EXAMINING THE ROLES OF THALAMOCORTICAL AND FRONTOPARIETAL CIRCUITRY FOR WORKING MEMORY IN THE RAT

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ABSTRACT

Working memory (WM) is the cognitive capacity for short-term maintenance and manipulation of stimuli and goals for the purpose of guiding behaviour. Research in primates has indicated that WM relies on a large network including the prefrontal cortex, various posterior cortical areas, and subcortical nuclei. However, the circuit mapping of WM in rodents is incomplete as it pertains to the specific involvement of thalamocortical and frontoparietal circuitry across WM tasks. In this dissertation, I present the findings of three sets of experiments using two different rodent WM tasks: The odour span task (OST), an incrementing delayed non-matching-to-sample task using odours, and; the Trial-Unique Non-matching-to-Location (TUNL) task, a touchscreen-based visuospatial delayed non-matching-to-sample task. 1) I found evidence that the OST relies on a thalamocortical circuit connecting the medial prefrontal cortex (mPFC) and the mediodorsal thalamus (mdThal). Moreover, the mPFC and mdThal played dissociable roles in the foraging element of the OST, with mdThal inactivation causing a dramatic reduction in exploratory motor activity. 2) I examined the role of the PPC in the OST and found that it is not necessary for OST performance. 3) I found that the PPC is critical for TUNL, confirming that the rodent PPC plays an essential role in visuospatial WM. Additionally, I found that TUNL is independent of NMDA signalling in the PPC and instead depends only on AMPA/Kainate receptors in contrast to previous research showing an important role for NMDA receptors in WM. Overall, the results indicate that thalamocortical and frontoparietal pathways are differentially involved across WM tasks, with frontoparietal circuitry being more sensory modality-specific than thalamocortical circuitry.

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LIST OF ABBREVIATIONS

%AppE	Percentage of total approaches accounted for by error odour
5-HT	5-hyrdroxytryptamine; serotonin
ACh	Acetylcholine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
AP	Anteroposterior
AP5	2-amino-5-phosphonopentanoic acid
App/O	Approaches per odour
cAMP	Cyclic adenosine monophosphate
CMOR	Cross-modal object recognition
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
DA	Dopamine
dmSTR	Dorsomedial striatum
DMTS	Delayed match-to-sample
DNMTS	Delayed non-match-to-sample
dlPFC	Dorsolateral prefrontal cortex
DR	Discrimination ratio
DV	Dorsoventral
FAM	Formalin/acetic acid/methanol solution
FoA	Focus of attention
GABA	Gamma-aminobutyric acid
IOR	Inhibition of return
ITI	Intertrial interval
LTM	Long-term memory
mdThal	Mediodorsal thalamus
mEP	Mean position of errors
ML	Mediolateral
NE	Norepinephrine
NMDA	N-methyl-D-aspartic acid
OST	Odour span task

PBS	Phosphate buffered saline
PC	Parietal cortex
PFC	Prefrontal cortex
PKA	Protein kinase A
PPC	Posterior parietal cortex
SEM	Standard error of the mean
STM	Short-term memory
TTX	Tetrodotoxin
TUNL	Trial-Unique, Non-matching-to-Location
VTA	Ventral tegmental area
WM	Working memory

1. GENERAL INTRODUCTION

A fundamental aspect of cognition is the ability to store information in order to guide present and future behaviour. Stimuli must often be stored as internal representations so that they may be used to guide behaviour in the absence of their external referents. This general function is supported by a range of neural processes collectively known as "memory". Memory is traditionally subdivided into explicit and implicit (also known as declarative and non-declarative) memory (Squire & Dede, 2015). While implicit memory pertains to unconscious skills and habits, explicit memory refers to memories that are conscious and the content of which can be verbally conveyed. Long-term explicit memories are thought to be stored as "engrams", which are persistent changes in brain circuits corresponding to experiences that can be dormant until retrieval in the presence of cues (Josselyn, Köhler, & Frankland, 2015). Long-term memory (LTM) enables the encoding, storage, and retrieval of vast quantities of complex information.

Explicit memory can also be "short-term". This form of memory is generally referred to as short-term memory (STM) or "working memory". This terminology is often used interchangeably and STM and WM may be largely overlapping constructs, although more precise usage defines WM as being STM with active manipulation of stored information (Aben, Stapert, & Blokland, 2012). Unlike LTM, WM stores only very small quantities of information for short periods of time for immediate use and manipulation. However, this capacity is critical for normal cognition. Indeed, to borrow an example popular among WM researchers, the reader would not be capable of comprehending this sentence from start to finish without intact WM. Accordingly, WM impairment is a common and debilitating aspect of many neuropsychiatric conditions including schizophrenia (Rajji et al., 2015), Alzheimer's disease (Kumar et al., 2017), Parkinson's disease (Grogan et al., 2018), and major depressive disorder (Shan et al., 2017). Given the fundamental importance of WM function to normal cognition, as well as its relationship to various neuropsychiatric disorders, it is of great importance to have a detailed neurophysiological understanding of WM function.

1.1. The History and Cognitive Psychology of Working Memory

Working memory has been studied under various labels and definitions since the late 19th century. The earliest research that posited a division between LTM and STM was conducted by William James, in which he posited a division of memory involving "primary memory", which is roughly equivalent to STM or WM, and "secondary memory", which is equivalent to LTM

(James, 1890). The earliest use of the term "working memory" appeared in (Miller, Galanter, & Pribram, 1960).

1.1.1. Multicomponent Models of Working Memory

Likely the most widely known and influential model of WM was devised by Baddeley & Hitch (1974) who posited that WM was composed of several different components. The multicomponent model includes a "central executive", a global hub for executive control over WM processes, in addition to two buffer systems, the "phonological loop" and the "visuospatial sketchpad". The two buffer systems support online verbal rehearsal and maintenance of mental images of visual scenes, respectively. In a later update to the model, a "long-term memory buffer" was also added in order to account for representations activated from long-term memory (Baddeley, 2000).

1.1.2. State-based Models of Working Memory

Although multicomponent models of WM have been extremely influential for decades, a newer form of thinking has moved to so-called "state-based" models which consist either of "activated LTM" or "sensorimotor recruitment" (D'Esposito & Postle, 2015). Models involving activated LTM challenge the idea of a basic distinction between STM and LTM, arguing instead that the information held in WM exists as activated LTM representations (Jonides et al., 2008). Several versions of such a model posit that WM consists of activated LTM containing memory representations in addition to a "focus of attention" (FoA) (Oberauer, 2009, 2013). In contrast, "sensorimotor recruitment" hold that the WM representations are stored in the sensory cortices initially used to perceive the referents of the representations (Scimeca, Kiyonaga, & D'Esposito, 2018). There is still significant debate over which of these models best fits the neurophysiological evidence (Gayet, Paffen, & Van der Stigchel, 2018; Scimeca et al., 2018; Xu, 2018).

1.1.3. Limited Capacity, Interference, and Serial Position Effects

As I have already mentioned above, one of the defining characteristics of WM is its limited capacity. Precisely *how limited*, however, is still a topic of debate (Ma, Husain, & Bays, 2014; Oberauer, Farrell, Jarrold, & Lewandowsky, 2016). Early research found WM capacity to be equal to roughly 7 plus or minus 2 items (Miller, 1956) whereas more recent work finds it to

be closer to 3-4 discreet items (Cowan, 2000). Various theories have been proposed to account for the limited capacity of WM.

Some authors argue that capacity is limited by decay in the fidelity of representations over time such that only a limited number of representations can be adequately rehearsed before they are lost to decay (Schweickert & Boruff, 1986). Other accounts hold that WM capacity is a limited resource that can be exhausted as the number of items held in WM exceeds the allowable quantity. One account holds that WM capacity is a limited resource pool that can be subdivided by the addition of each subsequent WM item until the resource pool is too subdivided to afford sufficient resolution to each item for it to be effectively recalled (Ma et al., 2014). A popular interpretation of this flexible resource model is that the accuracy of recall when WM capacity is exceeding is reduced because of interference between items (Oberauer et al., 2016; Oberauer & Kliegl, 2006). Alternatively, a "discrete" account holds that WM capacity is determined by the presence of a discrete number of "slots" which cannot be exceeded by additional WM items (Cowan, Rouder, Blume, & Saults, 2012).

An additional feature of WM capacity is the presence of serial position effects. Items in a WM set are not equally likely to be recalled correctly. Recall of items in a sequence is often marked by both a primacy effect, meaning that the first item in a sequence has an enhanced likelihood to be recalled, as well as a recency effect, meaning that the most recent item in a sequence is also more likely to be correctly recalled. Combined, primacy and recency effects cause there to be a so-called serial position curve in which the items nearer the middle of a sequence are recalled with the least accuracy (Botto, Basso, Ferrari, & Palladino, 2014; Stephane et al., 2010).

1.2. The Neural Correlates and Organization of Working Memory in Humans and Non-human Primates

1.2.1. The Prefrontal Cortex

There is overwhelming evidence and widespread agreement that WM recruits a wide network of brain areas including high-level association cortices, lower-level sensory cortices, and a number of subcortical nuclei (Christophel, Klink, Spitzer, Roelfsema, & Haynes, 2017). Although WM is a highly distributed process, the prefrontal cortex (PFC) is of central importance and is considered a keystone in the hierarchy of brain regions involved in WM.

Lesions of the PFC reliably causes severe WM deficits in humans and non-human primates (Chafee & Goldman-Rakic, 2000; Levy & Goldman-Rakic, 1999; Tsuchida & Fellows, 2009; Voytek & Knight, 2010). Additionally, a number of neuroimaging and recording studies have found that the PFC is engaged during WM tasks (Courtney, Petit, Maisog, Ungerleider, & Haxby, 1998; D'Ardenne et al., 2012; Funahashi, Bruce, & Goldman-Rakic, 1989; Fuster & Alexander, 1971; Veltman, Rombouts, & Dolan, 2003). There has been considerable attention paid to the potentially dissociable roles of subregions within the PFC. There is convincing evidence that the lateral portions of the primate PFC are most consistently involved in WM. Furthermore, there is evidence of dissociations between the dorsal and ventral aspects of the lateral PFC which may correspond to the type of content being stored or the type of processing (e.g. maintenance vs manipulation) although it is not yet clear precisely how these functions are divided (D'Esposito, 2006). For example, a meta-analysis of 36 imaging studies found that WM function in the PFC may be between a dorsal area with enhanced sensitivity to spatial information and a more lateral region with enhanced sensitivity to non-spatial information, loosely recapitulating the what/where dissociation in the dorsal and ventral streams of the posterior cortices (Nee et al., 2013).

Related to the findings of PFC activity during WM, an extremely prominent area of research in WM pertains to the finding that individual neurons in the PFC remain active over the delay period of WM, a potential cellular mechanism for maintenance of memory representations (Funahashi et al., 1989; Fuster & Alexander, 1971). These initial observations resulted in the widely-held view that memory for WM stimuli were maintained, at the level of individual neurons, by persistent spiking activity that spanned the delay (Goldman-Rakic, 1995) This model of WM delay maintenance remains, to this day, one of the most influential and is still subscribed to by several leaders in the field (Constantinidis et al., 2018).

The role of persistent delay cells has, however, recently received heavy criticism. The "persistent" activity of delay cells has been argued to be the result of "averaging" the activity over multiple trials (Lundqvist, Herman, & Miller, 2018). Without this averaging, neurons in the PFC exhibit dynamic activity over the delay period inconsistent with the traditional persistent delay activity account (Stokes, 2015). An additional criticism is that persistent spiking is metabolically expensive and thus an inefficient mechanism in an evolutionary sense (Mongillo, Barak, & Tsodyks, 2008). Thus, persistent activity of delay cells is likely not fully sufficient to

explain memory maintenance over the delay period. An emerging view holds that delay maintenance of WM information may be mediated, at least in part, by short-term synaptic mechanisms (Erickson, Maramara, & Lisman, 2010; Mongillo et al., 2008; Stokes, 2015). However, much of the current literature is still dominated by the persistent firing model and hence, it will necessarily frame some of the discussion for the remainder of this document.

1.2.2. Posterior Cortices

In addition to the PFC, WM function has been localized to posterior cortices including the posterior parietal cortex (PPC) and visual cortex. Evidence from parietal lesions in humans and non-human primates indicates that it is critical for WM (Berryhill & Olson, 2008; Chafee & Goldman-Rakic, 2000; Shallice & Warrington, 1970). The PPC and visual cortex also show activity related to WM function (Supèr, Spekreijse, & Lamme, 2001; Vogel & Machizawa, 2004). Interestingly, these areas exhibit activity related to capacity limits in WM (Vogel & Machizawa (2004). Although persistent activity has been observed in both the PFC and posterior cortices (notwithstanding current criticisms of the persistent activity model), it is thought to play different roles between these regions. For instance, one model holds that the PFC controls access to WM and limits the quantity of information that is stored whereas the PPC mediates the fidelity or quality of the information (Ku, Bodner, & Zhou, 2015). Other types of distinctions in the type of processing may exist between the PPC and PFC such as a where/how distinction in which the PFC is more involved in representing methods of interacting with stimuli, whereas the PPC is more involved in representing the locations of those stimuli (Borowsky et al., 2005).

1.2.3. Subcortical Nuclei

In addition to neocortical areas, a number of subcortical nuclei have been implicated in WM function. The mediodorsal thalamus (mdThal), like the PFC, exhibits sustained activity during the delay period of WM and may play a role in driving delay activity in the PFC (Watanabe & Funahashi, 2012). Similarly, the basal ganglia also play a critical role in WM and are thought to gate sensory input in order to filter WM content (McNab & Klingberg, 2008). Combined, the evidence indicates that WM function relies on thalamocortical and corticostriatal loops involved the PFC (Christophel et al., 2017).

1.2.4. Synaptic Mechanisms

At the level of individual neurons and synapses, WM function has been shown to rely on signalling through several neurotransmitter systems. WM is a highly distributed cognitive function and is likely not amenable to a cleanly circumscribed list of necessary neurotransmitter systems, but specific roles have been identified for glutamate, GABA, dopamine (DA), norepinephrine (NE), and acetylcholine (ACh). The roles of each are briefly summarized below.

1.2.4.1. Glutamate and GABA

Unsurprisingly, WM function relies on glutamate and GABA, the main excitatory and inhibitory neurotransmitters in the brain, respectively. Glutamate binds to a family of both ionotropic and metabotropic receptors (Willard & Koochekpour, 2013). For the purposes of this review, I will discuss the ionotropic glutamate receptors which have been heavily implicated in WM function. The ionotropic glutamate receptors AMPA and NMDA share a set of biochemical characteristics including a tetrameric structure and cation permeability in response to glutamate binding (Traynelis et al., 2010). AMPA receptors are the primary receptor mediating fast excitatory signal transduction and are also necessary for synaptic plasticity due to their essential role in the removal of the Mg^{2+} ion blocking NMDA Ca^{2+} pores (Greger, Watson, & Cull-Candy, 2017). NMDA receptors require the binding of both glutamate and glycine in order to function and have slower kinetics than AMPA receptors (Vyklicky et al., 2014; Wang, 1999).

During WM, AMPA and NMDA receptors each play somewhat dissociable roles. NMDA receptors, particularly those containing the GluN2B subunit, provide the substrate for persistent delay cell activity due to their slow kinetics (Arnsten & Jin, 2014; Floresco, 2013; Murray, Jaramillo, & Wang, 2017; Wang et al., 2013; Wang, 1999). In contrast, blockade of AMPA receptors has only a subtle effect on delay cell activity, but causes significant disruption of "response cells" which mediate behavioural responses to WM stimuli (Wang et al., 2013). As mentioned previously, the entire "persistent activity" model of WM has come under heavy criticism and alternative, synaptic mechanisms have been proposed (Erickson et al., 2010; Stokes, 2015). Some research has proposed that the GluR1 subunit of AMPA receptors may play a role in short-term potentiation during WM (Erickson et al., 2010).

GABA is the main inhibitory neurotransmitter in the mammalian brain. In the cortex, GABA is synthesized and released by GABAergic interneurons of which there are a number of subtypes (Krienen et al., 2019). GABA signals through two main receptors, $GABA_A$ and

$GABA_B$ (Wu & Sun, 2015). The $GABA_A$ receptor is an inhibitory ionotropic receptor consisting of 5 subunits which opens a chloride ion channel when activated by GABA or another agonist such as muscimol, hyperpolarizing the affected neuron and reducing the probability of action potentials (Chua & Chebib, 2017). The $GABA_B$ receptor is a metabotropic receptor consisting of 2 subunits and is coupled to the $G_{i/o}$ signalling pathway which has a downstream inhibitory effect on neuronal activity by inhibiting adenylyl cyclase and voltage-gated Ca^{2+} channels (Padgett & Slesinger, 2010). GABA signalling is thought to be required in order to filter information processed by pyramidal neurons during WM by providing lateral inhibition (Goldman-Rakic, 1995). In patients with schizophrenia, there is a reduction in parvalbumin-expressing GABAergic interneurons with a commensurate reduction in cortical gamma oscillations and WM performance (Gonzalez-Burgos, Cho, & Lewis, 2015).

1.2.4.2. Neuromodulators

The actions of several neuromodulators are critical for modulating the actions of glutamatergic and GABAergic cortical neurons during WM. These include, but are not limited to, dopamine (DA), acetylcholine (ACh), and serotonin (5-HT). Generally-speaking, these neuromodulators affect WM function, and the physiology of the PFC, in an inverted U-shaped dose-response manner.

Dopamine (DA) is a monoamine neuromodulator produced by cell bodies in the ventral tegmental area (VTA) and substantia nigra (Gantz, Ford, Morikawa, & Williams, 2018). Projections from DA neurons in the VTA reach the PFC via the mesocortical dopaminergic pathway in order to affect PFC-dependent cognitive function including WM (Haber, 2014). DA signals through a family of five different G protein-coupled receptors which are grouped into the D1 and D2 subcategories (Beaulieu & Gainetdinov, 2011). Generally speaking, D1 receptors are coupled to the G_s signalling pathway and stimulate cAMP and PKA while D2 receptors are coupled to $G_{i/o}$ and inhibit adenylyl cyclase (Beaulieu & Gainetdinov, 2011). Prefrontal DA signalling is necessary for normal cognitive function and WM specifically (Arnsten, Cai, Murphy, & Goldman-Rakic, 1994). Depletion of PFC DA causes impairments in WM which can be reversed through administration of L-Dopa (Brozoski, Brown, Rosvold, & Goldman, 1979) whereas high levels of DA can also impair WM (Arnsten, 2000). Signalling through DA receptors has been shown to play an important role in modulating the inhibition of cortical

pyramidal neurons by GABAergic interneurons (Seamans, Gorelova, Durstewitz, & Yang, 2001).

NE is secreted from neurons located in the locus coeruleus (Schwarz & Luo, 2015). The NE receptor with the most prominent importance for WM function is the α2-adrenergic receptor, which plays an important role in the regulation of dendritic spine excitability in prefrontal pyramidal neurons (Arnsten & Jin, 2014). Antagonism of α2-adrenergic receptors impairs WM in monkeys (Li & Mei, 1994). Additionally, pharmacologically enhancing signalling through this receptor subtype enhances WM function and alleviates WM deficits in aged monkeys (Arnsten & Goldman-rakic, 2013).

ACh is produced by neurons in an array of brain structures including the basal forebrain and habenula (Ren et al., 2011; Zaborszky, Pang, Somogyi, Nadasdy, & Kallo, 1999). Selective lesions of cholinergic basal forebrain neurons innervating the primate PFC cause a selective impairment in spatial WM while, interestingly, sparing decision making and long-term memory (Croxson, Kyriazis, & Baxter, 2011). With respect to specific ACh receptors, ACh signals through ionotropic nicotinic receptors and metabotropic muscarinic receptors (Picciotto, Higley, & Mineur, 2012). Agonism of the nicotinic subclass of ACh receptor has been shown to enhance WM function whereas antagonism impairs it (Levin & Rose, 1995).

5-HT is secreted by neurons whose cell bodies are located in the dorsal and median raphe nuclei (Hornung, 2003) and binds to at least 14 different receptor subtypes (Celada, Puig, & Artigas, 2013) which are all metabotropic with the exception of the 5-HT_3 receptor which is ionotropic (Barnes, Hales, Lummis, & Peters, 2009). Relatively little work has been conducted to elucidate the role of serotonin in WM and the serotonergic system is extremely complex due to the relatively diverse array of 5-HT receptors (Puig & Gulledge, 2011). Pharmacological activation of 5-HT_{2A} receptors improve WM function and enhance the spatial tuning of PFC neurons in primates (Williams, Rao, & Goldman-Rakic, 2002). Additionally, computational modelling has suggested that 5-HT modulates WM function according to an inverted U dose-response relationship (Cano-Colino, Almeida, & Compte, 2013; Cano-Colino, Almeida, Gomez-Cabrero, Artigas, & Compte, 2014).

1.3. Working Memory in the Rodent

In addition to the large primate WM literature reviewed above, a significant amount of research has been dedicated to researching WM in rodent models (Dudchenko, 2004;

Dudchenko, Talpos, Young, & Baxter, 2013). Much of this research recapitulates findings in primates. However, research in the rodent is warranted and necessary for several reasons.

As a matter of basic science, it is of interest to gain a comparative understanding of cognitive function across species in order to observe how the neurophysiology underlying phenomena like WM are shaped by the evolutionary history of a model species. Efforts in comparative neuroscience have encountered significant difficulty defining functional and anatomical homologies and analogies between primates and rodents. Compared to the primate neocortex, the rodent neocortex is, obviously, a much smaller volume, less differentiated (Uylings, Groenewegen, & Kolb, 2003), and contains fewer interneuron subtypes (Krienen et al., 2019). There are also significant inconsistencies in the literature in how the rodent PFC is defined, both in terms of anatomical boundaries and functional subdivisions which have likely further complicated the understanding of rodent PFC function (Laubach, Amarante, Swanson, & White, 2018).

Another obvious challenge lies in the fact that rodents cannot linguistically describe their internal states. Although it is a popular convention in the literature to discuss rodent memory with the implicit assumption that they possess explicit memories, this cannot be verified with the introspective methods that initially enabled the categorization of human memory. Thus, rodent WM cannot truly be operationalized as a *psychological* construct possessing phenomenological characteristics like a "visuospatial sketchpad". As such, WM in rodents is operationalized based on behaviour in tasks with rules designed to require mechanisms approximating WM. The phenomenology of memory during these tasks, from the rodent's perspective, may or may not correspond to that of human WM.

The need to develop our knowledge of rodent cognitive function and its neural substrates is made all the more urgent for pragmatic reasons. Rodent models offer a number of advantages over human and non-human primate research. They are relatively inexpensive and easy to use in large numbers relative to primates. Transgenic and viral technologies are also much further along in their development in rodents than in primates. These attributes make it possible to perform of wide array of sophisticated, highly specific manipulations of circuits underlying WM which would be comparatively cumbersome and expensive to perform in primates. For these reasons, rodent models now comprise a very large proportion of preclinical research, including with respect to disorders affecting WM (Dudchenko et al., 2013), so it is imperative that WM in the

rodent be thoroughly understood in order to maximize the translational value of rodent models. This effort is complicated to some extent by the fact that "WM" is defined differently in rodents than in humans (Dudchenko, 2004), placing emphasis on the importance of carefully understanding their behaviour in WM tasks.

1.4. Behavioural Models of Working Memory in the Rodent

Dudchenko (2004) provides a clear and efficient definition of WM in rodents. Rodent WM is generally defined as memory for representations of stimuli that is used within sessions, but not between sessions, to guide behaviour. The stimulus-response associations are also flexible meaning that between trials, responding to a given stimulus could be either correct or incorrect. Additionally, this memory ought to be vulnerable to interference. These criteria could be summed up to mean that rodent WM pertains to trial-unique memories held in a dynamic state that can be quickly discarded between trials and is vulnerable to distraction and interference. Assessment of WM in rodents is performed through a wide array of delayed response tasks (Dudchenko, 2004; Dudchenko et al., 2013).

1.4.1. Delayed Non-Match-to-Sample Tasks

One of the most common behavioural paradigms for assessing WM in rodents is delayed non-match-to-sample (DNMTS). In its basic form, DNMTS involves sample, delay, and test phases. During the sample phase, animals are presented with one or more stimuli. Following the delay phase, animals are presented with the original stimuli as well as a new stimulus. In order to recognize the new stimulus as being novel, animals must have retained a memory representation of the original sample stimuli. The basic DNMTS paradigm has been adapted for use with discreet stimuli such as objects and odours, but also applies to various maze paradigms in which animals must non-match to a spatial location or cue.

The most popular maze paradigms using DNMTS include T-maze alternation and the radial arm maze. In T-maze alternation, as the name suggests, animals navigate from the distal end of a t-shaped maze to two goal arms. In order to receive rewards, animals must alternate between goal arms on each successive trial, meaning that they must non-match to their previous choice of goal arm (Dudchenko, Wood, & Eichenbaum, 2000). The radial arm maze is also a test of WM for spatial positions but uses a radially shaped maze generally including 8 arms. Rats begin each trial in the centre of the maze and must refrain from re-entering previously-explored

arms (Olton, 1987). This paradigm expands on T-maze alternation by providing a measure of WM capacity due to the inclusion of more than 2 spatial locations. This ability to measure WM capacity is relatively unique and also leads to another unique attribute, which is the ability to measure serial position effects including both primacy and recency effects in rodents (Bolhuis & van Kampen, 1988; Dimattia & Kesner, 1984; Harper, Dalrymple-Alford, & McLean, 1992; Kesner & Novak, 1982). The radial arm maze has also been adapted to be submerged in water to provide an aversive, rather than appetitive, motivation for maze performance (Burešová, Bureš, Oitzl, & Zahálka, 1985).

DNTMS can also be performed using discreet, nonspatial stimuli such as objects or odours. In many cases, DNMTS is used to assess the spontaneous exploration of novel stimuli, exploiting the natural propensity of rats and mice to preferentially explore novel stimuli. Memory is operationalized as the ratio of time animals spend exploring the novel versus familiar stimuli. Spontaneous exploration tasks are extremely advantageous in that they can performed easily and quickly. The earliest example of this type of task was the now-prolific novel object recognition task developed by Ennaceur & Delacour (1988). This paradigm has been adapted to research in WM by using short delay periods on the order of seconds or tens of seconds which is well within the typical timescales associated with human WM in addition to being well within the cellular consolidation processes which would convert the memory from short- to long-term (Rudy & Sutherland, 2008).

DNMTS is often also performed as an operant conditioning task. Many variants of this paradigm have been used to assess WM in rodents. Mumby, Pinel, & Wood (1990) developed a DNMTS task based on one previously used in monkeys (Mishkin, 1978) in which rats are first presented with an identical set of sample objects followed by one of the sample objects and a novel object. Rats received a reward for selecting the novel object. The same procedure can be performed with odours rather than objects (Otto & Eichenbaum, 1992).

Although operant DNMTS often requires weeks or months of training, this paradigm has several other advantages. Because animals are motivated by reward, performance does not rely on the novelty of stimuli meaning that the same stimuli can be reused in different configurations to produce larger volumes of data. There is a conceptual advantage as well in that under these conditions, memories must necessarily rely on the trial-unique configuration of stimuli and animals cannot mediate their performance by searching for novelty.

A potential criticism of the DNMTS paradigm, in particular when using spatial location, is that impairments in this paradigm can reflect a failure of inhibition of return (IOR), the tendency of animals not to return their spatial attention to already-explored stimuli (Castel, Pratt, & Craik, 2003; Posner, Rafal, Choate, & Vaughan, 1985), rather than a failure of memory *per se*. Although inhibition of return is at least partially mediated by spatial WM (Castel et al., 2003), its presence as a parallel and partly non-overlapping form of processing may confound purely WM-based interpretation of the DNMTS paradigm. One way to control for IOR is to use non-spatial DNMTS tasks. Additionally, experiments can be performed using a delayed match-to-sample paradigm. This approach would complement the use of delayed non-match-to-sample by allowing the verification that impairments are not simply due to a failure to inhibit returning to a previously rewarded stimulus.

1.5. Circuit Mapping of Delayed Non-Match-To-Sample Tasks in Rodents

1.5.1. The Prefrontal Cortex

Like primates, rodents possess a prefrontal cortex (Uylings et al., 2003). Although there is less differentiation within the rat PFC than compared to the primate PFC, the rat medial PFC (mPFC) appears to share anatomical and functional similarities to the primate dlPFC (Uylings et al., 2003). The mPFC is a subregion of the PFC which includes the medial as well as orbital and lateral regions (Heidbreder & Groenewegen, 2003). Moving from dorsal to ventral, the mPFC has traditionally been subdivided into the medial precentral, anterior cingulate, prelimbic, and infralimbic areas on the basis of cytoarchitecture and development (van Eden & Uylings, 1985). More recent anatomical work also supports a dorsal-ventral anatomical and functional distinction based on patterns of connectivity with other brain regions (Heidbreder & Groenewegen, 2003). However, differences in the definition of the mPFC have led to confusion and incongruent findings in the past (Laubach et al., 2018).

In addition to the evidence from human and non-human primates, an extensive literature has demonstrated that the rodent PFC, specifically the mPFC, is critically involved in DNMTS tasks. Impairments following disruption of the mPFC have been found in various delayed non-matching-to-sample tasks spanning multiple sensory modalities. The mPFC is necessary for performance in spatial DNMTS tasks such as the radial arm maze (Kolb, Sutherland, & Whishaw, 1983) and delayed t-maze alternation (Dias & Aggleton, 2000), and other DNMTS

tasks using spatial positions such as levers (Auger & Floresco, 2017) and visuospatial touchscreen stimuli (Davies, Hurtubise, Greba, & Howland, 2017). Additionally, mPFC disruption impairs non-spatial DNMTS using odours (Davies, Molder, Greba, & Howland, 2013).

1.5.2. Interactions between PFC and Subcortical Nuclei

An additional large volume of research has been aimed at testing the role of interactions between the mPFC and other areas known to be involved in DNMTS tasks. The mPFC shares reciprocal connections with multiple subcortical nuclei important for WM function. These include, but are not limited to, the mediodorsal thalamus (mdThal) (Alcaraz, Marchand, Courtand, Coutureau, & Wolff, 2016; Gabbott, Warner, Jays, Salway, & Busby, 2005) and different subregions of the striatum (Gabbott et al., 2005).

The mPFC has also been shown to critically interact with both the dorsomedial (dmSTR) and ventral (vSTR) striatum. For example, the mPFC critically interacts with the vSTR is critical for spatial WM during navigation of the radial arm maze (Floresco, Seamans, & Phillips, 1997). The mPFC also engages in critical interactions with the dmSTR during DNMTS with odours (Davies, Greba, et al., 2017).

The mdThal sends dense projections to the mPFC (Alcaraz et al., 2016) and plays a vital role in learning and memory (Mitchell, 2015; Sébastien Parnaudeau, Bolkan, & Kellendonk, 2018). Historically, the role of the mdThal in rodent WM has been fraught with contradictory evidence (Wolff, Alcaraz, Marchand, & Coutureau, 2015). A number of previous studies have found impairments in WM following disruption of the mdThal and its connections with the mPFC (Burk & Mair, 1998; Floresco, Braaksma, & Phillips, 1999; Young, Stevens, Converse, & Mair, 1996), but the results of these studies may have been affected by confounding variables such as lesions encroaching on neighboring thalamic nuclei in addition to non-mnemonic effects (Wolff et al., 2015). More recent studies using optogenetics, which allows the visual verification of affected cells and projections, have provided more convincing evidence that the mdThal is involved in rodent WM. An increasingly popular view holds that the PFC works in concert with the mdThal in order to produce the prefrontal neuronal activity necessary to support WM function (Christophel et al., 2017; Sébastien Parnaudeau et al., 2018). Previous research has demonstrated that pharmacological disconnection of this circuit impairs WM in the radial arm maze (Floresco et al., 1999). More recently, several studies have used optogenetic methods to

demonstrate the involvement of this circuit in WM. Specifically, the mPFC-mdThal circuit has been shown to be essential for spatial WM in a t-maze task (Bolkan et al., 2017) as well as a visual and auditory WM task (Schmitt et al., 2017).

1.5.3. Posterior Parietal Cortex

The PPC has been a central focus of primate WM research, but relatively little research has been conducted on the role of the rodent PPC in WM (Bucci, 2009). The research conducted to-date shows a mixed story with respect to the role of the PPC in DNMTS. Disruption of the PPC does not impair DNMTS using objects (Kolb, Buhrmann, McDonald, & Sutherland, 1994). In contrast, performance in the radial arm maze has been shown to rely on the PPC (Espina-Marchant et al., 2006; Kolb et al., 1983). Thus, the PPC may play a specific role in spatial DNMTS tasks. However, results of PPC disruption in t-maze tasks have been inconsistent (McDaniel, Compton, & Smith, 1994; Wörtwein, Mogensen, & Divac, 1993).

1.5.4. The Medial Temporal Lobe

Several structures in the rodent medial temporal lobe (MTL) play a role in certain DNMTS tasks. The HPC is necessary for performance of several spatial DNMTS tasks including the radial arm maze (Floresco et al., 1997), T-maze alternation (Hallock, Arreola, Shaw, & Griffin, 2013), DNMTS with visuospatial touchscreen stimuli (Josey & Brigman, 2015), as well as non-spatial DNMTS with objects (Wood, Mumby, Pinel, & Phillips, 1993). In contrast, the HPC is not necessary for DNMTS with odours (Dudchenko et al., 2000). The overlying perirhinal (PRh) and entorhinal (EC) cortices are necessary for DNTS with odours (Otto & Eichenbaum, 1992) and objects (Mumby & Pinel, 1994).

1.6. Neural Correlates of the Odour Span Task and TUNL Task

My dissertation focuses on the circuit mapping of two DNMTS tasks in particular which our lab has directed significant attention to in recent years: the odour span task (OST) and the Trial-Unique, delayed-Non-matching-to-Location (TUNL) task. Below, I present a review of the current state of knowledge of the neural correlates of these two tasks.

1.6.1. The Odour Span Task

An additional behaviour task for assessing rodent WM is the OST, an incrementing DNMTS task using odours (Dudchenko et al., 2000). Schemata illustrating the basic procedure

used in the OST are shown in Figure 2.1A and Figure 3.2A. The OST is one of two main behavioural tasks that can be used to measure WM capacity in rodents, the other being the radial arm maze (Olton, 1987; Olton & Samuelson, 1976). The OST is unique in its ability to provide a measure of rodent non-spatial WM capacity for multiple items.

Performance of the OST critically relies on the function of several neurotransmitter systems. There is extensive evidence that glutamatergic signalling is critical for memory in the OST. In line with evidence that WM function relies on NMDA receptor signalling, several studies have shown that OST performance is disrupted by NMDA receptor blockade (Davies, Greba, & Howland, 2013; Galizio, Deal, Hawkey, & April, 2013b; Galizio et al., 2019; MacQueen, Dalrymple, Drobes, & Diamond, 2016; Rushforth, Steckler, & Shoaib, 2011). Genetic overexpression of the GluN2B subunit in the mouse forebrain enhances performance in the OST (Cui et al., 2011). Additionally, AMPA receptor signalling is necessary for intact performance (Davies, Greba, et al., 2013). The OST can also be impaired via agonism or positive allosteric modulation of GABA receptors (Galizio et al., 2016, 2017). Signalling through ACh receptors is also necessary for the OST as lesions of cholinergic neurons in the basal forebrain impair performance (Turchi & Sarter, 2000) as well as antagonism via scopolamine (Galizio, Deal, Hawkey, & April, 2013a). Administration of nicotine rescues ketamine-induced impairments in OST performance (Rushforth et al., 2011). Performance of the OST is also impaired by various stimulant drugs (Galizio et al., 2016; Hawkey, April, & Galizio, 2014).

The brain areas underlying OST performance have also received attention. Experiments in our lab have demonstrated that inactivation of the rat mPFC impairs performance in the OST (Davies, Greba, et al., 2017; Davies, Molder, et al., 2013). Recently, our lab published a paper showing that the population activity of neurons in the mPFC predicted performance in the OST (De Falco et al., 2019). There has also been research examining the roles of connections. For instance, connections between the mPFC and various subregions of the striatum are critical in several WM tasks. The subregions relevant to WM mainly include the dorsomedial striatum (dmSTR) and ventral striatum, the latter of which is often interchangeably termed the nucleus accumbens (NAc). Disconnection of the mPFC and the dmSTR impair performance in both the OST (Davies, Greba, et al., 2017). In contrast to the mPFC and dmSTR, the OST is not dependent on the HPC. Lesions to the HPC do not impair performance in the OST, but do impair a spatial version of the task (Dudchenko et al., 2000).

1.6.2. The TUNL task

Recently, there has been development of touchscreen-based, automated behavioural tasks to assess a variety of cognitive functions in rodents (Hvoslef-Eide, Mar, et al., 2015). The TUNL task is a touchscreen-based, automated visuospatial WM task (Oomen et al., 2013; Talpos, McTighe, Dias, Saksida, & Bussey, 2010). A schematic showing the basic procedure is shown in Figure 4.1A. The TUNL task is particularly advantageous because it allows simultaneous assessment of visuospatial WM as well as controlled manipulation and assessment of spatial pattern separation, the ability to distinguish between spatial patterns with varying degrees of dissimilarity. Impairments in pattern separation can be isolated from overall WM impairments by comparing treatment versus control performance specifically on the least dissimilar patterns. The task also includes "correction trials" in which animals are allowed to repeat incorrect choices until they change their response to a correct one which can allow for assessment of cognitive flexibility (Hurtubise et al., 2017).

Like the OST, performance of TUNL is impaired by blockade of glutamatergic signalling and is impaired by NMDA receptor antagonists (Hurtubise et al., 2017; Kumar, Olley, Steckler, & Talpos, 2015). Genetic deletion of the GluN2B subunit throughout the cortex and dorsal CA1 also impairs performance (Kenton, Castillo, Holmes, & Brigman, 2018). Performance is also improved following intra-PFC infusions of an α1-adrenergic agonist (Hvoslef-Eide, Oomen, et al., 2015) demonstrating that TUNL performance is modulated by noradrenergic signalling.

Like other DNMTS tasks, performance of TUNL is impaired following disruption of the mPFC (Davies, Hurtubise, et al., 2017; McAllister, Saksida, & Bussey, 2013). A number of experiments have also examined the role of the HPC in TUNL. Lesions of the HPC impair TUNL in a delay-dependent manner in addition to impairing pattern separation. As previously mentioned, genetic deletion of the GluN2B subunit from the CA1 subfield of the HPC impairs performance (Kenton et al., 2018). Connections between the dmSTR and mPFC, as well as NMDA receptors within this circuit, are necessary for TUNL performance (Davies, Hurtubise, et al., 2017). TUNL performance also depends on intact hippocampal neurogenesis (Carrica et al., 2019)

1.7. The Present Studies

1.7.1. Knowledge Gaps in the Understanding of DNMTS Neurocircuitry

Extensive previous research has mapped the circuits underlying DNMTS tasks in rodents. However, the circuits underlying performance of the OST and TUNL, two DNMTS tasks with unique advantages, is incomplete. A number of knowledge gaps persistent in our understanding of the neural correlates of these tasks.

As discussed previously, thalamocortical connections between the mPFC and mdThal in WM are now considered to be essential for WM function in general. However, the role of these connections in WM has been controversial in the past (Wolff et al., 2015) and has never been examined in the OST. Disruption of the mdThal during olfactory learning and memory has also been inconsistent (Courtiol & Wilson, 2015). Thus, it is of interest to determine the exact impairments in olfactory learning resulting from mdThal disruption, including during olfactory WM. Additionally, little work has been done to examine the role of this circuit in WM capacity outside of the radial arm maze (Floresco et al., 1999), so the role of this circuit in WM capacity for multiple items has not been examined for nonspatial stimuli. Although the OST uses the presentation of a series of multiple stimuli, no study to date has performed detailed examination of the position of error in the odour sequence. Distinct serial position effects have been demonstrated in rodents using the radial arm maze (Harper et al., 1992; Kesner & Novak, 1982).

The role of the rodent PPC in DNMTS tasks remains unclear and no studies to date have investigated the role of the PPC in either the OST or TUNL. Relative to other brain regions, the rodent PPC has received relatively little attention for its role in WM and what studies have been conducted have yielded dramatically discrepant results (Espina-Marchant et al., 2006; Kolb et al., 1994; McDaniel et al., 1994; Wörtwein et al., 1993). These discrepancies may be a result of the engagement of different sensory modalities which seems to contradict the more domain-general view of the PPC expressed in the primate literature (Eriksson, Vogel, Lansner, Bergström, & Nyberg, 2015). Additionally, the relative roles of glutamate receptor subtypes within this region have been understudied relative to the mPFC and STR.

1.7.2. Temporary Inactivation as a Method for Circuit Mapping

The principle method of disrupting activity in target brain regions that I rely on throughout the experiments in this dissertation is temporary inactivation. Temporary inactivation

is a technique generally using pharmacological inhibition, but also thermal inactivation in some cases (Lomber, 1999), to reversibly silence action potentials in a region of brain tissue and assess any resulting losses of function. The technique is often characterized as a "reversible lesion", but there are important differences between temporary inactivation and lesions such as the reduced ability for homeostatic neuroplastic adaptation to injury that is associated with a lesion. Animals are implanted with cannulae targeting a region of interest to allow the infusion of inhibitory drugs. Often, local anaesthetics that block voltage-gated sodium channels such as TTX or lidocaine are used to inactivate brain tissue including cell bodies and fibers of passage (Floresco et al., 1999; Klement, Past'alková, & Fenton, 2005). Alternatively, high concentrations of drugs that bind to synaptically-located inhibitory receptors can inactivate cell bodies while sparing fibers of passage (Martin & Ghez, 1999).

In this dissertation, I use a cocktail of muscimol and baclofen which are $GABA_A$ and $GABA_B$ agonists, respectively (Chua & Chebib, 2017; Padgett & Slesinger, 2010), in order to inactivate neurons in target regions. As previously discussed, GABA receptors play a critical role in cognition, including WM (Auger & Floresco, 2015, 2017), and drugs that modulate GABA signalling can modulate cognitive function in complex ways when administered at subanaesthetic doses (Galizio et al., 2016, 2017). However, it was not my goal in these experiments to investigate the cognitive role of GABA receptors. Rather, the purpose of using these drugs was to achieve a dramatic degree of inhibition in the target structures such that neuronal activity was temporarily silenced.

1.8. General Aims

The series of experiments presented in this dissertation aimed to address the above knowledge gaps pertaining to the circuits underlying the OST and TUNL. Broadly, I aimed to clarify the roles of thalamocortical and frontoparietal circuitry in rodent WM. To this end, I pursued the following specific aims:

1. **Characterize serial position and rehearsal effects in the OST and examine how they are mediated by the mPFC.**

Although our lab has previously shown that the mPFC is critically involved in the OST our analysis has been limited to examining only memory capacity and response latency, neglecting the phenomenon of serial position effects and how they may be modulated by mPFC

disruption. Moreover, no study to my knowledge has ever attempted to behaviourally characterize error position effects in the OST. I predicted that I would replicate our previous finding that the mPFC is critically involved in the OST. My examination of error position effects, on the other hand, was of a highly exploratory nature. Based on previous research finding serial position effects in the radial arm maze, I predicted that I would detect a pattern of errors matching serial position effects. Although no research to my knowledge has examined the effects of mPFC disruption on error position effects in rodents, I predicted that inactivating the mPFC would cause some change in error position effects given that the PFC mediates a large portion of WM function.

2. **Examine the role of the mdThal, and its connections with the mPFC, in the OST and how it mediates both olfactory WM capacity, error position, and rehearsal effects.**

Although previous research has examined the role of the mdThal and its connections with the mPFC in other DNMTS tasks, its role in the OST pertaining to memory capacity and serial position effects has not been examined. Given the inconsistent findings in research on the role of the mdThal in olfactory memory, I anticipated some probability that mdThal or disconnection from the mPFC would not severely affect the OST. Moreover, the effects of mdThal disruption on serial position effects, if any, were of an exploratory nature.

3. **Examine the role of the PPC in the OST.**

The rodent PPC has received little attention for its potential role in DNMTS tasks, and no previous study has examined its role in the OST. The results of PPC disruption during DNMTS tasks has been inconsistent. For the most part, the PPC appears involved in WM for spatial locations and not objects, but the studies using objects have not involved any measure of capacity. Thus, I initially predicted that the PPC may be necessary for the OST as it incorporates a more taxing capacity component. After finding a null result from PPC inactivation in the OST, all further analyses such as serial position effects were rendered moot. I instead aimed to verify the efficacy of my PPC inactivation protocol by testing rats in a positive control task, cross-modal object recognition, which has been shown to rely on the PPC (Winters & Reid, 2010).

4. **Examine the role of the PPC in TUNL.**

Following the null result of Aim 3, I aimed to determine whether the PPC was involved in the TUNL task. I predicted that, given previous research showing a role of the rodent PPC in spatial WM tasks, as well as a wealth of primate literature showing likewise, that disruption of the PPC would impair performance of TUNL. Following a positive finding of impairment in TUNL from PPC inactivation, I further aimed to characterize the relative roles of intra-PPC ionotropic glutamate receptors in TUNL. Given the ample previous research showing critical involvement of NMDA receptors in WM function, I predicted that NMDA receptor blockade in the PPC would severely impair TUNL performance.

2. EXPERIMENT 1: RELATIVE ROLES OF THE MEDIAL PREFRONTAL CORTEX, MEDIODORSAL THALAMUS, AND THEIR COMBINED CIRCUIT FOR PERFORMANCE OF THE ODOUR SPAN TASK IN RATS: ANALYSIS OF MEMORY CAPACITY AND FORAGING BEHAVIOUR

The content in this chapter has been accepted for publication in *Learning & Memory* and is in press as of the date of submission of this dissertation. My specific contributions to this work included the training and supervision of coauthors performing behavioural training/testing as well as histology. I also performed a large proportion of intracranial drug infusions. I statistically analyzed all behavioural and histological data including designing the analysis strategy for the quantification of errors and foraging effects. Finally, I was the primary author and completed the majority of manuscript preparation, preparation of figures, and responses to reviewers. The following additional authors made significant contributions to this work: Nadine K. Zabder assisted in performing behavioural testing and intracranial infusions, Quentin Greba assisted in performing stereotaxic surgery, John G. Howland assisted in experimental design and manuscript preparation, and Max C. Liu, Nimra B. Tahir, and Yuanyi Song assisted in behavioural testing. Nimra Tahir also assisted in the quantification of errors and foraging.

2.1. Abstract

Working memory (WM), the capacity for short-term storage of small quantities of information for immediate use, is thought to depend on activity within the prefrontal cortex. Recent evidence indicates that the prefrontal neuronal activity supporting WM is driven by thalamocortical connections arising in mediodorsal thalamus (mdThal). However, the role of these connections has not been studied using olfactory stimuli leaving open the question of whether this circuit extends to all sensory modalities. Additionally, manipulations of the mdThal in olfactory memory tasks have yielded mixed results. In the present experiment, we investigated the role of connections between the rat medial prefrontal cortex (mPFC) and mdThal in the odour span task (OST) using a pharmacological contralateral disconnection technique. Inactivation of either the mPFC or mdThal alone both significantly impaired memory performance in the OST, replicating previous findings with the mPFC and confirming that the mdThal plays an essential role in intact OST performance. Contralateral disconnection of the two structures impaired OST performance in support of the idea that the OST relies on mPFC-mdThal connections, but ipsilateral control infusions also impaired performance, complicating this interpretation. We also performed a detailed analysis of rats' errors and foraging behaviour and found a dissociation between mPFC and mdThal inactivation conditions. Inactivation of the mdThal and mPFC caused a significant reduction in the number of approaches rats made per odour, whereas only mdThal inactivation or mPFC-mdThal disconnection caused significant increases in choice latency. Our results confirm that the mdThal is necessary for performance of the OST and that it may critically interact with the mPFC to mediate OST performance. Additionally, we have provided evidence that the mPFC and mdThal play dissociable roles in mediating foraging behaviour.

2.2. Introduction

Working memory (WM) is the ability to maintain small amounts of information over a brief delay period for use and manipulation. The prefrontal cortex (PFC), particularly the primate dorsolateral and rodent medial (mPFC) prefrontal cortices, has been shown to play a critical role in maintaining WM information over a delay period (Kamigaki & Dan, 2017). The physiological mechanism by which prefrontal neurons maintain stimulus representations over a delay is a contested issue (Lundqvist et al., 2018; Silvanto, 2017; Stokes, 2015), but a large body of evidence has led to the popular view is that the information held in WM is encoded in the form of prefrontal activity manifested either in persistent firing of individual neurons (Fuster & Alexander, 1971) or transient activity of groups of neurons that collectively "tile" the delay period (Constantinidis et al., 2018).

In addition to cortical contributions to WM function, recent work has begun to highlight the critical role played by subcortical structures. The mediodorsal thalamus (mdThal) plays a significant role in learning and memory (Mitchell, 2015; Mitchell & Chakraborty, 2013; Sébastien Parnaudeau et al., 2018) and has connections with the prefrontal cortex that also play an important role in learning and memory (Alcaraz et al., 2016; Mitchell, 2015). Deficits in these connections are also present in psychiatric disorders. For instance, functional connectivity between the PFC and thalamus, as well as WM performance (Lee & Park, 2005), is reduced in people with schizophrenia (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). However, the role of the mdThal in WM has been historically controversial (Wolff et al., 2015) with some studies showing minimal effects of mdThal lesions on WM (Alexinsky, 2001; Zhang, Burk, Glode, & Mair, 1998), only transient impairments (Mumby, Pinel, & Dastur, 1993), and impairments that could have resulted from damage to adjacent thalamic nuclei (Young et al., 1996) demonstrating that the mdThal may be only be selectively involved in certain tasks or non-WM aspects of task performance (Wolff et al., 2015).

Several recent and prominent studies have demonstrated that WM performance as well as persistent activity during WM is supported by thalamocortical loops between the PFC and mdThal (Bolkan et al., 2017; Bray, 2017; Floresco et al., 1999; Guo et al., 2017; Sebastien Parnaudeau et al., 2013; Schmitt et al., 2017). Bolkan et al (2017) optogenetically inhibited projections from the mdThal to the mPFC of mice during a delayed non-match-to-sample T-maze task and found that spatial WM was impaired when these connections were inhibited

specifically during the delay period. Schmitt et al (2017) optogenetically inhibited mdThal projections to the mPFC during a task in which mice were required to use an auditory cue presented before a delay to use one of two "rules" to select either a visual or auditory stimulus for a reward. Optogenetic inhibition of the mdThal during the delay period impaired performance in addition to dampening rule-selective responses in the PFC. Collectively, these recent studies convincingly show a role for mdThal projections to the mPFC in maintaining WM representations over a delay.

However, no study has yet examined the role of PFC-thalamus connections in an olfactory WM task. The odour span task (OST) is an incrementing delayed-non-match-to-sample (DNMTS) task with olfactory stimuli which is used in rodents to measure olfactory WM capacity (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013; Dudchenko et al., 2013, 2000; Scott, Zabder, Greba, & Howland, 2018). Previous work in our lab has shown that performance of the OST relies on the mPFC and dorsomedial striatum (Davies, Greba, et al., 2017; Davies, Molder, et al., 2013). We have also recently found that delay period spiking of mPFC neurons is predictive of WM performance in the OST (De Falco et al., 2019), showing an involvement of delay period activity in the mPFC. However, we found in a separate experiment that performance of the OST is independent of the parietal cortex (Scott et al., 2018), in contrast to ample previous research showing an important role for the PC in WM. This result brings into question whether other circuits well known to be involved in WM might be unnecessary for olfactory WM.

Moreover, there remains considerable doubt regarding exactly how the mdThal itself participates in olfactory perception, learning, and memory. The mdThal receives olfactory projections (Powell, Cowan, & Raisman, 1965) and has been referred to as the "olfactory thalamus" (Courtiol & Wilson, 2015, 2016), but the precise role of the mdThal in olfactory processing remains unclear (Courtiol & Wilson, 2015). Neurons in the mdThal show single unit activity and increased expression of c-Fos in response to olfactory stimuli (Courtiol & Wilson, 2014; Fredericksen, McQueen, & Samuelsen, 2019) as well as task-related activity during olfactory discrimination (Courtiol & Wilson, 2016). However, lesions targeting the mdThal do not cause anosmia in rats (Eichenbaum, Shedlack, & Eckmann, 1980), and results involving the effects of mdThal lesions on odour discrimination have produced mixed findings in which impairments are typically seen in more difficult odour discriminations, are more severe with larger lesions, and manifest as a slower learning curve which can be overcome with intensive

training (Eichenbaum et al., 1980; Slotnick & Risser, 1990; Staubli, Schottler, & Nejat-Bina, 1987). With respect to the role of the mdThal in olfactory learning and memory, lesions of the mdThal impair odour reversal learning (McBride & Slotnick, 1997; Slotnick & Kaneko, 1981; Slotnick & Risser, 1990; Staubli et al., 1987), but produce discrepant results in olfactory DNMTS (Koger & Mair, 1994; Zhang et al., 1998).

In light of the discordant literature regarding the role of the mdThal in WM and in olfactory processing, it stands as an open question whether performance of the OST relies on the mdThal-mPFC circuit. In the present experiment, we sought to investigate the role of interactions between the mdThal and the mPFC in rats during the OST. We examined the behavioural effects of mPFC or mdThal inactivation alone, as well as connections between the two structures. In order to test the role of mdThal-mPFC connections, we used a contralateral disconnection technique which has been previously used to investigate the roles of interareal connections while preserving partial function in each targeted structure (Davies, Greba, et al., 2017). Additionally, we performed a detailed analysis of rats' errors and foraging behaviour in order to further distinguish the types of impairments caused by disruption of each structure. To the best of our knowledge, these types of behavioural analyses have not been reported for the OST to date.

2.3. Materials and Methods

2.3.1. Subjects

Nineteen male Long-Evans rats (Charles River Laboratories, Kingston, NY) weighing 300-500g were used. Rats were individually housed in standard ventilated cages and kept on a 12-hour light/dark cycle (lights on at 0700). Individual housing was used throughout the experiment to prevent cage mates from tampering with one another's surgical implants and to preclude the possibility of feeding complications such as dominant cage mates depriving submissive cage mates of food. Rats were maintained at 85-90% of their free-feeding weight with water available *ad libitum*. All experiments were approved by the University of Saskatchewan Animal Research Ethics Board and conformed to the guidelines of the Canadian Council on Animal Care.

2.3.2. Odour Span Task

The OST was performed in a similar fashion to previous experiments from our lab (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013; Murray, Davies, Molder, & Howland, 2017;

Scott et al., 2018) and used a “lid-flipping” procedure adapted from MacQueen, Bullard, & Galizio (2011). Training was conducted on a table (plywood painted black; 0.84 m^2) with a 2.5 cm high border around the edges and elevated 95 cm above the floor with a metal frame and casters. Between trials, the rats were placed in an opaque Plexiglas enclosure (32 cm W x 50 cm H x 35 cm D) located on one side of the table. During trials, the rats were let out of the enclosure and allowed to explore the table. Rewards were presented in plastic Dixie cups (59 mL) containing a small amount of sand with 2 reward pellets (45 mg rodent purified dustless precision pellets; Bio-Serv, Flemington, NJ) sitting on top of the sand. The portion cups were covered with scented, opaque plastic Dixie cup lids sitting loosely on top of them. The table was lined with 24 holes (5.5 cm diameter) drilled into the plywood tabletop along the borders of the open field 3.5 cm from the table edge and 7.5 cm from one another. During testing, the portion cups were placed inside of these holes. The lids were scented with odours by storing them in Tupperware containers containing various spices (Allspice, Anise, Basil, Cacao, Caraway, Celery Salt, Cinnamon, Clove, Coffee, Cumin, Dill, Fennel, Garlic, Ginger, Lemon, Marjoram, Mustard, Nutmeg, Onion, Orange, Oregano, Paprika, Sage, and Thyme). Lids were replaced each time rats made physical contact with them in order to prevent rats from mediating their performance by leaving olfactory cues on the stimuli.

Training consisted of 3 phases: The first stage was initial shaping, in which rats were habituated to the table and trained to flip lids off the cups to obtain a reward. This was done by first presenting an open cup with a reward and, after each correct trial (the rat successfully obtaining the reward within 5 min), an unscented lid was placed on the cup covering incrementally more of the opening (1/2 covered, ¾ covered, fully covered). This stage continued with each rat receiving 3 trials per day until they could successfully obtain 3 rewards in one day from fully covered cups.

The second phase of training was delayed nonmatching-to-sample (DNMTS) training. This stage was conducted by presenting a cup with a sample odour and allowing the rat to obtain the reward. After a delay of approximately 40 s (the time between stimulus presentations), a second cup containing a reward with a novel odour (S+) along with an unbaited cup with the original sample odour (S-) were placed on the table. Importantly, the locations of the novel and familiar odours were shuffled to remove any spatial cues from the task and to prevent rats from visually tracking where the novel odour was placed on the table. Rats were given 6 trials per day

(with an intertrial interval (ITI) of 60 s) of DNMTS until they performed with 80-100% accuracy for 3 out of 4 consecutive days with the 4th day being no less than 50% accuracy, or until 10 days of DNMTS training had elapsed, at which point rats were moved to OST training regardless of whether they had reached criterion in DNMTS Rats that were slow learners in DNMTS learned the OST after extensive training even if they had not mastered DNMTS within 10 days.

An illustration of the basic procedure used in the OST is shown in Figure 2.1. The OST was performed in the same manner as DNMTS; however, after each correct choice, an additional odour was added to the table in serial fashion until the rat made an error at which point that particular trial was ended and an ITI began before commencing the next trial. As with DNMTS, the locations of the odours were thoroughly shuffled during the ~ 40 s delay to remove spatial cues from the task and prevent visually-guided mediating strategies. Each rat was trained in this task for a maximum of 3 trials per day (with no repetition of odours across different trials) or a maximum of 30 min, whichever criterion was met first. Rats were trained for 6-7 weeks ~5 times/week before being tested with infusions. During training, the span performance, errors, and latency to make a choice were recorded by hand, and latency was tracked manually with a stopwatch. Additionally, video recordings of OST sessions were obtained using a camera located above the arena.

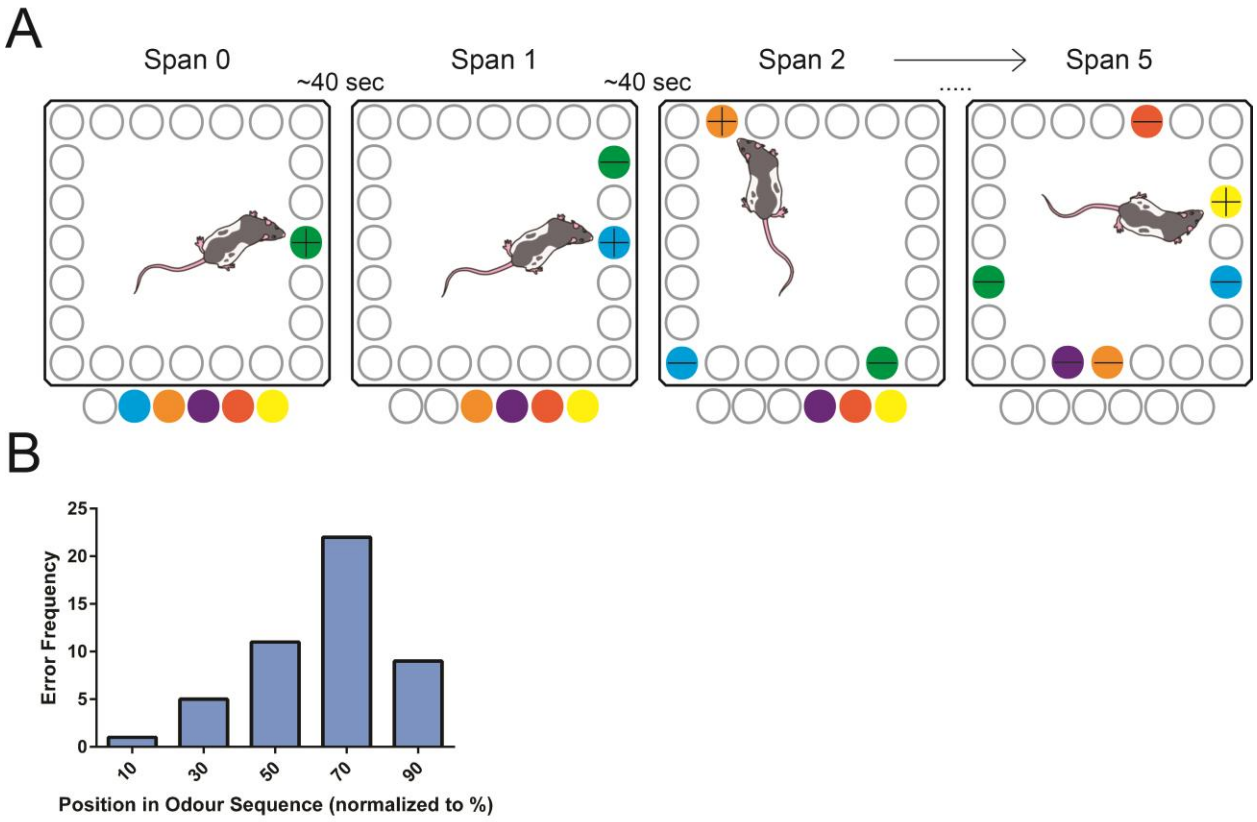

Figure 2.1. A) Illustration showing the basic procedure used in the OST. The OST is an incrementing delayed non-matching-to-sample task using olfactory stimuli that is used as a measure of olfactory WM capacity in rodents (Dudchenko et al., 2013). B) Frequency histogram of errors in the OST as a function of percentage of progress through the sequence of presented odours compiled from pooled data from Saline-infused rats. The OST exhibits a striking pattern of error frequency as a function of the sequential position of the incorrectly chosen odour in odour presentations. Incorrectly chosen odours are typically those located approximately 70% of the way through the odour sequence, while odours occurring nearer the beginning or end of the sequence are less likely to be incorrectly chosen.

2.3.3. Stereotaxic Surgery and Intracranial Infusions

Intracranial cannulae implantation as well as the basic infusion procedure were conducted in the same manner described in our previous papers (Davies, Greba, et al., 2013; Davies, Hurtubise, et al., 2017; Scott et al., 2018). Briefly, rats were implanted with 2 sets of 23-gauge stainless steel guide cannulae with 1 set aimed bilaterally at the mPFC (AP +3.00 mm; ML ±0.70 mm; DV -3.20 mm) and another set aimed bilaterally at the mdThal (AP -2.90 mm; ML ±0.70

mm; DV -5.00 mm). Before infusions were conducted, rats were habituated to the infusion procedure for 3 consecutive days. Habituation consisted of bringing rats into the infusion room, removing and replacing their dummy stylets, and performing "sham" infusions in which a shortened infusion needle was inserted into the guide cannulae without piercing the target area and the infusion pump (Harvard Apparatus, Holliston, MA, USA) was run. On infusion days, rats received infusions of combined muscimol and baclofen (M/B; each 0.5 mg/mL, mixed together in a 1/1 ratio (Davies, Molder, et al., 2013; Scott et al., 2018)) at a rate of 0.3 µL/min to a total infusion volume of 0.3 µL. The infusions were conducted using 30-gauge stainless steel infusion needles connected to Hamilton syringes with PE50 tubing. The tips of the infusion needles extended 1 mm past the tips of the guide cannulae when fully inserted. Infusions into the mdThal were performed with M/B diluted to half the concentration of that used for the mPFC (0.25 mg/mL) due to our observation that the 0.5 mg/mL concentration used in the mdThal frequently resulted in severe locomotor impairments characterized by mild ataxia and hypotonia with a drastic reduction in exploratory behaviour. Contralateral disconnections were performed according to the general procedure used in Davies et al. (Davies, Greba, et al., 2017). M/B was infused unilaterally into contralateral sides of the mPFC and mdThal simultaneously, leaving half of each structure undisturbed, but incapable of communicating with the other structure within hemispheres. The hemisphere (left or right) was counterbalanced between rats. Ipsilateral control infusions were performed by unilaterally infusing M/B into ipsilateral sides of the mPFC and mdThal simultaneously in order to control for potential effects of unilateral inactivation.

Infusions were conducted in a semi-counterbalanced fashion over a period of 7 days in the following order: 1) Two days of mdThal infusions in which rats received Saline or M/B in counterbalanced order; 2) Two days of mPFC infusions with counterbalanced Saline and M/B conditions; 3) Two days of contralateral disconnections in counterbalanced order, and; 4) One day of ipsilateral control infusions. This protocol resulted in a total of 7 infusions per rat with up to 4 infusions conducted at any one infusion site. Although a portion of the subjects were administered infusions on consecutive days, we interspersed infusion days with washout days involving normal OST training in a subset of rats and observed no significant loss of performance on the washout days. We have used this general protocol in previous experiments involving pharmacological disconnection (Davies, Greba, et al., 2017; Davies, Hurtubise, et al., 2017).

2.3.4. Perfusions and Histology

After the conclusion of behavioural testing, rats were perfused intracardially with either saline (0.9%) followed by 30% formalin or with a solution of formalin, glacial acetic acid, and methanol (FAM). Although two different methods of perfusion were used, we expected there to be no confound caused by the difference in perfusion procedure as we were examining only cannulae placement and did not perform any volumetric analyses. Before sectioning, brains were transferred to a cryoprotectant solution of 0.1% sodium azide and 30% sucrose and left until they were no longer buoyant. Brains were sectioned coronally at 40 µm on a freezing sliding microtome (Leica) and the sections were mounted to glass slides. Assessment of infusion placements was performed by comparing mounted sections to a rat brain atlas (Paxinos & Watson, 2006).

2.3.5. Analysis of Behaviour

2.3.5.1. *Span Capacity*

Memory capacity in the odour span task was defined as the mean number across trials of odours rats could reach minus the first odour presented and the final odour if the rat made an error on that trial, resulting in a "mean span" variable. Additionally, the variable "best trial" was used to express span capacity on rats' best trial during a given testing session.

2.3.5.2. *Choice Latency*

Choice latency was measured from the beginning of a trial iteration to when the rat made a choice (flipped a lid off of a cup) and reported as an average across all trial iterations for a given session.

2.3.5.3. *Error Position and Foraging*

In addition to our analysis of span capacity and latency, we sought to interrogate more subtle effects on rats' exploration of odours, by quantifying the number of times rats "approached" each odour. An "approach" was operationally defined as any instance in which a rat's nose was within ~2 cm of an odour cup with a heading within ~180° of the odour cup without displacing the lid and was identified visually by human raters. A rat's nose entering this zone and leaving was counted as a single approach irrespective of how long the rat's nose remained within the zone and a second approach was not counted until the rat's nose left the zone and then subsequently re-entered it.

We measured several variables related to rats' foraging and errors: 1) As shown in Figure 2.1B, our particular variant of the OST exhibits a striking "error frequency curve" in which rats under control conditions most often make errors by incorrectly choosing odours that are presented ~60-70% of the way through the odour sequence. We compared the mean error position (mEP) by standardizing the position of "error odours" (the odour that was incorrectly chosen) as a percentage of progress through the full sequence of presented odours. For instance, if a rat made an error during the 4th trial iteration by incorrectly choosing the 2nd odour from the sequence, the serial position of this error would be 50%; 2) We quantified what percentage of total odour approaches was comprised by approaches to the error odour (%AppE) as a measurement of how much rats were benefitting from repeated "rehearsal" of familiar odours in the sequence; 3) We quantified the average number of approaches per odour (App/O) in the sequence as a measure of how much rats were revisiting/comparing between odours in the sequence. Sessions in which rats achieved a Best Trial of less than 2 were excluded from these analyses case-wise because of the extremely restricted variability in serial position on span lengths any shorter than this. Additionally, some sessions contained no errors and thus could not be used for any error analysis but were included in the analysis of App/O. Unfortunately, the location of the error odour was also not clear in a small subset of videos, making these unusable for the %AppE analysis.

2.3.6. Statistical Analysis

Mean span and Best Trial were analyzed as 1-way repeated measures ANOVAS with sham infusion performance (taken 1 day prior to beginning of infusions), saline infusion performance, and M/B infusion performance. Choice Latency was analysed using dependent t-tests for mPFC and mdThal bilateral inactivations comparing only Saline and M/B conditions, whereas a 1-way repeated measures ANOVA was used to analyse the disconnection experiment to compare Saline, Ipsilateral Control, and Disconnection groups. Due to unequal sample sizes in the mEP, %AppE, and App/O analyses, the Saline and M/B conditions were treated as a between-subjects, rather than paired, conditions and were analysed using independent t-tests in the case of mPFC and mdThal inactivations and using 1-way between-subjects ANOVAS for the disconnection experiment. The distribution of data within conditions frequently violated assumptions of normality (as determined by D'Agostino-Pearson tests) and homogeneity of variance (as determined by Bartlett's tests), particularly in the inactivation conditions, so

applicable nonparametric tests were performed and those results reported in cases where the outcome differed from the parametric tests.

2.4. Results

2.4.1. Cannulae Placements

All rats had infusion sites acceptably within the mPFC and mdThal (Figure 2.2). Infusions in the mPFC mainly targeted the prelimbic area. All rats had damage to the cortex overlying the mPFC infusion sites as well as damage to the cortex and dorsal hippocampus overlying the mdThal.

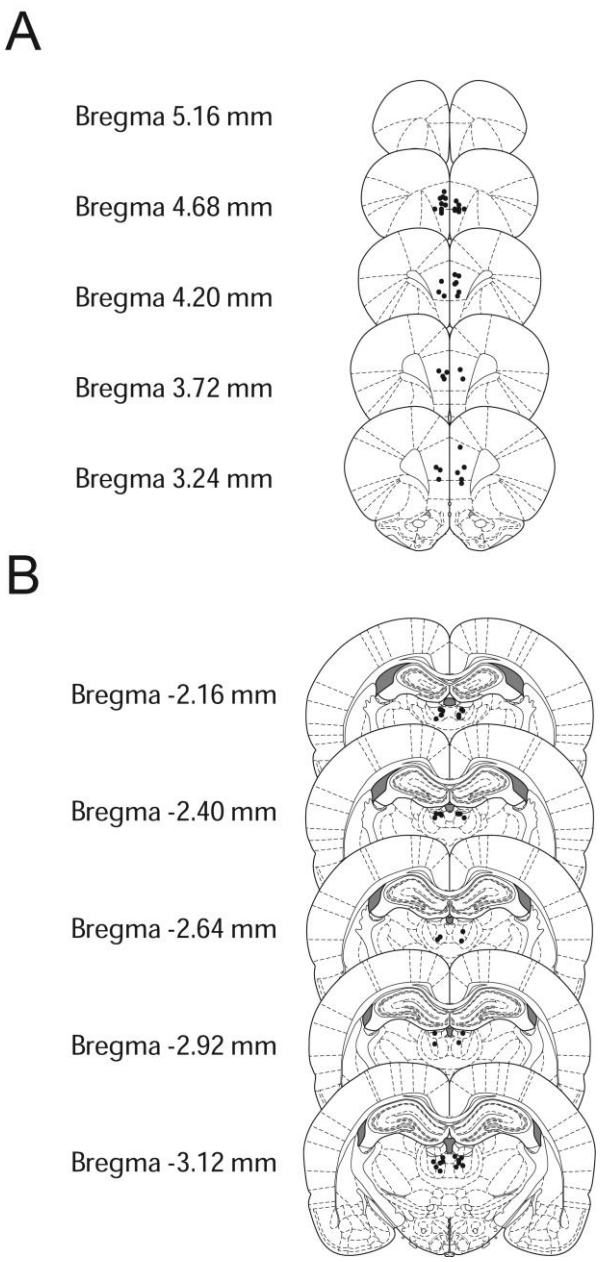

Figure 2.2. Illustrations showing the approximate locations of infusions into the mPFC and mdThal. Illustrations were adapted from Paxinos & Watson (2006).

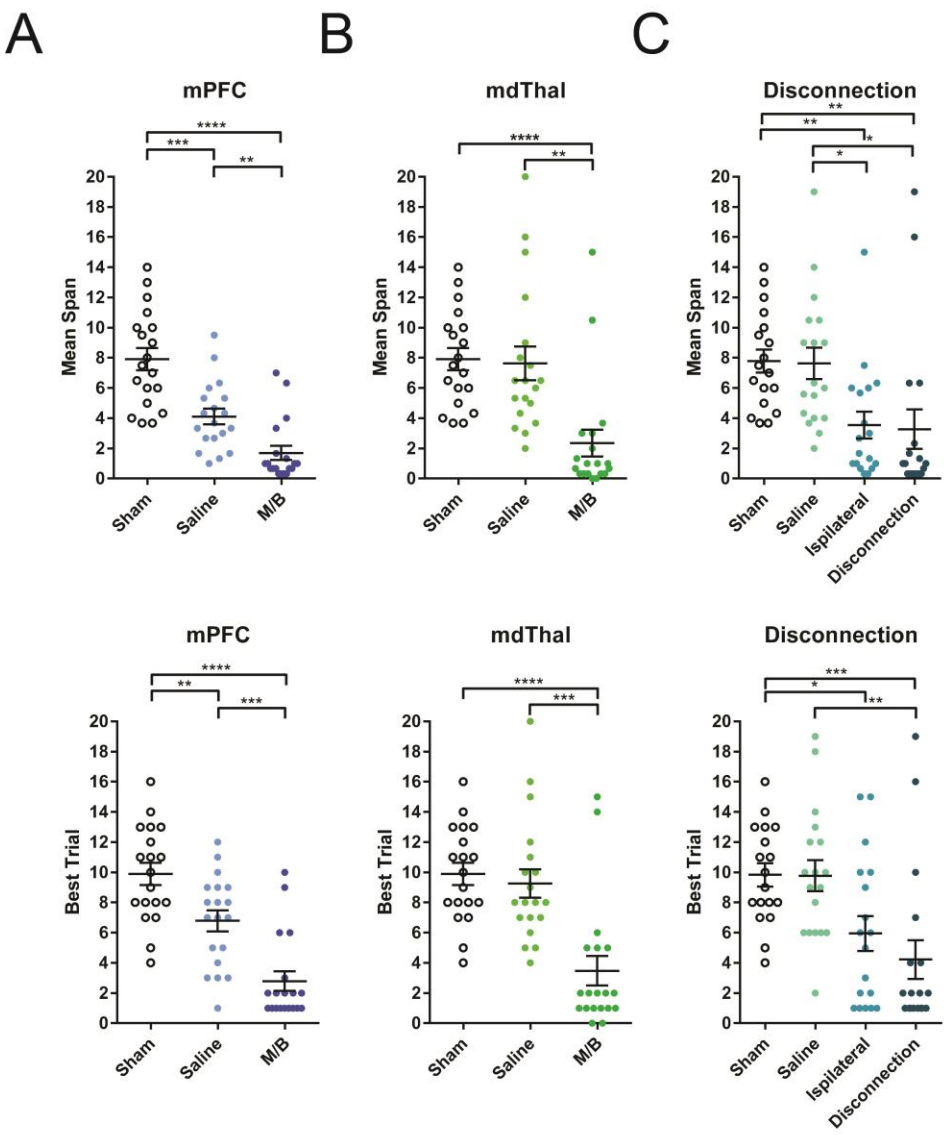

Figure 2.3. A) Mean (±SEM) Span and Best Trial after Sham, Saline, and after M/B infusions into the mPFC. Infusions of M/B into the mPFC significantly impaired Mean Span and Best Trial, replicating previous findings that the mPFC is critical for OST performance. Interestingly, Saline infusions also caused a significant impairment relative to Sham infusions, demonstrating that the Saline control is not, itself, entirely inert; B) Mean (±SEM) Span and Best Trial after Sham, Saline, and M/B infusions into the mdThal. Infusions of M/B into the mdThal significantly reduced both Mean Span and Best Trial, demonstrating that the mdThal is critical for intact OST performance. C) Mean (±SEM) Span and Best Trial after Saline infusions, Ipsilateral M/B infusions, or disconnection of the mPFC and mdThal. Disconnection caused a significant impairment in both Mean Span and Best Trial, whereas Ipsilateral infusions caused a

significant impairment in Mean Span and Best Trial. The results are not inconsistent with the interpretation that mPFC-mdThal connections are necessary for the OST, but the impairment in the Ipsilateral control condition means other explanations cannot be ruled out.

2.4.2. Inactivation of the medial prefrontal cortex

2.4.2.1. *Memory Performance*

Mean (±SEM) Span and Best Trial for mPFC inactivations are shown in Figure 2.3A. A repeated measures 1 -way ANOVA conducted on Mean Span between Sham performance, Saline, and M/B revealed a significant main effect of Drug treatment ($F_{(2,56)} = 35.44$, $p<.0001$). Tukey-corrected multiple comparisons revealed that mean span was significantly lower in the M/B condition than in the Sham condition ($p <.0001$) and the Saline condition ($p <.01$). Interestingly, saline-treated rats were significantly worse than the Sham condition ($p <.005$). A repeated measures 1 -way ANOVA conducted on Best Trial between Sham performance, Saline, and M/B revealed a significant main effect of Drug treatment ($F_{(2,56)} = 33.83$, $p <.0001$). Tukey-corrected multiple comparisons revealed that mean span was significantly lower in the M/B condition than in the Sham condition ($p <.0001$) and the Saline condition ($p <.001$). Best trial in the Saline condition was also significantly lower than in the Sham condition ($p <.01$).

2.4.2.2. *Error Position and Foraging Effects*

Mean (±SEM) Choice Latency, mEP, %AppE, and App/O after Saline or M/B infusions into the mPFC are shown in Figure 2.4. A dependent t-test revealed no significant difference in Choice Latency between Saline and M/B infusions ($t_{(18)} = 1.1$, $p = .29$). After filtering by exclusion criteria for our error analysis, the sample size was n = 18 for Saline and n = 10 for M/B for the mEP and App/O analyses and, for the %AppE analysis, n = 17 for Saline and n = 10 for M/B. Independent t-tests revealed no statistically significant differences between Saline and M/B on mEP ($t_{(26)} = 0.59$, $p = .56$) or %AppE ($t_{(25)} = 1.66$, $p = .11$). In contrast, mean App/O was significantly lower in the M/B condition than the Saline condition ($t_{(26)} = 3.06$, $p<.01$), indicating that in the M/B condition, rats made significantly fewer approaches to each odour.

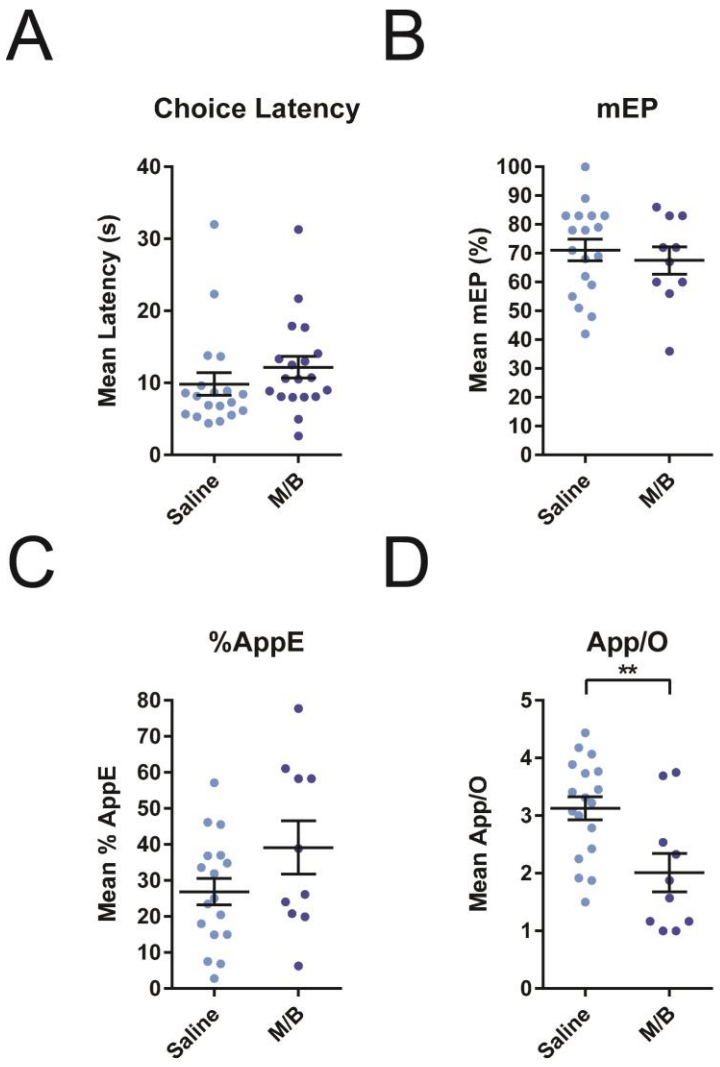

Figure 2.4. A) Mean (±SEM) Choice Latency following infusions of Saline or M/B into the mPFC. Rats' choice latency was unaffected by mPFC inactivation. B) Mean (±SEM) mEP following infusions of Saline or M/B into the mPFC. There was no shift in the serial position of errors caused by mPFC inactivations. C) Mean (±SEM) %AppE following infusions of Saline or M/B into the mPFC. Inactivation of the mPFC did not cause a statistically significant change in the percentage of total odour approaches accounted for by the error odour. D) Mean (±SEM) App/O following infusions of Saline or M/B into the mPFC. Inactivation of the mPFC caused rats to make significantly fewer approaches per presented odour.

2.4.3. Inactivation of the mediodorsal thalamus

2.4.3.1. *Memory Performance*

Mean (±SEM) Span and Best Trial are shown in Figure 2.3B. A repeated measures 1 - way ANOVA revealed a significant main effect of Drug treatment ($F_{(2,56)} = 13.92$, $p < .0005$). Tukey-corrected multiple comparisons revealed that mean span was significantly lower in the M/B condition than in the Saline ($p < .005$) or Sham ($p < .0001$) conditions and the Saline and Sham conditions were not significantly different ($p = .98$). A 1-way repeated measures ANOVA revealed a main effect for Best Trial ($F_{(2, 56)} = 18.98$, $p < .0001$). Tukey post hoc tests revealed that rats' best trial in the M/B condition was significantly lower than both the Saline condition ($p < .001$) and the Sham Infusion condition ($p < .0001$) while there was no difference between the Saline and Sham Infusion conditions ($p < .84$).

2.4.3.2. *Error Position and Foraging Effects*

Mean (±SEM) Choice Latency, mEP, %AppE, and App/O for mdThal inactivations are shown in Figure 2.5. Choice Latency following mdThal infusions were found to have A dependent t-test on revealed that rats had significantly higher choice latency in the M/B condition than the Saline condition ($t_{(18)} = 3.04$, $p < .01$). Due to this significant increase in Choice Latency, we sought to determine whether the impaired mean span may be related to the increased latency. The relationship between Mean Span and Choice Latency is shown in Figure 2.6A. Due to the fact that the distribution of choice latencies following mdThal inactivation violated normality ($K2 = 7.51$, $p < .05$), a Spearman correlation was conducted and revealed that Mean Span was significantly negatively correlated with Choice Latency ($r_s = -.57$, $p < .05$). After applying exclusion criteria to the error analysis, the sample size was n = 17 for Saline and n = 11 for M/B for the mEP analysis, Saline = 14 and M/B = 11 for the %AppE analysis, and Saline = 19 and M/B = 11 for the App/O analysis. Independent t-tests revealed no significant change in mEP ($t_{(26)} = 1.86$, $p = .07$) or %AppE ($t_{(23)} = 1.74$, $p = .09$). The distribution of App/O following mdThal Saline infusions violated normality ($K2 = 8.94$, $p < .05$), so a Mann-Whitney test was performed to compare the Saline and M/B conditions and revealed that App/O was significantly lower in the M/B condition ($U = 55$, $p < .05$), indicating the rats made significantly fewer approaches per odour in the M/B condition.

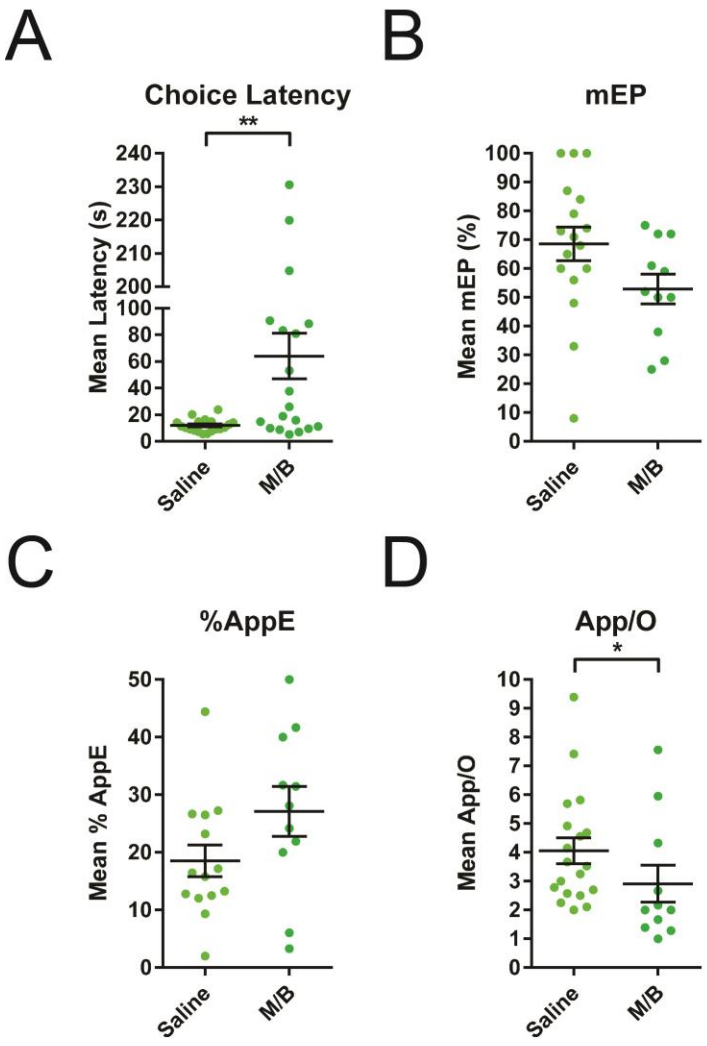

Figure 2.5. A) Mean (±SEM) Choice Latency following infusions of Saline or M/B into the mdThal. Inactivation of the mdThal caused a large and significant increase in choice latency. B) Mean (±SEM) mEP following infusions of Saline or M/B into the mdThal. Inactivation of the mdThal caused no significant change in mean error position. C) Mean (±SEM) %AppE following infusions of Saline or M/B into the mdThal. Inactivation of the mdThal caused no significant change in the percentage of total approaches accounted for by the error odour. D) Mean (±SEM) App/O following infusions of Saline or M/B into the mdThal. Inactivation of the mdThal caused rats to make significantly fewer approaches per odour.

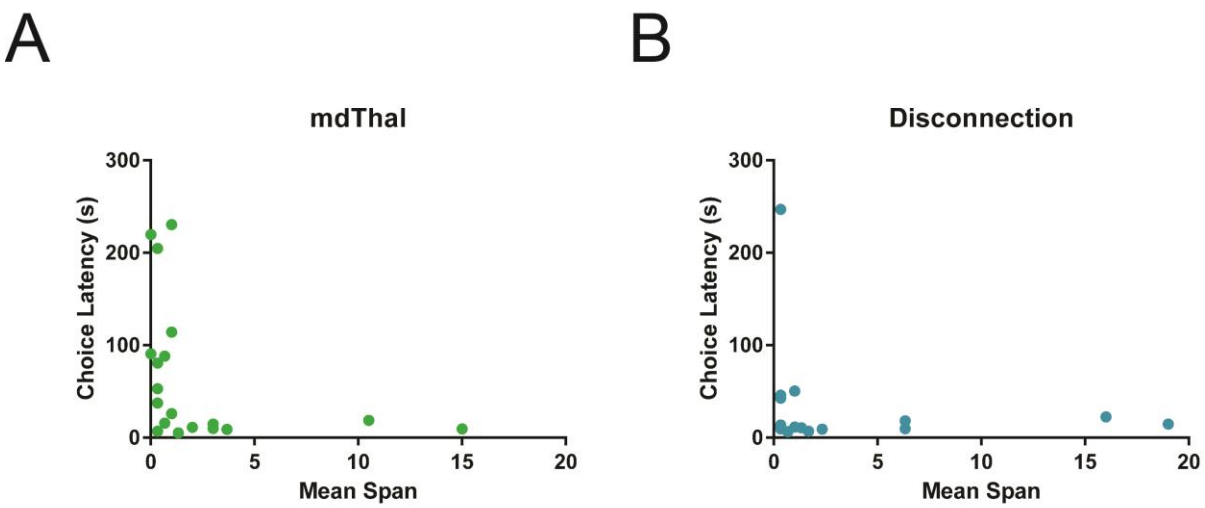

Figure 2.6. A) A scatterplot showing the relationship between Mean Span and Choice Latency following mdThal inactivation. Mean Span was significantly negatively correlated with Choice Latency suggesting that the memory impairment following mdThal inactivation may be partly explained by memory decay over increased latencies. B) A scatterplot showing the relationship between Mean Span and Choice Latency following disconnection of the mPFC and mdThal. Mean Span and Choice Latency were not significantly correlated.

2.4.4. Contralateral disconnection of the medial prefrontal cortex and mediodorsal thalamus

2.4.4.1. *Memory Performance*

Mean (±SEM) Span and Best Trial for mPFC-mdThal disconnection is shown in Figure 2.3C. One rat which had been included in the previous analyses was excluded from analyses of memory performance and foraging due to a lack of responding during OST testing following disconnection infusions. A repeated measures 1 -way ANOVA conducted on Mean Span revealed a significant main effect of Drug treatment ($F_{(3,71)} = 9.26$, $p < .001$). Tukey-corrected multiple comparisons revealed that mean span was significantly lower in the Disconnection condition than in the Sham Infusion condition ($p < .01$) and the Saline condition ($p < .05$). The Sham Infusion and Saline conditions were not significantly different ($p = .99$). Additionally, the Ipsilateral Control had significantly worse performance than both the Sham Infusion ($p < .01$) and Saline ($p < .05$) conditions. There was also no significant difference between the Disconnection and Ipsilateral Control conditions ($p = .99$). A 1-way repeated measures ANOVA conducted on Best Trial revealed a significant main effect for Best Trial ($F_{(3,71)} = 11.16$, $p < .0001$). Tukey post

hoc tests revealed that the Disconnection condition was significantly impaired relative to Saline (p <.01) and Sham Infusion (p <.001) conditions. The Sham Infusion and Saline conditions were not significantly different (p >.99). Although Best Trial was significantly lower in the Ipsilateral Control condition than the Sham Infusion condition (p <.05), it was not significantly lower than the Saline condition (p = .07) despite a trend towards impairment. Finally, there was no significant difference between the Disconnection and Ipsilateral Control conditions (p = .29).

2.4.4.2. *Serial Position of Errors and Foraging Effects*

Mean (±SEM) Choice Latency, mEP, %AppE, and App/O for mPFC-mdThal disconnection are shown in Figure 2.7. The distribution of choice latency data violated normality in all conditions ($K2s \geq 6.11$, ps <.05), so a Friedman test was conducted to compare latencies across conditions and produced a Friedman value of 8.44 (p <.05), indicating a significant change in Choice Latency. Dunn's multiple comparisons revealed that Choice Latency was only increased in the Disconnection condition relative to the Saline condition (p <.05) with no differences between Saline and Ipsilateral (p =.99) or Ipsilateral and Disconnection (p = .06). The relationship between Mean Span and Choice Latency for the Disconnection condition is shown in Figure 2.6B. To determine whether Mean Span was correlated with Choice Latency in the Disconnection condition, a Spearman correlation was conducted and found no significant correlation (r_s = -.19 , p = .45). After applying exclusion criteria to the error analysis, the sample size was Saline = 18, Disconnection = 10, and Ipsilateral = 14 for the mEP analysis, Saline = 15, Disconnection = 10, and Ipsilateral = 12 for the %AppE analysis, and Saline = 18, Disconnection = 10, and Ipsilateral = 13 for the App/O analysis. 1-way between subjects ANOVAS revealed no significant main effects for mEP ($F_{(2,41)}$ = 0.46, p = .64), %AppE ($F_{(2,36)}$ = 2.28, p = .12), or App/O ($F_{(2,40)}$ = 1.47, p = .24).

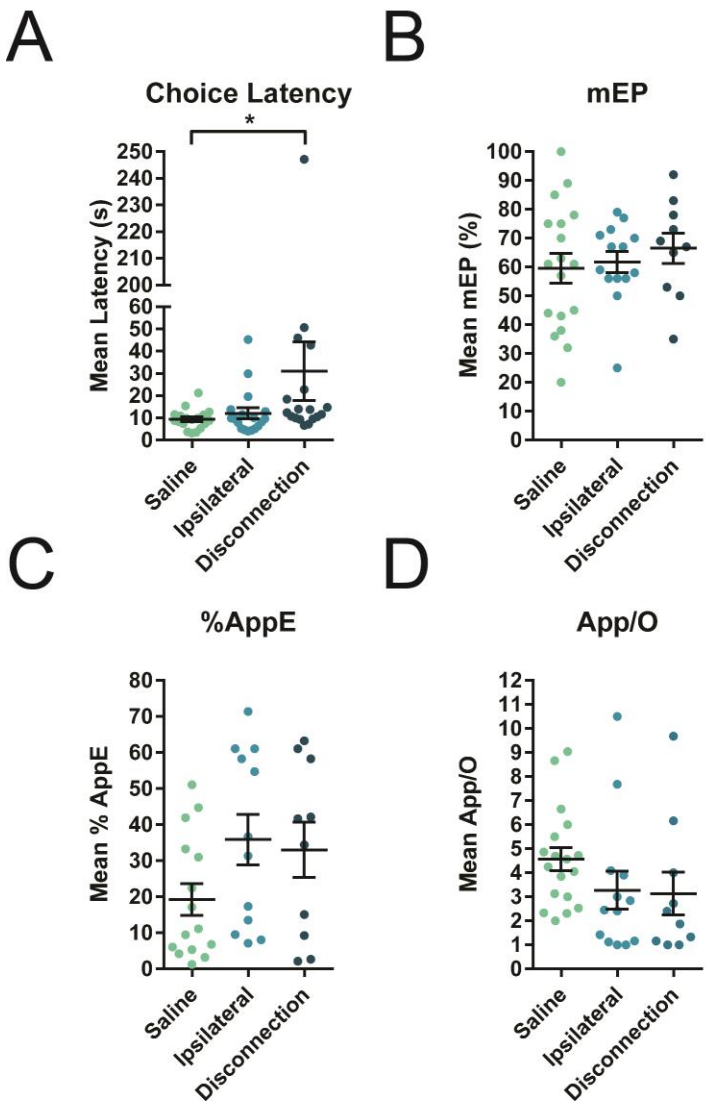

Figure 2.7. A) Mean (±SEM) Choice Latency following disconnection of the mPFC and mdThal. Choice Latency was significantly higher in the Disconnection condition than in the Saline condition. B) Mean (±SEM) mEP following disconnection of the mPFC and mdThal. There was no shift in the mean error position caused by disconnection of the two structures. C) Mean (±SEM) %AppE following disconnection of the mPFC and mdThal. Disconnection of the two structures did not cause a statistically significant change in the percentage of total odour approaches accounted for by the error odour. D) Mean (±SEM) App/O following disconnection of the mPFC and mdThal. Disconnection of the two structures caused no significant change in the number of approaches rats made per odour.

2.5. Discussion

In the present experiment, we found that bilateral inactivation of the mPFC or mdThal both produced severe impairments in memory capacity in the OST. The results replicate previous findings from our lab involving mPFC inactivation (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013). We also provide the first finding, to our knowledge, that the mdThal mediates OST performance, providing more evidence for the uncertain role of the mdThal in WM and olfactory processing (Courtiol & Wilson, 2015; Wolff et al., 2015). OST performance was also significantly impaired by contralateral disconnection of the mPFC and mdThal, but ipsilateral control infusions also produced a similar impairment to disconnection. Thus, the present results are more or less in line with previous research demonstrating a role for this circuit in WM (Bolkan et al., 2017; Floresco et al., 1999; Guo et al., 2017; Schmitt et al., 2017), but cannot be conclusively explained as a result of disrupted mPFC-mdThal connections. Additionally, we performed detailed analysis of rats' foraging behaviour and errors and revealed putative differences in the respective roles of the mPFC and mdThal in mediating rats' search strategies during the OST. Specifically, mPFC and mdThal inactivations both significantly reduced the number of odour approaches rats made before making a choice, while only mdThal inactivation and disconnection of the mdThal from the mPFC caused a significant increase in choice latency. Neither contralateral disconnection or ipsilateral control infusions had any significant effects on errors or foraging.

The present results of mPFC inactivation in the OST replicate previous findings from our lab (Davies, Greba, et al., 2017; Davies, Molder, et al., 2013) as well as a large literature demonstrating the critical role of the PFC in WM. Rats' span capacity was significantly reduced following infusions of M/B, confirming the finding that the mPFC is critical for OST performance. We have previously demonstrated that the impairment following mPFC inactivation is not due to a loss of olfactory sensitivity (Davies, Molder, et al., 2013), meaning that the present results are very unlikely to be the result of a deficit in olfactory perception. Additionally, we saw no change in latency following mPFC inactivation. Our lab has found that changes in latency are associated with larger infusion volumes (Davies, Molder, et al., 2013) whereas smaller infusion volumes like the one used presently do not increase latency (Davies, Greba, et al., 2017). We did not manipulate the delay period in the OST during this experiment, but previous research has produced inconsistent findings with respect to the delay-dependence of

mPFC disruption-induced impairments with some authors finding impairments at short/no delays (Davies, Hurtubise, et al., 2017; Joel, Weiner, & Feldon, 1997) and others finding delay-dependent effects (McAllister et al., 2013).

Interestingly, rats were impaired by both Saline and M/B infusions into the mPFC relative to preinfusion performance. We take this as evidence that the mPFC is extremely sensitive to any amount of manipulation including Saline infusions. Our Saline was room temperature at the time of infusion, meaning that it was not isothermic with the brain tissue into which it was being infused and thus could have achieved some thermal inactivation as a result (Lomber, 1999). Additionally, the insertion of steel infusion needles also causes minor tissue disruption as well as aberrant neuronal activity and immediate early gene expression (Gulbrandsen & Sutherland, 2014). Thus, it should not be surprising that, in a particularly vital and sensitive brain region, a behavioural impairment can be elicited even from a "control" infusion.

A large literature has previously demonstrated that the mdThal plays a critical role in cognition (Mair et al., 2015; Mitchell, 2015; Mitchell & Chakraborty, 2013). Although there has been controversy regarding the role of the mdThal in WM with many discrepant results (M. Wolff et al., 2015). Recent evidence supports the view that it mediates persistent activity in the PFC during WM tasks (Sebastien Parnaudeau et al., 2013; Schmitt et al., 2017). However, olfactory stimuli have been shown to bypass some circuitry typically associated with WM function such as the posterior parietal cortex (Scott et al., 2018) and hippocampus (Dudchenko et al., 2000), and research on the role of the mdThal in olfactory memory has been produced inconsistent findings (Koger & Mair, 1994; Slotnick & Risser, 1990; Zhang et al., 1998). The present results confirm that the mdThal plays a role in the OST, contributing to the emerging understanding of the role of the mdThal in olfactory learning and memory tasks (Courtiol & Wilson, 2015). Of note, we did not perform any experiments to measure olfactory sensitivity or discrimination, and some previous research has found impaired olfactory discrimination for difficult odour comparisons following mdThal disruption (Eichenbaum et al., 1980; Slotnick & Risser, 1990). In the present study, mdThal inactivation did not reduce memory span completely to zero, suggesting that rats at least had sufficient olfactory discrimination abilities to achieve extremely low spans.

Additionally, choice latency was significantly increased by both bilateral mdThal inactivation and disconnection. Choice latency was also negatively correlated with mean span in the mdThal inactivation condition. This presents a potential problem in interpreting the impairment in mean span given that increased response latency could presumably decrease accuracy by overburdening maintenance processes. While not every rat with a low mean span had high choice latency following mdThal inactivation, not one rat with high latency obtained a high span. This would suggest that at least some of the impairment in memory performance could be accounted for by memory decay over extended choice latencies. However, it may also be the case that rats achieving higher spans following mdThal inactivation are simply also less impaired in their exploratory foraging behaviour and these variables are independent. Previous research has shown that the effects of mdThal disruption on WM tasks can be independent of delay (Chauveau, Célérier, Ognard, Pierard, & Béracochéa, 2005; Mumby et al., 1993).

Despite this potential confound with respect to memory performance, the result points to an interesting role of the mdThal in foraging behaviour. Previous research has found that inactivations affecting the mdThal can cause reductions in exploratory motor behaviour. For example, Swerdlow, Pitcher, Noh, & Shoemaker (2002) found that inactivation of the mdThal with TTX caused a reduction in motor activity following prepulse inhibition testing, but no reduction in startle magnitude, possibly indicating impaired exploratory motor activity. Additionally, lesions of the mdThal have been shown to attenuate the hyperlocomotor response to apomorphine is rats with 6-hydroxydopamine denervation of the nucleus accumbens (Swerdlow & Koob, 1987). This involvement of the mdThal in locomotor behaviour is not limited to spontaneous exploration but appears to be involved in goal-directed motor activity as well. An early study by Mogenson & Wu (1988) examined the effects of mdThal inactivation on food hoarding behaviour during an open field foraging task and found that mdThal inactivation reduced the number of food pellets hoarded within a 30 minute period. This evidence, combined with the present findings, suggests that a significant component of mdThal involvement in the OST may be in mediating goal-directed foraging behaviour.

Importantly, a cause of past controversy over the role of the mdThal in WM has been the fact that the degree of impairment is related to the spread of lesions or infusions to neighbouring brain structures with the most specific disruptions sometimes causing minimal impairments (Wolff et al., 2015). Although we did not directly verify the spread of our infusions, our

inactivation protocol used similar coordinates and even smaller infusion volumes than what has been previously shown to result in infusate spread confined to the mdThal (Swerdlow et al., 2002). Nonetheless, we cannot entirely rule out the possibility that some adjacent brain structures could have been affected. Furthermore, the role of the mdThal has been additionally controversial due to effects of lesions that are not specific to WM impairments (Wolff et al., 2015). In the present study, we observed significant changes in rats' foraging behaviour which could have in turn affected memory performance including a large increase in latency which correlated with memory capacity. Thus, our results should be viewed conservatively as evidence of mdThal involvement in overall performance of the OST, but the involvement is likely not specific to purely mnemonic processing in the OST.

This is the first study, to our knowledge, to examine the role of mdThal-mPFC connections in an olfactory WM capacity task. Rats had equally impaired mean span in the disconnection and ipsilateral control conditions. Although Best Trial for the ipsilateral control was not significantly different than Saline, the trend is clearly towards an impairment nearly as severe as the disconnection. Hence, the present results certainly do not contradict the interpretation that the mPFC-mdThal circuit is necessary for performance of the OST, but the failure of our ipsilateral control leaves open the possibility of several competing interpretations.

It is possible that contralateral projections between mdThal and mPFC play as important a role as ipsilateral connections, unilateral infusions were able to diffuse across the midline to have some affect on the contralateral hemisphere, or that even a unilateral infusion in one or both structures is sufficient to impair performance. The first possibility appears highly unlikely given that tracing studies have found that connections between the two structures appear to be exclusively ipsilateral (Alcaraz et al., 2016). Impairments from interhemispheric diffusion or unilateral inactivation seem unlikely in the case of the mPFC given our lab's previous finding that ipsilateral control infusions which include the mPFC do not significantly impair OST performance (Davies, Greba, et al., 2017). Similarly for the mdThal, Floresco et al. (1999) pharmacologically disconnected the mPFC and mdThal and found no impairment in the radial arm maze following unilateral mdThal inactivation at an infusion volume greater than ours. The remaining possibility is that the OST is so sensitive to mdThal disruption that unilateral inactivation possibly including some interhemispheric diffusion is sufficient to impair performance and this would require further experiments to rule out. In any case, the impairments

in our disconnection experiment are not conclusively tied to a disruption of mPFC-mdThal connections *per se* and it is even possible that the two structures are both independently critical for OST performance.

The impairments we observed in OST memory performance may be explained by factors other than deficits in memory capacity *per se*. Our version of the OST involves a procedure in which odour stimuli are added after each successful choice such that the ratio of familiar odours to the correct novel choice increases in every trial iteration. This attribute has the effect of increasing the number of distractors in addition to increasing memory load such that distractor susceptibility may confound the analysis of memory capacity in addition to underestimating rats' actual memory capacity, a problem that can be overcome by holding constant the number of presented comparison stimuli and computing memory accuracy and distractor susceptibility independently (April, Bruce, & Galizio, 2013). For example, Galizio et al. (2019) recently demonstrated that the MK-801 impairment in the OST is partly explained by increased distractor susceptibility. Therefore, it is possible that the present results might similarly be a combined result of impaired memory capacity in addition to increased distractor susceptibility. This interpretation is perhaps supported by our finding that both mPFC and mdThal inactivation caused a reduction in the mean number of approaches per odour which could, in our case, be explained by rats falsely detecting novelty in familiar odours and ending their search. Alternatively, this pattern is consistent with an increase in impulsivity or decrease in behavioural inhibition, an effect previously observed following mPFC disruption (Feja & Koch, 2014; Hardung et al., 2017), which could manifest in the OST as a tendency to prematurely choose an odour before searching sufficiently.

As previously mentioned, it is unclear whether the OST impairments caused by mPFC or mdThal inactivation are delay-dependent, which may also be problematic for the interpretation that the impairments are specific to mnemonic processes (Wolff et al., 2015). Given the changes in foraging behaviour and the possibility that rats were afflicted with increased distractor susceptibility, the present findings may be in line with the interpretation that the mPFC-mdThal circuit is playing an "executive", as opposed to "mnemonic" role consistent with the model of this circuit as an "executive" system (Wolff et al., 2015). Memory capacity for odours may be mediated by the relevant sensory areas, as is suggested by sensory recruitment accounts of WM (Scimeca et al., 2018). It is not known precisely where odour representations in the OST are

stored, but candidate structures include primary olfactory cortex such as the piriform (Stettler & Axel, 2009) and/or other areas receiving olfactory projections that are relevant to the reward value of stimuli such as the orbitofrontal cortex (Illig, 2005) and amygdala (Sosulski, Bloom, Cutforth, Axel, & Datta, 2011).

We attempted to elucidate whether disruption of the mPFC or mdThal affected which odours rats were likely to incorrectly choose when making an error as a function of their position in the sequence of presented odours and, while finding no effect of our manipulations on mEP, we feel this aspect of the OST warrants discussion. Rats are most likely to incorrectly choose odours that are ~70% of the way through the odour sequence and considerably less likely to incorrectly choose odours occurring nearer the beginning or end of the sequence. It is tempting to make an analogy here to primacy and recency effects present in human WM tasks (Botto et al., 2014; Stephane et al., 2010) and in rats during performance of the radial arm maze (Dimattia & Kesner, 1984; Kesner & Novak, 1982).

However, our version of the OST allows early-presented odours to be revisited by the rat during each trial iteration violating the serial order of odour presentation. Due to the fact that the most recent odour presented before rats' make an error is only presented in a single trial iteration, it could be argued that the "recency effect" we observe is truly comparable to the recency effect found in humans (Botto et al., 2014; Tronel et al., 2010) and in the serial position curve findings in the radial arm maze (Dimattia & Kesner, 1984; Kesner & Novak, 1982). In contrast, the "primacy effect" is very likely confounded by repeated rehearsal of familiar odours located early in the sequence. This is evidenced by the prominent skewing of the curve in which the "primacy effect" appears to be of a greater magnitude than the "recency effect". Indeed, previous research controlling when and how often rats are able to revisit familiar odours suggests that the relative familiarity of odours contributes significantly to whether an error is made (April et al., 2013). As such, it is likely inappropriate to make a direct comparison to serial position effects. Nonetheless, we believe this phenomenon in the OST may be of interest to future research.

2.6. Conclusions

The present study examined the relative contributions of the mPFC and mdThal to performance of the OST, an olfactory WM task. Although our lab has previously shown that the mPFC is critical for OST performance (Davies, Greba, et al., 2017; Davies, Molder, et al., 2013),

no other study to our knowledge has examined how the mdThal, or its connections with the mPFC, contributes to the OST, and the role of the mdThal in WM and olfactory processing has been controversial (Courtiol & Wilson, 2015; Wolff et al., 2015). Inactivation of either the mPFC or the mdThal significantly impaired odour span capacity, as did contralateral disconnection of the two structures. However, ipsilateral control infusions caused an almost equal impairment in memory performance, meaning that impairments in the disconnection may be a result of either interrupted thalamocortical communication or the result of unilateral disruption of one or both structures in an independent fashion. Nonetheless, the results add to previous literature disrupting these connections in other WM tasks and support the view that these connections participate in WM function across sensory modalities. Additionally, these results provide some evidence of dissociations in the effects of mPFC or mdThal inactivation on rats' foraging behaviour whereby mdThal inactivations or disconnection cause a reduction in exploratory motor activity in the task similar to previous research on the role of the mdThal in foraging behaviour (Mogenson & Wu, 1988).

3. EXPERIMENT 2: PERFORMANCE OF THE ODOUR SPAN TASK IS NOT IMPAIRED FOLLOWING INACTIVATIONS OF PARIETAL CORTEX IN RATS

The content in this chapter is published in *Behavioural Brain Research*. My specific contributions to this work included performing the majority of behavioural training/testing, the majority of intracranial surgeries, a large proportion of intracranial drug infusions, all histology, and all statistical analyses of behaviour. Finally, I was the primary author and completed the majority of manuscript preparation, preparation of figures, and responses to reviewers. The following additional authors made significant contributions to this work: Nadine K. Zabder assisted in performing behavioural testing and intracranial infusions, Quentin Greba assisted in performing stereotaxic surgery, and John G. Howland assisted in experimental design and manuscript preparation.

Scott, G. A., Zabder, N. K., Greba, Q., & Howland, J. G. (2018). Performance of the odour span task is not impaired following inactivations of parietal cortex in rats. *Behavioural Brain Research, 341,* 181–188.

3.1. Abstract

Working memory (WM) is the ability to temporarily store information for use and manipulation. Working memory is thought to depend on a distributed set of higher cortical areas including the prefrontal and parietal cortex in primates while relatively little research has been conducted in rodents to elucidate the exact role of the parietal cortex (PC) in WM, particularly in relation to the construct of working memory capacity. Previous work in our lab demonstrates that performance of the odour span task (OST), an olfactory incremental delayed nonmatching-to-sample task, relies on the medial prefrontal cortex (mPFC). However, the effects of inactivating the PC on the OST has not been studied. Therefore, the present experiment assessed the effects of inactivating the PC with the GABA receptor agonists muscimol/baclofen on performance of the OST. Infusions of muscimol/baclofen did not disrupt working memory performance, assessed by the mean number of odours each rat could remember before making an error on each day of testing. In contrast, performance of a positive control task, spontaneous cross-modal object recognition, was impaired by inactivating the PC. These results suggest that performance of the OST does not depend on the PC in rats. Our results are notable given past research demonstrating the importance of the parietal cortex for attentional processes and working memory in other tasks.

3.2. Introduction

Working memory (WM), the ability to temporarily store information for use and manipulation, is believed to depend in primates and rodents on a distributed set of higher cortical areas involved in executive function and attention such as the prefrontal and parietal cortices (D'Esposito & Postle, 2015; Eriksson et al., 2015). Considerable evidence has linked WM function with the parietal cortex in humans and non-human primates (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey, Devinsky, Doyle, Golfinos, & Curtis, 2016; Öztekin, Mcelree, Staresina, & Davachi, 2009; Ravizza, Delgado, Chein, Becker, & Fiez, 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). However, relatively little research has been conducted in rodents to elucidate the role of the parietal cortex (PC) in WM (Bucci, 2009), and in particular, the construct of WM capacity – the number of items that can be simultaneously held in WM with enough accuracy for recall. Two previous studies found working memory impairments following manipulations of the PC. McDaniel, Compton and Smith (1994) performed lesions of the posterior PC and found a WM impairment in a multiple T-maze; Espina-Marchant et al. (2006) found WM impairments in an Olton 4x4 maze after temporary inactivation of the posterior and anteromedial parietal cortex with lidocaine. However, given that both of these studies used spatial WM tasks, the effects of parietal cortex manipulations on spatial navigation cannot be fully separated from the effects on WM capacity *per se*. Kolb, Buhrmann, McDonald and Sutherland (1994) investigated the effects of posterior PC lesions in rats on WM and found no impairment in two nonmatching-to-sample tasks using objects with delays from 0 to 20 s. Importantly, these experiments required rats to maintain information over a delay period, but did not assess the effects of PC lesions on WM capacity, as there was no requirement to remember multiple items. Thus, it is possible that rats would be impaired in remembering a larger amount of information over a delay period after disruption of the PC.

The odour span task (OST), an incrementing olfactory delayed nonmatching-to-sample (DNMTS) task that requires rats to maintain an increasingly large number of odours over a delay period (Dudchenko et al., 2000), serves as a test of WM capacity in rodents (Dudchenko et al., 2013; Moore, Geyer, Carter, & Barch, 2013). Previous work from our lab demonstrates that the medial prefrontal cortex (mPFC) and striatum in rats are critical for WM capacity in this task (Davies, Greba, et al., 2017; Davies, Molder, et al., 2013), which complements the primate

literature with respect to the role of both structuresin WM (Champod & Petrides, 2007; Curtis, 2006; Funahashi et al., 1989; Olesen, Westerberg, & Klingberg, 2004).

In the present experiment, we assessed the effects of temporarily inactivating the PC on performance of rats in the OST to determine if the rat PC is involved in WM capacity (Brigadoi et al., 2017; Vogel & Machizawa, 2004). In addition, we assessed the effects of these same inactivations on performance of cross-modal object recognition (CMOR), a task requiring rats to rely on multisensory integration to recognize objects, as well as tactile- and visual-only recognition tests (Cloke, Jacklin, & Winters, 2015; Marks, Cain, Snutch, & Howland, 2016; Reid, Jacklin, & Winters, 2012; Winters & Reid, 2010). The tactile and cross-modal sub-tests of the CMOR battery rely on the PC in rats (Winters & Reid, 2010) and as a result, acted as a positive control to determine the effectiveness of the PC inactivations. We anticipated that if the rat PC is necessary for normal WM capacity (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004), performance of rats in the OST would be diminished. Performance in the CMOR and tactile tests should be similarly be reduced, in line with previous findings showing a role of the rat PC in the task (Winters & Reid, 2010).

3.3. Materials and Methods

3.3.1. Subjects

Thirty-one male Long Evans rats (Charles River Laboratories, Kingston, NY) weighing 300-500g were used. Rats were individually housed in standard ventilated cages and kept on a 12-hour light/dark cycle. For experiments requiring food restriction, rats were maintained at 85-90% of their free-feeding weight with water available *ad libitum*. For experiments not requiring food restriction, both food and water were available *ad libitum*. All experiments were approved by the University of Saskatchewan Animal Research Ethics Board and conformed to the guidelines of the Canadian Council on Animal Care.

3.3.2. Odour Span Task

The OST was performed in a similar fashion to previous experiments from our lab (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013; B. G. Murray et al., 2017) but used a "lid-flipping" procedure adapted from MacQueen, Bullard, & Galizio (2011). Training was conducted on a table (cream-coloured corrugated plastic; 91.5 cm^2) with a 2.5 cm high border

around the edges and elevated 95 cm above the floor with a metal frame and casters. Between trials, the rats were placed in a transparent Plexiglas enclosure (32 cm W x 50 cm H x 35 cm D) located in one corner of the table. During trials, the rats were let out of the enclosure and allowed to explore the table. Rewards were presented in plastic Dixie cups (59 mL) containing a small amount of sand with 2 reward pellets (45 mg rodent purified dustless precision pellets; Bio-Serv, Flemington, NJ) sitting on top of the sand. The portion cups were covered with scented plastic Dixie cup lids sitting loosely on top of them. The lids were scented with odours by storing them in Tupperware containers containing various spices (Allspice, Anise, Basil, Cacao, Caraway, Celery Salt, Cinnamon, Clove, Coffee, Cumin, Dill, Fennel, Garlic, Ginger, Lemon, Marjoram, Mustard, Nutmeg, Onion, Orange, Oregano, Paprika, Sage, and Thyme). The lids were repeatedly reused in training so that rats could not rely on marking individual 'fresh' lids with their own scents as a mediating strategy.

Training consisted of 3 phases: The first stage was initial shaping, in which rats were habituated to the table and trained to flip lids off the cups to obtain a reward. This was done by first presenting an open cup with a reward and, after each correct trial (the rat successfully obtaining the reward within 5 min), an unscented lid was placed on the cup covering incrementally more of the opening (1/2 covered, ¾ covered, fully covered). This stage continued with each rat receiving 3 trials per day until they could successfully obtain 3 rewards in one day from fully covered cups.

The second phase of training was delayed nonmatching-to-sample (DNMTS) training. This stage was conducted by presenting a cup with a sample odour and allowing the rat to obtain the reward. After a delay of approximately 40 s (the time between stimulus presentations), a second cup containing a reward with a novel odour (S+) along with an unbaited cup with the original sample odour (S-) were placed on the table. Importantly, the locations of the novel and familiar odours were shuffled to remove any spatial cues from the task and to prevent rats from visually tracking where the novel odour was placed on the table. Rats were given 6 trials per day (with an intertrial interval (ITI) of 60 s) of DNMS until they performed with 80-100% accuracy for 3 out of 4 consecutive days with the 4th day being no less than 50% accuracy, or until 10 days of DNMS training had elapsed, at which point rats were moved to OST training regardless of whether they had reached criterion in DNMTS Rats that were slow learners in DNMTS learned the OST after extensive training even if they had not mastered DNMTS within 10 days.

The OST was performed in the same manner as DNMTS; however, after each correct choice, an additional odour was added to the table in serial fashion until the rat made an error at which point that particular trial was ended and an ITI began before commencing the next trial. As with DNMTS, the locations of the odours were thoroughly shuffled during the ~ 40 s delay to remove spatial cues from the task and prevent visually-guided mediating strategies. A schematic of this procedure is shown in Figure 3.2A. Each rat was trained in this task for a maximum of 3 trials per day (with no repetition of odours across different trials) or a maximum of 30 min, whichever criterion was met first. Rats were trained for 6-7 weeks ~5 times/week before being tested with infusions. During training, the span performance, errors, and latency to make a choice were recorded by hand, and latency was tracked manually with a stopwatch.

3.3.3. Cross-modal Object Recognition

The cross-modal object recognition task was administered in the same basic fashion as previous literature (Marks et al., 2016; Winters & Reid, 2010). Training was conducted in a Y-shaped maze (white corrugated plastic; 10 x 27 cm) with one entrance arm and two object arms. The entrance arm was blocked by a sliding plastic divider except during the task. Transparent Plexiglas dividers separated the objects from the rest of the maze for visual-only training. Objects consisted of a variety of plastic toys, Lego towers, and assorted ornamental items made of plastic, glass, or porcelain.

Training consisted of 4 phases: habituation, tactile object recognition, visual object recognition, and cross-modal object recognition. Habituation took place over 2 days and consisted of 2 10-min sessions that included a counterbalanced mix of the following conditions: Testing room lights on for 50% of each session and the Plexiglas dividers in for 50% of each session. Thus, rats were habituated to both the lit and unlit testing room and the presence/absence of the Plexiglas dividers. Training and testing for the tactile, visual, and cross-modal phases consisted of a 3-min sample phase, in which the rat was allowed to explore the maze with identical copies of an object in each object arm, followed by a 2-min test phase 1 h later. The tactile, visual, and cross-modal phases took place across 4 consecutive days, with the cross-modal test being conducted twice for each rat with two different object pairs in order to maximize the sample size in this test.

A schematic depicting the phases of behavioral training and testing is shown in Figure 3.3. During the tactile phase, the room lights were turned off, and the rats were only able to

explore the objects using touch (the objects were thoroughly cleaned with ethanol to minimize any of their olfactory features). During the visual phase, the room lights were on, and Plexiglas dividers blocked the objects from direct contact, requiring the rats to explore using vision only. In the cross-modal phase, the sample phase was conducted with the lights off and dividers out so that the objects would be explored using touch only, and the test phase was conducted with lights on and dividers in so that rats would be tested on their memory using vision only. To recognize objects during the cross-modal test phase, rats must rely on multisensory integration, extrapolating from a tactile representation of the familiar object to recognizing it from its visual features alone (Cloke et al., 2015; Winters & Reid, 2010). In addition, different copies of the objects were used in the sample and test phases to minimize the influence of olfactory cues on performance.

During both the sample and test phases, the order of objects presented as 'familiar' and 'novel' was counterbalanced, as was the location of the novel object in either the left or right arm of the maze. Across the tactile and visual phases of the task, the rats receiving saline or drug was counterbalanced such that rats receiving saline during the tactile phase received drug during the visual phase and vice versa. The cross-modal phase of the task was run twice with two different sets of objects to allow all rats to perform the task under both the saline and drug conditions. As such, the order that rats received saline or drug was counterbalanced in addition to the other counterbalancing described above.

3.3.4. Surgery and Intracranial Drug Infusions

3.3.4.1. *Stereotaxic Surgery*

Surgeries were performed under isoflurane anesthesia (Janssen, Toronto, Ontario). After shaving the head and placing the rat in a stereotaxic frame (Kopf Instruments, Tajunga, CA), the scalp was cleaned and incised along the midline. Five to six small burr holes were drilled to accommodate jeweler's screws in order to secure the head caps. Larger holes were then drilled to allow the lowering of 4 11-mm 23-gauge guide cannulae into the PC. Each set of guide cannulae was fashioned into "double barrel" cannulae by soldering them side by side with a steel spacer in between (small section of 23-gauge steel tubing; ~1 mm). These double barrel cannulae were implanted -3.9 mm posterior and 2.2 and 3.4 mm lateral to bregma, with the mediolateral coordinate being measured from the inside cannulae of each set. Dorsoventral placement was based on lowering the cannulae tips to the brain surface and then advancing them ~0.2 mm

deeper. Coordinates were derived from the Rat Brain Atlas (Paxinos & Watson, 2006). The anatomical definition of the PC in rats varies somewhat in the literature. Our coordinates correspond to what some authors consider the posterior PC (Licata et al., 2017; Rosner & Mittleman, 1996), whereas other authors consider the posterior PC as slightly more posterior to our coordinates (Winters & Reid, 2010). In at least one case, the area we targeted is also defined as containing an "anteromedial" area (Espina-Marchant et al., 2006). Evidence from the tracing of cortical connectivity reveals little difference in the cortical inputs to the PC along its anteroposterior axis; however, differences in cortical connectivity along its mediolateral axis exist (Wilber et al., 2014), of which our coordinates covered the full extent. Given previous evidence that 0.5 µL of infusate can spread 1-2 mm from the infusion site (Izquierdo et al., 1997), and allowing for the aforementioned variation in the anatomical definition of the rat PC, we anticipated that our infusions would affect the full mediolateral extent of the PC, and likely between 50% and 100% of its anteroposterior axis.

Steel dummy stylets were placed in the guide cannulae to plug them between infusions. Importantly, due to the fact that the tips of the guides were very close to the brain surface the dummy stylets were either of equal length to the guides or were left ~1 mm longer than the guides and beveled to a point in order to prevent repeated irritation of the dura mater in some rats. After fashioning a head cap from dental acrylic, rats were given a single subcutaneous injection of Anafen (0.5 mg/kg) for analgesia, the scalp was sutured, and the rats allowed to recover for a minimum of a week before recommencing behavior testing.

3.3.4.2. *Intracranial Drug Infusions*

Rats were first habituated to the infusion procedure through handling in the room in which infusions were to take place. This included removal of their dummy stylets and mock infusions, which consisted of inserting shortened infusion needles that were not connected to the microinfusion pumps and running the pumps to simulate the noise and sensation of the infusions. Approximately 20-30 min before behavioral testing (prior to sample phase in CMOR), rats received intracranial drug infusions of either 0.9% saline or muscimol/baclofen (0.5 µg/µL, respectively; dissolved in saline and mixed together (Davies, Molder, et al., 2013; Sangha, Robinson, Greba, Davies, & Howland, 2014)). Infusions were performed using two microinfusion pumps (Harvard Apparatus, Holliston, MA) at all 4 sites simultaneously at a flow

rate of 0.5 µL/min for 1 min. Infusion needles (27 gauge; 12 mm) were left in the guide cannulae for 1-2 min to allow the drug to diffuse, and rats were returned to their cages to await testing.

3.3.5. Perfusions and Histology

Rats were overdosed with isoflurane and perfused transcardially with 200 mL of 0.1 M PBS followed by 200 mL of 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde for 24 h before being transferred to a cryoprotectant solution of 0.1% sodium azide/30% sucrose. After the brains had sunk in the cryoprotectant, they were sectioned coronally at 40 µm on a freezing sliding microtome (Leica Biosystems, Concord, Ontario). Sections containing cannula tracks were mounted to glass slides and cannulae placement was determined with the aid of a stereoscope and brain atlas for reference (Paxinos & Watson, 2006).

3.3.6. Data Analysis

3.3.6.1. *Odour Span Task*

The mean span of each animal on each training day was calculated by taking the number of correct choices minus 1 within each trial and averaging the scores between all trials completed. To analyze the effects of infusions into the PC, rats' performance (mean span capacity) was compared in a dependent t-test between Saline and M/B days. Performance in the Saline condition was also compared to performance the day before infusions, and performance on 9 days of pre-infusion training was compared to ensure that performance was stable across days.

3.3.6.2. *Cross-modal Object Recognition*

Performance in CMOR was assessed separately for each phase of the task (tactile, visual, and cross-modal). A discrimination ratio (DR) was calculated for each animal using the following equation:

$$\frac{(Exploration\ Time^{Novel} - Exploration\ Time^{Sample})}{(Exploration\ Time^{Novel} + Exploration\ Time^{Sample})}$$

Importantly, rats were excluded from the analyses of each day of training if they 1) had 0 exploration of the sample objects, or 2) did not explore both objects in the test phase (i.e., a DR of 1 or -1), or had a DR that was ±2 standard deviations or greater from the mean. The first minute of the test, as well as both combined minutes of the test, were used in the analysis.

3.4. Results

3.4.1. Location of Infusion Sites

The infusion sites are shown in Figure 3.1. Twenty-one rats had injections that were deemed acceptably within the PC. Ten rats had injections that were anterior to the parietal cortex, located in the somatosensory cortex, or were too ventral and affected the dorsal hippocampus, and were thus removed from all subsequent analyses.

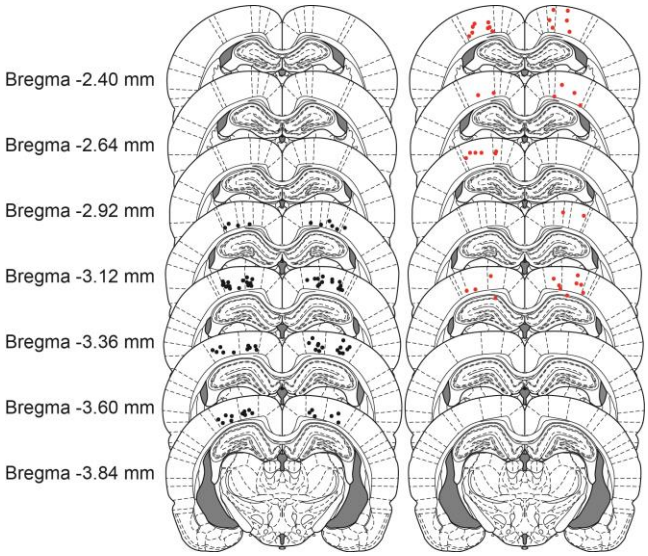

Figure 3.1. Diagram showing the locations of correctly-placed (black, left) and incorrectly-placed (red, right) infusions. Images were adapted from Paxinos & Watson (2006).

3.4.2. Odour Span Task

A one-way within-subjects ANOVA was conducted on 9 days of pre-infusion training (Figure 3.2B) to verify that performance was stable on the days before infusions. No main effect of training day was found ($F_{(8,72)} = 0.40$, $p = .74$), indicating stable performance across the days leading up to infusions. A significant main effect of individual rat was found ($F_{(9,72)} = 11.28$, $p < .05$), revealing that performance significantly differed between rats. However, we do not consider this to be a concern, as the two infusion treatments were compared within subjects. Mean span in the saline condition also did not significantly differ from performance the day before infusions ($t_{(9)} = 0.29$, $p = .78$), indicating that performance was not affected by the infusion procedure. The mean spans (Figure 3.2C) achieved by each rat in either the Saline or M/B conditions were compared in a dependent t-test. Mean span was not affected by M/B

infusions ($t_{(9)}$ = 0.88, p = .40, 2-tailed). In addition, there was no difference in the latency (Saline = 8.42 s, SEM = 1.98; M/B = 13.46 s, SEM = 5.62) to make a choice ($t_{(9)}$ = 0.99, p = .35, 2-tailed). Due to the mean span being lower than previously-published studies (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013; MacQueen et al., 2011), a single-sample t-test was performed to confirm that the mean span of rats in the saline condition was significantly higher than a hypothetical mean span of 0 ($t_{(9)}$ = 3.14, p <.05, 2-tailed).

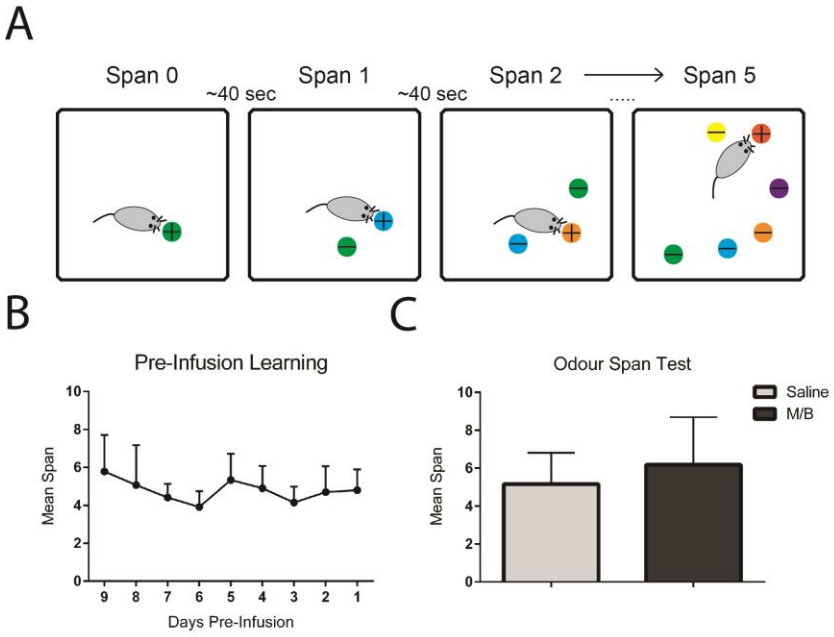

Figure 3.2. A) A schematic illustrating the basic learning procedure taking place in the OST; B) Mean (±SEM) OST performance of all rats during the 9 days of training before infusion (due to rats receiving different amounts of training, only the final 9 days of training before infusions represent the learning data of every rat). Group performance was stable across these training days, although the performance of individual rats tended to fluctuate; C) Mean (+SEM) OST performance of rats with either Saline or M/B infusions in the posterior parietal cortex. Mean span was unaffected by infusions of M/B, suggesting that memory capacity in the OST does not depend on the PC.

3.4.3. Cross-modal Object Recognition

Exploration times during the sample phases for tactile, visual, and cross-modal object recognition for Saline and M/B conditions are shown in Table 1. No significant differences in sample phase exploration between infusion conditions was found for tactile ($t_{(19)}$ = 1.23, p = .24,

2-tailed), visual ($t_{(18)} = 1.76$, $p = .10$), or cross-modal ($t_{(9)} = 0.17$, $p = .87$), indicating that any potential differences in the test phase could not be accounted for by differences in exploration time during the sample phase. Total exploration times during test phases are shown in Table 2. The analysis of the DRs from test phases is described below.

Table 3.1. Mean (SEM) Total Exploration Time During CMOR Sample Phases (s)

Infusion	Tactile	Visual	Crossmodal
Saline	36.14 (5.22)	7.86 (0.52)	35.11 (4.21)
M/B	27.30 (4.54)	11.16 (1.64)	34.19 (3.71)

3.4.3.1. *Tactile Object Recognition*

The mean DRs for Saline and M/B conditions is shown in Figure 3.3A. Single sample t-tests (2-tailed) revealed that a significant DR for the Saline condition in Minute 1 ($t_{(11)} = 8.37$, $p < .05$) and Minutes 1 & 2 ($t_{(11)} = 5.47$, $p < .05$), and the M/B condition in Minute 1 ($t_{(7)} = 2.55$, $p < .05$) and Minutes 1 & 2 ($t_{(7)} = 3.59$, $p < .05$), indicating that rats explored the novel object significantly more than the familiar object in both infusion conditions. An independent t-test was conducted on the DRs between Saline and M/B rats and showed no significant group difference on Minute 1 ($t_{(18)} = 1.49$, $p = .15$, 2-tailed) or Minutes 1 & 2 ($t_{(18)} = 0.31$, $p = .76$, 2-tailed), indicating that infusions of M/B into the PC had no effect on Tactile OR.

3.4.3.2. *Visual Object Recognition*

The mean DR for Saline and M/B conditions is shown in Figure 3.3B. Single sample t-tests (2-tailed) revealed that a significant DR for the Saline condition in Minute 1 ($t_{(5)} = 2.80$, $p < .05$) but not Minutes 1 & 2 ($t_{(7)} = 2.25$, $p = .06$). In the M/B condition, a significant DR was found in Minutes 1 & 2 ($t_{(9)} = 4.83$, $p < .05$), but not in Minute 1 ($t_{(8)} = 1.93$, $p = .09$), indicating that rats explored the novel object more than the familiar object in at least part of the test in both conditions. An independent t-test was conducted on the DRs between Saline and M/B rats and showed no significant group difference in Minute 1 ($t_{(13)} = 0.55$, $p = .59$, 2-tailed) or Minutes 1 & 2 ($t_{(16)} = 0.40$, $p = .70$, 2-tailed).

3.4.3.3. *Cross-modal Object Recognition*

The mean DR for Saline and M/B conditions is shown in Figure 3.3C. Single sample t-tests (2-tailed) revealed that significant DRs in the Saline condition for Minutes 1 & 2 ($t_{(16)} =$

3.10, p <.05), but not Minute 1 ($t_{(16)}$ = 1.36, p = .19). In the M/B condition, no significant DR was found in Minute 1 ($t_{(17)}$ = 0.05, p = .96) or Minutes 1 & 2 ($t_{(17)}$ = 0.24, p = .81), indicating that in the M/B condition, rats did not explore the novel object significantly more than the familiar object. An independent t-test was chosen to compare Saline and M/B due to the fact that some rats were excluded unequally from one drug condition or the other and the analysis could therefore not be run as a true repeated measures design. A significant difference in the DRs was found between Saline and M/B condition on Minutes 1 & 2 ($t_{(33)}$ = 2.07, p <.05, 2-tailed), indicating that infusions of M/B significantly impaired cross-modal OR across the whole duration of the test, but not Minute 1 ($t_{(33)}$ = 0.93, p = .36, 2-tailed), although rats in the Saline condition did not exhibit a significant novel object preference during this minute of the test.

Table 3.2. Mean (SEM) Total Exploration Time During CMOR Test Phases (s)

Infusion		**Tactile**	**Visual**	**Crossmodal**
Saline	*Minute 1*	18.89 (2.70)	1.86 (0.39)	3.39 (0.41)
	Minutes 1 & 2	32.17 (4.03)	3.63 (0.63)	5.31 (0.55)
M/B	*Minute 1*	12.66 (3.43)	3.60 (0.48)	3.84 (0.48)
	Minutes 1 & 2	21.69 (4.59)	5.46 (0.65)	5.93 (0.62)

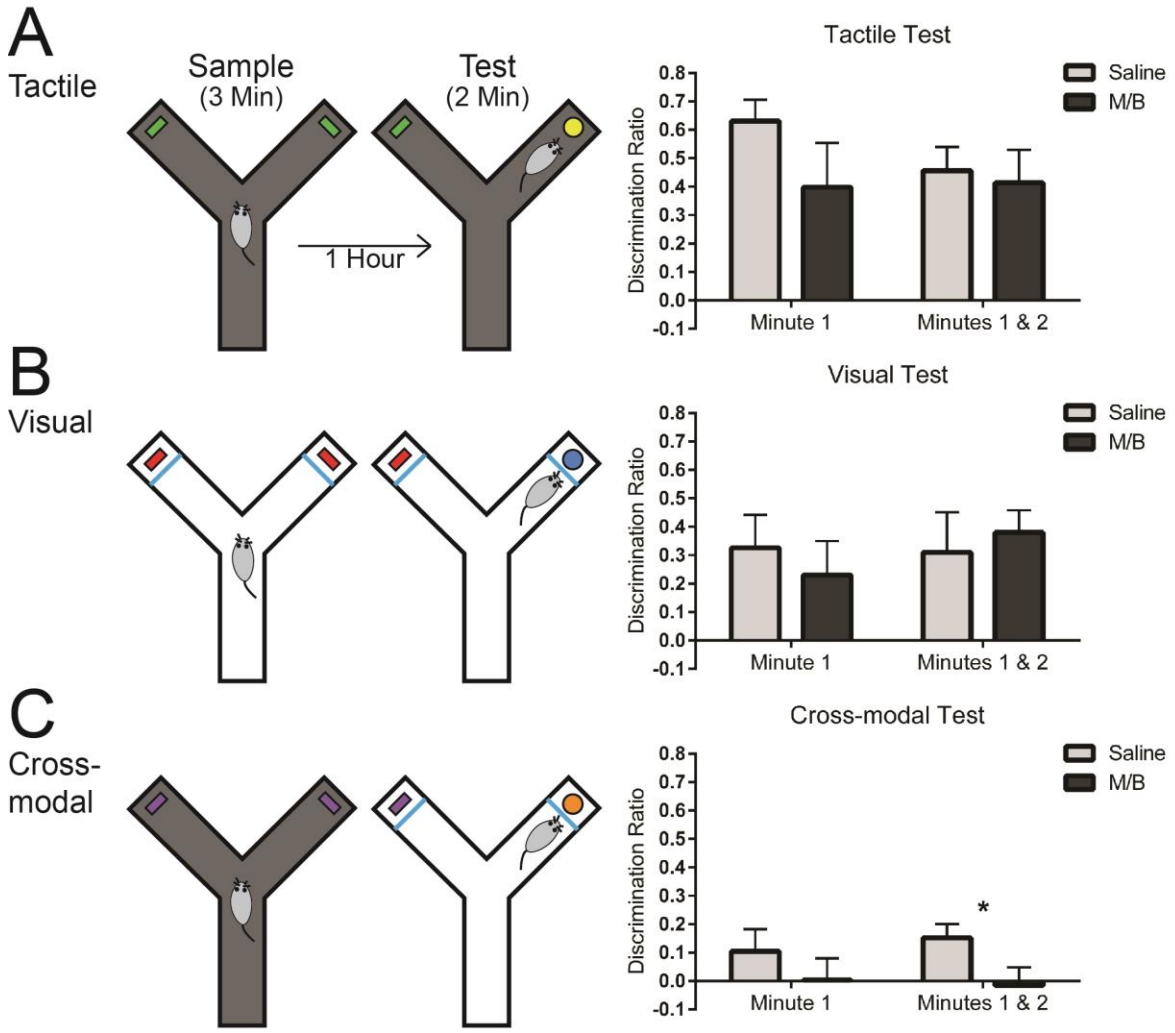

Figure 3.3. A) A schematic illustrating the behavioural procedure for Tactile OR and behavioural performance (Mean DR ±SEM). Rats in both conditions showed a significant novel object preference across the duration of the test and there were no group differences B) A schematic illustrating the behavioural procedure for Visual OR and behavioural performance (Mean DR ±SEM). Rats in the Saline condition showed a significant novel object preference only during Minute 1 of the test, whereas M/B rats showed a significant novel object preference over both minutes of the test, but not during Minute 1 specifically. No group differences were observed. C) A schematic illustrating the behavioural procedure for Cross-modal OR and behavioural performance (Mean DR ±SEM). Rats in the Saline condition showed a significant novel object preference across the duration of the test, but not during Minute 1 specifically, whereas rats in the M/B condition showed no novel object preference. Additionally, a significant group

difference was found where rats in the M/B condition showed a significantly lower DR across the duration of the test than in the Saline condition.

3.5. Discussion

The present experiment showed that temporary inactivations of the PC in rats did not disrupt span capacity in the OST, whereas the same inactivation procedure impaired performance in CMOR. Although previous studies have investigated the role of the rat PC in working memory (Espina-Marchant et al., 2006; Kolb et al., 1994; McDaniel et al., 1994), this was the first study, to our knowledge, that tested the role of the rat PC in mediating WM capacity for multiple non-spatial items. These results suggest that performance of the OST is independent of the PC in rats. Our findings provide evidence that the PC may not be a critical brain structure mediating WM capacity with all types of stimuli, which is surprising given that the PC is frequently implicated in WM in humans and non-human primates in many studies (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004).

The present results are also in contrast to previous experiments in which the PC was disrupted in rats during performance of a WM task (Espina-Marchant et al., 2006; Kolb et al., 1994; McDaniel et al., 1994). Importantly, McDaniel et al. (1994) used a multiple T-maze and Espina-Marchant et al. (2006) used an Olton 4x4 maze, both WM procedures that are spatial in nature, and found impairments in WM. Kolb et al. (1994) tested rats in two DNMTS tasks, the Aggleton DNMTS (Aggleton, 1985) and the Mumby DNMTS (Mumby et al., 1990), both of which are tests of non-spatial working memory. Rats with lesions to the PC performed normally in both tasks. However, the present experiment differs from Kolb et al. (1994) in a critical way in that the OST measures a greater range of WM capacity than DMNTS. Even given this difference, we did not find any effect of PC inactivations, indicating that even when the load on WM capacity is increased, the PC does not play a critical role in mediating it.

Of note, the mean spans achieved by the rats in the current study were lower than previously observed in our lab (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013). Importantly, we used a procedure adapted from MacQueen, Bullard, & Galizio (2011) to administer the OST that requires the rats to flip scented plastic lids off cups, rather than dig in bowls of scented sand used previously in our laboratory (Davies, Greba, et al., 2013; Davies, Molder, et al., 2013). Nonetheless, we are confident that the spans are high enough to have

revealed an impairment from our manipulation and that the present results do not appear to be an issue of floor effects. In the saline condition, rats performed significantly better than a hypothetical mean span of 0, and indeed, half of the rats performed better under the M/B condition than the Saline condition, with one of the rats achieving a span of 23 (the maximum possible score) after M/B infusions. Performance was also stable across 9 days before infusions began, and performance following saline infusions did not differ from performance on the day before infusions.

A caveat to our interpretation of the span data is that we considered only mean span as a measure of capacity in the OST, whereas other authors have considered percent accuracy or "longest run" (the largest span achieved by a rat within a session) as measures of OST performance (Galizio et al., 2016, 2013b). It is therefore possible that deficits in some other such metric of OST performance may be detected after PC disruption. What is clear from our interpretation, however, is that mean WM capacity in the OST – arguably the most basic metric for performance - does not depend on an intact PC.

We used CMOR as a positive control to verify that our inactivations sufficiently disrupted PC function. This task assesses the ability of rats to use multisensory integration to recognize objects (Cloke et al., 2015; Marks et al., 2016; Winters & Reid, 2010), and relies in part on the PC (Winters & Reid, 2010). The present findings showed an impairment in the cross-modal phase of CMOR after inactivations of the PC, in line with previous findings (Winters & Reid, 2010), and confirm the efficacy of our inactivations. However, in contrast to these previous findings, infusions of M/B did not impair either the Tactile or Visual phases of the task. An additional caveat with respect to our cross-modal test is that the Saline DR, despite being significantly above chance across both minutes of the test, is noticeably lower than controls in previously published papers (Marks et al., 2016; Reid et al., 2012; Winters & Reid, 2010), which may be a result of damage to the PC caused by the implantation of the 4 guide cannulae. Nonetheless, we observed that rats in the M/B condition showed no novel object preference during the cross-modal test and were significantly impaired relative to the Saline condition, providing evidence that our PC inactivations significantly affected behaviour.

It should also be noted that our null finding with respect to the PC and WM capacity pertains only to performance after extensive OST training. The OST and CMOR differ in that OST requires weeks of behavioural training, whereas CMOR is a spontaneous task involving no

skill or rule learning on the part of the rat. The findings therefore do not preclude a role of the PC in initial learning of task-related rules in the OST. There is some evidence from human neuroimaging studies that the PC is engaged during initial learning of task rules (Dumontheil, Thompson, & Duncan, 2011; Ruge & Wolfensteller, 2016). It is therefore possible that manipulations of the PC during early shaping of the task would impair acquisition. However, this would likely be an impairment specifically related to the non-matching *rule* rather than an impairment in maintaining task stimuli.

The lack of involvement of the PC in the OST may reflect functional differences between the rodent and primate PC. In contrast to findings with primates (Buschman & Miller, 2007), rats with lesions to the PC do not appear impaired in various tests of attention (Broussard, Sarter, & Givens, 2006; Maddux, Kerfoot, Chatterjee, & Holland, 2007; Muir, Everitt, & Robbins, 1996; Rosner & Mittleman, 1996; Ward & Brown, 1997; but see Fox, Barense, & Baxter, 2003). The OST in humans also depends on the hippocampus (Levy et al., 2003) whereas it does not in rats (Dudchenko et al., 2000). However, it is noteworthy that a spatial span task (Dudchenko et al., 2000) and other spatial (Olivito et al., 2016) and object-based tasks (Sugita, Yamada, Iguchi, & Ichitani, 2015) for measuring memory capacity involve the hippocampus in rats. Hence, there may be evolutionary differences in the in the circuitry underlying the OST, including the relative importance of the PC for attention, between primates and rodents which, in light of the present results, could extend to WM capacity.

A more likely reason for the discrepancy between the present results and the majority of previous literature on the role of the PC in WM is the type of stimuli used. Human and non-human primate studies have predominantly used visuospatial or phonological stimuli (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). The PC is well-known as a major component of the dorsal visual processing stream (Cooper & O'Sullivan, 2016). The evidence of involvement of the human PC in olfactory processing is mixed. Although a few neuroimaging studies find activation of parts of the PC during olfactory tasks (Frasnelli et al., 2012; Karunanayaka et al., 2014; Savic, Gulyas, Larsson, & Roland, 2000), patients with lesions to the PC are not impaired in in odour identification (Jones-Gotman & Zatorre, 1988). Rather, olfactory stimuli are processed in the piriform, entorhinal, and amygdalar cortices (Chapuis et al., 2013; Miyamichi et al., 2011). If the items held in WM are non-spatial olfactory

stimuli, as they were in the present study, the PC may simply not be engaged. Hence, the present results may reflect that the PC performs a sensory role in visual or visuospatial WM, and is not involved in non-spatial olfactory WM. This interpretation is bolstered by results from other labs showing evidence for sensory modality-specific processing in the PC (Licata et al., 2017; Pisella, Berberovic, & Mattingley, 2004).

3.6. Conclusions

Previous studies of the role of the rat PC in WM have found involvement of the PC in tests of spatial WM (Espina-Marchant et al., 2006; McDaniel et al., 1994) but not non-spatial WM (Kolb et al., 1994). Importantly, these studies have not investigated the role of the PC in WM in a non-spatial test requiring WM capacity for multiple items. The present study found no disruption of performance in the OST by PC inactivations with combined muscimol/baclofen, whereas cross-modal object recognition was impaired by the same manipulation. These results suggest that WM in rats, at least for non-spatial olfactory stimuli, is independent of the PC. The discrepancy between the present findings and the findings in humans and non-human primates may be a result of an evolutionary difference between primates and rodents in the type of information processing in the PC, or a reflection of differences in the types of sensory stimuli used in primate studies and the present study.

4. EXPERIMENT 3: PERFORMANCE OF THE TRIAL-UNIQUE, DELAYED NON-MATCHING-TO-LOCATION (TUNL) TASK DEPENDS ON AMPA/KAINATE, BUT NOT NMDA, IONOTROPIC GLUTAMATE RECEPTORS IN RATS

The content in this chapter is published in *Neurobiology of Learning and Memory*. My specific contributions to this work included performing the majority of behavioural training/testing, the majority of intracranial surgeries, a large proportion of intracranial drug infusions, all histology, and all statistical analyses of behaviour. Finally, I was the primary author and completed the majority of manuscript preparation, preparation of figures, and responses to reviewers. The following additional authors made significant contributions to this work: Andrew J. Roebuck assisted in behavioural training and manuscript preparation, Quentin Greba assisted in performing stereotaxic surgeries and intracranial infusions, and John G. Howland assisted in experimental design and manuscript preparation.

Scott, G. A., Roebuck, A. J., Greba, Q., & Howland, J. G. (2019). Performance of the trial-unique, delayed non-matching-to-location (TUNL) task depends on AMPA/Kainate, but not NMDA, ionotropic glutamate receptors in the rat posterior parietal cortex. *Neurobiology of Learning and Memory, 159,* 16–23.

4.1. Abstract

Working memory (WM), the capacity for short-term storage and manipulation of small quantities of information, depends on fronto-parietal circuits. However, the function of the posterior parietal cortex (PPC) in WM has gone relatively understudied in rodents. Recent evidence calls into question whether the PPC is necessary for all forms of WM. Thus, the present experiment examined the role of the rat PPC in the Trial-Unique Non-matching-to-Location (TUNL) task, a touchscreen-based visuospatial WM task that relies on the rat medial prefrontal cortex. Temporary inactivation of the PPC caused via bilateral infusions of muscimol and baclofen significantly impaired accuracy and increased the number of correction trials performed, indicating that the PPC is necessary for performance of TUNL. Additionally, we investigated the effects of blocking NMDA or non-NMDA parietal ionotropic glutamate receptors on TUNL and found that, in contrast to the prefrontal cortex, NMDARs in the PPC are not necessary for TUNL performance, whereas blockade of AMPA/Kainate receptors significantly impaired accuracy. These results indicate that performance of the TUNL taskdepends on the PPC but that NMDA signaling within this brain area is not necessary for intact performance.

4.2. Introduction

Working memory (WM) is the ability to temporarily store small quantities of information for use or manipulation and is likely mediated by a distributed network of brain areas including a fronto-parietal circuit involved in attention and executive control (D'Esposito & Postle, 2015; Eriksson et al., 2015). The neural correlates of WM have been understudied in rodents relative to primates and reveal a less consistent story with respect to the role played by the parietal cortex (PC). In primates, the PC has been consistently shown to participate in WM tasks (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). In rodents, however, disruption of the PC has led to inconsistent results. Tasks involving spatial stimuli seem to engage the PC (Espina-Marchant et al., 2006; McDaniel et al., 1994) whereas tasks with non-spatial stimuli are independent of the PC (Kolb et al., 1994; Scott et al., 2018).

In a recent paper, we demonstrated that the odor span task (OST), a non-spatial olfactory WM task (Dudchenko et al., 2013), does not rely on the posterior parietal cortex (PPC) in rats (Scott et al., 2018), a finding contrary to the view that the PPC is critical for WM performance. We hypothesized that the PC in both rodents and primates plays a sensory modality-specific role in WM that has been overlooked due to the heavy emphasis in primate research on visual or visuospatial WM tasks (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). Hence, further investigation is required to delineate the role of the rodent PPC in WM.

In the present experiment, we investigated role of the rat PPC in the Trial-Unique Non-matching-to-Location task (TUNL), a visuospatial WM task for rodents (Dudchenko et al., 2013; Hvoslef-Eide, Mar, et al., 2015). The TUNL task offers several advantages over other WM tasks typically used in rodents. It is fully automated, allowing the rapid administration of large numbers of trials with minimized bias or variability introduced by heavy experimenter interaction with animals. Additionally, it closely mimics human visuospatial WM testing in order to provide high construct and predictive validity (Hvoslef-Eide, Mar, et al., 2015). The stimuli are also presented at varying degrees of physical separation in TUNL, thereby varying the level of difficulty from trial to trial which may engage hippocampal-dependent pattern separation processes (McAllister et al., 2013). Performance of TUNL relies on the rodent mPFC (Davies,

Hurtubise, et al., 2017; Kim et al., 2015; McAllister et al., 2013) in line with studies in human and non-human primates.

In addition to investigating the role of the PPC in visuospatial WM with temporary inactivations, we investigated the relative contributions of NMDA and non-NMDA ionotropic glutamate receptors in the PC. WM performance relies heavily on NMDA receptors in the PFC (Davies, Greba, & Howland, 2013; MacQueen, Bullard, & Galizio, 2011; Wang et al., 2013) and the TUNL task specifically requires NMDA receptor activation in the mPFC (Davies, Hurtubise, et al., 2017; Hurtubise et al., 2017; G. Kumar et al., 2015). The role of NMDA receptors in the PPC for WM has received very little study, but some research suggests that NMDA receptors in the rat PC are not necessary for short-term retention in a one trial step-down inhibitory avoidance task (Izquierdo et al., 1998, 1997). Thus, we were interested in assessing the involvement of PPC ionotropic glutamate receptors in the TUNL task.

4.3. Methods

4.3.1. Subjects

Fifteen male Long Evans rats (Charles River Laboratories, Kingston, NY) weighing 300-500g were used in the experiment. Rats were individually housed in standard ventilated cages and kept on a 12-hour light/dark cycle (lights on at 0700). Rats were maintained at 85-90% of their free-feeding weight with water available *ad libitum*. All experiments were approved by the University of Saskatchewan Animal Research Ethics Board and conformed to the guidelines of the Canadian Council on Animal Care.

4.3.2. Behavioural Training

4.3.2.1. *Training Apparatus*

An illustration of the training apparatus for TUNL is shown in Figure 4.1B. Training in TUNL was conducted within 8 touchscreen-equipped operant conditioning chambers controlled by ABET II Touch software (Lafayette Instruments, Lafayette, IN) as we have used previously (Davies et al., 2017; Roebuck et al., 2018). Briefly, each chamber was housed within a sound-attenuated, vented box, with live video feed. Touchscreens were fitted with an interchangeable mask which obscured the screen except for the response windows. For TUNL, response windows consisted of an array of 14 2x2 cm square holes in a 7x2 arrangement. The response windows sat above a spring-loaded response shelf requiring the animal to stand when responding.

4.3.2.2. *Touchscreen Habituation and Pretraining*

All habituation, pretraining and training stages were conducted according to protocols developed by Lafayette, and previous experiments conducted in our lab (Davies et al., 2017). All stages of TUNL occurred once daily, 5 days per week. Behavioral training began with several days of habituation, which first involved 1 h in the touchscreen room with all equipment on. On days 2 and 3 of habituation, rats were placed in the touchscreen chambers for 30 min with reward pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, NJ, USA) placed in the food dispenser.

Following habituation, animals progressed through 4 pretraining stages upon reaching intermediate criterion. The first pretraining stage was Initial Touch where one square is illuminated pseudorandomly. Correct touches during this stage were followed by illumination of the food port and delivery of 3 reward pellets. If the animal failed to contact the illuminated square within 30 s, the food port was illuminated, and a single reward pellet dispensed. Each trial was separated by a 20 s inter-trial interval (ITI) which was consistent across all stages. Criterion for initial touch was completion of 100 selection trials in 1 h. Following Initial Touch, animals progressed to Must Touch on the next training day. Must touch was administered similarly except that the rat must touch the illuminated square to receive a single reward pellet. There was no reward if the animal did not contact the illuminated square and the trial continued until a response was recorded. Criterion for Must Touch was completion of 100 selection trials in 1 h. Must Initiate followed and added the requirement that the animal nose poke the food port to initiate each trial. Criterion for Must Initiate is 100 selection trials in 1 h. The final pretraining stage, Punish Incorrect, was administered similarly to previous stages wherein animals must initiate by nose poking the food port and correctly touch the illuminated square to receive a food reward. However, in Punish Incorrect, incorrect touches were punished by a 5 s timeout and illumination of the house lights. Following the timeout period, a ‘correction trial’ began where the animal must repeat the same trial configuration until it was successfully completed, at which point the animal was rewarded with a food pellet and a selection trial was completed. Criterion for Punish Incorrect was completion of 100 selection trials in 1 h, with >80% accuracy for two consecutive days. Accuracy was computed from the initial presentation of a trial only. Correction trials did not count toward accuracy or the number of completed selection trials.

4.3.2.3. *TUNL Task Acquisition*

Following pretraining, animals began TUNL task acquisition (Figure 4.1A and C). TUNL is a non-match to sample task consisting of multiple trials. Trials were composed of a sample phase and a test phase. During the sample phase one of the 14 squares was illuminated and the animal was required to touch the square. After the lit square was touched, there was a delay period (2 s in Initial TUNL, 6 s in Full TUNL), before the test phase began. During the test phase the same square from the sample phase was illuminated, as well as a new separate square. The animal was required to touch the new square to complete the trial and receive a food reward. The distance between the sample stimuli and test stimuli varied (ranging from 2-6 squares apart). These distances were categorized as Large (least challenging), Medium, and Small (most challenging). If the sample stimuli were incorrectly chosen during the test phase, the animal was punished with a 5 s timeout and illumination of the house lights. This also triggered a correction trial where the animal had to repeat the same trial configuration until it was successfully completed, at which point the animal was rewarded with a food pellet and a selection trial was completed. Accuracy was computed from the initial presentation of a trial only. Correction trials did not count toward accuracy or the number of completed selection trials.

Acquisition of TUNL was completed in 2 stages: Initial TUNL and Full TUNL. During Initial TUNL animals were trained for 1 h daily until they were able to complete 42 selection trials in 40 min with a 2 s delay between the sample and test phase. After completing initial TUNL animals were moved to Full TUNL where the maximum is 84 selection trials in 1 h, with a 6 s delay between the sample and test phase. Before undergoing intracranial surgeries, rats were trained to a criterion of at least 75% accuracy at Large distances for 2 consecutive days. On infusion days, rats were tested using the same Full TUNL protocol as above, including correction trials. Although the inclusion of correction trials makes for a less pure assessment of memory performance, it confers the major benefit of simultaneously measuring behavioural flexibility (Hurtubise et al., 2017; Kumar et al., 2015; Lins & Howland, 2016; Lins, Phillips, & Howland, 2015). Additionally, accuracy on test phases is calculated only from rats' first attempt at choosing the correct test stimulus, so correction trials do not influence rats' accuracy score.

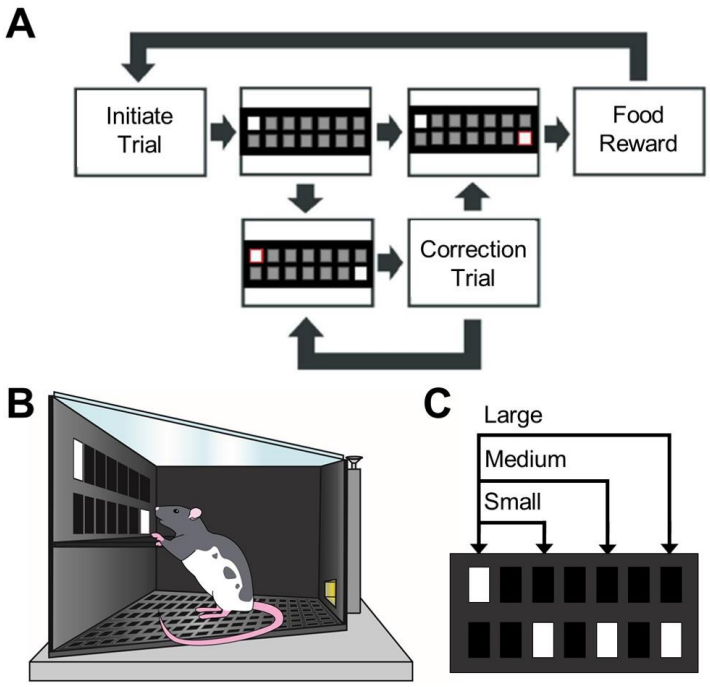

Figure 4.1. A) Schematic showing the general progression of a TUNL trial. B) A representation of the interior of a touchscreen chamber used in the experiment. C) A schematic representation of example stimulus distances used in TUNL.

4.3.3. Surgery and Intracranial Infusions

Intracranial cannulae implantation as well as the basic infusion procedure were conducted in the same manner described in Scott et al. (2018). Briefly, rats were bilaterally implanted with 2 23-gauge stainless steel guide cannulae per hemisphere aimed at the PPC (AP -4.0mm, ML ±2.2 and 3.4, DV -0.2 from brain surface). On infusion days, rats received infusions of combined muscimol and baclofen (M/B; each 0.5 mg/mL, mixed together in a 1/1 ratio (Davies, Molder, et al., 2013; Scott et al., 2018)), the competitive NMDA antagonist AP5 (5.90 mg/mL (Bett et al., 2013)), the competitive AMPA/Kainate receptor antagonist CNQX (disodium salt; 828.36 µg/mL (Bett et al., 2013)), or vehicle (0.9% physiological saline) in counterbalanced order over 4 consecutive days of testing. Drugs were infused through 30-gauge stainless steel needles connected to PE50 tubing using Hamilton syringes and a microinfusion pump (Harvard Apparatus, Holliston, MA, USA). Needles were lowered 1 mm past the end of the guide cannuale. Each drug was infused at 0.5 µL/min to a final infusion volume of 0.5 µL per site and

a total of 1 µL per hemisphere. Infusions were given 20-60 minutes before rats were placed in the touchscreen chambers.

4.3.4. Perfusions and Histology

Following the conclusion of testing, rats were perfused intracardially with 0.1 M PBS followed by 30% formalin. Brains were removed and post-fixed in 30% formalin and cryoprotected in 0.1% sodium azide/30% sucrose until brains were no longer buoyant in the cryoprotectant solution. Brains were sectioned at 40 µm on a freezing sliding microtome (Leica Biosystems, Concord, ON). Sections were mounted to glass slides and assessed for cannulae placement with the help of a rat brain atlas (Paxinos & Watson, 2006).

4.3.5. Statistical Analyses

All data were collected automatically and are presented as group means +/- SEM. Analysis consisted of several measures of task performance: selection trial completion (number of completed selection trials in a session), percent accuracy on initial test stimuli presentation, correction trial completion (number of correction trials in a session), total trial completion (number of completed selection trials + correction trials in a session), correct response latency (latency to respond during the test phase for correct choices), incorrect response latency (latency to respond during the test phase for incorrect choices), and reward collection latency (latency from reward dispensation to reward collection) which were all obtained using the in-software analysis tools included in Abet Touch II. Each drug condition was separately compared to Saline because some rats did not complete all drug conditions (fewer rats were administered CNQX) meaning that a combined within-subjects analysis was not possible. Percent accuracy was analyzed in a 2x3 mixed-design factorial ANOVA with a Drug factor with 2 repeated measures levels and a Distance factor with 3 independent levels representing large, medium, and small stimulus separations.

The number of selection trials completed, the number of correction trials, and the total number of trials (including both selection and correction trials) were each compared between Saline and drug conditions in within-subjects t-tests. Latency for correct choices and latency for incorrect choices were compared between Saline and Drug in 2x2 mixed-design ANOVAs with a repeated measures Drug factor and an independent Trial Outcome factor (correct versus incorrect

choice), and latency for reward collection were also compared between Saline and Drug conditions in within-subjects t-tests.

4.4. Results

4.4.1. Histology

Approximate placements of the infusion needles, as well as a representative photomicrograph of the infusion sites, are depicted in Figure 4.2. All 15 rats had infusion sites that were deemed acceptable. All rats had some damage to the superficial layers of the cortex proximal to the location of the guide cannulae.

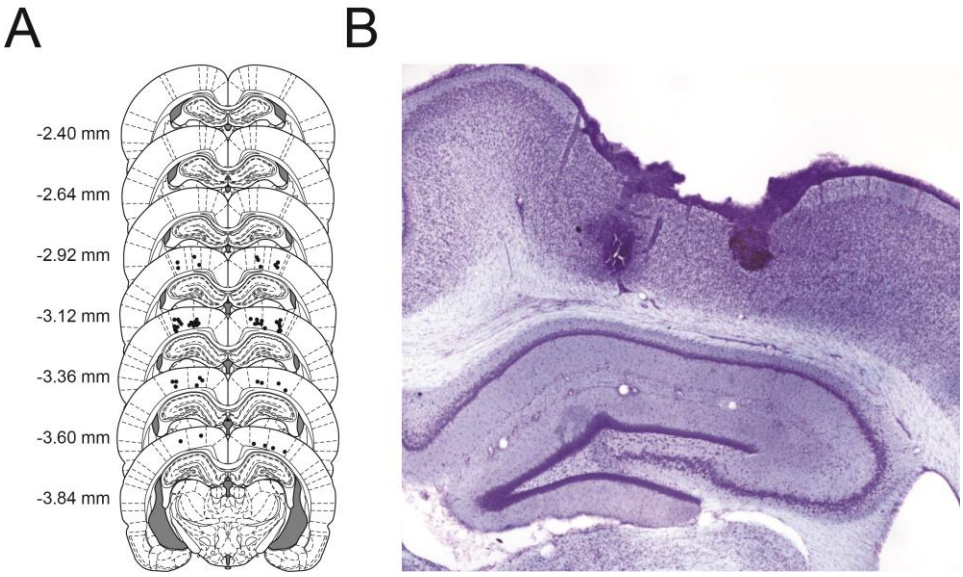

Figure 4.2. A) Diagram showing approximate locations of infusion sites. Images were adapted from Paxinos & Watson (2006). B) Photomicrograph of a representative cresyl violet-stained section showing the locations of infusions.

4.4.2. Percent Accuracy

Percent accuracy for all 3 drug conditions are shown in Figure 4.3. One rat was removed from the M/B analysis due to excessive motor impairment and response latency after M/B infusions while another rat was euthanized due to illness before completing all drug conditions and was not included in the CNQX or AP5 conditions. Additionally, a smaller subset of the total sample was administered CNQX. Thus, the final sample sizes for each drug condition were n = 14 for M/B, n = 10 for CNQX, and n = 14 for AP5. A 2x3 mixed design ANOVA comparing Saline with M/B infusions revealed a main effect of drug ($F_{(1,39)} = 21.47$, $p < .0001$) indicating

that rats had significantly impaired accuracy following inactivation of the PPC. There was also a significant interaction of drug by distance ($F_{(2,39)} = 6.08$, $p < .01$), but no main effect of distance ($F_{(2,39)} = 2.45$, $p = .10$). Fisher's LSD post-hoc tests revealed that percent accuracy was significantly reduced at large ($p < .05$) and medium ($p < .0001$) distances but not at small distances ($p = .67$). To test for a possible floor effect at small distances that might obscure differences between drug conditions, we performed single-sample t-tests comparing rats' accuracy to chance (50%) which revealed that rats still performed significantly better than chance in the Saline ($t_{(13)} = 7.22$, $p < .0001$, 2-tailed) and M/B ($t_{(13)} = 4.86$, $p < .001$, 2-tailed) conditions. CNQX infusions also yielded a significant main effect of Drug ($F_{(1,27)} = 9.98$, $p < .005$) indicating a significant impairment in accuracy following blockade of AMPA/Kainate receptors in the PPC. There was also a significant interaction of drug by distance ($F_{(2,27)} = 5.15$, $p < .05$), but no main effect of distance ($F_{(2,27)} = 1.60$, $p = .22$). Fisher's LSD post-hoc tests revealed that percent accuracy was reduced at medium distances ($p < .001$) but not at large ($p = .35$) or small ($p = .90$) distances. Single-sample t-tests were again run to test for a floor effect at small distances and revealed that rats performed significantly better than chance in the Saline ($t_{(9)} = 7.11$, $p < .0001$, 2-tailed) and CNQX ($t_{(9)} = 4.20$, $p < .01$) conditions. In contrast, infusions of AP5 exhibited no main effect of drug ($F_{(1,39)} = .04$, $p = .85$) or any interaction ($F_{(2,39)} = .57$, $p = .57$), indicating that blockade of NMDA receptors in the PPC did not affect accuracy. However, a significant main effect of distance was found ($F_{(2,39)} = 18.91$, $p < .0001$). Fisher's LSD post-hoc tests revealed that rats in both conditions had significantly lower accuracy at small distances than at large ($p < .001$) and medium ($p < .0001$) distances with no difference in accuracy between large and medium distances ($p = .11$).

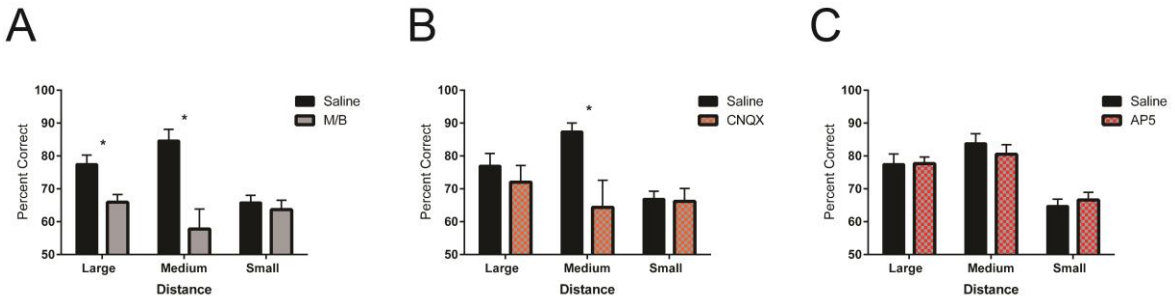

Figure 4.3. A) Percent accuracy (Mean ±SEM) in TUNL after Saline or M/B infusions. Accuracy was significantly reduced by M/B infusions at large and medium distances, indicating that intact PC function is necessary for TUNL. B) Percent accuracy (Mean ±SEM) in TUNL after Saline or

CNQX infusions. Accuracy was significantly reduced at medium distances, indicating that AMPA/Kainate receptors are necessary for intact TUNL performance. C) Percent accuracy (Mean ±SEM) in TUNL after Saline or AP5 infusions. Accuracy was completely unaffected by AP5, indicating that TUNL performance is independent of NMDA signaling in the PC.

4.4.3. Trial Completion

Trial completion for all 3 Drug conditions and Saline is shown in Figure 4.4. A within-subjects t-test revealed a significant increase in the number of correction trials performed in the M/B condition ($t_{(13)} = 3.30$ $p < .01$), indicating an increase in perseverative behaviour. Additional within-subject t-tests revealed no difference between Saline and M/B in the number of selection trials completed ($t_{(13)} = -1.99$, $p = .07$) or the total number of trials completed ($t_{(13)} = -0.16$, $p = .88$). Infusions of CNQX, by contrast, did not increase correction trials relative to Saline ($t_{(9)} = .64$, $p = .54$), nor did it affect completion of selection trials ($t_{(9)} = -0.95$, $p = .37$) or the total number of trials ($t_{(9)} = -0.46$, $p = .66$). Infusions of AP5 had no effect on correction trials ($t_{(13)} = .71$, $p = .49$), selection trials ($t_{(13)} = -0.27$, $p = .79$), or total trials ($t_{(13)} = 0.14$, $p = .89$).

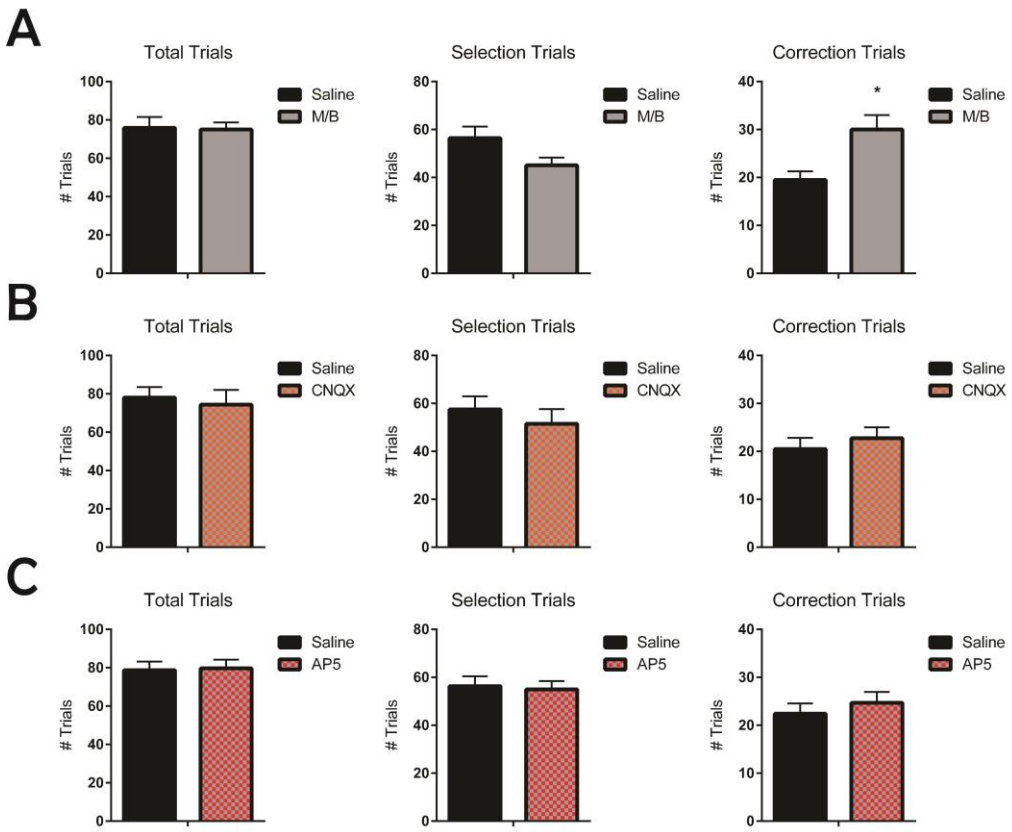

Figure 4.4. A) Mean (±SEM) number of completed trials in TUNL after Saline or M/B infusions. Neither the number of selection trials or the number of total trials completed were affected by M/B. However, M/B caused a significant increase in the number of correction trials performed, indicating a possible impairment in behavioral flexibility. B) Mean (±SEM) number of completed trials in TUNL after Saline or CNQX infusions. CNQX had no effect on selection, correction, or total trials completed. C) Mean (±SEM) number of completed trials in TUNL after Saline or AP5 infusions. AP5 had no effect on selection, correction, or total trials completed.

4.4.4. Response Latency

Response latency for all 3 drug conditions and Saline is shown in Figure 4.5. A 2x2 mixed design ANOVA on latencies for correct versus incorrect choices after M/B infusions revealed no main effect of Drug ($F_{(1,26)} = 1.48$, $p = .24$), Trial Outcome ($F_{(1,26)} = 2.09$, $p = .16$), or any interaction ($F_{(1,26)} = 0.01$, $p = .93$), suggesting that PPC inactivation did not affect rats' speed of responding during test phases. However, a small but significant increase in reward collection latency was observed after M/B infusions ($t_{(13)} = 3.94$, $p < .01$). Similarly, infusions of CNQX also yielded no main effect of Drug ($F_{(1,18)} = 0.57$, $p = .46$), Trial Outcome ($F_{(1,18)} = 2.40$, $p =$

.14), or any interaction ($F_{(1,18)}$ = 0.002, p = .97) but, unlike M/B infusions, did not cause a change in reward collection latency ($t_{(9)}$ = -.24, p = .81). Infusions of AP5 produced no main effect of Drug ($F_{(1,26)}$ = 0.77, p = .39), Trial Outcome ($F_{(1,26)}$ = 1.39, p = .25), or any interaction ($F_{(1,26)}$ = 0.38, p = .54), or difference in reward collection latency ($t_{(26)}$ = .26, p = .80).

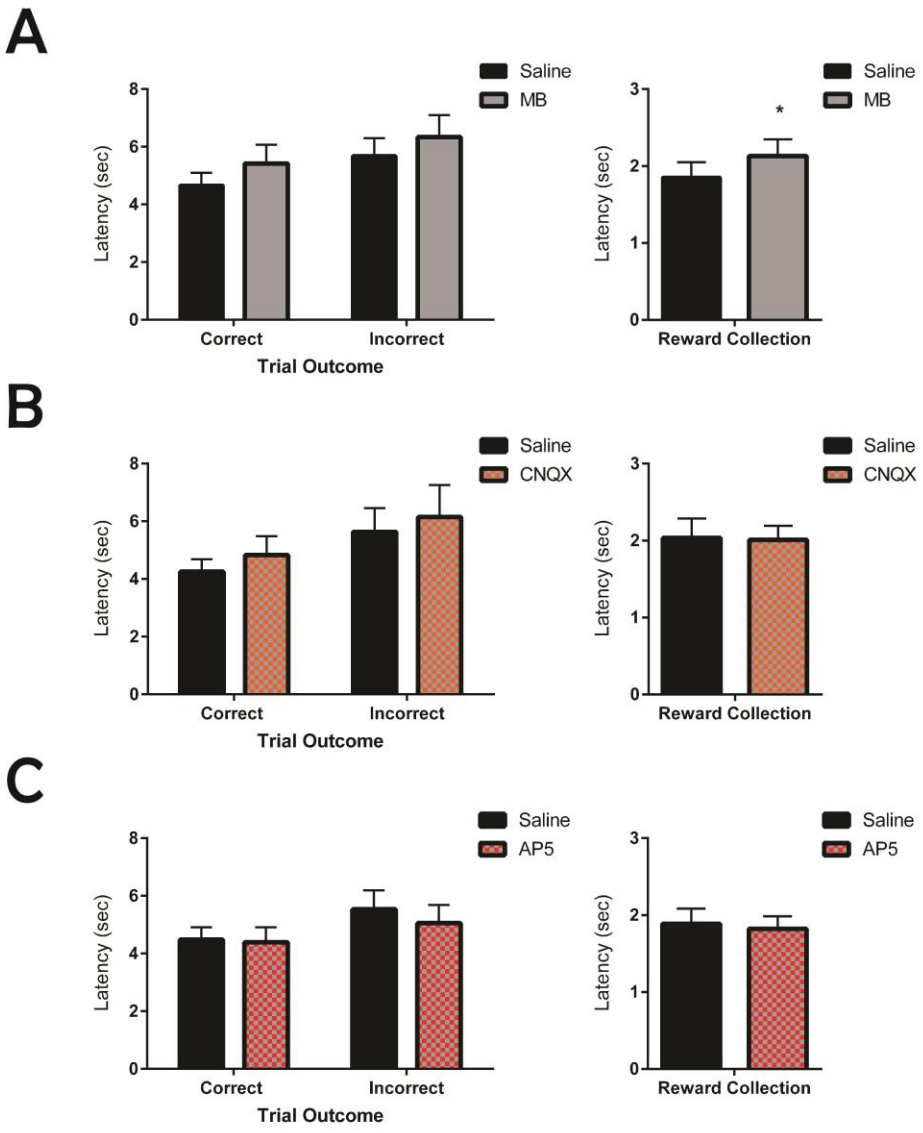

Figure 4.5. A) Mean (±SEM) latency in TUNL after Saline or M/B infusions. Neither correct choice latency or incorrect choice latency were affected by M/B. However, M/B caused a small but statistically significant increase in reward collection latency which may be related to the PPC's role in task-reward associations. B) Mean (±SEM) latency in TUNL after Saline or CNQX infusions. CNQX had no effect on latency. C) Mean (±SEM) latency in TUNL after Saline or AP5 infusions. AP5 had no effect on latency.

4.5. Discussion

The present experiment aimed to determine whether the rodent PPC plays a role in the TUNL task, a rodent model of visuospatial WM. After PPC inactivation with M/B or AMPA/Kainate blockade with CNQX, rats performed with significantly impaired accuracy. Thus, the present results indicate that the rat PPC is necessary for intact performance of the TUNL task. The results are similar to previous studies which have demonstrated an involvement of the rodent PPC in spatial WM including a multiple T-maze task (McDaniel et al., 1994) and the Olton 4 x 4 maze (Espina-Marchant et al., 2006). Additionally, they are in agreement with the ample evidence from primates that the PPC is involved in visuospatial WM (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). In contrast, disruption of the rat PPC during WM tasks using odours (Scott et al., 2018) or objects (Kolb et al., 1994) does not cause impairments. Overall, the combined findings strongly suggest that the PPC plays a sensory modality-specific role in WM that includes visual/visuospatial stimuli. Indeed, the PPC is a part of the dorsal visual stream (Cooper & O'Sullivan, 2016), which is involved in the perception of the location of objects in space. Recent evidence also confirms that the rodent PPC is connected to auditory and tactile sensory areas (Hovde, Gianatti, Witter, & Whitlock, 2018) although manipulation of the PPC has been shown to affect the visual modality more than the auditory modality (Licata et al., 2017). The PC is also a crucial part of the frontoparietal attention network along with the PFC that has gained significant recent interest for its role in the executive control of WM and attention (Fiebelkorn, Pinsk, & Kastner, 2018; Johnson et al., 2017; Murray et al., 2017; Wallis, Stokes, Cousijn, Woolrich, & Nobre, 2015).

The impaired accuracy that we observed after infusing M/B or CNQX was specific to large and medium stimulus distances. Rats performed small distances with less accuracy than medium or large distances, although the main effect of distance only reached significance in the AP5 comparison. The lack of an effect of M/B and CNQX appears to reflect a drop-off in performance in the Saline condition at these distances. Under all drug conditions, however, rats still performed with accuracy significantly better than chance, making it highly unlikely that this was a floor effect. Rather, it appears that M/B and CNQX caused rats to perform with mostly equally poor accuracy across all stimulus distances while performance in the Saline and AP5

conditions benefited from the less-challenging large and medium stimulus distances and that our manipulations, while causing significant impairment, still spared some intact performance. This pattern of results is very similar to Talpos, McTighe, Dias, Saksida, & Bussey (2010) who found that, using a 6-s delay (the same as the present experiment), hippocampal lesions only resulted in a difference in percent accuracy at large and medium distances, with control performance dropping to the level of lesion performance at small distances.

An additional dimension to our accuracy results is pattern separation. The TUNL task, in addition to measuring visuospatial WM, has been used to measure hippocampal-dependent pattern separation (Talpos et al., 2010). Specifically, rats are inferred to have impaired pattern separation when they are more severely impaired at small distances than large distances. There is also some evidence from humans that the parietal cortex additionally participates in pattern separation (Pidgeon & Morcom, 2016). The present CNQX results could be argued to partially fit this pattern given that rats are impaired at medium distances but not at large distances (although there is no drug effect at minimum distances). Conversely, the impairment from M/B does not satisfy this criterion as rats are impaired at maximum and medium distances. Hence, AMPA/Kainate blockade within the PPC may have a specific effect on pattern separation, leaving memory intact on trials with more distinct choices, whereas complete inactivation with M/B causes a more global impairment in accuracy.

In addition to impairing accuracy, other impairments were observed after infusions of M/B. Correction trials were significantly increased, indicating that rats perseverated more on incorrect choices, an effect which has previously been used to infer impaired behavioral flexibility (Hurtubise et al., 2017; Kumar et al., 2015; Lins & Howland, 2016; Lins et al., 2015). This finding is in general agreement with previous research that has shown that lesions of the rodent PPC can impair attentional set-shifting (Fox et al., 2003), indicating that the PPC plays some role in mediating behavioral flexibility. Of note, the inclusion of correction trials during the tests, while providing a measure of behavioural flexibility, may have contributed some bias to the results in that they comprise a practice element that rats may benefit more from under control conditions. However, given that the TUNL task is trial-unique, this practice would ostensibly introduce a bias in rule acquisition rather than accuracy on individual trials as accuracy was only computed from rats' first choice on a set of test stimuli. Another interesting finding was the increased reward collection latency following M/B infusions. Although the difference is

extremely small (~0.3 sec), previous research has shown that the human PPC contains representations of task-reward associations (Wisniewski, Reverberi, Momennejad, Kahnt, & Haynes, 2015) meaning that this finding could be interpreted as an impairment in task-reward association.

Interpretation of WM impairments can be confounded if changes in latency are found along with changes in accuracy. Longer latencies may fatigue the maintenance mechanisms of WM and impair performance even if the given manipulation has no direct effect on WM accuracy or capacity. Despite the increased reward collection latency following infusions of M/B, we did not observe significant increases in latency for correct or incorrect choices during test phases, so the reduction in accuracy after M/B infusions likely had little to do with increased latency. Additionally, there was no significant reduction in the total number of trials rats completed after M/B infusions compared with Saline infusions. We therefore deem it unlikely that the results of M/B infusions are confounded by locomotor deficits, as rats evidently completed trials with the same efficiency after M/B or Saline.

Interestingly, the impairment caused by CNQX infusions was specific to accuracy and was not associated with alterations in latency or correction trials. This result may simply reflect a less severe impairment due to our chosen CNQX dose. We deliberately used a CNQX dose similar to what has been previously used in experiments dissociating NMDA from non-NMDA signaling (Bett et al., 2013). However, other groups have used considerably higher CNQX doses when comparing NMDA and non-NMDA signaling (Agrawal & Fehlings, 1997; Barker & Warburton, 2011). Alternatively, it is possible that this apparent dissociation reflects the pharmacological differences between M/B and CNQX. While CNQX would primarily exert its effects post-synaptically as the majority of AMPA receptors are located post-synaptically (Feligioni, Holman, Haglerod, Davanger, & Henley, 2006; Fujiyama et al., 2004), the baclofen in our M/B mixture would presumably bind to GABAb receptors on the presynaptic terminals of afferent connections arising from other areas of cortex or from neuromodulator-secreting nuclei and inhibit neurotransmitter release (Misgeld, Bijakt, & Jarolimek, 1995). For example, it has been demonstrated that norepinephrine signaling in the human PPC is involved in task switching under memory load (Wolff, Mückschel, Ziemssen, & Beste, 2018) which could be said to be similar to the rats in the present experiment having to switch their response during correction trials.

The present findings confirm a role for the PPC in TUNL, but do not indicate during which epochs of the task (i.e. sample, delay, test, and reward collection) it is critically engaged. The impairment we found could reflect the disruption of stimulus encoding/delay period maintenance (Christophel, Cichy, Hebart, & Haynes, 2015; Christophel, Hebart, & Haynes, 2012; Ester, Sprague, & Serences, 2015; Vogel & Machizawa, 2004) or retrieval (Berryhill & Olson, 2008; Öztekin et al., 2009). Further experimentation with more temporally precise methods will be necessarily to clarify this issue. Additionally, we did not include variable delay periods in this experiment meaning that we cannot rule out the possibility that performance would still be impaired by our manipulations under short delays or no delay, which would reflect an impairment in choice accuracy rather than WM maintenance *per se*. Therefore, although the present results fit well with the literature showing a role for the PPC in visuospatial WM (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004), we cannot rule out the possibility that our findings reflect impairments other than WM maintenance.

The lack of an effect of blocking NMDA receptors is interesting given that major models of WM require NMDA signaling (specifically NR2B-containing NMDA receptors) for maintenance of delay period persistent activity (Wang et al., 2013; Wang, 1999). Additionally, a considerable body of research, including from our own lab, has found that systemic or PFC-specific blockade of NMDA receptors impairs WM (Arnsten & Jin, 2014; Davies et al., 2017; Hurtubise et al., 2016; Monaco, Gulchina, & Gao, 2015; Wang et al., 2013). NMDA receptors have been localized in the rat PPC using binding assays (Bean, Zheng, Patel, & Monaghan, 2006) demonstrating the presence of available AP5 binding sites within the PPC. Hence, it is unlikely that the null effect in our experiment is the result of our drug treatment lacking a pharmacological target. Additionally, we used a high dose of AP5 (Davies, Hurtubise, et al., 2017). These results are similar to previous findings using a step-down inhibitory avoidance task that showed NMDA receptors in the PPC not to be necessary for "short-term" memory, but rather to become engaged after longer intervals following training (Izquierdo et al., 1998, 1997).

4.6. Conclusions

The present experiment showed that inactivation of the PPC and intra-PPC AMPA/Kainate blockade impaired performance in the TUNL task, a visuospatial WM task for rodents. In contrast, blockade of NMDA receptors in the PPC with AP5 did not impair TUNL,

whereas CNQX infusions impaired performance, suggesting that, unlike in the PFC, WM function in the PPC relies only on non-NMDA ionotropic glutamate receptors. The results may have implications for models of WM involving NMDA receptors as a fundamental mechanism for maintaining stimulus representations over a delay (Wang et al., 2013; Wang, 1999). These findings, along with previous research (Izquierdo et al., 1998, 1997), suggest that this involvement of NMDA receptors in WM does not extend to all areas of the cortex, and that NMDA receptor function in the PPC is not necessary to support visuospatial WM.

5. GENERAL DISCUSSION

The set of experiments described in this dissertation contribute several general findings to the circuit mapping of two DNMTS tasks, the OST and TUNL, which are behavioural assays of WM in rodents. In the first experiment (Chapter 2), I showed that the mPFC and mdThal are both necessary for performance of the OST. Although my experiment was not able to conclusively show that it was the connections between the structures that were necessary, the results certainly show an independent involvement of the two structures at the very least. This circuit is critical for other WM tasks, but no data has been generated for the OST and previous work disrupting the mdThal has yielded mixed results with respect to olfactory learning and WM in general. Furthermore, I provided novel analyses of foraging behaviour and error position effects in the OST which are the first such analysis of the OST to my knowledge. This analysis showed no change in the position of errors but demonstrated a dissociation in the roles of the mdThal and mPFC in foraging behaviour in which the mdThal mediates exploratory motor behaviour during foraging consistent with previous research. In the second set of experiments (Chapter 3), I showed that performance of the OST is independent of the rat PPC. In the third set of experiments (Chapter 4), I showed that, unlike the OST, performance of TUNL critically relies on the PPC. I also showed that, within the PPC, NMDA receptors were unnecessary for TUNL performance whereas AMPA/Kainate receptors were necessary. This series of experiments advances the current understanding of the thalamocortical and frontoparietal correlates of the OST and TUNL. Overall, the results demonstrate that thalamocortical and frontoparietal pathways play dissociable roles in the OST and TUNL tasks.

5.1. Thalamocortical Circuitry in the OST

The results of Chapter 2 confirm that both the mPFC and mdThal are necessary for performance of the OST. Unfortunately, the failure of my ipsilateral control infusions makes it impossible to conclude purely on the basis of the present results that *connections* between the two structures are what mediated performance. However, the results are very consistent with previous research showing that these connections mediate WM performance (Bolkan et al., 2017; Floresco et al., 1999; Sebastien Parnaudeau et al., 2013).

Combined with previous research, there seems to be good evidence that this pathway is necessary across DNMTS tasks using different sensory modalities. However, a previous criticism of research on this pathway is that disruptions cause non-mnemonic impairments including delay-independent reductions in accuracy (Wolff et al., 2015). The results of my

experiment with the OST fall into this category. I observed significant changes in foraging behaviour which correlated with memory performance. Thus, the impairments in the OST that I observed in the OST following inactivation of the mdThal or disconnection of the mPFC and mdThal are partially explained by non-mnemonic aspects of the task.

A previous model has proposed that the mPFC-mdThal circuit mediates the "executive" aspects of task performance, whereas mnemonic aspects of task performance are mediated by a circuit including the anterior thalamic nuclei and hippocampus (Wolff et al., 2015). Application of this model to the OST is complicated by the fact that the OST is independent of the HPC (Dudchenko et al., 2000). Future research will be required to elucidate which circuits formed with the mPFC mediate the mnemonic aspects of the OST or if such a pathway is even fully dissociable from the executive/foraging components of the OST.

The results highlight the importance of conducting thorough and sophisticated analyses of behaviour in neuroscientific research. Rodent behavioural tasks involve many components including mnemonic, attentional, motivational, and motor aspects of behaviour. A simplistic analysis of "memory capacity" fails to capture these various aspects and can erroneously consign a neural circuit to memory function without considering these other aspects of behaviour.

5.2. Frontoparietal Circuitry in the OST and TUNL

Over the course of Chapters 3 and 4, I showed a clear dissociation between the OST and TUNL with respect to the role of the PPC. The PPC is not necessary for performance of the OST. In contrast, performance of TUNL critically relies on intact PPC function. This general finding suggests that frontoparietal circuitry does not universally mediate WM function. It also begs an explanation for precisely what difference between these tasks explains the dissociation. Several possible explanations are discussed below.

5.2.1. Differences Between the Cognitive Demands of the OST and TUNL

There is a stark dissociation between the OST and TUNL with respect to the role of the PPC and several differences between the tasks may explain this. Perhaps the most obvious is the particular sensory modality engaged by each task. The TUNL task engages the visuospatial modality(ies) whereas the OST engages olfaction. Hence, a straightforward interpretation of this pattern of results is that the PPC serves a sensory role specific to visual/spatial stimuli and the OST simply doesn't engage this modality. Such an interpretation is well-supported by a large

body of literature locating the PPC in the dorsal visual stream which has been referred to as the "where" pathway (Cooper & O'Sullivan, 2016; Ungerleider & Mishkin, 1982). However, the PPC is at times treated by prominent theorists as a domain-general "executive" brain structure in WM (Eriksson et al., 2015). My own results with PPC inactivation in CMOR support the view of the PPC as being involved in multisensory integration (Winters & Reid, 2010). Indeed, the primate PPC, or at least subregions within it, does appear to be engaged in WM across a variety of stimuli including non-spatial (e.g. phonological) stimuli (Vallar & Baddeley, 1984). My findings are strong evidence that, at least in the rodent, the role of the PPC is modality specific. However, the OST and TUNL differ in other important ways such that the sensory modality is far from the only difference between the tasks that could be relevant to which neurocircuitry underlies them.

For the most part, the rodent PPC appears to be involved in WM tasks with a spatial navigation component. Lesions or temporary inactivation such as a multiple water T-maze (McDaniel et al., 1994), a memory-guided cued virtual T-maze (Harvey, Coen, & Tank, 2012), and the radial arm maze (Espina-Marchant et al., 2006). In contrast, the PPC may not be necessary for WM involving objects as evidenced by the lack of an impairment in object delayed non-match-to-sample following PPC lesions (Kolb et al., 1994). Akrami, Kopec, Diamond, & Brody (2018) optogenetically silenced the PPC during an auditory WM task in which rats were presented with two tones sequentially and, after a delay, were required to respond via a nose-poke to indicate which tone was louder. Surprisingly, silencing of the PPC *improved* performance. Combined, these findings paint an inconsistent picture of the effects of PPC disruption on WM tasks that appears to differ by sensory modality or presence of spatial information.

However, an additional way in which the tasks differ is with respect to pattern separation. Pattern separation was not specifically manipulated in OST and I performed no experiments to measure the distinctiveness of the odours. In contrast, TUNL provides a precise means to manipulate the distinctiveness of visuospatial patterns (Talpos et al., 2010). A closely related point is that rats' olfactory sense is simply far better than their vision (Burn, 2008). Hence, there may be differences in the difficulty of pattern separation between the two tasks. Combined, these aspects of rat perception and the nature of these two tasks might mean that rats' performance on

the OST is simply more robust because the stimuli in the OST are easier to perceive and encode with high precision and less difficult to remember.

Another potential difference between the OST and TUNL is the arguably differential amount of intertrial interference. In the OST, rats perform a maximum of 3 trials during a test day with none of the odour stimuli being reused on that day. During a TUNL test session, rats perform dozens of trials and the same locations of test stimuli likely reappear in multiple trials. Few studies in rodents have intentionally manipulated intertrial interference during WM (Dudchenko et al., 2013). Likewise, I unfortunately did not intentionally manipulate intertrial interference during any of my experiments. This could have been accomplished by reusing odour stimuli over multiple trials in the OST. There is also evidence that percent accuracy in olfactory DNMTS is reduced if the size of the set of odours used is reduced (Otto & Eichenbaum, 1992). In the TUNL task, interference could be induced by shortening the intertrial interval. As such, there may be an intrinsic difference between the versions of OST and TUNL that I used such that this aspect of behaviour may have been less challenging during OST and TUNL. Hence, I cannot rule out that an OST impairment following PPC inactivation could have been achieved by introducing greater intertrial interference.

5.2.2. Role of Glutamate Receptors within Frontoparietal Circuitry

In Chapter 4, I showed that TUNL performance does not rely on NMDA receptors in the PPC. Based on previous results from our lab blocking NMDA receptors in the mPFC during TUNL (Davies, Hurtubise, et al., 2017) and my results blocking NMDA receptors in the PPC (Scott, Roebuck, Greba, & Howland, 2019) in appears that the frontoparietal circuit only requires intact NMDA receptor signalling within the mPFC in order to effectively mediate TUNL performance. These results place constraints on some influential synaptic theories of WM function in that this physiological mechanism does not apply to the PPC (Murray et al., 2017; Wang et al., 2013; Wang, 1999). Additionally, some activity-silent synaptic theories of WM that are NMDA receptor dependent are contradicted by my results. For instance, a recent model proposes that a population of PFC neurons alters their connectivity through NMDA receptor-dependent Hebbian synaptic weight changes with neurons in posterior cortices to select and maintain WM information (Manohar, Zokaei, Fallon, Vogels, & Husain, 2019).

5.3. The Dissociable Roles of Corticostriatal, Thalamocortical, and Frontoparietal Circuitry in Rodent Working Memory

The data I have presented add to our knowledge about the respective roles of corticostriatal, thalamocortical, and frontoparietal pathways in the rodent brain during performance of WM tasks. Based on previous findings as well as my own findings, the corticostriatal and thalamocortical pathways mediate WM across sensory modalities. Previous experiments conducted in our lab have shown the connections between the mPFC and dmSTR are necessary for both the OST (Davies, Greba, et al., 2017) and TUNL (Davies, Hurtubise, et al., 2017). Similarly, connections between the mPFC and mdThal mediate WM for spatial/navigational information (Bolkan et al., 2017; Floresco et al., 1999) and auditory information (Schmitt et al., 2017). Combined with the results I present in Chapter 2, the overall role of this pathway also appears to be sensory modality-general. However, I also found evidence of profound impairments in foraging behaviour which correlated with memory performance. Hence, it is possible that the thalamortical pathway between the mPFC and mdThal is mediating executive or goal-oriented aspects of task-related cognition rather than memory representations *per se* (Wolff et al., 2015).

Based on the findings I present in Chapters 3 and 4, as well as previous research, the combined evidence points to the frontoparietal pathway as having a more sensory modality-specific role in WM. Notwithstanding the other potentially consequential differences between the OST and TUNL other than sensory modality, my results indicate that the PPC may be involved in visuospatial, but not olfactory WM, although my results may also be explained by other important differences between the OST and TUNL tasks including differential degrees of interference and pattern separation. Additionally, there were minimal non-mnemonic effects of PPC inactivation on TUNL performance and the effects of AMPA blockade were completely specific to memory accuracy. Thus, the frontoparietal pathway appears to play a more memory-specific role in rodent WM tasks than the thalamocortical pathway between the mPFC and mdThal.

5.4. Limitations and Future Directions

5.4.1. Validity and Translatability of Rodent WM Tasks

As I discuss in Chapter 1, WM is defined differently in rodents than in humans because of the inaccessibility of a rodent's internal subjective state. A psychological construct such as WM can only be inferred from behaviour. Therefore, it is reasonable to critically question the validity of rodent "WM" tasks as they pertain to the human psychological construct by the same name.

The OST is currently accepted as a valid test of WM in rodents (Dudchenko, 2004; Dudchenko et al., 2013). Over the course of OST shaping and training, rats gain extensive familiarity with the odour stimuli through repeated exposure across training days, meaning that novelty alone cannot mediate task performance. Additionally, every single odour is likely to be both baited and unbaited multiple times over the course of training. Therefore, it is only the trial-unique information about which odour in a sequence has already been visited that can mediate task performance. These attributes satisfy the operational definition of rodent WM in that olfactory information is useful only within trials and not between trials (Dudchenko, 2004). Additionally, the OST has high ecological validity in that it mimics natural foraging behaviour and relies on olfaction, the preferential sensory modality for rodents (Burn, 2008). However, the OST also has attributes that do not fit well within traditional conceptions of WM. Rats typically achieve span lengths of ~8-12 odours (Davies, Molder, et al., 2013; Murray et al., 2017) which is considerably larger than the typical WM capacity of ~4 items (Cowan, 2000). Moreover, this capacity can be increased to dozens of odours if distractors are minimized (April et al., 2013). Therefor, the OST is ecologically relevant and satisfies some of the characteristics of WM, but rats typically have much higher memory capacity in this task than would be expected of human WM.

Modern touchscreen-based behaviour testing confers a number of advantages. These systems are extremely high-throughput and require little interaction between experimenters and animals, allowing the rapid acquisition of large amounts of behavioural data with high consistency (Hvoslef-Eide, Mar, et al., 2015). The behaviour paradigms are also directly based off human neuropsychiatric tests, allowing preclinical testing of the same cognitive domains in rodents as in humans. Ostensibly, this approach maximizes the construct validity of rodent models (Hvoslef-Eide, Mar, et al., 2015). However, there is a tension here between construct

validity and ecological validity. As previously discussed, rodents rely far more on their olfactory sense (Burn, 2008), whereas touchscreen-based testing is all conducted in the visual (and to a small extent, auditory) modalities. Investigation of cognition using ecologically-relevant behavioural tasks is crucial for understanding the effects of various perturbations of the brain (Gerlai & Clayton, 1999; Kingstone, Smilek, & Eastwood, 2008). Additionally, perturbations of WM circuitry can differentially affect performance based on the modality that is engaged, as I have shown.

5.4.2. The Lesion Method

Throughout the experiments presented in this dissertation, my sole method for perturbing neural activity was the use of temporary pharmacological inactivation. This method has several advantages. The effect on brain function is rapid, avoiding any compensatory changes in the targeted circuits that may be of concern when using irreversible lesions. This method is also reversible, allowing the use of within-subjects experimental designs. Perhaps what remains the most important capability of intracranial infusion is that it is the only available method to disrupt a wide array of neurotransmitter systems reversibly and in a region-specific manner. As such, temporary inactivation and related pharmacological manipulations are still well-suited for use in systems-level neuroscience research of the kind I have conducted (Vaidya, Pujara, Petrides, Murray, & Fellows, 2019). However, possible inferences made from this methodology are constrained by a number of factors.

5.4.2.1. Region Specificity

A primary and obvious concern is that quantifying and controlling the spread of the drug infusion so that it both encompasses and is confined by the boundaries of a target region is difficult. I used previously published estimates of the spread of a given volume of infusate to calibrate my drug infusions. Although this approach is routinely accepted as sufficient, it leaves some obvious uncertainty as to the exact effect of a given infusion when one considers individual differences in brain volume or infusion quality. Various methods have been used to quantify the spread of infusions. A relatively common method is the use of an injected dye (Gaskin & White, 2010; Izquierdo et al., 1997) or a drug (e.g. muscimol) conjugated with a fluorescent and radioactive marker to allow imaging of the real spread of the drug (Davies, Greba, et al., 2017). This method allows the direct and objective quantification of tissue that contained the drug;

however, this is distinct from quantification of the disruption of neuronal activity per se, which could vary based on local concentrations of the drug as a function of the distance from the infusion site. Other methods to directly quantify the direct effect of infusions on neuronal activity include simultaneous electrophysiological recordings (Klement et al., 2005). This method certainly allows the immediate and temporally-precise detection of infusion-related changes in neuronal activity, but the measured changes may result from downstream effects in tissue not directly contacted by the drug.

Targeting brain regions with lesions or temporary inactivation are crucial for establishing a causal relationship between brain activity and behaviour. Recordings or imaging of brain activity can provide insight into which brain areas are active during a task, and task-relevant information can often be decoded from brain activity (Christophel et al., 2012). This information alone, however, is only correlational and does not distinguish between necessary and sufficient brain activity during a task. Conversely, lesion methods alone provide causal information, but comparatively less detail about precisely *how* a brain region might be involved in a task because it is limited essentially by the number of dissociable independent variables present in the behavioural analysis.

To relate this to my experiments, this leaves open questions regarding exactly what information related to WM is actually being represented within the PPC. Currently, there are no published studies that examined neuronal activity in the PPC during the TUNL task. However, a considerable literature has examined how activity in the rodent PPC is related to evidence accumulation during decision making. Several papers have demonstrated that as animals encounter sensory evidence to guide a decision, neurons in the PPC exhibit a "ramping up" of their firing rate (Hanks et al., 2015). A recent paper by Harvey, Coen, & Tank (2012) imaged activity in the mouse PPC during a virtual spatial navigation task that involved WM. The authors found that the neurons in the PPC exhibited choice-specific sequences of firing. The role of the PPC in TUNL may be essentially related to its role in evidence accumulation during decision making, including memory-guided decision making.

5.4.2.2. Temporal Specificity

As I repeatedly discuss in my data chapters, a fundamental limitation of the research I present here is that none of my manipulations are temporally-specific. The pharmacological approach used throughout my experiments disrupts its targets for the entire duration of the

behavioural tasks. This is especially limiting in the research of WM which is a construct that manifests over much shorter timescales than long-term memory. Working memory tasks are typically comprised by several temporal epochs including initial encoding, maintenance (as well as manipulation and updating) over a delay, and then retrieval. Although lesions, temporary inactivation, and pharmacological manipulations can allow inferences about the importance of brain areas or neurotransmitter systems over the entire course of a WM task, they stop short of being able to dissociate the temporal epochs of WM tasks. Previous research using optogenetics in WM task have demonstrated that, in spite of the evidence of the role of the mPFC in WM, the mPFC is not necessary for delay period maintenance of WM in well-trained animals (Liu et al., 2014).

5.4.2.3. Cell-Type Specificity

The lesion method is also incapable of dissociating the relative contributions of different neuronal subtypes to cognition. This is particularly relevant to WM research given the computational models of WM function involving specific roles of excitatory versus inhibitory neurons (Fiebig & Lansner, 2017). Moreover, some research using optogenetics has found differential roles of somatostatin and parvalbumin interneurons (Kim et al., 2016).

5.4.3. Potential Future Experiments

In Chapter 4, I show that the PPC is critical for performance of TUNL (Scott et al., 2019). Additionally, previous experiments performed in our lab show that the mPFC is also critical for TUNL performance (Davies, Hurtubise, et al., 2017). Although I did not specifically investigate the role of connections between the mPFC and PPC, there is a very high likelihood that disrupting these connections would also impair TUNL. However, an interesting future question pertains to exactly which connections between these structures are most critical for mediating different aspects of task performance. The PFC and PPC communicate through cortico-cortical connections via the superior longitudinal fasciculus (Klingberg, 2006). In the rat, there is specific evidence of projections to the medial agranular, ventral orbital, and medial orbital regions of the PFC (Chandler, King, Corwin, & Reep, 1992).

There are also indirect connections between these structures via thalamic nuclei. Specifically, the PPC is connected with the lateral dorsal, lateral posterior, and posterior thalamic nuclei (Reep, Chandler, King, & Corwin, 1994). Similar to thalamocortical connections between

the mdThal and mPFC, a number of interesting experiments could be directed at understanding the roles of thalamocortical connections between the PPC and these thalamic nuclei in the performance of the TUNL task. Ekman, Fiebach, Melzer, Tittgemeyer, & Derrfuss (2016) performed diffusion MRI in human subjects and correlated the integrity of white matter tracts connecting lateral PFC and PPC with WM capacity. They found that maintaining WM items without updating or manipulation only correlated with the density of corticocortical connections, whereas a task requiring maintenance and updating of WM was correlated with corticocortical connections as well as a subcortical pathway involving the striatum and thalamus. Although these findings point to an interesting functional dissociation between "direct" and "indirect" frontoparietal pathways in WM, the results of this study are only correlational. Following from the results of Ekman et al. (2016), an interesting follow up study would entail attempting to recapitulate this functional dissociation in the TUNL task using selective opto- or chemogenetic inhibition of corticocortical vs subcortical connections between the mPFC and PPC.

5.5. Final Conclusions

In the set of experiments presented in this dissertation, I expanded on the current state of circuit mapping of rodent WM tasks and particularly the roles of thalamocortical and frontoparietal networks. With respect to the role of thalamocortical connections between the mPFC and mdThal, I showed that these areas, and possibly the circuit connecting them, are necessary for olfactory WM despite previous contradictory reports in the role of the mdThal in olfactory learning and memory. However, these results may also be partly explained by impairments in the non-mnemonic foraging aspects of the OST. I have demonstrated that the PPC is necessary for TUNL but not the OST. This difference may be explained by the different sensory modalities involved, both by virtue of which sensory modalities the PPC processes as well as the fact that rats have a more robust olfactory sense, as well as differences between the tasks in the difficulty of pattern separation and differences in the amount of intertrial interference. Additionally, I showed that WM function does not depend on NMDA receptors in the PPC. This result is significant because of the extensive research emphasizing the importance of NMDA receptor signalling in WM. Overall, the results add to the general picture that there are dissociable contributions of corticostriatal, thalamocortical, and frontoparietal pathways in rodent WM with respect to sensory modality and the involvement of NMDA receptors.

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