containing NMDA receptors in mPFC neural activity is under investigation. In rodent hippocampus, GluN2B-containing NMDA receptors may be more frequently localized to extrasynaptic areas and become activated when extracellular glutamate levels are elevated such as during acute stress (Yang et al., 2005; Wong et al., 2007; Howland and Wang, 2008). In rodent mPFC, NMDA receptors containing GluN2B subunits are found on pyramidal cells and interneurons and thus may be important for cognitive functions, including working memory. Broad spectrum NMDA receptor antagonists alter the firing properties of mPFC pyramidal cells in rodents by reducing burst firing (Jackson et al., 2004) while increasing basal firing rate (Jackson et al., 2004; Homayoun and Moghaddam, 2007). Interestingly, GluN2A receptors have been shown to critically modulate the increased gamma oscillations

observed in cortex following NMDA receptor blockade (Kocsis, 2012). In monkeys, GluN2B-containing receptors are located in the synapses of pyramidal neurons (Wang et al., 2013). Using electrophysiological recordings in freely behaving monkeys, direct application of Ro 25-6981 to the dorsolateral PFC was shown to impair performance of the delayed occulomotor response task and reduce firing of delay neurons in dorsolateral PFC (Wang et al., 2013). Similar results were observed following the systemic administration of ketamine (Wang et al., 2013). Whether similar effects of GluN2B antagonists on neural activity are observed in rodents is difficult to predict as response neurons are more commonly found in the rodent mPFC (Caetano et al., 2012; but see Devilbiss et al., 2012).

In the final experiment, we show that blocking mPFC AMPA receptors impairs span capacity (Figure 4.3C) while also reducing the latency for rats to make a choice. In previous studies, the effects of manipulating AMPA receptor activity in the mPFC on working memory have been inconsistent. Medial PFC infusions of CNQX impair working memory assessed by delayed alternation (Romanides et al., 1999) while systemic administration of the AMPA receptor antagonist with YM90K failed to alter performance of a radial arm maze task with or without a delay (Li et al., 1997). Application of CNQX to the dorsolateral prefrontal cortex in monkeys produced mixed results on neural activity in the delayed oculomotor response task (Wang et al., 2013). CNQX significantly reduced the firing rate of cue cells; however, it had varied results on response and delay cells. In addition, when rats were treated with CNQX in the OST, they showed reduced latency to dig, which may result from a psychomotor effect from CNQX; thus, caution is warranted when interpreting the impaired span capacity data. Whether this finding reflects increased impulsivity is unclear; however, AMPA receptor blockade in the

infralimbic sub-region of the mPFC does not cause an impulsive phenotype in the five choice serial reaction time task (Murphy et al., 2012).

4.5.3 Conclusion

Previous research using various tasks including the OST implicates the mPFC as an essential substrate for working memory (Kolb, 1991; Seamans et al., 1995; Floresco et al., 1997; Aujla and Beninger, 2001; Davies et al., 2013b). The present results suggest a role for GluN2B-containing NMDA receptors and AMPA receptors in the mPFC for span capacity. Caution is warranted regarding the involvement of AMPA receptors in the mPFC during the OST given that both span capacity and latency decreased with CNQX infusions. The CNTRICS group has specified working memory capacity as a construct that needs more basic research before being incorporated into translational sequence for drug development (Barch and Smith, 2008; Dudchenko et al., 2012). Thus, these results may aid in novel therapeutic development for persons with schizophrenia.

Chapter 5: Interactions between medial prefrontal cortex and dorsomedial striatum are necessary for odour span capacity in rats: role of GluN2B-containing NMDA receptors

The content of this chapter is in preparation for submission to *Cerebral Cortex*. I gratefully recognize the contributions of Quentin Greba, Jantz C Selk, Jillian K Catton, Landon D Baillie, Sean J Mulligan and John G Howland to this work. Any redundant information provided elsewhere in the dissertation has been removed.

5.1 Abstract

Working memory is involved in the maintenance and manipulation of information essential for complex cognition. While the neural substrates underlying working memory capacity have been studied in humans, considerably less is known about the circuitry mediating working memory capacity in rodents. Therefore, the present experiments tested the involvement of mPFC and dorsal striatum (STR) in the OST, a working memory capacity task used in rodents. Initially, Long Evans rats were trained to dig in scented sand for food following a serial-delayed-non-match-to-sample rule. Temporary inactivation of dorsomedial (dm) STR significantly reduced span in well trained rats. Inactivation of mPFC or contralateral disconnection of the mPFC-dmSTR circuit also reduced span. Infusing the GluN2B-containing NMDA receptor antagonist Ro 25-6981 into mPFC had no effect on span; however, span was significantly reduced following bilateral Ro 25-6981 infusions into dmSTR or contralateral disconnection of mPFC (inactivation) and dmSTR (Ro 25-6981). These results suggest that span capacity in rats depends on GluN2B-containing NMDA receptor-dependent interactions between the mPFC and the dmSTR. Therefore, interventions targeting this circuit may improve the working memory capacity impairments in patients with schizophrenia and Parkinson's disease.

5.2 Introduction

Working memory is a type of short-term memory necessary for storage, maintenance, and manipulation of information for higher order cognition (Goldman-Rakic, 1996;Baddeley, 2003;D'Esposito, 2007) that is impaired in individuals with brain disorders including schizophrenia (Barch and Smith, 2008), Alzheimer's disease (Huntley and Howard, 2010), and Parkinson's disease (Owen et al., 1992;Gabrieli et al., 1996;Bublak et al., 2002). Experiments using working memory tasks in rodents with strong translational potential to humans may provide insight into the neural circuitry underlying working memory and the development of novel therapeutics for treating working memory impairments (Barch et al., 2012;Dudchenko et al., 2013). Working memory is often divided into a number of constructs including goal maintenance, interference control, and capacity (Barch and Smith, 2008;Moore et al., 2013). Most working memory tasks used with rodents do not include a component related to capacity (Dudchenko, 2004;Dudchenko et al., 2013), although the OST first developed by Dudchenko and colleagues (2000) has received attention in this regard (Moore et al., 2013;Dudchenko et al., 2013). The OST is an incremental delayed-non-match-to-sample task (Figure 3.1A) in which rodents receive food reward for choosing a bowl of sand scented with a novel odour, either by digging in the sand (Dudchenko et al., 2000;Young et al., 2007b;Rushforth et al., 2010;Rushforth et al., 2011;Davies et al., 2013a;Davies et al., 2013b) or by flipping a lid covering the sand (MacQueen et al., 2011;April et al., 2013;Galizio et al., 2013). Since limited information exists regarding the neural circuitry involved in odour span, the goal of the present experiments was to assess the role of a corticostriatal circuit in performance of the OST by rats.

In humans, working memory involves the frontal cortex and striatum (Frank et al., 2001;McNab and Klingberg, 2008) and working memory capacity correlates with fronto-striatal

connectivity and its modulation by dopamine during task performance (Wallace et al., 2011). We have recently shown that the mPFC is required for performance of the OST in rats using temporary lesions (Davies et al., 2013b); however, it is unknown which brain areas interact with the mPFC to support span capacity. In rodents, the mPFC projects strongly to the dorsal STR (Voorn et al., 2004) and lesions of the dmSTR impair performance in working memory tasks without a capacity component (White, 2009). Given these studies, we tested whether odour span capacity in rats depends on the dorsal STR. As the rodent dorsal STR can be functionally divided into dmSTR, which receives substantial projections from the prelimbic area of mPFC, and the dorsolateral striatum (dlSTR), which receives substantial projections from the sensory-motor cortical areas (McGeorge and Faull, 1989;Voorn et al., 2004), we performed bilateral inactivations of the mPFC, dmSTR, and dlSTR separately and also used a disconnection design (Floresco et al., 1997;Hannesson et al., 2004;Baker and Ragozzino, 2014) to test whether functional interactions between areas were necessary for span.

Research examining the neurochemical modulation of working memory capacity in humans has focused on dopamine (Cools et al., 2008;Landau et al., 2009). We and others have shown that ionotropic glutamate receptors are involved in performance of the OST in rats. In particular, reduced and increased span capacity have been noted following either blockade of GluN2B-containing NMDA receptors with the antagonist Ro 25-6981 (Davies et al., 2013a) or genetically overexpressing GluN2B-containing NMDA receptors in the forebrain (Cui et al., 2011), respectively. Therefore, we also tested whether GluN2B-containing NMDA receptors in the mPFC and dorsal STR were involved in the OST.

5.3 Methods

5.3.1 Animals

Three groups (total $n = 24$) of adult male Long Evans rats (265-415 g; Charles River, Quebec, Canada) were tested in the experiments using a within subjects design. The rats were individually housed in clear plastic cages in a colony room on a 12 h light/dark cycle (lights on at 07:00) with ad libitum access to water. Except for several days after arrival and surgery, rats were food restricted to maintain 85% of their free feeding weight. Experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board. Previously published protocols were followed closely (Davies et al., 2013a; Davies et al., 2013b).

5.3.2 Surgery and Infusions

Rats were anesthetized with isoflurane and prepared for surgery using previously reported procedures (Davies et al., 2013a; Davies et al., 2013b). Rats from each squad were implanted with guide cannulae (23 Ga) bilaterally to target two of the following three brain areas: mPFC (AP + 3.00 mm; ML ± 0.70 mm; DV −3.20 mm from bregma), dmSTR (AP + 0.80 mm; ML ± 2.20 mm; DV −3.40 mm), or dlSTR (AP + 0.80 mm; ML ± 3.60 mm DV −3.40 mm). Obdurators (0.033 cm diameter stainless steel wire) were placed into the cannula to prevent obstruction. Following surgery, rats were allowed to recover for at least a week before training resumed. Rats were habituated to the infusion procedure on three separate days during the week before infusions were administered. Bilateral infusions were performed by inserting custom made needles (30 Ga stainless steel tubing) linked via PE-50 tubing to an infusion pump (PHD 2000, Harvard Apparatus, Holliston, MA) 1 mm past the end of the cannula. Drugs were infused

over 1 min and the infusion needles remained in place for an additional minute after the infusion to allow diffusion of the drug. Rats were tested on the OST 15 min following brain infusions. On treatment days, rats were tested for approximately 30 min (1–3 spans) without a break between spans.

Experiment 1: Bilateral inactivation of dmSTR or dlSTR. Rats (n = 9) for this experiment had cannulae implanted over the dmSTR and dlSTR. Infusion needles were inserted into one area bilaterally and either the GABA receptor agonists muscimol (Abcam, Cambridge, MA) and baclofen (Abcam, Cambridge, MA) or vehicle (PBS) was delivered to the dmSTR or dlSTR. The agonists were dissolved separately in PBS at a concentration of 500 ng/µl and then mixed together before infusion (McFarland and Kalivas, 2001;St Onge and Floresco, 2010;Davies et al., 2013b;Sangha et al., 2014).

Experiment 2: Bilateral inactivation of mPFC, bilateral inactivation of dmSTR, or disconnection of mPFC and dmSTR. The same infusion method was used as for experiment 1. Seven rats were tested following three treatments: bilateral mPFC inactivation, bilateral dmSTR inactivation, and contralateral disconnection (unilateral infusions in mPFC and dmSTR of opposite hemispheres). The disconnection procedure was used to block transmission of information within the mPFC-dmSTR pathway in each hemisphere. This procedure has been used to define the route of serial information transfer between different brain regions in a number of tasks (Floresco et al., 1997;Hannesson et al., 2004;Baker and Ragozzino, 2014).

Experiment 3: Role of GluN2B-containing NMDA receptors in mPFC and dmSTR in the OST. GluN2B-containing NMDA receptors were selectively targeted with Ro 25-6981. Eight rats were tested in a counterbalanced order following either vehicle (12% DMSO; 88% PBS) or Ro 25-6981 (2.5 µg/0.5 µl; (Zhang et al., 2008;Davies et al., 2013a), delivered bilaterally to

mPFC or dmSTR. In the contralateral disconnection treatment, Mus/Bac was infused into the mPFC and Ro 25-6981 was infused into the dmSTR. One rat was not tested in the disconnection experiment (bilateral or ipsilateral) as it failed to perform the task reliably on training days. Post-mortem examination of its brain revealed evidence of an infection in the mPFC.

5.3.3 Histology

After testing on the OST was complete, rats were sacrificed with isoflurane and perfused with saline. Brains were removed and post-fixed in a 10% formalin-10% sucrose solution. Brains were sectioned on a sliding microtome and infusion sites were determined using standard protocols with reference to a rat brain atlas (Paxinos and Watson, 1997).

5.3.4 Florescent muscimol infusions

In order to assess the spread of muscimol in the mPFC and dorsal STR following infusions, two rats were anesthetized and fluorescent muscimol (BODIPY TMR-X Conjugate, Life Technologies, Burlington, ON; (Allen et al., 2008) was infused using the stereotaxic coordinates described above. One rat was infused unilaterally into the mPFC while the other had unilateral infusions into the dmSTR and dlSTR of different hemispheres. Sixty minutes following the infusions, the rats were anesthetized with isoflurane, perfused with saline, and brain slices (200 μm) were cut with a vibratome. Images (Figure 5.1B) of the fluorescent muscimol conjugate were captured using a Zeiss Discovery V8 stereoscope equipped with a 16-bit, 1344 × 1024 ORCA-R2 CCD camera (C10600-10B, Hamamatsu) cooled to −35 C. BODIPY TMR-X muscimol conjugate was excited using a filtered (HQ 535/50) Schott KL 1600 wide

spectrum LED light source and epifluorescence filtered with an ET 605/70 installed just prior to the camera.

5.3.5 Data Analysis

Odour spans and latencies to choose the bowls were manually recorded during testing and entered into Microsoft Excel (2010) and Statistical Package for the Social Sciences (SPSS version 19) for analysis. All descriptive values are reported as means ± standard error of the mean. Comparisons were performed using paired t-tests. Statistical tests were considered significant if p values were < 0.05.

5.4 Results

5.4.1 Training

Rats from all three experiments were initially trained to dig in a bowl of sand for a buried Froot Loop (dig training phase). This phase of training took an average of 5.13 days to complete (range = 3 to 9 days). After dig training, rats were trained for an average of 7.13 days (range = 3 to 12 days) in the non-matching-to-sample task until they got 5/6 trials correct for three sessions. Rats were then trained on the OST for an average of 14.87 days (range, 11 – 23 days). Figure 5.1A displays the average spans obtained during the 7 training days before the first infusion for rats in all experiments.

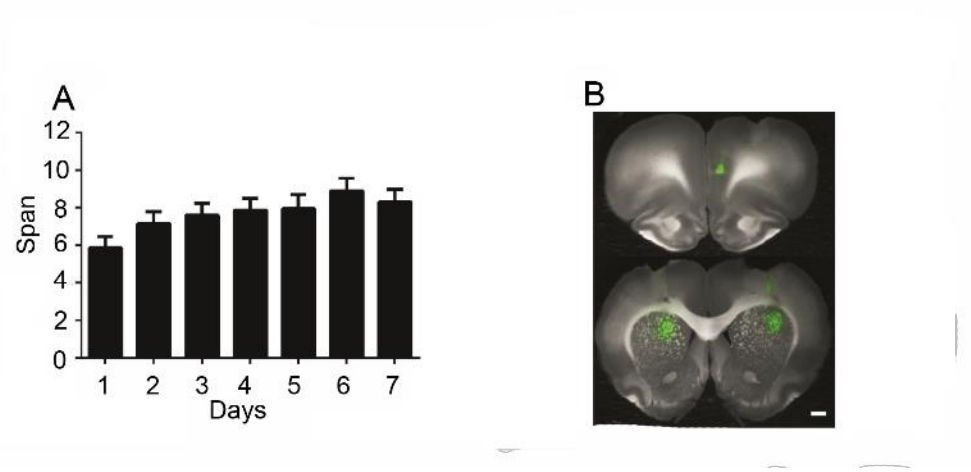

Figure 5.1. **(A)** Mean odour spans during the 7 days of training prior to the first treatment for all rats in the three experiments (n=25). **(B)** Infusions sites of florescent muscimol in the right hemisphere of the mPFC (top), left hemisphere of the dmSTR (bottom left), and right hemisphere of the dlSTR (bottom right).

5.4.2 Inactivation of dmSTR impairs odour span

In this experiment, we inactivated the dmSTR or dlSTR as these regions receive distinct projections from the prelimbic and sensory-motor areas of frontal cortex (Voorn et al., 2004). Following bilateral inactivation of dmSTR with Mus/Bac (Figure 5.2A), rats had a significantly lower span (1.65 ± 0.66 odours) than when they were treated with vehicle (7.20 ± 1.31 odours; $t(8) = 5.82$, $p < 0.001$). Latency to initiate digging did not differ between the treatments (Mus/Bac = 11.92 ± 4.59 s; vehicle 3.59 s ± 0.66 s; $t(8) = 1.70$, $p = 0.13$). Inactivation of dlSTR also impaired span without affecting latency of response (Figure 5.2B). Following the bilateral inactivation of dlSTR, rats had a span of 2.80 ± 1.15 odours, lower than the average span following vehicle infusions (6.15 ± 0.83 odours). However, this effect failed to reach significance ($t(8) = 2.19$, $p = 0.060$). Latency to begin digging did not differ between treatments (Mus/Bac = 10.12 ± 3.31 s; vehicle = 5.10 s ± 2.73 s; $t(8) = 1.49$, $p = 0.17$). Figure 5.2C displays representative infusion sites for the dmSTR and dlSTR and Figure 5.1B displays images of the spread of fluorescent muscimol following infusion into either site.

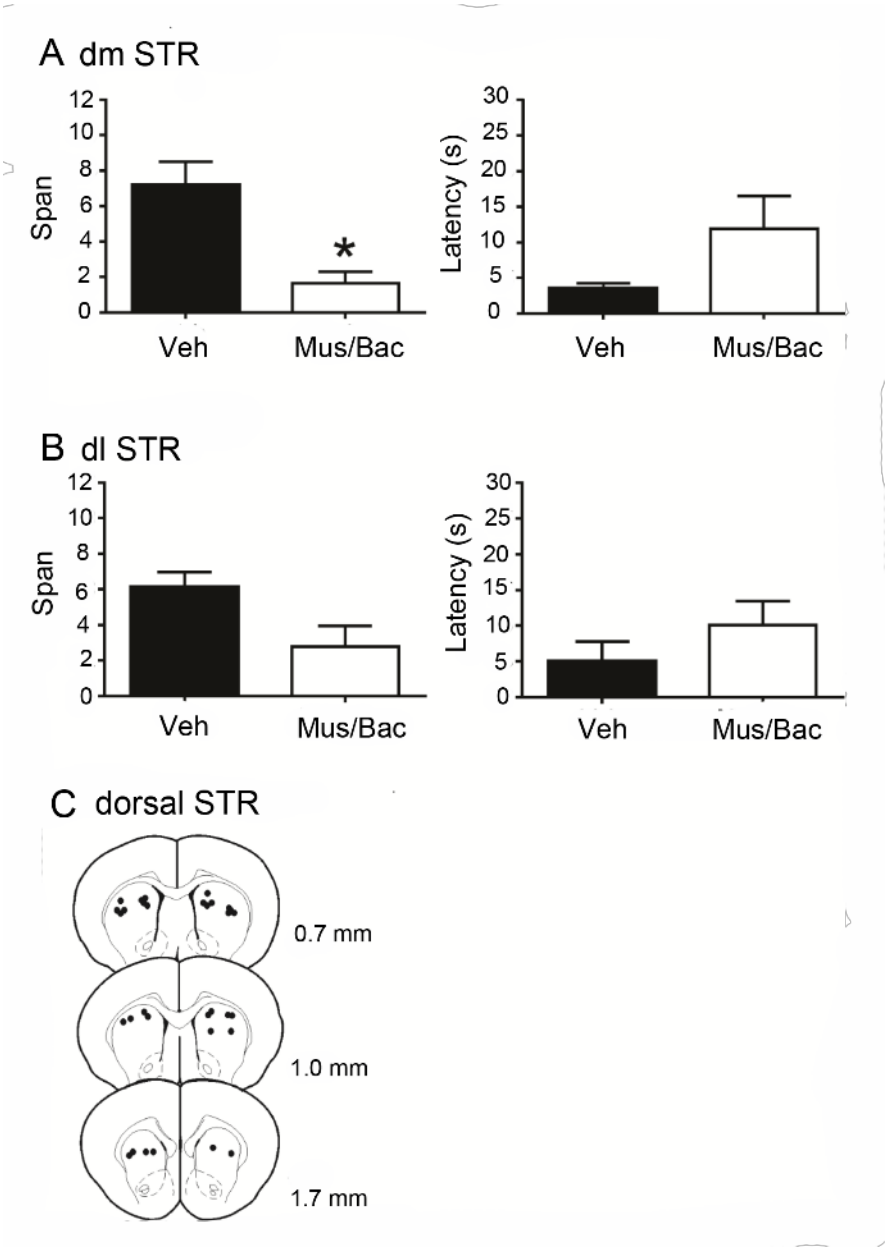

Figure 5.2. **(A)** Mean spans of rats following vehicle (Veh) or muscimol and baclofen (Mus/Bac) infusions into dmSTR (left). Mean latency of the rats to begin digging in a bowl for the dmSTR treatments (right). **(B)** Mean spans of rats following Veh or Mus/Bac infusions into dlSTR (left). Mean latency of the rats to start digging in a bowl for the dlSTR treatments (right). **(C)** Representative infusions sites in the dmSTR and dlSTR for the experiments that occurred in A and B. Numbers refer to the anterior-posterior location of the plates relative to bregma. * Refers to a significant difference between treatments ($p < 0.05$).

5.4.3 Projections from mPFC to dmSTR are necessary for odour span capacity

We have previously shown that the mPFC is necessary for performance of the OST (Davies et al., 2013b). Results of the present experiment replicate this finding as inactivation of mPFC impaired span without significantly affecting latency to dig during the task (Figure 5.3A). Following bilateral mPFC inactivation with Mus/Bac, rats had a span of 2.67 ± 0.94 odours, which was significantly lower than following mPFC vehicle infusions (7.76 ± 1.28 odours; $t(6) = 4.21$, $p = 0.006$). Latency to start digging did not differ between the treatments ($t(6) = 1.06$, $p = 0.33$). In the second part of this experiment, we also confirmed the role of the dmSTR in the OST in a separate group of rats. In this group, inactivation of dmSTR with Mus/Bac significantly reduced span without affecting latency to dig during the task (Figure 5.3B). Following bilateral inactivation of dmSTR (Mus/Bac), rats had a span of 5.64 ± 1.40 odours which was significantly lower than following vehicle infusions (10.95 ± 1.51 odours; $t(6) = 2.58$, $p = 0.042$). Latency to begin digging did not differ between treatments ($t(6) = 0.23$, $p = 0.82$).

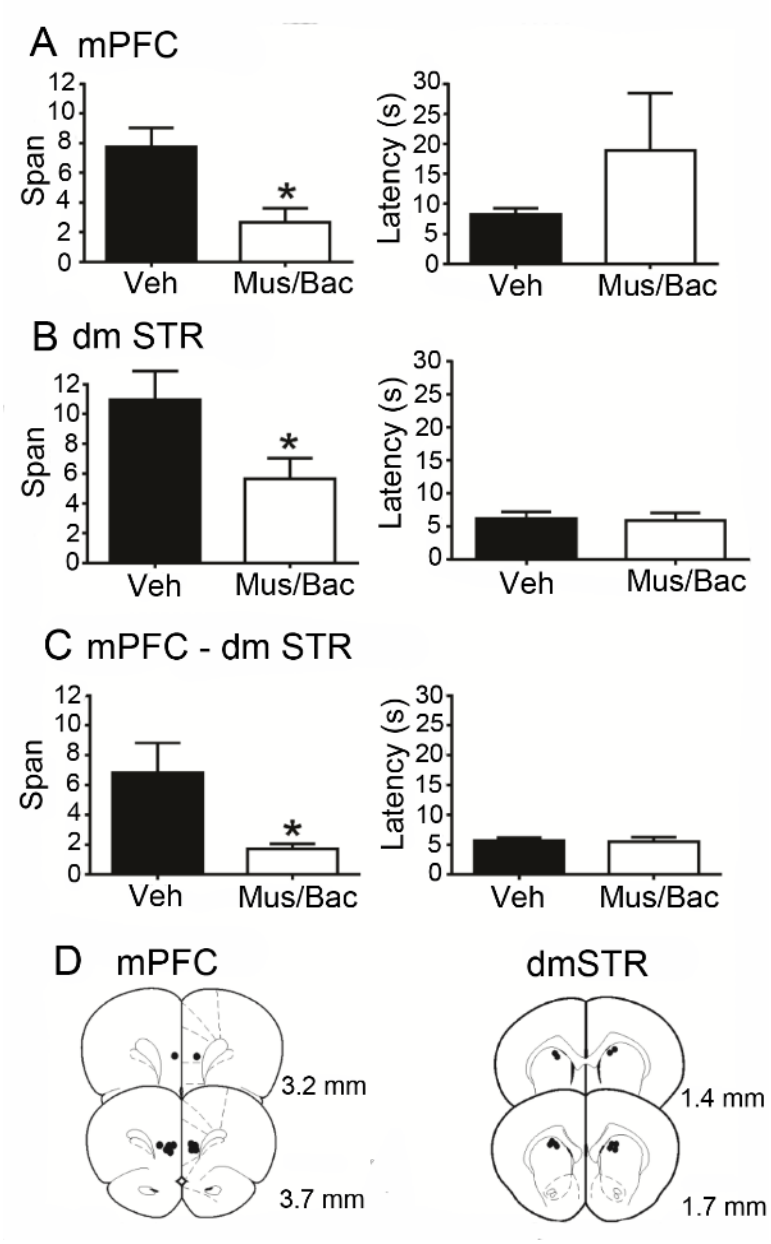

Figure 5.3. **(A)** Mean spans of rats following vehicle (Veh) or muscimol and baclofen (Mus/Bac) infusions into the mPFC (left). Mean latency of the rats to begin digging in a bowl for the mPFC treatments (right). **(B)** Mean spans of rats following Veh or Mus/Bac infusions into the dmSTR (left). Mean latency of rats to start digging in a bowl for the dmSTR treatments. **(C)** Mean spans of rats following Veh or Mus/Bac for contralateral disconnection of mPFC and dmSTR (left; see text for details). Mean latency of rats to start digging in a bowl for the contralateral disconnection treatments (right). **(D)** Representative infusions sites in the mPFC (left) for experiments A and C, and dmSTR (right) for experiments B and C. Numbers refer to the anterior-posterior location of the plates relative to bregma. * Refers to a significant difference between treatments ($p < 0.05$).

Previous research has confirmed that projections from mPFC to dmSTR are involved in behavioural flexibility (Baker and Ragozzino, 2014) and attention (Christakou et al., 2001) using disconnection procedures. Therefore, we tested whether temporary disconnection of mPFC and dmSTR would impair performance of the OST. Unilateral infusions of Mus/Bac into the mPFC of one hemisphere and the dmSTR of the opposite hemisphere significantly impaired span without affecting latency to dig during the task (Figure 5.3C). Following the disconnection with Mus/Bac, rats had a span of 1.74 ± 0.34 odours which was significantly lower than vehicle-treated rats (8.00 ± 2.05 odours; $t(6) = 3.45$, $p = 0.013$). Latency to dig did not differ between treatments ($t(6) = 0.22$, $p = 0.83$). Figure 5.3D displays the representative infusion sites for the mPFC (5 prelimbic infusion sites; 2 infralimbic infusion sites) and dmSTR for this experiment.

5.4.4 Odour span capacity depends on activation of GluN2B-containing NMDA receptors in the mPFC-dmSTR circuit

NMDA receptors containing GluN2B subunits have been implicated in the OST in two studies. Cui and colleagues (2011) showed increased working memory capacity in mice that overexpressed GluN2B-containing NMDA receptors in the forebrain. Previous research from our laboratory (Davies et al., 2013a) showed that blocking GluN2B-containing NMDA receptors with systemic injections of Ro 25-6981 impaired span. Direct infusion of Ro 25-6981 into mPFC impaired odour span in the majority (6/7) of rats tested, although this effect failed to reach significance. Therefore, we assessed whether Ro 25-6981 infusions into mPFC or dmSTR affected odour span in a new sample of eight rats. Following Ro 25-6981 infusions in mPFC, span did not differ significantly in this sample with a mean span of 8.19 ± 1.42 odours while vehicle treatment resulted in a mean span of 9.23 ± 1.65 (Figure 5.4A, left side; $t(7) = 0.50$, $p =$

0.63). Latency to respond did not differ between treatments (Figure 5.4A, right side; $t(7)= 0.57$, $p = 0.59$).

In contrast, bilateral infusions of Ro 25-6981 into dmSTR significantly impaired span without affecting response latency (Figure 5.4B). Following blockade of GluN2B-containing NMDA receptors with Ro 25-6981 rats had an average span of 6.21 ± 0.89 odours while vehicle treated rats had an average span of 10.31 ± 1.64 odours ($t(7) = 2.87$, $p = 0.024$). Latency to respond did not differ between treatments (Ro 25-6981 = 4.95 ± 0.93 s, vehicle = 5.15 ± 1.11 s; $t(7) = 0.24$, $p = 0.82$).

To assess the role of glutamatergic mPFC inputs in activating postsynaptic GluN2B-containing NMDA receptors in dmSTR, we performed contralateral disconnections of the mPFC infused with Mus/Bac and dmSTR infused with Ro 25-6981 as reported previously (Baker and Ragozzino, 2014b). Contralateral disconnection of mPFC (Mus/Bac) and dmSTR (Ro 25-6981) reduced span without affecting digging latency (Figure 5.4C). Following disconnection rats had a span 2.43 ± 0.56 odours which was significantly lower than the vehicle treatment of 9.45 ± 0.85 ($t(6) = 7.44$, $p < 0.001$). Latency to dig did not differ between treatments; contralateral disconnection 2.97 s ± 0.94 compared to vehicle 4.55 ± 0.80 ($t(6) = 1.56$, $p = 0.17$). A unilateral infusion into mPFC (Mus/Bac) and dmSTR (Ro 25-6981) was also performed to confirm the specificity of the disconnection procedure. Following unilateral infusions ipsilaterally into both sites, rats displayed a mean span of 6.88 ± 1.56, which was not significantly different from their vehicle treatment ($t(6) = 1.80$, $p = 0.12$). Figure 5.4D displays the representative infusion sites for the mPFC (4 prelimbic infusion sites; 4 infralimbic infusion sites) and dmSTR.

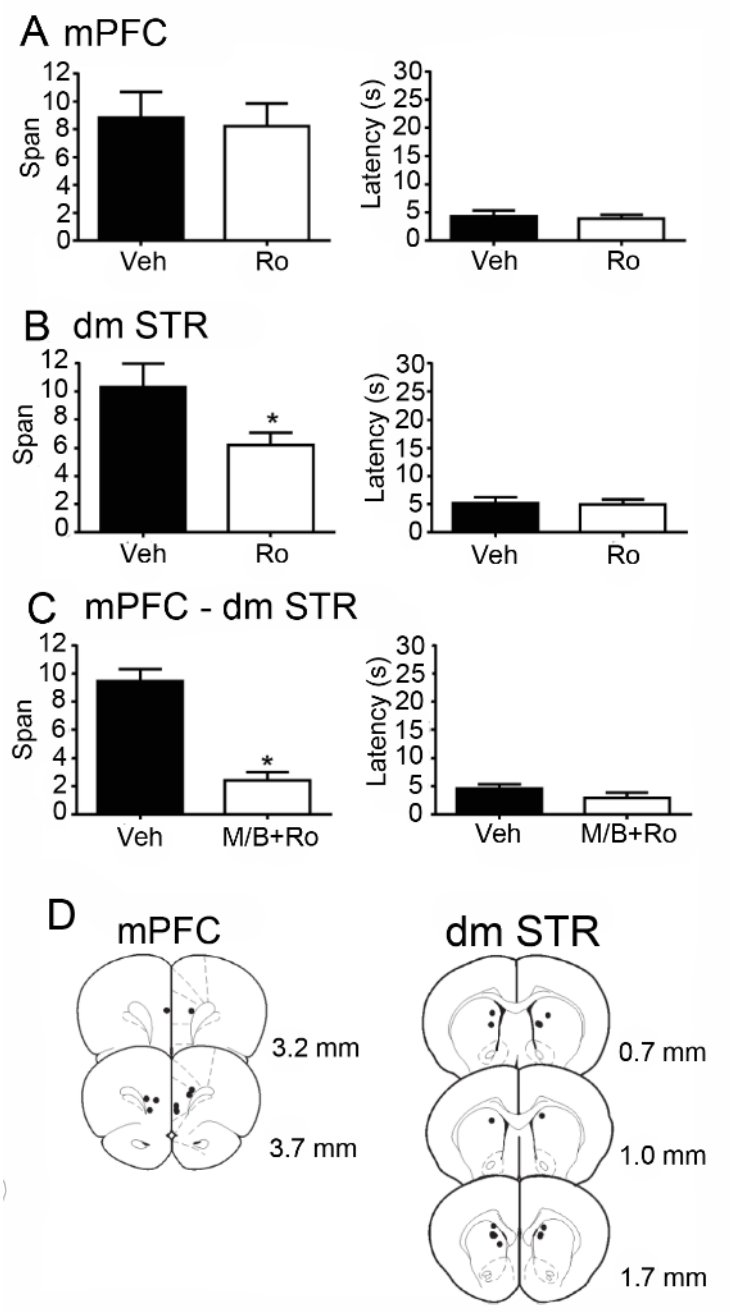

Figure 5.4. **(A)** Mean spans of rats following vehicle (Veh) or Ro 25-6981 (Ro) infusions into the mPFC (left). Mean latency of the rats to begin digging in a bowl for the mPFC treatments (right). **(B)** Mean spans of rats following Veh or Ro infusions into the dmSTR (left). Mean latency of the rats to begin digging in a bowl for the dmSTR treatments (right). **(C)** Mean spans of rats following Veh or muscimol and baclofen+Ro 25-6981 (M/B+Ro) for contralateral disconnection in mPFC (M/B) and dmSTR (Ro) (left; see text for details). Mean latency of rats to start digging in a bowl for the contralateral disconnection treatments (right). **(D)** Representative infusions sites in the mPFC (left) for experiments A and C, and dmSTR (right) for experiments B and C. Numbers refer to the anterior-posterior location of the plates relative to bregma. * Refers to a significant difference between treatments ($p < 0.05$).

5.5 Discussion

5.5.1 Contribution of a corticostriatal circuit to odour span capacity

To the best of our knowledge, the present experiments are the first to assess the role of a corticostriatal circuit for working memory capacity in rats. Temporary inactivation of dmSTR and dlSTR reduced span in the OST (Figure 5.2; 5.3B), although the reduction was only significant following dmSTR inactivation. Previous studies have shown effects of dorsal striatum lesions on a number of working memory tasks in rodents without a capacity component. Lesions of the medial aspect of the dorsal striatum impair performance of delayed alternation (Dunnett et al. 1999; Moussa et al. 2011) and win-shift tasks (Winocur, 1980), while small lesions centered in the middle of the dorsal striatum impair reference but not working memory in a radial arm maze task (Colombo et al. 1989; Packard and White 1990). The dlSTR is required for stimulus-response learning as demonstrated in a T-maze habit learning task, and a win-stay task, which involves repeated reinforcement of responding to a light stimulus (Gornicka-Pawlak et al. 2015; White 2009; Moussa et al. 2011; DeCoteau et al. 2004) but not a win shift task, which measures spatial working memory (Packard and White 1991). Dopamine within dlSTR may be involved with the consolidation of stimulus-response learning (White, 2009). Packard et al., (1989) failed to show a working memory impairment when the dorsal striatum was lesioned (Packard et al. 1989). Upon further investigation, Packard et al. (1992) revealed that dorsal striatum lesions produced impairments when the rats are given food on the maze arms during their adaptation condition to the radial arm maze, but no impairment was observed when the maze adaptation is performed without food in the maze arms (Parkard et al., 1992). These results suggest that the appetitive component of the OST may have been necessary for the observed reduction in working memory capacity following the dmSTR and dlSTR inactivations.

A recent study from our lab demonstrates that the mPFC is essential for intact working memory capacity (Davies et al. 2013b). However, Davies et al., (2013b) also showed that mPFC inactivation increased the latency to respond while in the current study, there was no significant difference in latency to respond between the inactivation treatment compared to the vehicle treatment. One possible reason for this discrepancy in the latency results is that the Davies et al., (2013b) study used a higher volume (0.5µl) of Mus/Bac compared to our current study that used 0.3µl of Mus/Bac. Importantly, mPFC inactivation does not alter performance on an olfactory sensitivity test, in which rats are presented with two bowls, scented and unscented. Rats with mPFC inactivation or vehicle infusions spend equal amounts of time at the scented bowl (Davies et al. 2013b). Our current experiment supports the notion that the inactivation of mPFC impairs working memory capacity. The involvement of mPFC is associated with a variety of working memory tasks (Holmes and Wellman 2009) including delayed alternation (Baeg 2003) and the delayed win-shift task on the radial arm maze (Seamans et al. 1995, 1998; Floresco et al. 1997; Aujla and Beninger 2001; Lapish et al. 2008), although these tasks do not measure working memory capacity.

The mPFC sends substantial projections to the dmSTR (Voorn et al., 2004;McGeorge and Faull, 1989). Contralateral disconnection with inactivation of mPFC and dmSTR reduced span on OST. Disconnection of prefrontal cortex to striatum impaired delayed alternation performance in rats (Dunnett et al., 2005;White and Dunnett, 2006). Our results are the first to demonstrate that contralateral disconnection in the corticostriatal circuit disrupts working memory capacity in rats.

5.5.2 Contribution of GluN2B-containing NMDA receptors to span capacity

Blocking GluN2B-containing NMDA receptors in the dmSTR reduced working memory capacity (Figure 5.4B). This impairment is consistent with Smith-Roe et al., (1999), which blocked NMDA receptors in the dmSTR prior to a radial arm maze task that measures working memory. Blockade of NMDA receptors in the dmSTR is associated with a reversal learning impairment in rats (Watson and Stanton, 2009b) and behavioural switching is impaired in a behavioural flexibility task (Baker and Ragozzino, 2014). In the striatum, NMDA receptors are enriched in the medium spiny projection neurons and interneurons (Standaert et al., 1994). Blockade of striatal NMDA receptors reduces spontaneous firing of medium spiny neurons in anesthetized rats (Pomata et al., 2008) and awake rats (Sandstrom and Rebec, 2003). GluN2B-containing NMDA receptors are abundant in the striatum, whereas GluN2A-containing NMDA receptors are found in low levels in the striatum (Standaert et al., 1994). The striatum receives glutamatergic projections from many sites such as the mPFC, hippocampus, amygdala, and thalamus (Standaert et al., 1994).

Our previous study, which blocked GluN2B-containing NMDA receptors in mPFC did not significantly alter OST performance; however, Ro 25-6981 injected systemically at 10mg/kg impaired span, which suggests GluN2B-containing NMDA receptors are necessary for working memory capacity (Davies et al. 2013a). Our current experiment with GluN2B-containing NMDA receptor blockade in mPFC did not alter span. The systemic Ro 25-6981 impairment may have resulted from blockade of GluN2B-containing NMDA receptors in dmSTR and possibly other structures responsible for working memory capacity.

Cui et al., (2011) increased working memory capacity in mice with an overexpression of GluN2B-containing NMDA receptors in the forebrain and the striatum (Tang et al., 1999). The

improvement observed with the GluN2B-containing NMDA receptors may have occurred due to the overexpression in the striatum rather than the forebrain. In rodent mPFC, GluN2B-containing NMDA receptors are found on pyramidal cells and interneurons and thus may be important for cognitive functions, including working memory. Broad spectrum NMDA receptor antagonists alter the firing properties of mPFC pyramidal cells in rodents by decreasing burst firing (Jackson et al., 2004) while increasing basal firing rate (Homayoun and Moghaddam, 2007;Jackson et al., 2004). In monkeys, GluN2B-containing NMDA receptors are located in the synapses of pyramidal neurons (Wang et al., 2013). Electrophysiological recordings in freely behaving monkeys with direct application of Ro 25-6981 to the dorsolateral PFC was shown to reduce firing of delay neurons in dorsolateral PFC in the delayed occulomotor response task (Wang et al., 2013).

Contralateral disconnections of mPFC (inactivation) and dmSTR (Ro 25-6981) reduced OST span (Figure 5.3C; 5.4C), which is consistent with Cui et al., (2011) that found GluN2B-containing NMDA receptors contribute to working memory capacity in the corticostriatal circuit. Importantly, to confirm that only the contralateral disconnection reduced span, unilateral infusions were performed ipsilaterally into both sites, which did not affect span. In a behavioural flexibility study, contralateral disconnection of mPFC (Mus/Bac) and dmSTR (NMDA receptor antagonist) impaired conditional discrimination performance by reducing the ability to shift behavioural responses, and an increase in perseverative errors (Baker and Ragozzino, 2014b). Dorsal STR is active during choice learning, whereas reversal of the learned choice activates the prefrontal cortex (Brigman et al., 2013). Deletion of GluN2B-containing NMDA receptors on GABA interneurons in mice impair discrimination learning on a touchscreen visual learning task (Brigman et al., 2015).

We used the compound Ro 25-6981 which is 5,000 times more selective for GluN2B subunits compared to GluN2A subunits (Fischer et al., 1997). While we cannot eliminate the possibility that some GluN2A-containing NMDA receptors were affected, it is highly likely that the effects we observed on odour span were due to effects of Ro 25-6981 on GluN2B-containing NMDA receptors. Our experimental data are consistent with other OST studies that block NMDA receptors with CPP (Davies et al., 2013b), MK-801 (acute treatment) (MacQueen et al., 2011b;Galizio et al., 2013b), and ketamine (repeated injections) (Rushforth et al., 2011b). Other working memory tasks, which do not measure capacity, show impairments following treatment with NMDA receptor antagonists (Li et al., 1997;Moghaddam and Adams, 1998;Aura and Riekkinen, Jr., 1999).

5.5.3 Functional implications

Working memory capacity is a complex cognitive construct (Barch and Smith, 2008;Dudchenko et al., 2013) that requires proper allocation of attentional resources (Leonard et al., 2013). Rats with mPFC lesions are impaired in attentional tasks such as the 5-choice-serial-reaction-time-task, which raises the potential that an attentional impairment may underlie the reduction in working memory capacity in the current experiment (Chudasama and Robbins, 2004). Christakou et al., (2005) disconnected mPFC to dmSTR, which produced impairment on an attention task. Working memory impairments from mPFC lesions are proposed to result from impaired inhibitory response control (Holmes and Wellman, 2009). This raises the possibility that an inhibitory impairment may contribute to the reduction in working memory capacity in the current study.

Previous research using various tasks implicates the mPFC and dmSTR as essential substrates for working memory (Kolb, 1990;Floresco et al., 1997;Seamans et al., 1995;Davies et al., 2013a;Davies et al., 2013b;White, 2009;Moussa et al., 2011). The present series of experiments suggest that the mPFC interacts with the dmSTR to support working memory span capacity. Since the capacity component of working memory in rodents is understudied, tasks such as the OST have a strong translational potential for humans and may offer novel therapeutic developments in schizophrenia (Barch and Smith, 2008;Dudchenko et al., 2013).

Schizophrenia is associated with frontal-striatal dysfunction and impaired working memory (Pantelis et al., 1997). In schizophrenia the dysfunctional prefrontal cortex is a result of altered NMDA receptor neurotransmission (Lewis and Hashimoto, 2007). Working memory impairments across the domains of goal maintenance, interference control, and capacity are consistently observed in schizophrenia patients (Barch et al., 2012; Fleming et al., 1995; Honey and Fletcher 2006; Keefe et al., 1995; Lett et al., 2014; Morris et al., 1997; Park and Holzman; Park and McTigue 1997; Weinberger and Cermak 1973). Gold et al. (2010) observed that schizophrenia patients show reduced working memory capacity (number of items recalled) but spared precision and maintenance (recall after short and long delays) of working memory (Johnson et al., 2013). In a rat model of schizophrenia (sub chronic ketamine exposure) nicotine restored OST performance (Rushforth et al., 2011). Wing et al., (2010) found visual spatial working memory was impaired in schizophrenia patients who abstained from smoking and the reinstatement of smoking reversed the impairment. Neuroimaging studies show that neural activation occurs within the human PFC (Courtney et al., 1997; Zarahn et al., 1997) and striatum (Wager and Smith, 2003) for relevant information during working memory tasks. The PFC controls top-down attention of neural substrates where items are stored whereas the striatum is

involved with the gating mechanism that allows relevant information into working memory (D'Esposito and Postle, 2015). Parkinson's disease patients have reduced updating of working memory for relevant information but improvements with distractor stimuli, which suggest that the gating mechanism is in a closed state more often relative to neurologically normal people (Cools et al., 2010). Parkinson's disease patients show reduced capacity in digit span tasks (Cooper et al., 1991; Dalrymple-Alford et al., 1994). However, another study did not find deficits in word span, visual span, or spatial span (Fournet et al., 1996). Frontal-striatal dysfunction in Parkinson's disease patients is identified and associated with impaired spatial working memory (Owen et al., 1992). In humans, increasing working memory capacity strengthens frontal-parietal phase synchrony (Palva et al., 2010). Recently, there has been much progress in identifying the neural substrates underlying working memory capacity in humans (Barch et al., 2012). Rodent experiments provide more precision over brain manipulations, which provides fine details about the neural substrates involved with working memory capacity. The results obtained in the OST contributes to a greater understanding of the neural substrates involved with working memory capacity, which could aid in novel therapeutic development for patients with schizophrenia or Parkinson's disease.

Chapter 6: General Discussion

6.1.1 Overview of the Main Results

In this thesis I showed NMDA receptors within the mPFC and dmSTR of rats are involved with working memory, and I demonstrated this through a series of experiments using two working memory tasks. First, I used systemic administration of the NMDA receptor antagonist CPP, which impaired TUNL accuracy; however, the GluN2B-containing NMDA receptor antagonist Ro 25-6981 did not affect TUNL accuracy (Chapter 2). CPP and Ro 25-6981 increased correction trials, which suggests that NMDA receptors are involved with perseverative behaviour on the TUNL task. Systemic administration of CPP or Ro 25-6981 impaired OST capacity (Chapter 4). There are differences between the tasks that produced the discrepant finding of Ro 25-6981 that impaired accuracy on the OST but not the TUNL task. Although both are working memory tasks the OST uses several stimuli, presumably requiring a higher working memory load than the TUNL task, which uses two stimuli. The TUNL task has a pattern separation component since distance between stimuli are varied. The OST uses olfaction to guide correct behaviour on a platform, whereas the TUNL task uses visual cues in a smaller operant conditioning chamber. The OST delay length was approximately 30-45 s, whereas the TUNL task delay length was 2 or 6 s.

Secondly, I implanted cannulas into mPFC, dmSTR and dlSTR and showed inactivation of these neural substrates reduced OST span (Chapters 3 and 5). The NMDA receptor antagonist AP5 30mM in the mPFC or dmSTR impaired TUNL accuracy (Chapter 2). In two batches of rats Ro 25-6981 was infused into the mPFC, with the first batch of rats showing reduced OST span that was not statistically significant, and the second batch of rats showing no change in OST span (Chapter 4 and 5). Ro 25-6981 infused into dmSTR impaired OST span. Contralateral

disconnections of the projections from mPFC to dmSTR reduced span when both sites were inactivated and when the mPFC was inactivated and the dmSTR received Ro 25-6981. Importantly, the unilateral disconnection did not affect working memory capacity. Taken together, my results suggest that NMDA receptors within the mPFC and dmSTR contribute to working memory.

6.1.2 Summary of the TUNL task

The TUNL task is a unique paradigm that assesses working memory and pattern separation in touchscreen equipped operant conditioning chambers. The strong translatability of this task makes it promising to study potential treatment of cognitive impairment in neurodevelopmental disorders such as schizophrenia (Bussey et al., 2012). Kumar et al. (2015) examined the role of NMDA receptors in the TUNL task. Chapter 2 obtained similar results to the Kumar and colleagues (2015) experiment. Specifically, systemic administration of an NMDA receptor antagonist impaired TUNL accuracy, and a GluN2B-containing NMDA receptor antagonist had minimal effect on TUNL performance. Kumar et al. (2015) used the non competitive NMDA receptor antagonist MK-801, which blocks the channel (Traynelis et al., 2010), whereas I used the competitive antagonist CPP, which binds to the glutamate site of the NMDA receptor (Monaghan and Jane, 2009). Kumar et al. (2015) used the GluN2B-containing NMDA receptor antagonist CP 101-606, which binds to the GluN1-GluN2B N-terminal domain. I used Ro 25-6981, which also binds to the GluN1-GluN2B N-terminal domain (Karakas et al., 2011). Future studies should attempt to reverse MK-801 induced TUNL impairment with nicotine and govadine since previous studies of these compounds reversed a working memory impairment and a visuospatial learning and memory impairment (Lins et al., 2015; Rushforth et

al., 2011). Nicotine receptors and NMDA receptors can be located on the same neuron (Marchi et al., 2015). The α7 nicotinic receptor provides presynaptic regulation of neurotransmitter release (Nomikos et al., 2000). The α7 nicotinic receptors within the DLPFC are proposed to contribute to working memory (Wang and Arnsten, 2015). Govadine has been assessed as a racemic mixture and as separate enantiomers, D- and L-govadine (Zhai et al., 2012). D- and L-govadine share a high affinity for dopamine D1 receptors and enhance dopamine efflux in mPFC (Lapish et al., 2014). L-govadine has a high affinity for dopamine D2 receptors and increases dopamine efflux in the nucleus accumbens (Lapish et al., 2014).

There is limited information about the neural substrates involved in the TUNL task. Mice with dorsal hippocampal lesions had impaired TUNL accuracy when sample locations were presented in the center (Kim et al., 2015), and had impaired pattern separation while working memory was unaffected (Josey and Brigman, 2015). Svensson et al. (2015) examined whether hippocampal neurogenesis affects TUNL performance. Rats treated with electroconvulsive therapy showed a strong increase in neurogenesis in the subgranular zone and dentate gyrus (Madsen et al., 2000; Malberg et al., 2000; Scott et al., 2000). Electroconvulsive therapy did not affect TUNL accuracy. Hippocampal lesions in rats impaired working memory and pattern separation (Talpos et al., 2010), while mPFC lesions in rats impaired working memory but spared pattern separation (McAllister et al., 2013). To my knowledge, previous studies only examined the hippocampus and mPFC on the TUNL task. Chapter 2 is the first study to show that the dmSTR is involved with the TUNL task.

6.1.3 Summary of the OST

Working memory is divided into different components including goal maintenance, interference control and capacity (Barch and Smith, 2008; Moore et al., 2013). Most working memory tasks used in rodents do not include a capacity measure (Dudchenko, 2004; Dudchenko et al., 2013), although the OST does. Many pharmacological manipulations impair OST, including several amnestic agents known to impair working memory, such as a positive GABA-A modulator, an anticholinergic, and the serotonin, dopamine, and norepinephrine agonist 3,4-methylenedioxymethamphetamine (MDMA; Galizio et al., 2013; Hawkey et al., 2014; Liechti and Vollenweider, 2001). Various NMDA receptor antagonists such as ketamine, MK-801, and CPP impair the OST (Davies et al., 2013a; MacQueen et al., 2011; MacQueen et al., 2016; Rushforth et al., 2011; Galizio et al., 2013). Nicotine reversed ketamine-induced impairment; however, the antipsychotic clozapine, and an mGlu2/3 agonist did not reverse ketamine induced impairments (Rushforth et al., 2011). Chapter 3 adds a novel contribution to the OST pharmacology literature since the GluN2B-containing NMDA receptor antagonist impaired span.

Limited information exists regarding the neural substrates involved with the OST. Dudchenko et al. (2000) showed that hippocampal lesions in rats do not affect OST span; however, hippocampal lesions impaired a spatial span task where each bowl was left in place after a rat made a response, and a spatial delayed-non-match-to-sample task across multiple delays. Others have manipulated the neurotransmitter system within the forebrain, including decreased acetylcholinesterase fibre density in the basal forebrain, which reduced OST span (Turchi and Sarter, 2000). Mice with overexpressed GluN2B-containing NMDA receptors in the forebrain and STR (Tang et al., 1999) had increased OST span (Cui et al., 2011). The neural substrates examined on the OST was limited to the above studies that examined the

hippocampus, forebrain and STR. Chapter 3 showed that the mPFC, a specific sub-structure within the forebrain, is involved with OST span. Chapter 5 expanded our knowledge of the neural substrates involved in the OST to include the mPFC, dmSTR and dlSTR.

Young has conducted experiments using various genetic manipulations in mice implicated in schizophrenia and Alzheimer's disease. The α7-nicotinic acetylcholine receptor is implicated in a range of cognitive impairments in schizophrenia. Mice with α7-nicotinic acetylcholine receptor knock out were impaired in acquiring the OST (Young et al., 2007a). Impaired odour recognition is one of the earliest symptoms of schizophrenia and Alzheimer's disease, and may serve as a biomarker for these disorders. Caspase-3 is highly expressed throughout the olfactory system, and mice over expressing human caspase-3 were impaired on the OST, which was reversed by nicotine (Young et al., 2007b). Young et al. (2009b) used a mouse model of Alzheimer's disease with mice that accumulated β-amyloid at 6 months of age (Kawarabayashi et al., 2001) and β-amyloid plaques at 8-9 months of age (Hsiao et al., 1996; Kawarabayashi et al., 2001). At 4 months of age the mice with the Alzheimer's disease model showed normal acquisition on a 12 odour OST but were impaired with 22 odours (Young et al., 2009b). OST span was impaired at 8 months of age when 12 odours were used, and at 1 year of age acquisition was impaired. These studies may result in the development of novel therapeutics for schizophrenia and Alzheimer's disease to reverse working memory impairments.

While all the studies that used the OST measured working memory capacity in rodents, there are differences in how experimenters run the task. Chapters 3-5 used sand filled bowls that rats dig to uncover a food reward (Dudchenko et al., 2000; Rushforth et al., 2010; Rushforth et al., 2011), while others used bowls with lids that the rat flip to reveal a food reward (MacQueen et al., 2011; Galizio et al., 2013). The maximum number of bowls on the platform are

manipulated in some studies such as having only 2, 5, or 10 bowls on the platform (April et al., 2013; MacQueen et al., 2011). This paradigm controls for the amount of stimuli on the platform and results in higher spans than the paradigm that adds unlimited bowls to the platform incrementally. Another paradigm incrementally adds all bowls to the platform and measures the number of errors (Dudchenko et al. 2000; Young et al. 2007a, 2007b). The paradigm I used measured span length by an incremental addition of bowls until a rat made its first error, which ended the trial. The OST is analogous to human working memory capacity studies since human tasks use several stimuli.

6.2.1 Critique of working memory tasks in rodents

The concept of working memory in rodents originates in the experiments of Olton and Honig. In the radial arm maze rats would consume the food reward from each arm and learn to visit all the arms without re-entering the previously entered arms (Olton and Samuelson, 1976). Subsequent experiments ruled out whether rats were using mediating strategies such as entering the arms in a specific order, marking the entered arms, or other intramaze cues. Since mediating strategies were ruled out, Olton et al. (1979) proposed that the radial arm maze measured working memory. Since arms were baited each day with food reward, the memory from the previous day was irrelevant to the current session.

Honig defined working memory as a representation of a cue over time in the absence of an external cue (Honig, 1978). The radial arm maze does not control the order the rat entered the arms or the duration of the delay since the rat is freely moving. Factors such as motivation and impulsiveness may influence the results of the radial arm maze more than other working memory tasks that standardize the order of stimulus presentation and delay duration.

Another maze paradigm that measures working memory is the alternation t-maze task, which takes advantage of the rodent's tendency to alternate maze arms without food reward (Richman et al., 1986). Additionally, rodents will alternate with food reward in the arms of the t-maze, which contrasts with Thorndike's law of effect that proposes an animal that receives food reward in a specific location should be more rather than less inclined to repeat that behaviour (Dudchenko, 2004). However, reactive inhibition proposes that when a rat turns one way into the maze arm, the rodent is less likely to repeat a re-entry into the same arm (Hull, 1943). The reactive inhibition theory was challenged in a study where rats on a plus-shaped maze alternated spatial locations, and not body turns (Montgomery, 1952). Others argue that alternation is due to stimulus satiation (Glanzer, 1953) or attention to stimulus change (Dember and Earl, 1957), which brings into question the validly of the t-maze to measure working memory. Support for the validly of the t-maze measuring working memory comes from delay dependant decreases in performance, and lesions of the hippocampus reducing performance. The alternation behaviour in rats is based on memory for many different types of information. Most researchers assume that rats solve the t-maze by remembering its spatial relationship with extramaze cues, and studies confirm that rats can sense their position in space (Douglas, 1966; Dudchenko and Davidson, 2002). Another study showed that rats trained with or without visual landmarks performed the same, suggesting that rats used more than extramaze spatial relationships to solve the t-maze (Dudchenko, 2001).

Maze tasks represent one category of working memory assessment while the delayed-non-match-to-sample tasks represent another category of working memory assessment. Delayed-non-match-to-sample tasks are conducted in operant conditioning chambers, which involve a presentation of a lever (sample), followed by the retraction of the lever during the delay phase.

After the delay phase, a rat is presented with both levers and is rewarded if it presses the novel lever. Operant chamber tasks do not use allocentric spatial working memory in the same way as a t-maze and radial arm maze. Unfortunately, rodents on the delayed-non-match-to-sample task are able to bridge the delay phase with mediating strategists (Dudchenko and Sarter, 1992). Rats that were required to nosepoke a port located between the levers during a delay phase positioned their nose into the side of the port closest to one of the levers during the delay (Chudasama and Muir, 1997). Researchers that viewed only the delay phase could accurately predict the response a rat made during the test phase.

A version of the delayed-matching-to-position task arranges the reward port on the opposite side of the levers, which requires a rat to turn 180 degrees to place their head in the reward port to initiate the delay phase (Hampson et al., 1999). This operant chamber layout may make mediating behaviours to bridge the delay difficult, but motor strategies may be used to bridge delays since rats are more accurate when the-to-be remembered stimulus was located on the periphery rather than the center (Gutnikov et al., 1994). The greater accuracy for periphery stimulus relative to center stimulus may result from a rat's body position when their head enters the reward port on the opposite wall and turn their body towards the correct stimulus during the delay phase.

The advantage of the TUNL task is that stimuli are presented in different locations, which makes the location of the to-be-correct stimulus unpredictable. However, elimination of orienting towards the-to-be correct stimulus may result in the use of other mediating strategies such as orienting towards the sample location. Talpos et al. (2010) stringently examined whether rats trained on the TUNL task displayed meditating behaviours during the delay phase. Trials with stimuli presented on the periphery were analysed since those trials have the greatest likelihood of

a rat displaying an orientation bias. Two behaviours produced significant benefit, only when uncorrected t-tests were used, which were a 0.68% improvement for orienting left and a 1.65% improvement for touching the screen where the sample was presented. It is unclear why orienting left was significant, whereas orienting right was non-significant. Some rats would touch the screen during the delay phase where the sample was presented, thereby learning to avoid the sample location on the test phase. However, this behaviour only accounted for a 1.65% improvement, which was not significant when the authors corrected for the number of comparisons.

While I cannot exclude the possibility that the rats trained on the TUNL task used mediating strategies such as orienting left or touching the screen where the sample stimulus was presented, these behaviours result in minimal improvements. If the rats used mediating behaviours they were not effective during NMDA receptor blockade systemically, and for infusions in mPFC and dmSTR. It is possible that pharmacological treatments impair mediating strategies, and are interpreted as an impairment in working memory (Chudasama and Muir, 1997). The drastic drop in accuracy from systemic NMDA receptor blockade, 68% for CPP and 80% for Veh at the 2 s delay, cannot be account for by a 1.65% improvement from touching the screen where the sample stimulus was presented. Similar decreased performance was obtained with NMDA receptor antagonist infusions into the mPFC or dmSTR. While the rats I trained may have used mediating behaviours, the effects of the mediating behaviours on the results should have minimal impact on the overall findings.

While the TUNL task uses a delayed-non-match-to-sample paradigm, the OST uses an incremental delayed-non-match-to-sample paradigm to measure capacity. It is necessary to compare the OST to human working memory tasks, and outline the strengths and limitations of

the OST. The majority of working memory studies in humans use vision to solve working memory tasks and few are conducted using odours (however, see human odour working memory experiments; MacQueen, 2015; Jonsson et al., 2011; Levy et al., 2003). Vision is the primary sensory modality in humans, and odour is the primary sensory modality in rodents, which is important for translatability since both species are using their primary sensory modality. Therefore, working memory tasks involving odour may require less training than visual working memory tasks. Rats on the OST took approximately 6 weeks of pre-training to obtain criterion whereas rats on the TUNL task took approximately 14 weeks of pre-training to obtain criterion. Therefore, more data may be obtained using odour working memory tasks than visual working memory tasks.

Diffusion of an odour bowl was not controlled for when rats performed the OST since scented bowls were placed on the platform without ventilation to control odour diffusion. Therefore, I was unable to measure when a rat could smell a specific bowl or if many bowls on the platform influenced behaviour by lowering the signal-to-noise ratio of odour. The incremental addition of stimuli in the OST is different than working memory tasks conducted in humans and monkeys that typically use an array of items. Therefore, some researchers question whether the OST measures working memory capacity. The OST satisfies the definition most researchers agree on, which is maintenance of information over a delay, and manipulation of information since a rat that encounters a specific odour (for example basil) will either dig (novel) or not dig (familiar) into a bowl. Therefore, the OST measures working memory capacity since it incorporates maintenance and manipulation of information for storage of multiple representations.

Differences in the working memory literature exist between humans and rodents. Baddeley and colleagues proposed a working memory model in humans that included a central executive controlling two sub-systems, a visual-spatial sketch pad and a phonological loop (Baddeley, 1986; Baddeley, 1992), which is not involved with rodent working memory. Baddeley's model of working memory gave way to the recent slot and resource pool models in humans. The slot model proposes that each item within working memory is encoded and retrieved within a fixed number of slots (D'Esposito and Postle, 2015). The resource pool model proposes that items are divided across one pool of resources, and large numbers of items spreads resources thin and results in error. There is a species disconnect since working memory models in humans are not applied to rodents. However, the OST measures capacity in rodents, which is extensively studied in humans, and tasks are refined such as the TUNL task that has minimal mediating strategies.

6.2.2 Critique of pattern separation tasks

While many independent research laboratories use behavioural tasks to measure pattern separation there is debate about whether a behavioural task can measure pattern separation. This debate stems from the disagreement over the operational definition of pattern separation and how to study this behaviour. Santoro (2013) argues that pattern separation should not be used across computational research, cell ensemble activity research, and behavioural neuroscience. Additionally, Santoro (2013) argues that pattern separation should never be used to describe behaviour, and should be replaced with the term discrimination. Santoro (2013) argues that pattern separation should only be used for computational models and neural electrophysiology. I disagree that the term discrimination is the most specific term to use since discrimination

between similar stimuli involves different neural processes than discrimination between distinct stimuli (Kent, 2015).

While the TUNL task does measure the ability of a rodent to discriminate between similar stimuli such as spatially close squares, Kent (2015) disagrees that the term discrimination is the most specific term to use. Discrimination is a general concept and in the case of some behavioural paradigms the specific term pattern separation is proper to use when examining the discrimination between similar items. Discrimination between similar spatial locations involves different neural processes than discrimination between distant spatial locations, which suggests that different types of discrimination processes are engaged (Kent, 2015). Therefore, it is beneficial to use the more specific term of pattern separation when referring to discrimination between similar stimuli.

I am unable to know if rats in the TUNL task engaged in pattern separation at the cellular level when presented with small separations. Electrophysiological recordings from the dentate gyrus when rodents are presented with small separations on the TUNL task could assess whether pattern separation is engaged at the cellular level. Increasing stimuli similarity could increase the overlap of neural inputs on a cellular level and satisfy the accepted definition of pattern separation. While behavioural tasks cannot directly measure pattern separation, they can measure behaviours consistent with a theory of pattern separation in computational models and cell ensembles. The term pattern separation is beneficial to use across different lines of research since it allows for interdisciplinary investigation into the mechanisms underlying pattern separation, and may lead to experiments that could examine behavioural pattern separation directly.

Neunuebel and Knierim (2014) provided the strongest evidence of pattern separation at the behavioural and cellular level. The authors measured single unit activity in the dentate gyrus

and CA3, which showed that the dentate gyrus outputs are less correlated than the inputs while manipulating cues to cause graded changes in sensory input in behaving rats. This finding provides direct evidence that pattern separation occurs in the dentate gyrus and that rodents performing pattern separation tasks such as the TUNL task may have dentate gyrus outputs that are less correlated than the inputs.

Aimone et al. (2011) outlined several critiques to pattern separation research including performance changes from alternations in the dentate gyrus or neurogenesis could be from impairment of inhibitory learning rather than pattern separation (McHugh et al., 2007; Sahay et al., 2011). Another critique of pattern separation research are tasks that incorporate a working memory component, since it is unclear whether an alteration in performance is due to pattern separation or working memory (Clelland et al., 2009; Creer et al., 2010; Gilbert et al., 2001; Hunsaker and Kesner 2008; Saxe et al., 2007). The TUNL task measures pattern separation and working memory with varying degrees of pattern separation. While the components of working memory and pattern separation cannot be completely teased apart in the data analysis of the TUNL task, we can examine different spatial separations while keeping the delay phase constant. Therefore, the separation is the only variable changing while the delay is constant across the different separations.

Using the term pattern separation poses a potential problem for circularity in the interpretations across research in computational models, cellular ensembles and behaviour (Aimone et al., 2011). In addition, Aimone et al. (2011) argued that if a full body of evidence existed without previous assumptions, it may result in another explanation for the evidence that does not involve pattern separation. Since behavioural tests are unable to measure pattern separation directly, it is inferred that pattern separation contributes to behaviours measured in

pattern separation tasks. Therefore, it is possible that the pattern separation proposed by computational models may not be related to pattern separation measured in behavioural studies. However, computational models provide theories that fit with behavioural pattern separation research (Kent, 2015). As mentioned above, there are potential beneficial effects of using the term pattern separation since it would facilitate interdisciplinary research across different fields of study. Aimone et al. (2011) proposed that the term memory resolution is a more appropriate term than pattern separation. Since perceptual pattern separation does not have a memory component (Kent et al., 2016), the term memory resolution is an incorrect term. As experiments extend the pattern separation literature, the validity of critiques will be determined.

6.2.3 Critique of behavioral pharmacology

While behavioral pharmacology has produced many significant contributions to the study of drugs effects on behaviour, there are limitations in behavioural pharmacology (Branch, 2006). The off target effect of drugs is a major limitation of behavioural pharmacology, especially with systemic administration since the drug can affect the whole body. Systemic administration of NMDA receptor antagonists were performed in chapters 2 and 4. NMDA receptors are located in the brain, spinal cord, peripheral glial cells, endothelium, kidney, bone, pancreas, and other regions of the body (Hogan-Cann and Anderson, 2016). Therefore, systemic administration of NMDA receptor antagonists may affect multiple locations in the body. While I am unable to exclude the possibility of NMDA receptor blockade in regions other than the brain affecting performance on behavioural tasks, I am able to compare latencies between vehicle treatment and NMDA receptor antagonist treatment. Similar latencies between vehicle treatment and NMDA receptor antagonist treatment would show that motor performance, motivation, and

impulsiveness are similar between treatments. In the TUNL task, correct response latency was altered in the AP5 mPFC treatment (chapter 2), in the OST, response latency was increased when the mPFC was inactivated (chapter 3), and AMPA receptor blockade increased latency (chapter 4). However, in the vast majority of experiments latency was not different between vehicle and drug treatments.

In addition to a drug affecting off target body regions, another critique of behavioural pharmacology is blockade of off target receptors. In the series of experiments in the previous chapters, I used the NMDA antagonist CPP and AP5. CPP did not interact with 21 putative neurotransmitter receptors including the putative AMPA and kainate receptors (Lehmann et al., 1987). AP5 has minimal effects on AMPA receptors and is a potent NMDA receptor antagonist (Davies et al., 1981; Monaghan and Jane, 2009). The GluN2B-containing NMDA receptor antagonist Ro 25-6981 is 5,000 times more selective for GluN2B subunits relative to GluN2A subunits (Fischer et al., 1997). While I cannot exclude the possibility that some off target receptors were affected, it is very likely that the effects observed for each treatment were due to the blockade of the targeted receptors.

To achieve direct access to the brain, cannula were implanted into specific regions of the brain. While the rats received 1 week to recover from the surgery of cannula implantation, the rats may have acquired lasting effects into the testing phase. This includes creating a permanent lesion above the target brain region. A permanent lesion from cannula implantation was made in the cortex above the mPFC and the dorsal STR. During the infusion treatments, needles were inserted into the cannula and placed approximately 1 mm below the end of the cannula, which delivered the drug to the target region and created a brain lesion from the needle. However, the

vehicle treatment was a proper control to eliminate the possibility that the lesions from the cannula and needles into the brain significantly reduce performance on working memory tasks.

The stress of treatment administration may have an effect on the results of the experiments. During an intraperitoneal administration, a rat is restrained to eliminate movement and picked up and tilted. The needle is quickly inserted into the abdomen and depressed to release the contents within the syringe. During a brain infusion, a rat is restrained to eliminate movement by placing a hand around the rat. This allows a researcher to insert needles into the cannula and run the infusion pump that administers the treatment. Proper controls were built into each experiment since all of the systemic injections and brain infusions from chapters 2-5 were composed of vehicle and drug treatments. The duration of a brain infusion is approximately 3 min and is much longer than systemic injections, which take approximately 5-10 s. The amount of stress that a rat will experience from treatments is partly due to the experience and comfort level of the researcher with the technique. While there are limitations in behavioural pharmacology, proper controls are in place to mitigate the limitations.

6.3 Future directions

The future directions include the improvement of the OST with an automated chamber, and extending the neural circuitry and pharmacology research, and using electrophysiology in the TUNL and OST. An automated OST would provide many advantages over the current OST. The OST requires an experimenter to devote approximately 30 min to each training session per rat. In contrast, on the TUNL task I used several operant chambers to train several rats at a time, which makes the automated TUNL task much more efficient. An automated version of the OST would eliminate the lack of control for diffusion of the odours. An automated version that

contains odour ports where scent in an airstream within the port would provide better control of the diffusion of odour. An automated version of the OST would likely occur in an operant chamber, which reduces the amount of movement from a rat relative to the current platform. Several operant chambers may be used in one room for an automated OST rather than one platform in one room. An automated OST may advance the working memory capacity literature faster since more rodents can be trained by one experimenter and other laboratories may become interested in an automated OST that requires less time investment than the current OST.

There are many questions that remain unanswered, which could be experimentally examined. In the TUNL and OST the hippocampus, frontal cortex and STR were the only neural substrates that were examined. Future experiments should elucidate more neural substrates related to working memory in the TUNL and OST. The posterior parietal cortex is involved with working memory, spatial motor planning, and spatial navigational tasks (Harvey et al., 2012; Whitlock, 2014). Since the TUNL task is a spatial working memory task the posterior parietal cortex may contribute to TUNL task performance. The OST uses odour rather than spatial cues to guide correct behaviour, and as a result the posterior parietal cortex may not be involved with OST performance. Scott et al. (2016) inactivated the posterior parietal cortex prior to the OST, which did not affect span. However, the sample size was small and more rats are required to make stronger conclusions about the effects of posterior parietal cortex lesions on the OST. Studies show that the dlSTR is associated with stimulus-response learning, but not involved with working memory (Packard and White, 1991; White, 2009). Since dlSTR inactivation impaired span on the OST, it is important to investigate the role of the dlSTR on the TUNL task. The ventral STR is involved with working memory (Baiardi et al., 2007) and inactivation may impair working memory on the TUNL task and OST. Many pharmacological manipulations were

performed in the TUNL task and OST; however, dopamine manipulations have yet to be examined on the tasks. Dopamine has an inverted U function on working memory where too little dopamine impairs performance and too much dopamine also impairs performance (Arnsten et al., 2016).

The examination of neural correlates on the TUNL task and OST using electrophysiology is limited. Electrophysiological recordings in the mPFC of freely moving rats on the OST showed transient firing early in the delay phase, late in the delay phase, and persistent firing throughout the delay phase (An et al., 2015; 2016). These results are similar to others that have used working memory tasks and found transiently and persistent firing in the mPFC during a delay phase (Batuev et al., 1990; Chang et al., 2002; Yang et al., 2014). The projections of the mPFC such as the dmSTR should be examined simultaneously, which allows for a measure of synchronization between the mPFC and dmSTR. Highly synchronized brain regions are associated with effective neural communication whereas desynchronized brain regions are associated with ineffective neural communication. Electrophysiology would reveal the neural mechanisms of the TUNL and OST since specific neural activity correlates with behaviour.

6.4 Conclusions

In my dissertation I showed that the mPFC and dmSTR are involved in working memory. Specifically, NMDA receptor blockade within the mPFC or dmSTR impaired TUNL task performance, GluN2B-containing NMDA receptor blockade in dmSTR impaired OST span, and contralateral disconnection of mPFC and dmSTR impaired OST span. Taken together, my findings show that the mPFC and dmSTR are involved with visual working memory and odour span capacity working memory. Since each behavioural task has limitations and provides an

incomplete picture of working memory, it is necessary to use multiple tasks that assess various facets of working memory. My series of experiments adds support to the literature that the mPFC and dmSTR contribute to working memory. My findings may contribute to the development of novel therapeutics in disorders with impaired working memory such as schizophrenia and Alzheimer's disease.

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